Abnormal phospholipids distribution in the prefrontal cortex from a patient with schizophrenia revealed by matrix-assisted laser desorption/ionization imaging mass spectrometry

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Abstract Schizophrenia is one of the major psychiatric disorders, and lipids have focused on the important roles in this disorder. In fact, lipids related to various functions in the brain. Previous studies have indicated that phospholipids, particularly ones containing polyunsaturated fatty acyl residues, are deficient in postmortem brains from patients with schizophrenia. However, due to the difficulties in handling human postmortem brains, particularly the large size and complex structures of the human brain, there is little agreement regarding the qualitative and quantitative abnormalities of phospholipids in brains from patients with schizophrenia, particularly if corresponding brain regions are not used. In this study, to overcome these problems, we employed matrix-assisted laser desorption/ionization imaging mass spectrometry (IMS), enabling direct microregion analysis of phospholipids in the postmortem brain of a patient with schizophrenia via brain sections prepared on glass slides. With integration of traditional histochemical examination, we could analyze regions of interest in the brain at the micrometric level. We found abnormal phospholipid distributions within internal brain structures, namely, the frontal cortex and occipital cortex. IMS revealed abnormal distributions of phosphatidylethanolamine molecular species particularly in the cortical layer of frontal cortex region. In addition, the combined use of liquid chromatography/electrospray ionization tandem mass spectrometry strengthened the capability for identification of numerous lipid molecular species. Our results are expected to further elucidate various metabolic processes in the neural system.

Keywords Schizophrenia · Postmortem brain · Imaging mass spectrometry (IMS) · Matrix-assisted laser desorption/ionization (MALDI) · Phospholipids

Introduction

Schizophrenia is one of the major psychiatric disorders, producing symptoms such as hallucinations and delusions...
Despite considerable worldwide research for over a century, the etiology of this illness remains incompletely understood. There are several hypotheses regarding the cause of schizophrenia, and a widely accepted one is the neurotransmitter hypothesis, which involves abnormal synaptic functions due to defects in neurotransmitter regulation, including their production, release, reuptake, and reception by neurotransmitter receptors [2]. Since the neuronal membrane is an important structural and functional site for the receptors, identification of neural lipid abnormalities in postmortem brains of schizophrenia patients has been also intensively pursued.

In the central nervous system, phospholipids are the most abundant lipids. They comprise a molecular family in which phosphoric acid is present in the ester form. Some classes of phospholipids are structurally distinct based on the head group linked to the phosphate attached at the sn-3 position of the glycerol backbone, such as phosphatidycholines (PCs) and phosphatidylinositols (PIs). Depending on the compositions of the two fatty acyl residues linked at the sn-1 and sn-2 positions, phospholipids of the same class are further subdivided into molecular species. In particular, regarding stored fatty acyl residues in the phospholipids, polyunsaturated fatty acyl residues have been paid special attention in schizophrenia research because they are essential to maintaining normal membrane structure and function as well as to normal brain development [3]. Two types of PUFAs, the n-6 and n-3 series, primarily arachidonic acid [arachidonate, 20:4(n-6)] and docosahexaenoic acid [DHA, 22:6(n-3)], are designated by the location of the first double bond from the methyl group (see also Electronic Supplementary Material Fig. S1).

So far, the combined use of thin-layer chromatography (TLC) and gas chromatography (GC) has been widely adapted for analyzing lipids in schizophrenia research, which allows the separation of each phospholipid class by TLC, and then quantifying the fatty acid composition of each phospholipid class by GC. In early researches, Horrobin et al. reported defects in the fatty acid composition of phosphatidylethanolamines (PEs), particularly in the frontal cortex of patients with schizophrenia [4]. More recently, Schmitt et al. [5] reported decreased PCs and sphingomyelin (SMs) levels and increased phosphatidylserine (PS) levels in the thalamus. Further, McNamara et al. reported decreased DHA and vaccenic acid levels in the orbitofrontal cortex [6, 7]. Mass spectrometry (MS) has been widely adopted in relatively recent schizophrenia research. By using LC-MS, Schwarz et al. reported that PCs and free fatty acids are increased in the frontal cortex of a schizophrenia patient's brain [8].

Despite such intensive studies, however, considerable disagreements exist regarding the qualitative and quantitative abnormalities of lipids in the brains from patients with schizophrenia. For example, Shimon et al. revealed that PI levels are decreased in the postmortem brain of patients [9]; however, Shapiro found no difference in the PI amount by the same method [10]. Hamazaki et al. reported that n-3 fatty acids such as DHA were not remarkably altered but that n-6 fatty acids, such as arachidonic acid, were decreased in the hippocampus of brains from schizophrenia and bipolar disorder patients [11]. On the other hand, a study focusing on the orbitofrontal area reported a 20% decrease in DHA in schizophrenia patients [6]. Although several well-recognized reasons lead to variations in the experimental results of postmortem human samples, i.e., ethnogenesis, personal drug and disease history, and postmortem time, the above-mentioned cases clearly indicate the importance of controlling experiments by using strictly corresponding brain regions. In fact, current lipid studies, including those by our group, demonstrate that brain lipids are distributed in a heterogeneous pattern even within small brain regions [12–14]: therefore, careful and precise dissection techniques are required in such studies, particularly when employing traditional biochemical methods.

Here, we utilized matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) for regional analysis of lipids on brain tissue sections. In this emerging imaging technology, tissue sections were directly scanned by MALDI laser, and mass spectra were recorded for each data point. Based on the ion intensities obtained from each tissue location, two-dimensional distribution maps for multiple lipids could be simultaneously obtained through one measurement [15]. The current spatial resolution of IMS, as defined by laser diameter and accuracy of sample stage movement [16], could examine tissues at micrometric level [17]; we could also directly conduct lipid quantification from microregions on the postmortem brain sections. Furthermore, analyzed tissue sections could be histochemically stained; therefore, the obtained lipid distribution maps could be integrated to traditional histochemical images, thus precisely recognizing the part of brain being analyzed.

In this study, we revealed abnormal PC distributions within internal brain structures, namely, the frontal cortex and occipital cortex of a postmortem brain from a patient with schizophrenia. In addition, the combined use of the LC electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) system strengthened the capability of the technique for molecular identification.

**Materials and methods**

**Human brain** The human brain samples including prefrontal cortex and occipital cortex were obtained following the protocols approved by the Fukushima Medical University. The postmortem brain of a patient with schizophrenia was supplied by the Postmortem Brain Bank of Fukushima for
Psychiatric Research. This Brain Bank specializes in psychiatric diseases in Japan. The patient with schizophrenia was a 70-year-old male, and the postmortem interval was 18 h. An age- and sex-matched normal sample was obtained from the Choju Medical Institute, Fukushima Hospital, Toyohashi. The patient characteristics are indicated in Table 1. Informed written consent was obtained from the families of both subjects. The patient with schizophrenia fulfilled the diagnostic criteria established by the American Psychiatric Association (Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)).

**Chemicals** Methanol, potassium acetate, and ultra pure water were purchased from Wako Chemical (Osaka, Japan). Calibration standard peptide and 2,5-dihydroxybenzoic acid (DHB) were purchased from Bruker Daltonics (Leipzig, Germany). All the chemicals used in this study were of the highest purity available. 9-Aminoacridine was purchased from Acronics (Pittsburgh, PA, USA). 1-Palmitoyl-2-oleyl-sn-glycero-3-phosphate, PC (diacyl-16:0/18:1), was purchased from Funakoshi Co. Ltd. (Tokyo, Japan).

**Tissue section preparation for IMS and ESI-MS/MS** Tissues blocks were sectioned at −18 °C using a cryostat (CM 1950; Leica, Germany) to a thickness of 8 μm, as described in previous reports [18, 19]. Although brain blocks were held by an optimum cutting temperature (OCT) polymer, they were not embedded in it because it was considered that any residual polymer on the tissue slices might degrade the mass spectra [19]. The frozen sections were thaw-mounted on indium-tin-oxide (ITO)-coated glass slides (Bruker Daltonics). For LC/ESI-MS/MS, several sections were collected into glass vials for lipid extraction (approximately 10 mg for each), and total lipids were extracted by the Folch method [20].

**Sample preparation for LC/ESI-MS/MS** The ESI-MS/MS analysis was performed using a 4000Q-TRAP quadrupole linear ion trap hybrid mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) with an ACQUITY Ultra Performance Liquid Chromatography (LC; Waters, Milford, MA, USA).

A chromatographic method was developed using an ACQUITY UPLCTM BEH C18 column (2.1×50 mm i.d., 1.7-μm particle), fitted with an identically packed guard column (2.1×5 mm; Waters, Milford, MA, USA). The column oven was maintained at 40 °C. The following gradient elution with mobile phase A (acetoniitrile:methanol:water=19:19:2 v/v/v, containing 0.1% formic acid and 0.028% ammonia) and mobile phase B (isopropanol, 0.1% formic acid, and 0.028% ammonia) was used at a flow rate of 0.4 mL/min:0–10 min:5% B→5% B; 10–15 min:5% B→50% B; 15–20 min:50% B→50% B, and 20–25 min:5% B.

**Precursor ion and neutral loss scanning for specific phospholipid classes** To identify the specific phospholipid classes, precursor ion scanning of the polar head group of PCs and SMs (m/z184), and neutral loss scan of that of PE (m/z141) were performed using the 4000Q-TRAP instrument; these methods are effective for detecting PCs because characteristic fragment ions are generated by collision-induced dissociation (CID) [21]. The level of collision energy is very important to the sensitive identification of focused molecules; therefore, optimal conditions for detecting the appropriate precursor ion and neutral loss were determined by preliminarily analyzing PC (diacyl-16:0/18:1) as standard lipid.

**Spray coating of the matrix solution for IMS** A DHB solution (40 mg/mL DHB, 20 mM potassium acetate, 70% MetOH, 0.1% TFA) was used as the matrix solution for imaging the PCs. Here, 9-aminoacridine (10 mg/mL, dissolved in 70% methanol) was used for imaging the PEs. The matrix solution was sprayed over the tissue surface using a 0.2-mm nozzle caliber airbrush (Procon Boy FWA Platinum; Mr. Hobby, Tokyo, Japan). Tissue sections that were to be compared were simultaneously spray-coated with each matrix solution to equalize analyte extraction and co-crystallization conditions. The distance between the nozzle tip and the tissue surface was 10 cm, and the spraying period was fixed at 5 min. Approximately 100 μL of matrix solution was sprayed onto each brain section.

**IMS conditions (single MS imaging)** Single MS imaging was performed using a MALDI TOF/TOF-type instrument (Ultraflex 2 TOF/TOF; Bruker Daltonics). This instrument was equipped with a 355-nm Nd:YAG laser. The data were acquired in the positive reflectron mode under an acceler-

|                      | Schizophrenia       | Normal control                  |
|----------------------|---------------------|---------------------------------|
| Age at death         | 74                  | 73                              |
| Gender               | Male                | Male                            |
| Cause of death       | Pneumonia and heart failure | Pneumonia and acute myocardial infarction |
| Brain hemisphere     | Left                | Left                            |
| Postmortem interval  | 18 h                | 2 h                             |

Table 1 Demographic information for subject with schizophrenia and matched control.
ating potential of 20 kV using an external calibration method. Signals between m/z 400 and 1,000 were collected. Raster scans on tissue surfaces were performed automatically using FlexControl and FlexImaging 2.0 software (Bruker Daltonics). The number of laser irradiations was 200 shots in each spot. Image reconstruction was performed using FlexImaging 2.0 software.

**MS/MS and MS/MS imaging** Molecular identification was performed with LTQ-XL (Thermo Fisher Scientific) equipped with an intermediate-pressure MALDI ion source. Since the ions corresponding to PEs contain multiple ions within the same nominal mass, we performed MS/MS imaging with LTQ-XL at a raster scan pitch of 40 μm in negative ion detection mode. Image reconstruction was performed using Image Quest (Thermo Fisher Scientific).

**Data analysis of IMS** We extracted the spectra in about 200 data point from gray matter by FlexImaging 2.0 software (Bruker Daltonics), and the intensities of assigned mass peaks were quantitated by FlexAnalysis 3.0 software (Bruker Daltonics). To relatively quantitative analyses, the mean intensity ratio of schizophrenia/normal of each assigned peaks was calculated in the frontal cortex and occipital cortex, and p value was calculated by t test using relative intensities of each data point in gray matter region to compare mean intensity ratio between schizophrenia and normal brain. Statistical differences between the data from the patient with schizophrenia and the control subject were assessed using an unpaired Student's t test. p<0.01 was considered to be significant.

**Results and discussion**

**Experimental scheme**

Figure 1 shows the experimental scheme employed in this study. In hospitals, including brain bank-network institutes, because of the large size and complex structure of human brains, dissected postmortem brains are generally sectioned into several blocks and then stored at −80 °C until further use. In this study, we focused upon the frontal and occipital parts of the cerebral cortex, particularly because the frontal cortex region is known to exhibit apparent anatomical defects in the brains of schizophrenia patients [22, 23]. Frozen brain blocks were thinly sliced, and for LC/ESI-MS/MS, several sections were collected into glass vials for lipid extraction. Further, few successive sections were thaw-mounted on the ITO-coated glass slides for the MALDI imaging experiment. Total lipids were extracted from the collected tissues in the vials, and molecular species compositions of specific phospholipid classes were selectively identified by MS/MS scans, provided by LC/ESI-MS/MS. Next, MALDI-IMS was performed on the successive tissue sections, and ion peaks corresponding to the pre-identified lipid molecular species by LC/ESI-MS/MS were selected. Since mass spectra obtained by imaging have spatial information, in the next step, we analyzed only spectra from corresponding brain regions of the normal control and patient, although the shapes of the dissected brain blocks differed considerably. Using mass spectra extracted only from the frontal and occipital cortex regions, we identified lipid species whose detected ion intensities changed between the control and diseased brains. Finally,
for the identified lipid species with abnormal expression amounts in the patient's brain, we validated them as
designated lipid species by MS/MS of the tissue section.

Identification of individual molecular species of PCs, SMs,
and PEs by LC/ESI-MS/MS

Before conducting MALDI-IMS experiments, we selectively
identified the composition of lipid molecular species, namely,
PCs, SMs, and diacyl-PEs in the total lipid extract from
normal human brain using LC/ESI-MS/MS. By precursor ion
scanning of the choline containing head group, we pre-
identified PC and SM molecular species; diacyl-PE molecular
species were identified by neutral loss scan of the phospho-
ethanolamine containing head group (Fig. 2). Such pre-
elucidation of the targeted phospholipid molecular species
was helpful in the IMS data interpretation described later.
Since IMS experiments directly subject tissue section samples that are complex mixtures of numerous biological
molecules, mass spectra obtained by IMS comprised hundreds of peaks derived from biological molecules as well as matrix clusters. In MALDI-IMS, molecular identification of such ions one by one is a time-consuming process.
In this study, by pre-identifying the existing lipid molecular species by LC/ESI-MS/MS, we could pre-define the target
mass peaks in the following IMS data analysis. This would be particularly important for a study with samples from
human patients because available sample amounts are severely limited.

For the identification of phospholipids containing the
choline head group, such as PC, lysoPC, and SM species, a
specific fragment at \( m/z \)184 (phosphorylcholine) is known
and has been used as a specific target in identifying choline-containing phospholipids using product ion and
precursor ion scanning methods [24–27]. Figure 2a shows a
two-dimensional (2D) abundance map of PC and SM
molecular species from the human brain obtained by LC/
ESI-MS using the C18 reverse-phase column in the positive
ion detection mode. The 2D map has the \( m/z \) value along
the vertical axis and the retention time along the horizontal
axis. The total ion chromatogram and averaged mass
spectra are shown on the upper and left sides of the 2D
map, respectively. When using a reverse-phase column,
PCs and SMs elute in order from the hydrophilic to the
more hydrophobic species. The length of fatty acyl chains
mainly influences the elution order, and the number of
double bonds in fatty acyl chains also influences the elution
order. Based on the \( m/z \) value and relative retention time
and previous reports [28, 29], we assigned 17 PC molecular
species and three SM species as abundant PC and SM
molecules in the human brain (Fig. 2b). In the same way,
we also identified 11 diacyl-PE species by neutral loss
scanning of \( m/z \)141 (phosphoethanolamine head group),
also in positive ion detection mode (Fig. 2c, d). One thing
we have to note is that plasmalogen-type PE were less
detected by this ESI-MS/MS method (J Am Soc Mass
Spectrom 15(10):[36]). Having obtained qualitative data
regarding molecular identification, we next proceeded to
MALDI-IMS of the normal control and schizophrenia brains.

Microregion analysis of phospholipid molecular species
in the brain sections

Next, we proceeded to microregion analysis of lipids in the
brain sections from the control subject and the patient with
schizophrenia. First, we performed MALDI-IMS of both
brain sections in the positive ion detection mode. We
employed an optimized experimental condition for PC and
SM detection including composition of matrix solution [14]
in order to selectively analyze PC and SM species. In
particular, potassium salts added to the matrix solution
caused merging of various lipid adducts (adducts with
proton, sodium, and potassium) into one single potassiated
species, thus making IMS data interpretation fairly easy
[30]. After the IMS measurement, applied matrix crystals
were removed by immersing the slides into methanol, and
the tissue sections were stained with hematoxylin and eosin
(HE). Stained sections were then observed by light
microscopy, and based on the obtained microscopic image,
we defined the cerebral cortex region of both sections
(Fig. 3a) and applied them to the IMS data set.

Because of the low availability of postmortem human
brain, it is generally difficult to acquire tissue blocks
containing complete corresponding brain regions. In this
case, the control brain block contained only gray matter
regions, while the brain block from the schizophrenia
patient contained both gray and white matter. We extracted
data only from the corresponding brain areas by the
imaging-based analysis. We defined regions of interest
(ROI) on the frontal and occipital lobes, according to the
microscopic images of HE-stained tissue sections. From the
defined regions of interest, approximately 200 spectra for
each were collected, and mass peaks were detected and then
their intensities were calculated. Electronic Supplementary
Material Fig. S2 shows the representative mass spectrum
obtained; from the numerous mass peaks detected, we
assigned 17 mass peaks as the pre-identified PC and SM
molecular species. Ion intensity values of the 17 ions from
the extracted spectra (800 spectra in total) were calculated
and compared between the control and patient samples.

The results are summarized in Table 2. In the table, the
green cells represent increase in the amount, while red
represents a reduced amount in the patient as compared to
the normal control. Table lines representing each lipid
species are arranged in order of the greatest increasing to
Fig. 2 Identification of individual molecular species of focused phospholipid classes by precursor ion and neutral loss scanning of their head groups in the positive ion mode. The extracted total lipid mixture from the control postmortem human brain was subjected to precursor ion scanning of $m/z$ 184 for detection of PCs and SMs (a, b) and neutral loss scan of $m/z$ 141 for detection of diacyl form PEs (c, d). The 2D map (a, c) has the $m/z$ value along the vertical axis and the retention time along the horizontal axis. The total ion chromatogram and averaged mass spectra were shown on the upper and left sides of the 2D map, respectively. From the 2D map, the relative ion counts of each PC, SM, and PE molecule species were calculated (The most intense species is shown as intensity at 1.) Detected PUFAs, namely, arachidonic acid, DHA, and docosatetraenoic acid, are shown as red, orange, and green characters, respectively.

(a) Precursor ion scan of $m/z$ 184

(b) Relative abundance ratio (Normalized the most abundant specie as 1)

(c) Neutral loss scan of $m/z$ 141

(d) Relative abundance ratio (Normalized the most abundant specie as 1)
decreasing trend in the frontal cortex of the patient. PC (diacyl-16:0/20:4) was the most increased species in the frontal cortex, while PC (diacyl-16:0/18:1) was the most reduced species in both the frontal and occipital cortex. Here, we particularly focused on the molecular species whose level was significantly altered in the frontal but not in the occipital cortex because such an abnormal pattern may reflect anatomical defects that may be attributed to schizophrenia [22, 23]; this molecule was identified as PC (diacyl-16:0/20:4).

In Fig. 3b, we have shown the distribution of the PCs. Ion images are arranged in the same order as in Table 2. These images demonstrated that each PC and SM molecular species have a rather heterogeneous distribution pattern even within cerebral cortex brain blocks, implicating the importance of precise tissue dissection when employing conventional biochemical analysis. On the other hand, taking advantage of the imaging analysis, we could adjust accurate corresponding regions for comparing differently shaped samples. In Fig. 4a, b, we plotted ion abundances of the PCs along with the distance from the cortical to the deep layer of the cerebral cortex. The graphs for PC (diacyl-16:0/20:4) and PC (diacyl-16:0/18:1) revealed that both—the increase in PC (diacyl-16:0/20:4) and the reduction in PC (diacyl-16:0/18:1)—in the patient were particularly observed in the cortical layer of the cortex (arrows). Regarding the PEs, since their ionization efficiency was relatively low in the positive ion detection mode when compared to the choline-containing lipids, we analyzed these in the negative detection mode using 9-aminoacridine as a matrix [31]. Furthermore, to achieve a high detection selectivity, we performed MS/MS imaging of the PE species for two abundant pre-identified species because PCs remain detectable as −CH3 ions if 9-aminoacridine is used as a matrix in the negative ion detection mode [32]. In fact, as Electronic Supplementary Material Fig. S3 shows, ions corresponding to PEs contained several ions other than PE, but we could successfully visualize PE (diacyl-18:0/22:6) and PE (diacyl-18:0/22:4) by visualizing ion transition in which specific fatty acyl residues were produced from their precursor ions (Fig. 4c and Electronic Supplementary Material Fig. S3), though significant alteration was not altered.

Finally, we validated the assignments of PC molecular species by tissue MS/MS, particularly for molecules demonstrating considerable alteration rates in Table 2. Figure 5 shows the product ion spectra for the ions corresponding to the six PC and SM molecules; in each spectrum, the presence of a choline containing head group...
and phosphate was confirmed. Neutral losses (NL) of 59 and 183 u from precursor ions, corresponding to trimethylamine and phosphocholine, respectively, were used as diagnostic ions in product ion mass spectra [33]. Furthermore, we confirmed detailed fatty acid constituents of each PC by replacing adducted alkali metal from potassium to lithium, by adding 20 mM of lithium acetate in the matrix solution [30]. MS/MS subjecting such lithium-adducted molecules provides increased acyl-chain loosed fragment ions; therefore, we could precisely identify the fatty acid moiety ([NL] of acyl-chain; see Electronic Supplementary Material Figs. S4 and S5). Overall, the six molecular species were identified as PC and SM molecules. On the other hand, the ion at m/z 848 was revealed to contain both PC (diacyl-18:0/20:4) and galactosylceramide, and therefore, its data was eliminated from this investigation (data not shown). We also note regarding lyso-phospholipids; we certainly detected the lyso-phospholipid species particularly of the lyso-PCs (LPCs) by both MALDI and ESI-MS. However, since their amounts are much smaller than diacyl-phospholipids, therefore, they are likely to be sensitive to postmortem degradation. Thus, we here omitted discussion on them.

### Table 2 Molecular species of PCs and SMs in order of the increased rate in the frontal cortex from the patient with schizophrenia as compared to the normal control, as revealed by MALDI-IMS

| Molecular weight | LC/ESI-MS/MS     | Rate of increase in frontal cortex compared to normal | Rate of increase in occipital cortex compared to normal |
|------------------|------------------|--------------------------------------------------------|--------------------------------------------------------|
|                  | Ratio            | p value                                                | Ratio                                                  |
| 820.53           | PC (diacyl-16:0/20:4) | 1.32 <0.01                                              | 1.09 <0.01                                             |
| 797.59           | SM (20:0)        | 1.26 <0.01                                              | 1.30 <0.01                                             |
| 744.49           | PC (diacyl-16:0/14:0) | 1.18 <0.01                                              | 1.13 <0.01                                             |
| 796.53           | PC (diacyl-16:0/18:2) | 1.12 <0.01                                              | 1.02 0.21                                              |
| 770.51           | PC (diacyl-16:0/16:1) | 1.10 <0.01                                              | 0.95 0.01                                              |
| 846.54           | PC (diacyl-18:1/20:4) | 1.08 <0.01                                              | 1.08 <0.01                                             |
| 772.53           | PC (diacyl-16:0/16:0) | 1.07 0.01                                               | 0.84 <0.01                                             |
| 844.53           | PC (diacyl-16:0/22:6) | 1.03 0.14                                               | 1.04 0.04                                              |
| 870.54           | PC (diacyl-18:1/22:6) | 1.00 0.95                                               | 1.27 <0.01                                             |
| 848.56           | PC (diacyl-18:0/20:4) | 0.99 0.57                                               | 0.77 <0.01                                             |
| 826.57           | PC (diacyl-18:0/18:1) | 0.98 0.39                                               | 0.93 <0.01                                             |
| 769.56           | SM (18:0)        | 0.98 0.30                                               | 0.82 <0.01                                             |
| 872.56           | PC (diacyl-18:0/22:6) | 0.97 0.04                                               | 1.13 <0.01                                             |
| 800.56           | PC (diacyl-16:0/18:0) | 0.97 0.06                                               | 0.86 <0.01                                             |
| 824.56           | PC (diacyl-18:1/18:1) | 0.94 <0.01                                              | 0.85 <0.01                                             |
| 822.54           | PC (diacyl-16:0/20:3) | 0.94 <0.01                                              | 0.94 <0.01                                             |
| 798.54           | PC (diacyl-16:0/18:1) | 0.86 <0.01                                              | 0.75 <0.01                                             |

These molecular species were identified by LC/ESI-MS/MS.

**Fig. 4** Regional analysis of PC and PE molecular species in the cerebral cortex of postmortem human brains. In-depth analyses of the most increased PCs (16:0/20:4) (a) and the most decreased one (16:0/18:1) (b), and PE (18:0/22:6) (c). Plots of ion abundances of the PCs and PE along with the distance from the cortical to the deep layer of the cerebral cortex, demonstrating that the cortical layer showed maximal changes in the lipid contents (arrows).
Lipid analysis linked to brain functional mapping in postmortem human brains

Through this study, we found that PC (diacyl-16:0/20:4) containing arachidonic acid was increased in the prefrontal cortex of the patient with schizophrenia; no such increase was found in the occipital cortex. As mentioned above, we particularly focused on the molecular species whose level showed a significant alteration in the frontal but not in the occipital cortex because such a defect pattern may reflect...
the anatomical defects attributable to schizophrenia [22, 23]. Our finding agrees with those of a previous study by McNamara et al. who found that among male schizophrenia patients, the arachidonic acid:DHA ratio was increased in the frontal cortex [6].

Our methodology in this study had the specific advantage of enabling small-region analysis of lipid molecular species along with spatial information. This could uncover abnormalities in local lipid metabolism within postmortem human brains. Each region of the human brain cortex is known to have heterogeneous functions, especially high-level ones, including social skills for communication [34]. Since schizophrenia patients exhibit abnormalities in such higher function, it is particularly important to link previous biochemical analyses—including lipid analyses—to brain functional mapping. We propose that our methodology would enable such evaluation. Although the present study is only a feasibility study, we are currently conducting large-scale case-control studies using postmortem brains provided by our brain bank network [35].

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