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# Widely targeted metabolomics of Alzheimer's disease postmortem cerebrospinal fluid based on 9-fluorenylmethyl chloroformate derivatized ultra-high performance liquid chromatography tandem mass spectrometry

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ABSTRACT

Confirmed biomarkers of postmortem cerebrospinal fluid (pCSF) are used to differentiate between Alzheimer's disease (AD) patients and healthy seniors with high diagnostic accuracy. However, the extent to which the performance of specific metabolic profiling facilitates reliable estimations of the concentrations of the different pCSF biomarkers and their ratios remains unclear. The interpretation of the lower levels of molecules of metabolic profiling and their concentration ratios in pCSF related to brain disorders could facilitate an unchallenging detection of peripheral biomarkers of AD stages and other dementia types. In this study, we proposed the use of widely targeted metabolomics for pCSF metabolic profiling using 9-fluorenylmethyl chloroformate- (FMOC) derivatized ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) to evaluate the diversity of 97 amine-mediated metabolic patterns and pathways from confirmed diagnosis based on AD brain pathology. Our results identified the metabolites that contributed toward and mutually influenced the principal component analysis plot with integrated analytes. Furthermore, the AD group showed a significant variation in several analyte concentration levels compared to those of control subjects. These trends of the concentration levels expressed by the amine metabolic pathways indicated the decreased activity of polyamine and tryptophan-kynurenine (Trp-Kyn) metabolisms. Moreover, increased metabolites such as methionine sulfoxide, 3-methoxy-anthranilate, cadaverine, guanine, and histamine were observed by widely targeted metabolomics of pCSF from the AD subjects. According to their metabolic pathway analysis using FMOC-derivatized UHPLC-MS/MS assay, we supposed that the involvement of polyamine and Trp-Kyn metabolisms was observed in the pCSF samples.

## 1. Introduction

Alzheimer's disease (AD) is a type of dementia causing neural difficulties involving memory, cognition and behavior among seniors. Two abnormal structures, referred to as plaques and tangles, have been identified in AD pathology as potential prime suspects in severely depressing metabolic mechanisms, killing crucial neurons, and effecting a lack of all of higher brain functions. Moreover, amyloid precursor protein metabolism, phosphorylation-stimulated tau protein, oxidative stress, mitochondrial dysfunction, inflammation, lipid deregulation, and neurotransmitter pathway disruption have been suspected in the presence of biological metabolisms associated with AD pathology [1,2]. Therefore, attention is focused on identifying biomarkers for the early diagnosis of AD and predicting its pathological progression. Since these histopathological manifestations cause no perceptible metabolic changes, a definitive AD diagnosis can only be determined at autopsy. AD is commonly defined based on the presence of senile plaques and neurofibrillary tangles (NFTs) in the brain tissue [3]. Moreover, for the diagnosis of mixed dementia, it is difficult to identify the disease type and the brain regions causing AD. To the best of our knowledge, these pathologies and metabolic mechanisms contribute to the distinct cognitive profile of the central nervous system (CNS) that is associated with AD. Ideally, the overall observation of the pathology associated with AD in the CNS should be accompanied by pathological profiling based on

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clinical diagnosis, tissue staining plaques and tangles accumulation, and metabolite detection. Presently, AD can be definitively diagnosed postmortem by direct examination of senile plaques and NFTs in several brain regions using techniques such Gallyas-Braak (GB) silver staining. Undoubtedly, the identification of various metabolites in the CNS tissue such as brain and cerebrospinal fluid (CSF) would facilitate a direct association with observed premortem brain pathology.

The monitoring of various candidate biomarkers in the CNS has formed a multilateral framework for the discovery of representative and next-step pathophysiological features in the neurogenic etiology of AD [4–7]. Recent reports have determined that the AD process is initiated > 30 years prior to the clinical onset of dementia. A series of pathophysiological changes occur over decades in the CSF biomarkers related to amyloid-beta (Aβ), tau protein tangles, brain Aβ deposition and progressive cognitive impairment [8–10]. We have previously investigated the metabolic changes of AD pathology, including Aβ deposition and tau protein tangles, in the human brain tissue employing a liquid chromatography-mass spectrometry (LC-MS) metabolomics technique [11]. Our results indicated that the polyamine metabolic pathway was altered in AD pathology. Recently, a gas chromatography–mass spectrometry technique was utilized to investigate the fluctuating metabolism in the AD brain tissue. Prominent metabolite alterations from several pathways involved in glucose clearance and utilization, the urea cycle, and amino acids were observed [12]. The combination of untargeted and targeted lipidomics and metabolomics using LC-MS reported a significant dysregulation in mitochondrial aspartate (Asp) metabolism in the AD brain, which correlated with dementia and AD pathology [13]. Matrix-assisted laser desorption/ionization mass spectrometry imaging was used in a transgenic AD mouse model, and it indicated an interesting shift toward pro-inflammatory molecules (uric acid) in the purinergic signal metabolism that was associated with a decrease in anti-oxidant levels [14].

The metabolic profiling of the pathways involved in AD pathology would need to represent various combinations reflecting the direct and indirect effects of AD on the brain tissue. However, it is impossible to use the brain tissue obtained at biopsy for the clinical diagnosis of early dementia. Thus, the combination of genomic, blood, and CSF analysis for AD diagnosis is the recommended approach to identify suitable and remarkable metabolites in the CNS [15]. Notably, the knowledge of CSF biomarkers increased the diagnostic confidence of clinicians in a local hospital [16,17]. At present, the CSF biomarkers are Aβ, total tau and phosphorylated tau [18]. However, deficient knowledge concerning the pathology and heterogeneity of AD and methodological challenges may hinder the implementation of these biomarkers in clinical practice, particularly in the early stages of AD. Thus, it may be necessary to include asymptomatic preclinical patients in the assessment of AD biomarkers to facilitate an earlier prevention of AD [18]. Exhaustive methodologies such as metabolomics should be explored to characterize metabolism-induced behavior in CSF related to AD or other dementia pathologies.

Previous studies investigating CSF biomarkers in AD pathological evaluation or clinical diagnosis have utilized chromatographic and mass spectrometric techniques. Kaddurah-Daouk et al. reported the first metabolomic study with liquid chromatography-electrochemical detection (LC-ECD) of postmortem CSF (pCSF) samples in autopsy-confirmed AD subjects. The alterations in tyrosine (Tyr), tryptophan (Trp), purine, and tocopherol pathways and reductions in norepinephrine were detected [19]. Liu et al. used Fourier transform ion cyclotron resonance mass spectrometry to survey the metabolomic effects of cholesterol in the etiology of AD-like progression using rabbit CSF [20]. Ibáñez et al. reported a non-targeted metabolomic approach based on capillary electrophoresis mass spectrometry to examine the metabolic differences in CSF samples from the subjects with different cognitive statuses related to AD progression [21]. Their results suggested that choline, arginine (Arg), valine (Val), proline (Pro), serine (Ser), histidine (His), creatine, carnitine, and suberylglycine were potential biomarkers for AD progression. Kaddurah-Daouk et al. used LC-ECD targeted metabolomics to profile CSF from AD, mild cognitive impairment (MCI), and control subjects [22]. The AD subjects displayed elevated methionine (Met), 5-hydroxyindoleacetic acid, vanillylmandelic acid, xanthosine and glutathione levels compared with those of the controls. Trushina et al. applied LC-MS non-targeted metabolomics to determine universal metabolic changes in the CSF from the same individuals [23]. Twenty altered canonical pathways were observed in CSF in MCI when compared with the control individuals (false discovery rate < 0.05), indicating that the Krebs cycle was significantly affected in MCI.

Recently, Guiraud et al. utilized a multi-analyte method by isotope dilution ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) to detect 17 metabolites in pCSF samples [24]. Their findings suggested that the homocysteine-Met pathway was crucial for understanding the relationship among neurological disorders. This analytical strategy has been employed in large-scale, observational metabolic pathway studies of pCSF. However, the process of AD pathology initiation and continuation and the association of metabolites present in CSF with the presence of senile plaques and NFTs in brain tissue remain unclear. Therefore, comprehensive information regarding the widely targeted metabolic profiling of pCSF employing a high-throughput method and simultaneous quantitative analysis of metabolites is essential. In this study, we propose widely targeted metabolomics for pCSF metabolic profiling using a 9-fluorenylmethyl chloroformate (FMOC) derivatized UHPLC-MS/MS assay to evaluate the diversity of amine-mediated metabolic patterns and pathways from confirmed AD cases diagnosed on brain pathology.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Widely targeted metabolites for 97 analytes and 33 internal standards (IS) were employed in this study. Glycine (Gly), β-alanine (β-Ala), cytosine, creatinine (CRE), valine (Val), threonine (Thr), taurine (Tau), 5-aminolevulinate (5-ALA), isoleucine (Ile), creatine, asparagine (Asn), aspartate (Asp), adenine, anthranilic acid (AA), tyramine, glutamine (Gln), histidine (His), methionine sulfoxide (MetSO), cysteate, arginine (Arg), citrulline (Cit), tyrosine (Tyr), $N^G$-monomethyl-L-arginine (NMMA), tryptophan (Trp), $N^1N^8$-diacetyl-spermidine (DAc-SPD), cytidine, adenosine, guanosine, 1, 3-diaminopropane (DAP), putrescine (PUT), acetyl-spermidine (Ac-SPD), cystathionine, carnosine, cystine, anserine, $N^1N^{12}$-diacetyl-spermine (DAc-SPM), spermidine (SPD), $N^1$-acetyl-spermine (Ac-SPM), spermine (SPM), taurine-$d_4$ (Tau-$d_4$), arginine-$^{13}C_6$, (Arg-$^{13}C_6$), 3-hydroxy-anthranilate-$d_3$ (3-hydroxy-AA-$d_3$), 9-fluorenylmethyl chloroformate (FMOC), sodium hydrogen carbonate ($NaHCO_3$), distilled water for amino acid sequence analysis, ethanol, acetonitrile and HPLC-grade formic acid (FA) were purchased from Wako Pure Chemical Co. (Osaka, Japan). 1-Amino-2-propanol, 4-aminobutanoic acid (GABA), 2-aminoethanesulfinic acid (Hypotaurine), hydroxyproline (ProOH), methionine (Met), cysteine sulfinic acid, phenylalanine (Phe), Pro-Ala, *N*-acetyl-lysine, $N^G$, $N^G$-dimethyl-L-arginine (ADMA), kynurenine (KYN), Leu-Ser, Leu-Pro, Pro-Leu, ophthalmate, Tyr-Leu, histidinol, 5-hydroxylysine, 3-methylhistidine, 3-hydroxykyurenine (3-HK), glutathione, *S*-adenosyl-homocysteine (SAH), glycine-$^{13}C_2{}^{15}N_2$, alanine-$^{13}C_3{}^{15}N$, GABA-$d_6$, serine-$^{13}C_3{}^{15}N$, proline-$^{13}C_5{}^{15}N$, valine-$d_8$, leucine-$d_{10}$, asparagine-$^{13}C_4{}^{15}N_2$ and tyrosine-$^{13}C_9{}^{15}N$ were purchased from Sigma-Aldrich (Buchs, Switzerland). Alanine (Ala), serine (Ser), proline (Pro), leucine (Leu), glutamate (Glu), cadaverine (CAD), lysine (Lys), and 1,6-diaminohexane (DAH) were purchased from Kanto Chemicals (Tokyo, Japan). Ethanolamine, *N*-methyl-glycine (Sarcosine), 2-aminobutyric acid (Homoalanine), 5-aminopentanoate, homoserine (Hse), phenethylamine, $N^1$-acetyl-putrescine (Ac-PUT), agmatine, 2-aminoacetophenone, phosphoethanolamine, *O*-acetyl-serine, guanine, 3-hydroxy-anthranilate (3-hydroxy-AA), tryptamine, 2-aminoadipate, *O*-phospho-serine, 4-methoxyanthranilate (4-methoxy-AA), glycyl-proline (Gly-Pro), serotonin,