Acta Med. Okayama, 2020
Vol. 74, No. 1, pp. 65–72

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Metabolic Profiling of the Cerebrospinal Fluid in Pediatric Epilepsy

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To characterize metabolic profiles within the central nervous system in epilepsy, we performed gas chromatography-tandem mass spectrometry (GC-MS/MS)-based metabolome analysis of the cerebrospinal fluid (CSF) in pediatric patients with and without epilepsy. The CSF samples obtained from 64 patients were analyzed by GC-MS/MS. Multivariate analyses were performed for two age groups, 0-5 years of age and 6-17 years of age, to elucidate the effects of epilepsy and antiepileptic drugs on the metabolites. In patients aged 0-5 years (22 patients with epilepsy, 13 without epilepsy), epilepsy patients had reduced 2-ketoglutaric acid and elevated pyridoxamine and tyrosine. In patients aged 6-17 years (12 with epilepsy, 17 without epilepsy), epilepsy patients had reduced 1,5-anhydroglucitol. Valproic acid was associated with elevated 2-aminobutyric acid, 2-ketoisocaproic acid, 4-hydroxyproline, acetylglycine, methionine, *N*-acetylserine, and serine. Reduced energy metabolism and alteration of vitamin B6 metabolism may play a role in epilepsy in young children. The roles of 1,5-anhydroglucitol in epilepsy in older children and in levetiracetam and zonisamide treatment remain to be explained. Valproic acid influenced the levels of amino acids and related metabolites involved in the metabolism of serine, methionine, and leucine.

Key words: antiepileptic drugs, gas chromatography-tandem mass spectrometry, metabolome analysis, metabolomics

Epilepsy is a chronic brain disorder marked by recurrent, unprovoked epileptic seizures. It affects 5-9% of the general population. Its etiology is heterogeneous, with structural, genetic, infectious, metabolic, and immune components [1]. Nevertheless, patients with epilepsy share common symptoms, *i.e.*, epileptic seizures, which are characterized by transient occurrence of signs and/or symptoms that result from abnormal excessive or synchronous neuronal activity in the brain [2]. It is not known how the various causes of epilepsy lead to the common phenomenon of excessive neuronal firing.

Epileptic seizures involve alterations in the concentration of ions (*e.g.*, sodium, calcium) and neurotransmitters (*e.g.*, glutamic acid, gamma-aminobutyric acid [GABA]), leading to an imbalance between excitation and inhibition in the central nervous system. Current antiepileptic drugs (AEDs) mainly target such mechanisms by suppressing neuronal excitation (*e.g.*, voltage-gated sodium and calcium channel blocking, glutamate receptor antagonism) or enhancing neuronal inhibition (*e.g.*, inhibition of GABA catabolism, chloride channel enhancement).

Recently, lactate dehydrogenase (LDH) has been reported to be a potential new target of AEDs, and

Received November 7, 2019; accepted December 19, 2019.
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Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

stiripentol, one of the currently available AEDs, has been shown to have an LDH-inhibiting effect [3]. Thus, understanding and targeting cellular metabolism may be a new approach to controlling epileptic seizures. Blood and urine are not ideal samples for investigating pathological metabolic processes in the brain because the blood-brain barrier and the blood-cerebrospinal fluid (CSF) barrier isolate the brain from the peripheral system. CSF samples have the disadvantage of invasive collection; however, if available, metabolic profiling of the CSF in patients with epilepsy may help elucidate changes in the metabolites in the brain that are associated with epileptic seizures, which may in turn provide clues for discovering new targets for AEDs.

In this study, we performed metabolome analysis of CSF samples obtained from pediatric patients with and without epilepsy using gas chromatography-tandem mass spectrometry (GC-MS/MS). We aimed to discover differences between the metabolic profiles of epileptic and non-epileptic patients in 2 age groups, 0-5 (<6) years of age and 6-17 (≥6 and <18) years of age. We also investigated which metabolites were affected by AEDs.

Materials and methods

Subjects. This study included 64 patients (34 patients with epilepsy and 30 patients without epilepsy) who were admitted to the Department of Child Neurology at Okayama University Hospital and underwent lumbar puncture to collect CSF samples for investigation of neurological symptoms from December 2012 to December 2016 and whose unused CSF samples were available. Patients with known inherited metabolic diseases were excluded, because their metabolic profiles would likely show a high degree of deviation. Patients with other comorbidities (*e.g.*, intellectual disability), underlying causes (*e.g.*, focal cortical dysplasia), and those receiving treatment (*e.g.*, AEDs) were not excluded. We chose to include a variety of patients to identify candidate metabolites that show common changes across diverse etiologies, and because the number of patients undergoing lumbar puncture in the clinical setting is limited. This study was approved by the Research Ethics Board at Okayama University Hospital. Written informed consent was obtained from the patients' guardians before the procedure.

Sample collection protocol. Lumbar puncture was conducted after 4-6 h of fasting. The CSF samples were aliquoted and used for routine laboratory tests (*e.g.*, cell count, protein, glucose) and for analysis of monoamine neurotransmitters, pyridoxal-5'-phosphate, and 5-methyltetrahydrofolate, as needed, at our laboratory. The unused CSF samples were stored at −80°C within 1 h after collection until metabolome analysis.

Metabolome analysis. The CSF samples were prepared according to a previously reported method [4]. GC-MS/MS analysis was performed using a GC-MS/MS TQ8040 (Shimadzu, Kyoto, Japan) system. Peak identification was performed automatically and then confirmed manually based on the specific precursor and product ions and retention time. The database used in this study includes data on 475 peaks from 333 metabolites [5].

Statistical analysis. Statistical analyses were performed using R 3.4.2 (https://cran.r-project.org/). For patient group comparison, Wilcoxon's test, Fisher's exact test, and chi-square test were performed. Correlation analysis was performed using Spearman's correlation coefficient. Metabolic profiles were analyzed in two age groups: 0-5 (<6) years of age (pre-school age) and 6-17 (≥6 and <18) years of age (school age), because the concentrations of many metabolites are known to be age-dependent. The concentration data of all metabolites were logarithmically transformed. To check for outliers, principal component analysis (PCA) was conducted after data scaling to a mean of zero and a variance of one. To investigate the effect of epilepsy (vs. non-epilepsy) and AEDs on individual metabolites, multiple regression analyses were performed. Because of the many multiple regression analyses for all metabolites, p-values were adjusted by controlling the false discovery rate using the R package "qvalue" to calculate q-values (http://genomics.princeton.edu/storeylab/qvalue/). The significance level was set at 0.05.

Results

Subject characteristics. Subject characteristics are presented in Table 1 and Table 2. There were 34 patients with epilepsy and 30 patients without epilepsy. The age group of 0-5 years consisted of 22 patients with epilepsy and 13 patients without epilepsy. The age group of 6-17 years consisted of 12 patients with epilepsy and 17 patients without epilepsy. There were no significant differences in age and sex between the

Table 1 Clinical characteristics of subjects

Case No.	Age	Sex	Antiepileptic drugs	Diagnosis
Patients with epilepsy				
1	0y 4m	M		FE, developmental delay
2	0y 4m	M	ZNS, PB	West syndrome, post-herpes simplex encephalitis
3	0y 5m	F	CBZ	Benign infantile epilepsy
4	0y 6m	F	LEV, CZP	West syndrome, FCD
5	1y 3m	M	PHT	FE, developmental delay, *KCNQ2* gene abnormality
6	1y 10m	F	PHT, TPM	FE, cavernous angioma
7	1y 11m	F	VPA	GE, developmental delay
8	2y 1m	F	VPA	GE, intellectual disability
9	2y 5m	F	VPA, ZNS	FE, FCD
10	2y 7m	F	LEV, CBZ	West syndrome, intellectual disability
11	2y 10m	M		GE
12	3y 2m	F	VPA, ZNS, LTG	West syndrome, intellectual disability
13	3y 3m	F	VPA	Epilepsy with myoclonic-atonic seizures
14	3y 4m	M	VPA, ESM	Lennox-Gastaut syndrome
15	3y 5m	M	VPA	Epileptic encephalopathy with CSWS
16	3y 6m	M	VPA, DZP	GFE
17	4y 0m	M	VPA, LEV, CBZ, TPM	GFE, intellectual disability
18	4y 4m	M	VPA, LEV	GFE, intellectual disability
19	4y 7m	M	VPA	FE, intellectual disability
20	4y 7m	F	VPA, LEV	FE, intellectual disability
21	5y 3m	M	ZNS, CBZ	FE, FCD
22	5y 10m	M	VPA, LTG	GE, intellectual disability, autism
23	6y 6m	F	ZNS	GE, intellectual disability
24	7y 5m	M	VPA, LEV, ESM, STM	Epileptic encephalopathy with CSWS, intellectual disability, autism, ADHD
25	9y 1m	F	VPA, TPM, ESM, RFN	Lennox-Gastaut syndrome, intellectual disability, autism
26	9y 3m	M	VPA, LTG	FE, subcortical band heterotopia
27	9y 7m	F	VPA, LEV, TPM, STM, CLB	FE, autism
28	9y 8m	M	ZNS, CBZ	FE, borderline intelligence
29	10y 11m	M	CBZ, CZP	FE, dystonia, intellectual disability
30	12y 10m	F	VPA, LEV	FE, FCD
31	13y 7m	M	LTG, PHT, CLB	FE, intellectual disability
32	14y 10m	F	LEV, ZNS, CZP, CLB, PB, ESM	GE, intellectual disability, autism
33	14y 10m	M	ZNS	FE
34	15y 4m	F	LEV, CBZ	FE, intellectual disability
Patients without epilepsy				
35	0y 4m	M		Hypertonia, developmental delay
36	0y 6m	F		Benign neonatal myoclonus
37	0y 9m	F		Cerebellar hypoplasia
38	1y 1m	M		Developmental delay
39	1y 1m	F		Congenital fiber-type disproportion myopathy
40	2y 0m	M	ZNS, CBZ	Suspected tic disorder
41	2y 0m	M		Intellectual disability
42	2y 1m	F		Intellectual disability
43	2y 2m	F		Intellectual disability, hypotonia
44	2y 6m	F		Benign congenital hypotonia
45	3y 0m	M		Autism
46	3y 7m	M		Intellectual disability
47	5y 6m	M	VPA, DZP	Autism
48	10y 4m	F		Equinus foot
49	10y 9m	F		Suspected restless legs syndrome
50	11y 2m	M		Intellectual disability, idiopathic basal ganglia calcification
51	11y 8m	M		ADHD, Tourette syndrome
52	11y 9m	F		Equinus foot
53	12y 7m	F		Somatoform disorder
54	12y 8m	M		Hypersomnia
55	12y 9m	M		Conversion disorder
56	13y 2m	M		Paroxysmal kinesigenic dyskinesia
57	13y 2m	F		Narcolepsy
58	14y 3m	F		Hypersomnia
59	14y 4m	F		Hypersomnia
60	14y 8m	F		Transient muscle weakness
61	15y 1m	M		Kleine-Levin syndrome
62	15y 6m	M		Hypersomnia
63	15y 7m	M		Narcolepsy
64	16y 7m	F		Myasthenia gravis

ADHD, attention-deficit hyperactivity disorder; CBZ, carbamazepine; CLB, clobazam; CSWS, continuous spike-waves during sleep; CZP, clonazepam; DZP, diazepam; ESM, ethosuximide; FCD, focal cortical dysplasia; FE, focal epilepsy; GE, generalized epilepsy; GFE, combined generalized and focal epilepsy; LEV, levetiracetam; LTG, lamotrigine; MDL, midazolam; PB, phenobarbital; PHT, phenytoin; RFN, rufinamide; STM, sulthiame; TPM, topiramate; VPA, valproic acid; ZNS, zonisamide.

Table 2 Comparison of clinical characteristics between epilepsy and non-epilepsy groups

Groups	Epilepsy	Non-epilepsy	*p*-value
Number	34	30	
Age	0y 4m–15y 4m (median, 4y 2m)	0y 4m–16y 7m (median, 11y 11m)	0.137
Sex (female)	16 (47%)	15 (50%)	0.814
Age group 0–5 years			
Number	22	13	
Age	0y 4m–5y 10m (median, 3y 0m)	0y 4m–5y 6m (median, 2y 0m)	0.177
Sex (female)	10 (45%)	6 (46%)	1
Antiepileptic drugs			
VPA	13 (59%)	1 (8%)	0.0039
LEV	5 (23%)	0	0.134
ZNS	4 (18%)	1 (8%)	0.630
CBZ	4 (18%)	1 (8%)	0.630
LTG	2 (9%)	0	0.519
PHT	2 (9%)	0	0.519
TPM	2 (9%)	0	0.519
BZDs	2 (9%)	1 (8%)	1
PB	1 (5%)	0	1
ESM	1 (5%)	0	1
CSF protein (mg/dL)	11–47 (median, 17)	10–32 (median, 18)	0.644
CSF lactate (mg/dL)	9.9–19.4 (13.5)	11.1–17.4 (12.5)	0.838
CSF glucose (mg/dL)	43–65 (51.5)	44–59 (51)	0.959
Plasma glucose (mg/dL)	72–108 (88)	65–103 (86)	0.918
CSF/plasma glucose	0.46–0.70 (0.59)	0.52–0.68 (0.59)	0.785
Age group 6–17 years			
Number	12	17	
Age	6y 6m–15y 4m (median, 10y 4m)	10y 4m–16y 7m (median, 13y 2m)	0.066
Sex (female)	6 (50%)	9 (53%)	1
Antiepileptic drugs			
VPA	5 (42%)	0	0.0067
LEV	5 (42%)	0	0.0067
ZNS	4 (33%)	0	0.0208
BZDs	4 (33%)	0	0.0208
CBZ	3 (25%)	0	0.0602
ESM	3 (25%)	0	0.0602
LTG	2 (17%)	0	0.163
TPM	2 (17%)	0	0.163
STM	2 (17%)	0	0.163
RFN	1 (8%)	0	0.414
PB	1 (8%)	0	0.414
PHT	1 (8%)	0	0.414
CSF protein (mg/dL)	18–63 (25)	12–65 (27)	0.673
CSF lactate (mg/dL)	8.9–15.8 (12.9)	10.0–15.7 (13.2)	0.965
CSF glucose (mg/dL)	45–64 (51.5)	50–70 (58)	0.0477
Plasma glucose (mg/dL)	80–115 (91.5)	87–110 (91)	0.328
CSF/plasma glucose	0.52–0.63 (0.57)	0.54–0.69 (0.60)	0.0416

p-values are derived from Wilcoxon's test, Chi-square test, and Fisher's exact test.
BZDs, benzodiazepines; CBZ, carbamazepine; CSF, cerebrospinal fluid; ESM, ethosuximide; LEV, levetiracetam; LTG, lamotrigine; PB, phenobarbital; PHT, phenytoin; RFN, rufinamide; STM, sulthiame; TPM, topiramate; VPA, valproic acid; ZNS, zonisamide.

patients with epilepsy and those without epilepsy in either age group. The proportions of patients treated with AEDs were significantly different, with patients receiving valproic acid (VPA) in both age groups, and patients receiving levetiracetam (LEV), zonisamide (ZNS), and benzodiazepines (BZDs) in the age group of 6-17 years. These AEDs were incorporated as independent variables for subsequent multiple regression analysis. Regarding routine laboratory tests, the concentrations of CSF protein, lactate, and plasma glucose were not significantly different between the patients with epilepsy and those without epilepsy in either age group. The CSF glucose concentration ($p = 0.0477$) and CSF/plasma glucose ratio ($p = 0.0416$) were lower in patients with epilepsy than in those without epilepsy.

PCA. The metabolome analysis using GC-MS/MS identified 180 metabolite derivatives in the CSF samples. PCA was performed initially to detect intrinsic

data clustering and outliers (Fig. 1). The PCA score plots did not show definite outliers in either age group; therefore, we included the data from all patients for subsequent analysis. There was no clear separation between epilepsy and non-epilepsy in either age group, suggesting that only a few metabolites had different concentrations in the epilepsy and non-epilepsy groups.

Effect of epilepsy on metabolites. Multiple regression analysis incorporating epilepsy (vs. non-epilepsy, categorical variable) and individual AEDs (categorical variables, only AEDs that were significantly different between the epilepsy and non-epilepsy groups in Table 2) as independent variables was performed to investigate the effect of these variables on individual metabolite concentrations (Table 3). In patients aged 0-5 years, there were 3 metabolites with q-values < 0.05 associated with epilepsy: 2-ketoglutaric acid (lower in epilepsy), pyridoxamine (higher in epilepsy), and tyrosine (higher in epilepsy). In patients aged 6-17 years, 1,5-anhydroglucitol (1,5-AG) showed a significant difference (lower in epilepsy).

Effect of AEDs on metabolites. The multiple regression analysis also demonstrated which metabolites were affected by AEDs (Table 3). In patients aged 0-5 years, VPA was associated with elevated concentrations of 2-propyl-5-hydroxypentanoic acid. In patients aged 6-17 years, VPA was associated with elevated concentrations of 2-aminobutyric acid, 2-ketoisocaproic acid, 2-propyl-5-hydroxypentanoic acid, 4-hydroxyproline, acetylglycine, methionine, *N*-acetylserine, and serine. LEV and ZNS were associated with reduced and elevated concentrations of 1,5-AG, respectively.

Relationship between glucose and 1,5-AG. Because the CSF concentrations of 1,5-AG as measured by GC-MS/MS and the CSF concentrations of glucose as measured by routine laboratory tests were different between the epilepsy and non-epilepsy patients in the age group of 6-17 years, we performed a correlation analysis between the concentrations of these metabolites and between the CSF 1,5-AG concentration and CSF/plasma glucose ratio. There were no significant correlations between the 1,5-AG and CSF glucose concentrations ($\rho = -0.0235$, $p = 0.904$) or between the CSF 1,5-AG concentration and CSF/plasma glucose ratio ($\rho = 0.0473$, $p = 0.808$).

Discussion

We conducted a study of GC-MS/MS-based metabolomics using CSF samples from pediatric patients with and without epilepsy. We demonstrated that GC-MS/MS analysis of the CSF samples was able to detect 180 metabolite derivatives.

In patients aged 0-5 years, there were 3 metabolites with significant differences in concentration between patients with epilepsy and those without epilepsy: 2-ketoglutaric acid, pyridoxamine, and tyrosine.

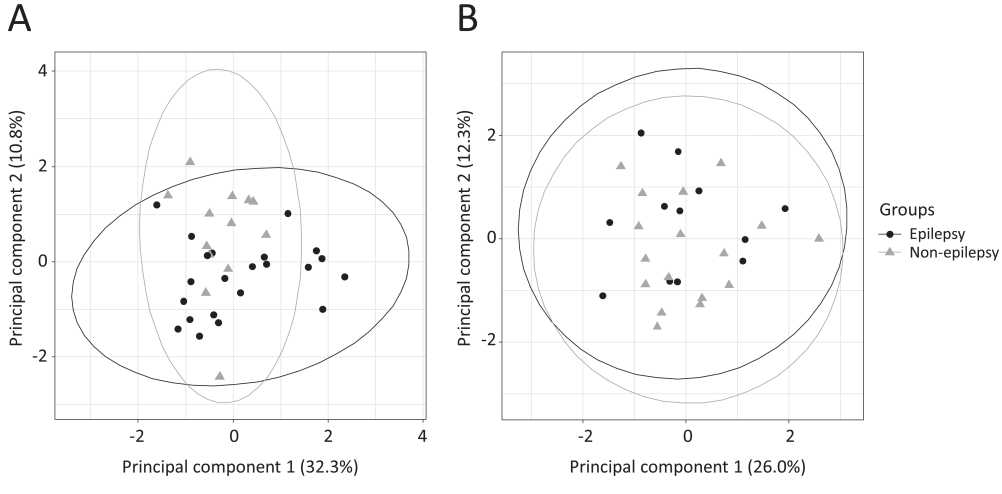

Fig. 1 Principal component analysis (PCA) of metabolome data. Score plots of PCA (first and second principal components) with 99% confidence ellipses in A) the age group 0–5 years and B) the age group 6–17 years. There were no outliers. No definite separation between the epilepsy and non-epilepsy groups could be established.

Table 3 Metabolites associated with epilepsy and antiepileptic drugs

Variables	Metabolites	*p*-value	*q*-value
Age group 0–5 years (n=35)			
Epilepsy	2-ketoglutaric acid (↓)	0.0003	0.0302
	Pyridoxamine (↑)	0.0011	0.0416
	Tyrosine (↑)	0.0009	0.0416
VPA	2-propyl-5-hydroxypentanoic acid (↑)	<0.0001	0.0038
Age group 6–17 years (n=29)			
Epilepsy	1,5-anhydroglucitol (↓)	0.0002	0.0292
VPA	2-aminobutyric acid (↑)	0.0025	0.0476
	2-ketoisocaproic acid (↑)	0.0007	0.0187
	2-propyl-5-hydroxypentanoic acid (↑)	0.0006	0.0187
	4-hydroxyproline (↑)	0.0013	0.0285
	Acetylglycine (↑)	0.0002	0.0115
	Methionine (↑)	0.0029	0.0491
	N-acetylserine (↑)	0.0003	0.0115
	Serine (↑)	0.0001	0.0115
LEV	1,5-anhydroglucitol (↓)	0.0002	0.0306
ZNS	1,5-anhydroglucitol (↑)	<0.0001	0.0050

Up and down arrows indicate the effect of the variables on metabolite concentrations.
VPA, valproic acid; LEV, levetiracetam; ZNS, zonisamide.

Pyridoxamine concentrations were higher in epilepsy patients than in non-epilepsy patients. The elevated pyridoxamine levels may have been due to reduced conversion from pyridoxamine to pyridoxal-5'-phosphate (PLP) via 2 enzymatic reactions catalyzed by pyridoxal kinase and pyridox(am)ine phosphate oxidase (PNPO). An extreme example of a condition in which this pathway is affected is PNPO deficiency, in which low CSF and plasma PLP and elevated plasma pyridoxamine concentrations have been reported [6,7]. PLP is essential for GABA synthesis. In a previous study, we reported lower CSF PLP concentrations in epilepsy patients than in non-epilepsy patients [8]. Collectively, these findings suggest that vitamin B6 metabolism is altered in epilepsy (Fig. 2).

In the tricarboxylic acid cycle, 2-ketoglutaric acid is a rate-determining intermediate with a crucial role in cellular energy metabolism [9]. 2-ketoglutaric acid is also produced from glutamate in many transamination reactions in the cytosol and mitochondria, and in oxidative deamination by glutamate dehydrogenase in mitochondria [10]. Reduced 2-ketoglutaric acid levels suggest impaired energy production in epilepsy and may in part be associated with altered vitamin B6 metabolism, because many aminotransferases are PLP-dependent (Fig. 2). Tyrosine is a conditionally essential amino acid, particularly in young children, and it is involved in dopamine synthesis. Tyrosine is degraded to 4-hydroxyphenylpyruvate by tyrosine aminotransferase, which is a PLP-dependent enzyme that uses tyrosine and 2-ketoglutaric acid as substrates. Congenital deficiency of this enzyme is known to cause tyrosinemia type II. In a previous study, we demonstrated that PLP administration is associated with a reduced concentration of tyrosine [11]. Reduction of PLP and 2-ketoglutaric acid together counteracts this enzymatic reaction and leads to elevation of tyrosine (Fig. 2).

In patients aged 6-17 years, 1,5-AG was significantly reduced in patients with epilepsy compared to patients without epilepsy. 1,5-AG is a metabolically stable sugar alcohol derived mainly from food. It has a high sensitivity to glycemic control. Serum concentrations of 1,5-AG are reduced by glucosuria, in which glucose in the urine inhibits the reabsorption of 1,5-AG in the proximal renal tubules. Concentrations of 1,5-AG in CSF and plasma are comparable [12]. The lower 1,5-AG levels found in patients with epilepsy in the present study are difficult to explain, because there was no patient with known glucosuria. There is also no clear

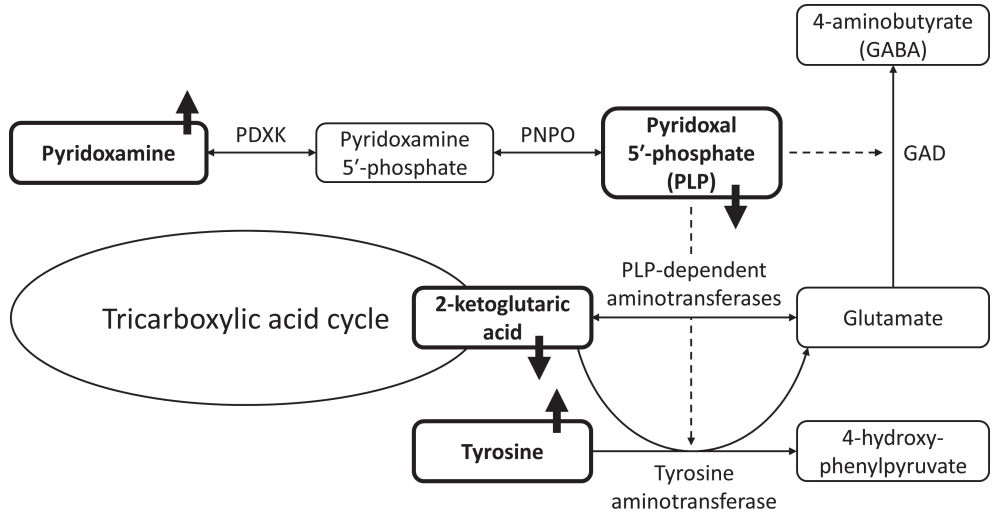

Fig. 2 Metabolic links between pyridoxamine, tyrosine, and 2-ketoglutaric acid. Elevated pyridoxamine may be due to reduced production of PLP from pyridoxamine via PDXK and PNPO. This leads to reduced generation of 2-ketoglutamic acid from glutamate and reduced degradation of tyrosine, because these reactions are catalyzed by PLP-dependent enzymes. PLP is also an essential cofactor for GABA biosynthesis in the central nervous system by GAD. GAD, glutamate decarboxylase; GABA, gamma-aminobutyric acid; PDXK, pyridoxal kinase; PLP, pyridoxal-5'-phosphate; PNPO, pyridox(am)ine phosphate oxidase.

explanation of the effect of LEV and ZNS on 1,5-AG concentrations. There may be a different mechanism to control 1,5-AG concentrations apart from glucose reabsorption in the kidney. For instance, a positive correlation between serum 1,5-AG and uric acid concentrations has been reported in non-diabetic patients, and 1,5-AG and uric acid may share a common transport system, independent of glucose excretion [13]. Interestingly, in our study, patients with epilepsy had lower CSF glucose concentrations and CSF/plasma glucose ratios than those without epilepsy, although these were not correlated with CSF 1,5-AG concentrations. This finding may be related to the cerebral glucose hypometabolism observed by interictal fluorodeoxyglucose positron emission tomography in epilepsy patients [14].

VPA was associated with elevated concentrations of 2-propyl-5-hydroxypentanoic acid, which is a metabolite of VPA. VPA is also associated with elevated concentrations of various amino acids and related metabolites. In a previous study, we reported an association between VPA administration and elevated serine and methionine [11]. VPA has been reported to impair methionine metabolism by inhibiting methionine adenosyltransferase, which synthesizes *S*-adenosylmethionine from methionine [15]. In our study, VPA was also associated with elevated levels of glycine, serine, and threonine metabolites: serine, *N*-acetylserine, acetylglycine, and 2-aminobutyric acid. 2-aminobutyric acid is also associated with methionine metabolism. *N*-acetylserine and acetylglycine are derived from the degradation of *N*-terminal acetylated proteins and are converted to serine and glycine, respectively, by aminoacylase 1. Elevated serine may contribute to elevation of *N*-acetylserine through negative feedback on this enzyme. The elevation of 2-ketoisocaproic acid, an intermediate metabolite of leucine degradation, can be explained by inhibition of branched-chain amino acid catabolism by VPA [16]. Elevation of 4-hydroxyproline is a marker of increased collagen turnover. The function of collagen in the central nervous system has recently been expanded—collagen is no longer considered a merely structural component, but rather a bio-active molecule playing a dynamic role within the central nervous system [17]. The role of VPA in collagen turnover remains to be elucidated.

This study is limited by the small sample size, and thus our results will require further investigation before conclusions can be drawn. We could not find metabolites associated with epilepsy that were common between the two age groups. Although we separated patients into two age groups to reduce the effect of age on metabolites, larger samples with fewer confounders (*e.g.*, variability of age, medications, and comorbidi-

ties) are required to obtain more reliable results. Finally, the metabolomic findings should be confirmed by quantitative assays optimized for individual metabolites.

In conclusion, metabolome analysis of CSF samples using GC-MS/MS detected 180 metabolites. In the age group of 0-5 years, epilepsy patients had reduced 2-ketoglutaric acid and elevated pyridoxamine and tyrosine concentrations, compared with those without epilepsy. These three metabolites were deemed to be associated with alterations in vitamin B6 metabolism. In the age group of 6-17 years, epilepsy patients had reduced concentrations of 1,5-AG, compared with those without epilepsy. The biological significance of these findings remains to be explained. VPA had an influence on various amino acids and related metabolites involved in the metabolism of serine, methionine, and leucine.

Acknowledgments. This study was supported by the Japan Epilepsy Research Foundation (JERF TENKAN 17001). This funding source had no involvement in the study design, the collection, analysis, and interpretation of data, the writing of the report, or the decision to submit this article. We thank Eibunkousei.net (http://www.eibunkousei.net/) for English language editing.

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