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Case Report

Lysine-restricted diet and mild cerebral serotonin deficiency in a patient with pyridoxine-dependent epilepsy caused by *ALDH7A1* genetic defect

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ABSTRACT

Pyridoxine dependent epilepsy (PDE) is caused by mutations in the *ALDH7A1* gene (PDE-*ALDH7A1*) encoding α-aminoadipic-semialdehyde-dehydrogenase enzyme in the lysine catabolic pathway resulting in an accumulation of α-aminoadipic-acid-semialdehyde (α-AASA).
We present the one-year treatment outcome of a patient on a lysine-restricted diet. Serial cerebral-spinal-fluid (CSF) α-AASA and CSF pipecolic-acid levels showed decreased levels but did not normalize. He had a normal neurodevelopmental outcome on a lysine-restricted diet. Despite normal CSF and plasma tryptophan levels and normal tryptophan intake, he developed mild CSF serotonin deficiency at one year of therapy. Stricter lysine restriction would be necessary to normalize CSF α-AASA levels, but might increase the risks associated with the diet. Patients are

Abbreviations: PDE, pyridoxine dependent epilepsy; α-AASAD, alpha-aminoadipic acid semialdehyde dehydrogenase; PDE-*ALDH7A1*, PDE caused by *ALDH7A1* genetic defect; α-AASA, alpha-aminoadipic acid semialdehyde; P6C, piperidine 6-carboxylic acid; PA, pipecolic acid; CNS, central nervous system; CSF, cerebral spinal fluid; CSF-α-AASA, CSF α-AASA; CSF-PA, CSF PA; MSEL, Mullen Scales of Early Learning; PDMS-2, Peabody Developmental Motor Scales — 2nd Edition; GA-I, glutaric aciduria type I; 5-HIAA, 5-hydroxyindolacetic acid; HVA, homovanillic acid levels; P5CR, pyrroline-5-carboxylate reductase; α AASAS, α-AASA synthase.

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at risk of cerebral serotonin deficiency and should be monitored by CSF neurotransmitter measurements.

1. Introduction

Pyridoxine dependent epilepsy (PDE) (OMIM#266100) was first described in 1954 [1]. Mutations in the *ALDH7A1* gene (PDE-*ALDH7A1*) encoding α-aminoadipic-semialdehyde-dehydrogenase (α-AASAD) (EC 1.2.1.31) enzyme in the lysine catabolic pathway were identified in 2006 [2]. α-AASAD enzyme deficiency leads to the accumulation of α-aminoadipic-acid-semialdehyde (α-AASA) and piperidine 6-carboxylic-acid (P6C); the latter inactivates pyridoxal-5-phosphate [2,3]. Pipecolic acid (PA) elevations in body fluids were reported as a secondary biomarker [4].

Classical presentation is neonatal onset intractable seizures with a dramatic response to pyridoxine, but later onset seizures (up to 3 years of age) initially responsive to antiepileptic-drugs have been also reported [3,5]. Pyridoxine supplementation alone does not normalize the accumulation of α-AASA and PA levels in the central nervous system (CNS), which might be the likely cause of developmental delays, necessitating new treatment modalities to improve neurodevelopmental outcomes. Lysine-restricted diet has been recently reported as an observational study with some evidence of improvements in biochemical and neurodevelopmental outcomes [6]. Here, we report a patient with PDE-*ALDH7A1* and his one-year treatment outcome on lysine-restricted diet as a case study.

2. Patient

This 22-month-old boy presented with neonatal intractable epilepsy and was diagnosed with PDE-*ALDH7A1* at the age of 3 months. Clinical, biochemical and molecular genetic features were reported previously [7]. The lysine-restricted diet therapy was approved by the Institutional Research Ethics Board.

He was exclusively breast fed until 6 months of age with weight and length following the 15th percentile (estimated lysine intake of 98 mg/kg/day) [8]. Due to his refusal of solid food intake along with episodes of vomiting, weight and length had fallen to the 3rd percentile between 6 and 7 months of age with an estimated lysine intake of 62 mg/kg/day. A small amount of lysine- and tryptophan-free medical food was started at 6 months of age (15 mL two times a day) to improve later acceptance of the medical food. While tryptophan restriction is not a dietary goal, the available medical food was devoid of both amino acids. The lysine-restricted diet was started at the age of 7 months, after partial improvement in weight gain and growth. He was continued on the same dose of pyridoxine (200 mg/day) throughout the diet therapy.

He underwent clinical and diet assessments and biochemical investigations for liver enzymes, albumin, protein, plasma amino acids, plasma PA and urine α-AASA every 3 months. Cerebral spinal fluid (CSF) neurotransmitters, CSF amino acids, CSF α-AASA (CSF-α-AASA) and CSF PA (CSF-PA) levels were measured at baseline, 6th month and 12th month of therapy. Developmental assessments were performed using the Mullen Scales of Early Learning (MSEL) and the Peabody Developmental Motor Scales — 2nd Edition (PDMS-2) at baseline and 6th and 12th months of therapy. The guidelines for dietary management of glutaric aciduria type I (GA-I) were applied for lysine intake [8]: 90 mg/kg/day, 7–12 months of age and 60–80 mg/kg/day, 1–3 years of age.

3. Results

Weight and length were followed at the 3rd–15th percentile until 16–19 months of age when the percentiles increased to the 50th–85th afterwards. He had significant difficulties drinking the medical food and had food aversions for the first 6 months of dietary restriction. These factors limited the amount of total protein intake and the extent of restriction of natural protein intake. Total protein intake was between 1.6 and 1.8 g/kg/day (average 1.7 ± 0.1), natural protein intake was between 1.3 and 1.7 g/kg/day (average 1.4 ± 0.1), lysine intake was between 80 and 116 mg/kg/day (average 96 ± 13) and tryptophan intake was between 15.6

and 23.4 mg/kg/day (average 19.43 ± 2.7). Nutrition parameters did not show any protein malnutrition including plasma amino acids, total protein, albumin, prealbumin, vitamin D, vitamin B12, folate levels and iron status.

Biochemical investigations were summarized in Table 1a. Plasma lysine and tryptophan levels were normal. CSF lysine was mildly low with normal CSF tryptophan levels. Urine α-AASA levels remained elevated. Plasma PA was normalized at the 6th month of therapy. CSF-α-AASA and CSF-PA levels were markedly elevated in the neonatal period, but decreased to moderately elevated levels prior to the initiation of the lysine-restricted diet. At the 6th month of therapy, CSF-α-AASA was increased from 7 to 16 times of normal and CSF-PA remained unchanged. At the 12th month of therapy, CSF-α-AASA level was mildly improved (11 times of normal) and CSF-PA was marginally improved (from 7.3 to 6.4 times of normal). CSF 5-hyroxyindolacetic acid (5-HIAA) (CSF-5-HIAA) was normal at the 6th month, but mildly low at the 12th month of the lysine-restricted diet therapy.

Developmental assessments performed at the age of 19 months revealed an age appropriate development for gross and fine motor, receptive and expressive language domains by MSEL and PDMS-2 (Table 1b). He remained seizure free on pyridoxine (200 mg/day; 16 mg/kg/day). At the age of 22 months during his last clinic visit, his weight, height and head circumference were at the 50th percentile. His neurological examination was unremarkable.

4. Discussion

We report the outcome of a lysine-restricted diet in a patient with PDE-*ALDH7A1*. Markedly elevated CSF-α-AASA and CSF-PA levels in the neonatal period were decreased to moderately elevated levels at the age of 7 months, prior to the initiation of the lysine-restricted diet. This might be due to the low lysine content of breast milk. Slightly higher lysine intake due to failure to thrive in the first 6 months of therapy resulted in an increase in CSF-α-AASA level (from 7 times to 16 times) for the first 6 months of therapy. Growth and dietary intakes improved over the second 6 months of therapy and lysine intakes were gradually decreased resulting in a decrease in CSF-α-AASA level (from 16 times to 11 times). CSF-PA levels remained moderately elevated at the 6th and 12th months of therapy. We were not able to achieve a normalization of CSF-α-AASA and CSF-PA despite low CSF lysine levels on the lysine-restricted diet in our patient. As long as CSF-α-AASA is elevated, the discontinuation of pyridoxine therapy poses as a high risk for status epilepticus development in patients with PDE-*ALDH7A1*.

According to the literature, lysine is catabolized by two pathways: 1) saccharopine pathway in liver; and 2) PA pathway in brain [2,3,9], however the latter pathway has not formally been proven to exist in humans.

Table 1a
Biochemical investigations in the neonatal period, at baseline and 6th and 12th months of lysine-restricted diet therapy.

Time of collection (age)	Urine α-AASA[a]	Plasma PA[b]	Plasma LYS[c]/TRP[d]	CSF LYS[e]/TRP[f]	CSF-α-AASA[g]	CSF-PA[h]	CSF 5-HIAA[i]/HVA[j]
Neonatal	39.6	31.2	NP	NP	5.8	8.390	NP
Baseline (7 mo)	3.1	6.2	48/42	8.9/5.4	0.7	0.880	NP
6th mo of therapy (13 mo)	11.6	4.2	58/25.3	8/2.3	1.6	0.902	146/365
12th mo of therapy (19 mo)	7.8	3.4	66/36	8.9/2.15	1.1	0.767	126/353

Abbreviations: mo = months; α-AASA = alpha-amino adipic acid semialdehyde; LYS = lysine; TRP = tryptophan; PA = pipecolic acid; CSF = cerebral spinal fluid; 5-HIAA = 5-hydroxyindolacetic acid; HVA = homovanillic acid; and NP = not performed.

[a] Age related reference ranges for urine alpha-AASA: newborn = 0–2 mmol/mol creatinine; <1 year of age = <1 mmol/mol creatinine; and >1 year of age = 0–0.5 mmol/mol creatinine.

[b] Age related reference ranges for plasma pipecolic acid: 0–1 month = 0.1–5.3; 1–6 months = 0.1–3.9; and 7 months–5 years = 0.1–4.2.

[c] Age related reference range for plasma lysine = 45–144 μmol/L.

[d] Age related reference range for plasma tryptophan = 12–69 μmol/L [11].

[e] Age related reference range for CSF lysine = 10.85–39.51 μmol/L.

[f] Age related reference range for CSF = 1.46–9.89 μmol/L.

[g] Age related reference range for CSF-α-AASA: 0.0–0.1 μmol/L.

[h] Age related reference range for CSF-PA: 0.009–0.120 μmol/L.

[i] CSF-5-HIAA reference range: 129–520.

[j] CSF-HVA reference range: 294–1115.

Table 1b
Neurodevelopmental assessments throughout lysine-restricted diet.

	Baseline (7 months of age)	6th months of therapy (13 months of age)	12 months of therapy (19 months of age)
Mullen Scales of Early Learning[a]	GMD = 34th percentile VRD = 76th percentile FMD = 16th percentile RLD = 27th percentile ELD = 42rd percentile	GMD = 4th percentile VRD = 10th percentile FMD = 46th percentile RLD = 46th percentile ELD = 38rd percentile	GMD = 18th percentile VRD = 16th percentile FMD = 58th percentile RLD = 73th percentile ELD = 73rd percentile
Peabody Developmental Motor Scales — 2nd Edition[b]	GMQ = 45th percentile FMQ = 58th percentile TMQ = 50nd percentile	GMQ = 55th percentile FMQ = 42th percentile TMQ = 50nd percentile	GMQ = 23th percentile FMQ = 50th percentile TMQ = 32nd percentile
Alberta Motor Scale[c]	25–50th percentile	25–50th percentile	NP

Abbreviations: GMD = gross motor domain; VRD = visual reception domain; FMD = fine motor domain; RLD = receptive language domain; ELD = expressive language domain; GMQ = gross motor quotient; FMQ = fine motor quotient; TMQ = total motor quotient; and NP = not performed.
[a] Mullen Scales of Early Learning Reference range = 1–99th percentiles.
[b] Peabody Developmental Motor Scales — 2nd Edition: average 25–75th percentiles.
[c] Alberta Motor Scales = average 5–95th percentiles.

The saccharopine pathway is the major pathway in cultured skin fibroblasts, but PA was formed as an unexpected finding likely from accumulated P6C through pyrroline-5-carboxylate (P5C) reductase (P5CR) enzyme [9]. The α-AASA synthase enzyme (α AASAS), which is the first step of lysine catabolism via saccharopine pathway, was absent in brain mitochondria in mice studies of GA-I and suggests that the saccharopine pathway has no role for cerebral lysine catabolism [10]. However, P5CR enzyme activity was not measured in mice brain tissue. Despite consistently low CSF lysine levels, fluctuations in lysine intake resulted in fluctuations in CSF-α-AASA with no fluctuations in CSF-PA levels in our patient. This might be due to limited the conversion of α-AASA to P6C in the CNS between two compartments, namely mitochondrion and peroxisome. Indeed, CSF-PA might be formed from P5C reductase enzyme through the accumulation of CSF-α-AASA in the brain. These findings might pose questions, if α-AASAS enzyme and saccharopine pathway exist in the human brain as major lysine catabolic pathways, or there would be an alternative third pathway for lysine catabolism.

Our patient developed mild serotonin deficiency (low 5-HIAA) with normal dopamine metabolites identified by CSF neurotransmitter analysis at the 12th month of therapy, despite normal plasma and CSF tryptophan levels and normal tryptophan intake in the diet. Serotonin deficiency can cause additional symptoms such as mood instability, sleep disturbances, loss of appetite, and difficulties in memory and learning [12]. CSF and plasma tryptophan levels are not sensitive to identify CNS serotonin deficiency and CSF neurotransmitters should be monitored in patients with PDE-*ALDH7A1* on a lysine-restricted diet. Development of a solely lysine-free (tryptophan-containing) formula would be essential to prevent extra L-tryptophan supplementation for future patients.

We applied standardized developmental assessments to our patient who was identified with borderline gross motor development at the 12th month of therapy by PDMS-2 with a normal fine motor quotient. MSEL revealed a decrease in the percentile for gross motor domain at 6th months of therapy, whereas improved from the 4th to the 18th percentile at 12th months of therapy. Fine motor domain improved consistently throughout therapy. Receptive and expressive language domains showed improvements at 12th months of therapy. We are not certain, if the lysine-restricted diet improved the neurodevelopmental outcome in our patient. A randomized control trial to assess effectiveness and to compare outcomes would be essential, if this diet is to be applied as standard care. However, due to limited number of patients and phenotypic variability for a very rare disease, even randomized control trials will be difficult to interpret.

5. Conclusions

We presented a patient with PDE-*ALDH7A1* and the one-year treatment outcome of a lysine-restricted diet as a case study. The lysine-restricted diet was well tolerated without major clinical side effects and normal growth, but mildly decreased CSF 5-HIAA level. As breast milk has low lysine content, if patients with PDE-*ALDH7A1* are on exclusive breastfeeding for the first 6 months of life, they would have physiologically low lysine intake and a lysine-restricted diet should be started at the time of solid food introduction. Normalization of CSF-α-AASA and CSF-PA would require stricter lysine restriction, but might increase the risks associated with the diet. Plasma PA can normalize on lysine restriction or pyridoxine monotherapy [13], while in fact, due to the unique availability of multiple CSF samples from this individual in this study, PA remained increased in the CNS compartment. Changes in lysine intake do not influence CSF-PA levels, but have an impact on CSF-α-AASA levels. This treatment outcome study poses new questions for brain lysine catabolism.

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