Research Paper

Development of targeted metabolomics for the determination of ornithine cycle compounds as possible biomarkers in cerebrospinal fluid regarding to Alzheimer's disease pathology using UHPLC-ESI-MS/MS

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Abstract An early diagnosis of Alzheimer's disease can lead to better and more targeted treatment and/or prevention for patients. Employing a pathological diagnosis by non-targeted metabolomics with ultra high performance liquid chromatography-electrospray tandem mass spectrometry, in our previous study, we found significant changes in polyamine metabolites arising from the ornithine cycle in human brain samples of patients with Alzheimer's disease. In this study, we develop a targeted metabolomics methodology for the separation and detection of compounds derived from the ornithine cycle in human cerebrospinal fluid samples. For the targeted metabolomics, two derivatization reagents (9-fluorenylmethyl chloroformate and 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole) are utilized for sensitive and accurate determination of amine analytes. Validation tests confirm a good linearity of $r^2 > 0.99$ or more in all calibration curves obtained using internal standards. The developed method is applied for the analysis of preliminary 15 metabolites including polyamine metabolites in cerebrospinal fluid samples classified according to brain autopsy Braak stages regarding to Alzheimer's disease and compared to a control samples. Utilizing the results obtained for the ornithine cycle, we expect to find a metabolic pathway that would be used as a potential biomarker in the future.

Key words: targeted metabolomics, liquid chromatography tandem mass spectrometry, Alzheimer's disease, ornithine cycle, cerebrospinal fluid

Introduction

Alzheimer's disease (AD) is a disorder that is seen frequently in the elderly and presents with symptoms of memory, orientation and behavioral disturbances. The classical hallmarks of AD are increased presence of senile plaques, composed mainly of amyloid β (Aβ), and abnormal phosphorylation of the tau protein in the brain tissue[1)]. The AD pathology has shown that accumulation of tau aggregates and deposition of Aβ cause neuronal cell death, resulting in damage to higher brain functions/process referred to as the "amyloid cascade hypothesis"[2)]. In recent years, clinical trials targeting new drugs to address the "amyloid cascade hypothesis" such as anti-Aβ antibodies and β-secretase inhibitor have continued to fail[3−7)]. Meanwhile, the importance of clinical treatment facilitated by early diagnosis has

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been highlighted, with particular importance given to useful biomarkers that enable definitive prenatal diagnosis over the world[8,9]. Interview scores, imaging, and biochemical methods have been reported as pathological indicators of AD in clinical diagnosis[10]. Cerebrospinal fluid (CSF) is also employed as a diagnostic tool that reflects biochemical changes in the nervous system[11]. Already, many researchers have investigated CSF biomarkers for the diagnosis of the onset of AD[12]. CSF metabolites in AD patients have been reported to change as early as several decades before the appearance of dementia symptoms, and therefore, these markers have attracted considerable attention as potential diagnostic markers for AD and mild cognitive impairment[13,14].

Polyamines are physiologically active amines, present widely in the world of biological organisms, from viruses to humans. Polyamines promote primarily protein nucleic acid synthesis by interacting with nucleic acids, especially RNA, and function as cell growth factors[15–18]. Polyamines are also involved as modulators of biological function through interaction with glycosamine glycans such as *N*-methyl-*D*-aspartate receptor and heparin[19]. In our previous study, we detected that spermidine (SPD) and spermine (SPM) are over-expressed in brain tissue affected by AD using a non-targeted metabolomics analysis of postmortem brains of AD patients[20]. We also found results that suggested activation of ornithine decarboxylase (ODC) by highly sensitive determination of ornithine (ORN), putrescine, SPD, SPM, and their acetylated forms. In addition, immunostaining of brain tissue sections confirmed that ODC was over-expressed in brains of AD patients when compared to non-AD patients[20]. Therefore, the polyamines regarding to ornithine cycle in central nervous system tissue such as CSF would be possible to use the preliminary biomarkers. On the other hands, there are few studies to investigate the relationship of polyamines and anticipated AD pathology with CSF. In this study, we develop the targeted metabolomic (auxiliary 15 analytes in ornithine cycle) using derivatized ultra high performance liquid chromatography-electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) and measure these analytes in conclusively diagnostic SCF based on AD pathology. On the basis of this cause effect behavior of the ornithine cycle in CSF, our evidence will hopefully provide next trials.

Material and Methods

Materials

Overall, 15 analytes were used. Spermidine (SPD), spermine (SPM), putrescine (PUT), diaminopropane (DAP), N^1-acetyl-spermidine (N^1-Ac-SPD), N^8-acetyl-spermidine (N^8-Ac-SPD), N^1N^8-diacetyl-spermidine (DAc-SPD), N^1-acetyl-spermine (Ac-SPM), N^1N^{12}-diacetyl-spermine (DAc-SPM), arginine (Arg), citrulline (Cit), and aspartate (Asp) were purchased from Wako Pure Chemical Co. (Osaka, Japan); ornithine (ORN) and N^1-acetyl-putrescine (Ac-PUT) were purchased from Tokyo Kasei Co. (Tokyo, Japan); and cadaverine (CAD) was purchased from Kanto Chemicals Co. (Tokyo, Japan). For internal standards (IS), SPD-d_8, SPM-d_8, aspartate-^{13}C, arginine-$^{13}C_6$, citrulline-d_4 and ornithine-d_7 were obtained from Sigma-Aldrich Co. (St. Louis, MO), and 1,6-diaminohexane (DAH) was purchased from Kanto Chemicals Co.. For derivatization, 9-fluorenylmethyl chloroformate (F-MOC) was obtained from Wako Pure Chemical Co.. 4-(*N*, *N*-Dimethylaminosulfonyl)-7-fluoro-2, 1, 3-benzoxadiazole (DBD-F) was obtained from Tokyo Kasei Co.. HPLC-grade water, acetonitrile and formic acid (FA, for HPLC 99%) were obtained from Wako Pure Chemical Co.. Sodium tetraborate (Borax) was purchased from Kanto Chemicals Co.. Purified water was obtained using a PURELAB flex 5 system from ELGA Co (Woodridge, IL). Stock solutions were adjusted using acetonitrile/water (50 : 50, v/v).

Human sample

Postmortem human tissues and CSF samples were collected from deceased bodies at the Choju Medical Institute Fukushimura Hospital. We obtained written informed consent for autopsy and the permission to use the obtained results for diagnosis, research, and genetic analysis from the patients' guardians. The utilized tissues and CSF samples from the Fukushimura Brain Bank were used for accurate, reliable, and detailed pathological evaluation of AD[21]. The volunteer's tissues and CSF samples were confined to specific brain region that is played visual pathology of plaques/tangles, and the patients had been diagnosed with gradual pre-mortem memory loss. The CSF samples were dispensed into several tube, stored at −80°C, and employed for analysis after gaining approval from the authorities, including the Ritsumeikan University ethics panel. On the basis of the pathological examinations, the CSF samples were classified into AD and control groups. These data are

Table 1. Characterization of the AD and Control study groups

Groups	Age	Sex	Pathological diagnosis	NFT braak stage	Grain stage
Control	71	Female	stroke, α-synuclein（—）	I	(—)
	86	Male	MI, LB（—）	I	I
	76	Male	MI, Bilateral Inferior olivary nuclear pseudohypertrophy, α-synuclein (—)	I	(—)
	96	Male	MI, arteriosclerosis, LB（—）	II–III	(—)
	82	Male	Dropping of cerebellar Purkinje cells, LB（—）	I	(—)
A	88	Female	MI, α-synuclein（—）	I	(—)
	77	Male	stroke, α-synuclein（—）, CAA (light)	I	(—)
	85	Male	MI, Cerebellar infarction, CAA (heavy)	I	(—)
	95	Female	MI, CAA (light), α-synuclein（—）	I	(—)
	86	Male	MI, brain arteriosclerosis, LB（—）, CAA（—）	I	(—)
B	71	Male	LB（—）, CAA, Cortical microinfarction	I	(—)
	78	Male	MI, Willis ring arteriosclerosis, α-synuclein（—）, TDP-43（—）	I	(—)
	84	Female	White thin arteriosclerosis	III–IV	n.d.
	90	Female	LB（—）, Mild compression of the cervical spinal cord	III	(—)
	89	Male	α-synuclein（—）, chronic subdural hygroma	II	n.d.
C	88	Male	AD, CAA (light), α-synuclein（+）	IV	n.d.
	88	Female	AD, CAA (heavy), α-synuclein（—）	VI	(—)
	82	Male	AD, CAA, α-synuclein（—）	V	(—)
	75	Female	AD, CAA, α-synuclein（—）	VI	(—)
	92	Female	stroke, AD, CAA, LB（—）, α-synuclein（+）	V	n.d.

Bottom note: MI: multiple infarct, CAA: cerebral amyloid angiopathy, LB: Lewy Body

shown in Table 1. In this study, we separated the four groups based on the neurofibrillary tangles (NFT) Braak stage. The Braak staging refers to classify the degree of pathology in AD. Thus, it is not direct diagnosis of clinical AD disease.

UHPLC-ESI-MS/MS

The UHPLC system was a Waters Acquity H Class (Waters Co., Milford, MA). The reverse phase (RP) analysis was performed using an Acquity BEH C_{18} column (1.7 μm, 2.1 × 100 mm) at 40°C. The injection volume was 5 μL. The mobile phase consisting of solvent A (0.1% FA in water) and solvent B (0.1% FA in acetonitrile), was delivered at a flow rate of 0.4 mL/min. The gradient elution was as follows: B%=20, 60, 90, 98, 98, 20, and 20 (0, 8, 10, 11, 12, 13, and 20 min). A Waters Xevo TQD triple quadrupole mass spectrometer was operated with an electrospray ionization (ESI) source in the positive mode. The ionization source conditions were as follows: capillary voltage, 2.00 kV; cone voltage, 20–70 V; collision energy, 10–40 eV; source temperature, 150°C; and desolvation temperature, 400°C. The cone and desolvation gas flows were 50 and 800 L/h, respectively, and were obtained using a nitrogen source (N_2 Supplier Model 24S, Anest Iwata, Yokohama, Japan).

Derivatization

Mixed standard (four analytes such as Asp, Arg, Cit, and ORN) and IS solutions were diluted by the addition of acetonitrile/water (50 : 50, v/v) to give solutions at 10 μM concentrations. The solvent was removed from 30 μL of each of these two solutions, and the residues were re-dissolved in 50 μL of 0.1 M Borax (pH 9.0) and reacted with an equal volume of 40 mM F-MOC in acetonitrile at room temperature for 10 min. Subsequently, 50 μL of 1% FA adjusted using acetonitrile/water (50 : 50, v/v) were added to the reaction mixtures to stop the derivatization reaction. Finally, the derivatization solutions (5 μL) were analyzed by UHPLC-ESI-MS/MS.

Second mixed standard (remaining 11 analytes) and IS solutions were diluted by the addition of acetonitrile/water (50 : 50, v/v) to give solutions at 10 μM concentrations. The solvent was removed from 30 μL of each of these two solutions, and the residues were dissolved in 150 μL of 0.1 M

Borax (pH 9.0) and subsequently reacted with an equal volume of 40 mM DBD-F in acetonitrile at 60°C for 30 min. Next, the reaction mixtures were removed and re-dissolved in 100 μL of mobile phase consisting of solvents A and B (each 50 μL). Finally, the derivatization solutions (5 μL) were analyzed by UHPLC-ESI/MS/MS.

Sample preparation

CSF samples were obtained from the Fukushimura Hospital (as described above in Section *Human sample*) and were classified according to the level of seriousness in terms of senile plaque levels (Non, n=5; CERAD-A, n=5; CERAD-B, n=5; and CERAD-C, n=5). The descriptive diagnosis of all study variables is summarized in Table 1. In the AD group, the Braak phase was shown in the pathological diagnosis after death lasted from 6 to 20 years[22]. In the pathological examination of the AD brain samples, we evaluated the functional characteristics of visual pathology by determining the presence of plaque/entanglements in brain tissues. After thawing, a CSF sample selected according to the pathological investigation was added to IS and mixed with an equal volume of acetonitrile, vortexed vigorously, and centrifuged at 15,000 rpm for 10 min. The supernatant solution was subsequently removed, and the remaining residue was re-dissolved in 0.1 M Borax and derivatized using the process described above for F-MOC and DBD-F. Thereafter, the sample was analyzed by UHPLC-ESI/MS/MS.

Results and Discussion

Derivatization of analytes involved in ornithine cycle with F-MOC and DBD-F

Accumulation of ammonia produced by protein metabolism is associated with significant neurotoxicity[23]. Since the ornithine cycle (also be known as the urea cycle) converts ammonia to non-toxic urea, disorders of this cycle can lead to various diseases[23]. Metabolites involved in ornithine cycle are contained in the body at high concentrations and play important roles in anti-inflammatory and anti-fatigue activities[23]. The term polyamine refers to a chemical structure that possesses two or more amino groups. In general, polyamines play important roles in cell proliferation and differentiation[15,17]. Additionally, in our previous study, we reported that the metabolism of polyamines, including ornithine, is involved in the pathology of AD[20]. On the basis of these discussions, in this study, we tried to develop a targeted metabolomics for preliminary 15 amine analytes (metabolites and polyamines, acetylated polyamine in the ornithine cycle; Fig. 1). For this experiment, the limited 15 analytes are selected for the wide-targeted metabolomics regarding to ornithine cycle. If these analytes have the characteristic values, we would expand the measurement ranges for various metabolic maps.

Free amine analytes such as amino acids and polyamines are difficult to separate and detect using RP-LC-ESI-MS/MS owing to their poor ionization efficiency, high polarity, and structural similarity. Derivatization of amine analytes is one way of modifying their hydrophobicity and improving RP-LC separation and detection sensitivity in ESI-MS[24]. F-MOC is a well-known derivatization reagent with the ability to improve LC analysis of amines and amino acids[25–27]. Moreover, in our study, the DBD-F-based derivatization method was reported previously to yield highly sensitive and simultaneous separation/detection of polyamines in saliva[28]. Based on our previous study, the DBD-F-derivatized method was investigated by RP-UHPLC-MS/MS. In this result, the four analytes such as Asp, Arg, Cit and ORN showed the incompetent sensitive, repeatability and detectable abilities to apply the pooled CSF samples. However, at any experiment, these analytes such as Asp, Arg, Cit and ORN should be monitored for the possible biomarkers in CSF regarding to AD pathology[29,30]. Thus, we tried to develop an analytical method based on two different derivatization reagents such as DBD-F for four analytes and F-MOC for others compounds[31]. The derivatization reaction with F-MOC and DBD-F generally proceeded efficiently under alkaline conditions. In this study, the alkaline conditions employed 0.1 M Borax (pH 9.0), and the reactions were carried out at room temperature for 10 min for the F-MOC process and at 60°C for 30 min for the DBD-F process.

UHPLC-ESI-MS/MS detection of derivatized analytes

In order to perform simultaneous separation and analysis of the 15 derivatized amine analytes, we first investigated the most optimal conditions for ESI-MS/MS. In particular, conditions such as product ions of F-MOC/DBD-F derivatives and collision energies were studied. In the fragment pattern of each F-MOC derivative, a product ion at *m/z* 179.09 was detected arising from the C-O bond of F-MOC cleavage in F-MOC. The ESI-MS/MS conditions for DBD-F polyamine derivatives were utilized as reported previously[28]. Table 2 shows the ESI-MS/MS measurement

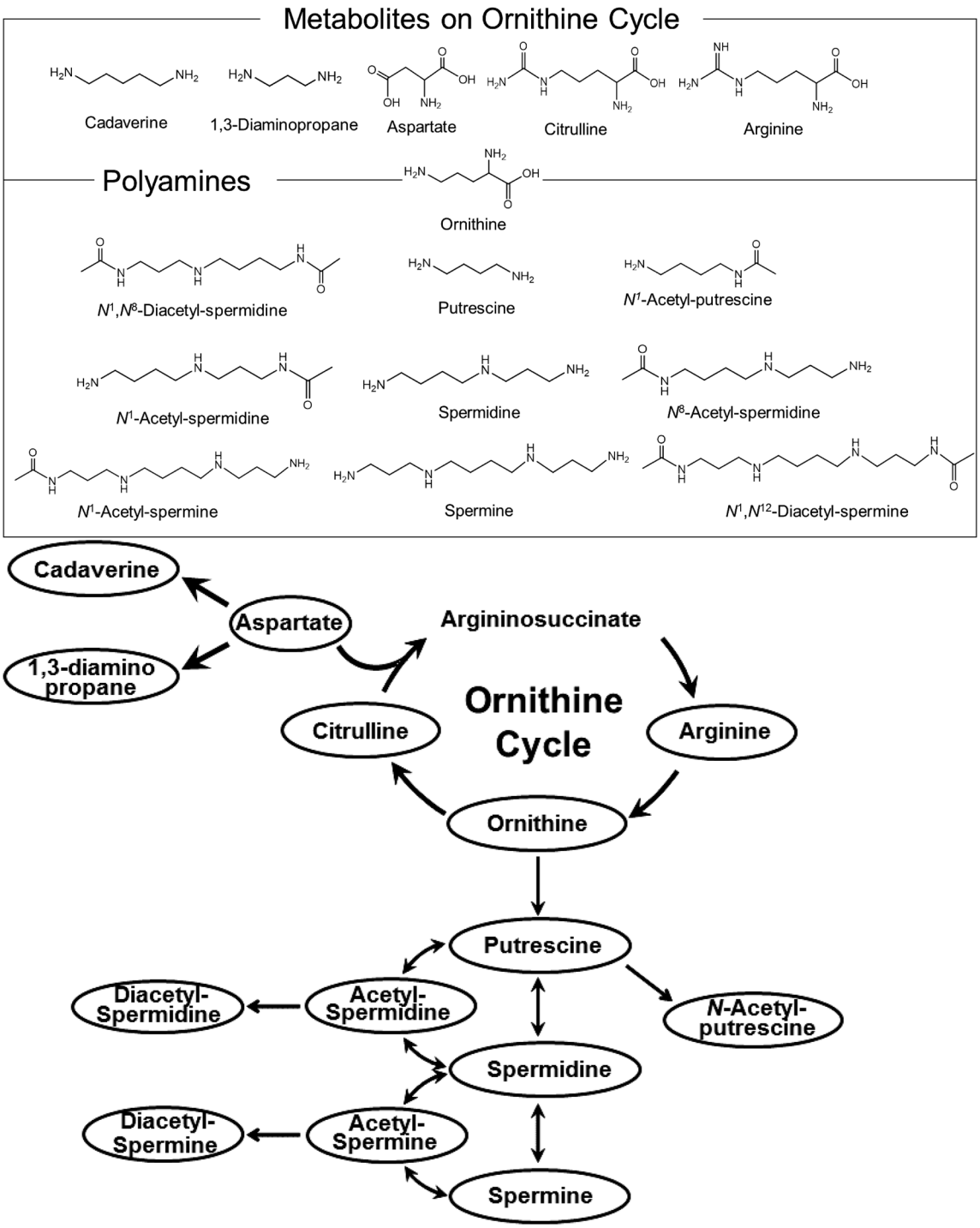

Fig. 1. Structures and metabolic pathway related to the metabolites from the ornithine cycle and polyamines.

conditions tested for 15 amine derivatives and IS. On the basis of the results presented in Table 2, precursor/product ions were set, and selective and sensitive analysis was performed using UHPLC-ESI-MS/MS in the selected reaction monitoring (SRM) mode. Next, in order to achieve simultaneous separation of the 15 targeted analyte peaks in the chromatogram, the UHPLC conditions were examined. Normally, when unmodified non-derivatized amines are separated by UHPLC in the RP mode using a C_{18} column, majority of them are eluted early in the polar mobile phase as a result of their high polarity, thus affording poor resolution. However, by labeling the amines with two derivatizing reagents, their hydrophobicity was increased, and the retention time on the C_{18} column was prolonged. In addition, by optimizing the mobile phase, it was possible to perform simultaneous separation and detection of 15 amines

Table 2. Detective condition of metabolites on ornithine cycle and polyamines derivatized with F-MOC and DBD-F

Analytes	RT (min)	MRM transitions (*m/z*)	Cone voltage (V)	Collision energy (eV)	Derivatization/ Internal standard
Asp	5.29	356 → 179	20	20	F-MOC/Asp-^{13}C
Asp-^{13}C	5.29	357 → 179	20	20	—
Arg	3.73	397 → 179	45	35	F-MOC/Arg-$^{13}C_6$
Arg-$^{13}C_6$	3.73	403 → 179	45	35	—
Cit	4.75	398 → 179	25	10	F-MOC/Cit-d_4
Cit-d_4	4.75	402 → 179	25	20	—
ORN	9.87	578 → 179	25	15	F-MOC/ORN-d_7
ORN-d_7	9.87	584 → 179	25	15	—
Ac-PUT	3.17	356 → 311	30	20	DBD-F/DAH
DAc-SPD	2.99	455 → 100	35	30	DBD-F/SPD-d_8
DAP	6.73	525 → 437	45	25	DBD-F/DAH
PUT	7.31	539 → 451	45	25	DBD-F/DAH
CAD	7.95	553 → 465	45	20	DBD-F/DAH
1,6-Diaminohexane (I.S.)	8.56	567 → 479	45	20	—
N^1-Ac-SPD	6.49	638 → 100	50	20	DBD-F/SPD-d_8
N^8-Ac-SPD	6.38	638 → 550	50	20	DBD-F/SPD-d_8
DAc-SPM	5.74	737 → 100	50	35	DBD-F/SPM-d_8
SPD	9.02	822 → 733	60	25	DBD-F/SPD-d_8
SPD-d_8	9.02	829 → 741	50	30	—
Ac-SPM	8.16	920 → 835	65	35	DBD-F/SPM-d_8
SPM	9.82	1,104 → 1,015	70	40	DBD-F/SPM-d_8
SPM-d_8	9.82	1,112 → 1,024	50	40	—

within a measurement time of 15 min as shown in Fig. 2(A).

Quantitative performance

Firstly, a validation test was carried out. Calibration curves [X: concentration (nM), Y: derivative/IS peak area ratio] of 15 amine analytes prepared using F-MOC and DBD-F as derivatization reagents were obtained. We obtained a calibration curve with high linearity and a correlation coefficient r^2 of 0.996−1.000 in the required concentration range for each analyte. Moreover, the limit of quantitation (LOQ) and the limit of detection (LOD) of each metabolite were determined to be in the level of several nM. For the application of CSF for various types, it is infrequently observed to detect the protrude concentration from the calibration range. In this case, a diluted liquid is prepared by adding not less than 10 parts of pure water to one part of the CSF sample. On the basis of prior studies for the direct/diluted methods, the recovery and repeatability were considered acceptable and useful values (more than 80% for recovery and less than 15% for repeatability, n=6). These results suggest that the developed analytical assay method is sufficiently sensitive to enable simultaneous detection of polyamines present in CSF (Table 3).

Targeted metabolomics for the ornithine cycle in CSF

The 15 selected amine analytes were measured in CSF samples using the UHPLC-ESI-MS/MS targeted metabolomics methodology developed specifically for the ornithine cycle. The obtained chromatograms are shown in Fig. 2(B). As observed in the chromatogram of the reference standard shown in Fig. 2(A), the results, with clearly observed sharp peaks, showed that simultaneous separation and detection is possible. The results from grouped CSF samples were shown in Table 4. In our previous study, the polyamine levels (SPD, SPM, PUT and Ac-SPD) in AD brain tissues were increased compared to control[20]. Based on the evidence, the polyamine metabolite pathway in a specific brain region is likely to be responsible for up-regulated polyamine metabolism and elevated ornithine decarboxylase (ODC) activity. On the other hand, this study showed that clear result is not observed compared with previous study. In the kruskal-wallis test, the ORN, PUT, N^8-AC-SPD and AC-SPM are less than 0.1 values. In addition, Arg is

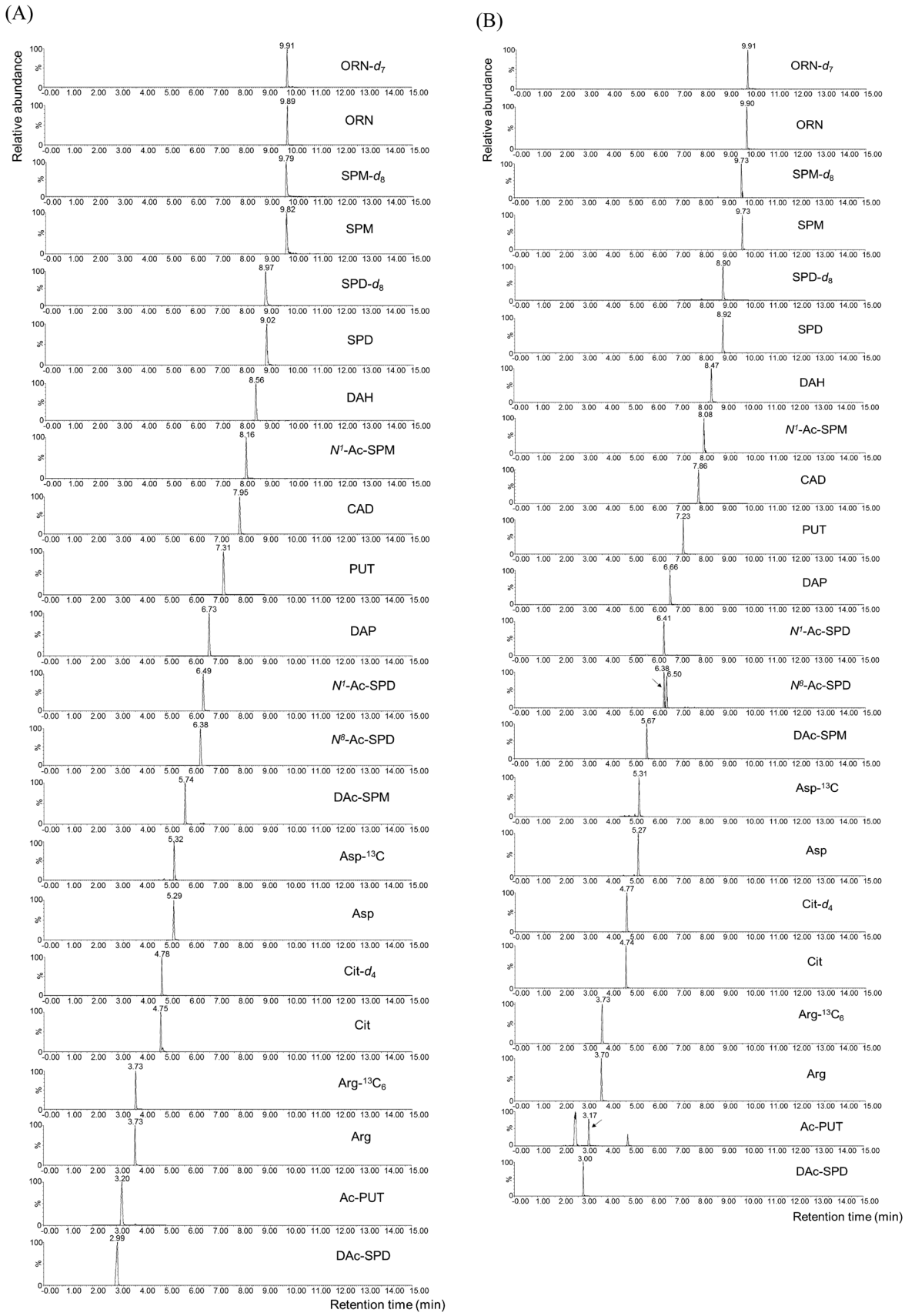

Fig. 2. (A) SRM chromatograms of amino acids and polyamines in standard solution. (B) SRM chromatograms of human CSF samples.

Table 3. Calibration curves and linearity of metabolites on ornithine cycle and polyamines

Analytes	Calibration range (nM)	Linearity (R2)	LOQ (nM)	LOD (nM)
Asp	1.00−100,000	0.999	1	0.1
Arg	10.00−100,000	1.00	3	1
Cit	1.00−100,000	0.999	1	0.1
ORN	10.00−100,000	0.999	1	0.1
Ac-PUT	6.25−2,000	0.998	1	0.2
DAc-SPD	6.25−2,000	0.996	2.5	1
DAP	6.25−2,000	0.997	1	0.1
PUT	6.25−2,000	0.996	0.5	0.1
CAD	6.25−2,000	0.997	0.5	0.1
N_1-Ac-SPD	50.0−2,000	0.998	50	25
N_8-Ac-SPD	50.0−2,000	0.998	50	25
DAc-SPM	25.0−2,000	0.997	25	20
SPD	25.0−2,000	0.997	25	10
Ac-SPM	50.0−2,000	0.996	50	25
SPM	50.0−2,000	0.998	50	25

Table 4. Concentration levels (μM) of metabolites on ornithine cycle and polyamines in CSF samples

Analytes	Group				*P* value (Kruskal−Wallis test)
	Control	A	B	C	
Asp	11.1±10.2	6.6±6.2	12.3±9.8	2.4±0.6	0.332
Arg	35.7±16.2	28.0±7.5	27.4±12.1**	27.2±2.8**	0.523
Cit	3.8±3.0	3.6±2.0	3.6±2.9	1.9±0.5	0.452
ORN	8.8±2.1	7.3±1.4	9.2±1.3	6.2±1.8	0.0871
Ac-PUT	0.3±0.9	0.3±0.2	0.4±0.2	0.2±0.1	0.354
DAc-SPD	0.2±0.3	0.3±0.1	0.2±0.1	0.3±0.1	0.617
DAP	0.4±0.2	0.2±0.2	0.5±0.2	0.3±0.1	0.257
PUT	1.6±1.0	0.7±0.4	1.5±0.7	0.6±0.2*	0.0498
CAD	0.2±0.1	0.2±0.1	0.3±0.2	0.4±0.1*	0.147
N^1-Ac-SPD	0.3±0.3	0.3±0.2	0.3±0.2	0.1±0.03	0.224
N^8-Ac-SPD	0.1±0.03	0.1±0.03	0.1±0.1	0.1±0.01	0.0968
DAc-SPM	0.9±0.3	1.3±1.0	0.7±0.3	1.2±0.5	0.501
SPD	12.3±11.1	4.8±5.7	20.9±19.4	2.3±0.6	0.161
Ac-SPM	5.9±2.9	9.0±13.2	7.5±1.1	9.5±3.9	0.0456
SPM	16.8±16.4	3.6±3.5	13.4±8.3	13.5±16.1	0.267

Mean（μM）±S.D.
*: $P<0.05$, **: $P<0.01$ (*vs.* Control)
The variance in a group was evaluated using the F test.
The difference between two groups were evaluated using Student's *t* test or the Wilcoxon−Mann−Whitney test.

decreased between control and other groups. Trushina et al. reported that the altered canonical pathways in CSF between the AD group *vs.* cognitively normal individuals showed the cholesterol and sphingolipids transport, energy metabolism, Krebs cycle, mitochondrial function, neurotransmitter and amino acid metabolism[32]. Thus, Arg is needed to investigate the exhaustive metabolic pathway in CSF based on AD pathology. Based on this result, PUT and CAD in CSF showed a minimal change between controls *vs.* group C. Recently, it is reported that hyperhomocysteinemia is a risk factor for development of dementia and AD based on the effects of homocysteine, polyamines and oxidative metabolism including PUT, CAD and others[33]. Moreover, the modulation of learning and memory by poly-

amine metabolism was discussed and figured to ODC and the sequential transfer of aminopropyl groups from *S*-adenosylmethionine to PUT by SPD/SPM synthases[34]. Already, we reported that the ODC in polyamine metabolism and ornithine cycle is an index enzyme-reaction for the AD pathology monitoring using biological samples[20]. In this study, it is unclear evidence to indicate the inevasible biomarker for AD severity. However, the possible pathway of polyamine metabolism and ornithine cycle can be presented using the targeted metabolomics of CSF samples. Future works would be needed to develop the wide-targeted metabolomics using UHPLC-MS/MS for various biological samples such as brain tissue, CSF, blood and others for AD diagnosis.

Conclusions

In this study, we developed amine-targeted metabolomics methodology employing UHPLC-ESI-MS/MS and two derivatization reagents (F-MOC and DBD-F). Furthermore, we used this targeted metabolomics in a pathological investigation to screen for CSF-based biomarkers suitable for diagnosis of AD. The results identified polyamine metabolism and ornithine cycle as candidate markers in CSF that exhibit marked changes dependent on the AD pathology. The methodology described here is demonstrated to be an effective method for the identification of possible AD markers in CSF.

Conflict of Interest

The authors have no conflicts of interest directly relevant to the content of this article.

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