



## First patient in Serbia with biochemically and genetically diagnosed pyridoxine-dependent epilepsy

Prvi bolesnik u Srbiji sa biohemijski i genetički dijagnostikovanom piridoksin zavisnom epilepsijom

Miloš M. Ješić\*<sup>†</sup>, Maja D. Ješić\*<sup>†</sup>, Svetlana Buljugić\*, Aleksandra Živanović\*

\*University Children's Hospital, Belgrade, Serbia; University of Belgrade, <sup>†</sup>Faculty of Medicine, Belgrade, Serbia

### Abstract

**Introduction.** Pyridoxine-dependent epilepsy (PDE) is a rare autosomal recessive inborn error of metabolism present with early-onset seizures resistant to common anticonvulsants. PDE has been shown to be caused by a defect of a  $\alpha$ -amino adipic semialdehyde dehydrogenase (also known as ALDH7A1 or antiquitin) in the cerebral lysine degradation pathway. Its deficiency results in accumulation of  $\alpha$ -amino adipic semialdehyde ( $\alpha$ -AASA), piperidine-6-carboxylate and pipercolic acid, which serve as diagnostic markers in urine, plasma and cerebrospinal fluid of the disease.  $\alpha$ -Amino adipic semialdehyde dehydrogenase is encoded by the ALDH7A1 or antiquitin gene and definite confirmation of diagnosis of PDE is made by genetic analysis. **Case report.** We present a first patient in Serbia who was diagnosed clinically, biochemically and genetically. We suspected PDE due to drug-resistant seizures in the seventh day of life when we attempted with pyridoxine. Since that time the patient has taken pyridoxine and the seizures have not recurred. Our patient had markedly elevated  $\alpha$ -AASA in urine while on treatment with individual dosages of pyridoxine. Molecular-genetical analysis identified mutations of the ALDH7A1 (antiquitin) gene. **Conclusion.**  $\alpha$ -AASA is reliable marker to select PDE patient for molecular analysis of the ALDH7A1 (antiquitin) gene. Diagnosis is confirmed by molecular-genetical analysis and pyridoxine withdrawal is no longer needed to establish the diagnosis of „definite“ PDE.

**Key words:** epilepsy; infant, newborn; vitamin B6; genetic diseases, inborn; diagnosis, differential; drug therapy; serbia.

### Apstrakt

**Uvod.** Piridoksin zavisna epilepsija (PZE) je redak, urođeni autozomno-recesivan poremećaj metabolizma sa ranom pojavom konvulzija rezistentnih na uobičajene antikonvulzivne lekove. Utvrđeno je da je PZE posledica poremećaja  $\alpha$ -amino adipin semialdehid dehidrogenaze (poznate i kao ALDH7A1 ili antikvitin) na putu degradacije cerebralnog lizina. Njegov nedostatak dovodi do nakupljanja  $\alpha$ -amino adipin semialdehida ( $\alpha$ -AASA), piperidin-6-karboksilata i piperkolične kiseline u urinu, plazmi i cerebrospinalnoj tečnosti, i oni se koriste kao dijagnostički markeri oboljenja.  $\alpha$ -Amino adipin semialdehid dehidrogenaza je kodirana ALDH7A1 ili antikvitin genom i definitivna dijagnoza PZE se utvrđuje genetičkom analizom. **Prikaz bolesnika.** Ovo je prikaz prvog bolesnika u Srbiji čije je oboljenje dijagnostikovano klinički, biohemijski i genetički. Na PZE smo posumnjali zbog konvulzija rezistentnih na lekove koje su se javile sedmog dana života kada smo kod bolesnika pokušali lečenje primenom piridoksina. Od početka primene piridoksina bolesnik više nije imao ponavljane konvulzije. Bolesnik je imao značajno povišen  $\alpha$ -AASA u urinu tokom lečenja pojedinačnim dozama piridoksina. Analizom na molekularno-genetičkom nivou identifikovane su mutacije ALDH7A1 ili antikvitin gena. **Zaključak.**  $\alpha$ -AASA je pouzdan marker za selekciju bolesnika sa PZE radi molekularne analize ALDH7A1 gena. Dijagnoza našeg bolesnika potvrđena je analizom na molekularno-genetičkom nivou i nije bilo potrebno prekidati terapiju piridoksinom radi potvrđivanja dijagnoze PZE.

**Ključne reči:** epilepsija; novorođenče; vitamin B6; genetičke bolesti, urođene; dijagnoza, diferencijalna; lečenje lekovima; srbija.

### Introduction

Pyridoxine-dependent epilepsy (PDE) is an autosomal recessive disease which occurs in 1 in 100,000 to 700,000 individuals. At least 100 cases have been reported

worldwide<sup>1</sup>. Typically, patients present with neonatal seizures, but atypical manifestations up to 3 years of age, as well as transient response to common anticonvulsants or poor initial response to pyridoxine have been reported<sup>2-4</sup>. Until recently, a definite diagnosis of PDE had been established

upon a successful therapeutic trial with pyridoxine and further proof of pyridoxine after a controlled withdrawal with recurrence of seizures<sup>5,6</sup>. Recently, PDE has been shown to be caused by a defect of  $\alpha$ -aminoadipic semialdehyde dehydrogenase (also known as ALDH7A1 or antiquitin, ATQ), in the cerebral lysine degradation pathway and to catalyze the conversion of  $\alpha$ -aminoadipic semialdehyde ( $\alpha$ -AASA) to  $\alpha$ -aminoadipic acid.  $\alpha$ -AASA is in chemical equilibrium with piperidine-6-carboxylic acid (P6C). P6C has been shown to inactivate pyridoxal phosphate (PLP), the active vitamer of pyridoxine, by a Knoevenagel condensation reaction, leading to a severe secondary PLP deficiency. As PLP is a cofactor of various enzymes in the central nervous system, seizures in PDE are more probably due to a perturbation in metabolism of cerebral amino acids and neurotransmitters<sup>3</sup>.

Biochemically, ALDH7A1 or ATQ deficiency is characterized by the accumulation of  $\alpha$ -AASA and P6C and by the accumulation of pipercolic acid (PA), which is formed proximally to the primary enzyme defect. Screening for ATQ deficiency is possible *via* determination of urinary or plasma  $\alpha$ -AASA and P6C, and of plasma PA. The diagnosis is confirmed by mutation analysis<sup>3</sup>.

Molecular analysis has revealed mutations of the ALDH7A1 or ATQ gene in all individuals with PDE and increased PA and/or  $\alpha$ -AASA in plasma or urine. Pyridoxine therapy is life-long, but despite treatment many patients with PDE have a disorder of psychomotor development. Gathering evidence on the usefulness of lysine restricted diet is in progress<sup>2,7</sup>.

We report the first patient in Serbia with biochemical and genetically diagnosed classic form of PDE.

### Case report

A 6-day old boy was referred to our Neonatal Unit from a maternity hospital due to recurrent episodes of seizures with shrieking, starting within the first days of life. The patient was born to a 23-year-old mother by spontaneous vaginal delivery after an uneventful pregnancy. A full term male neonate had 3,900 g, a length of 57 cm and head circumference 37 cm (97th percentile). At birth, his Apgar scores were 9 and 9, at 1 and 5 min, respectively. He was the first child of healthy parents without known consanguinity. In addition, there was no family history of epilepsy or neurological disorders.

The baby was incapable of tolerating oral feedings. The baby seizures were complex: flexion of the limbs, twisting and jerking of the body and limbs, jerks of facial muscles with blinking, and all accompanied by occasional screams. When applied anticonvulsants (phenobarbital and/or midazolam), the response was short-lived and seizures were repeated. The first electroencephalography (EEG) performed after a seizure that lasted 6 minutes showed a discharge of high voltage bizarre spike-wave complexes and multiple spikes. His lactate level was elevated (up to 12.7 mmol/L, normal < 2 mmol/L) during seizures and normal in the periods without seizures. His ammonia level was 28  $\mu$ mol/L (normal 42–144  $\mu$ mol/L), while urine ketones were negative. All other biochemical analyses (glycemia, calcium, magnesium, sodium, potassium) and ultrasound examinations did not indicate the

cause of drug-resistant seizures. After about 24 hours, an attempt was made to stop drug-resistant seizures with intravenous *iv* pyridoxine at a dose of 100 mg. Apnea did not occur during the administration of pyridoxine. EEG monitoring was not performed simultaneously due to technical reasons. He became seizure-free after the first dose. We continued with the administration of *iv* pyridoxine, 15 mg/kg/day over the next 7 days, and then moved on to oral administration. By day 10 cranial magnetic resonance imaging was performed. The significance of this finding was mild cerebellar hypoplasia and small subdural hemorrhage posteriorly.

Measurement of urinary  $\alpha$ -AASA by electrospray ionisation tandem mass spectrometry under pyridoxine treatment was performed at the Biochemistry Department, Institute of Child Health in London. Urinary  $\alpha$ -AASA level was markedly elevated (206.46 mmol/mol creatinine; normal at age < 6 months: < 2.0 mmol/mol creatinine). To make gene analysis and establish the cause of seizures we used the patient's DNA. The result of genetic analysis was performed at the Regional Molecular Genetic Laboratory at Great Ormond Street Hospital for Children in London. Sequence analysis for mutations associated with PDE identified that the patient was a heterozygous for the c.328 C > T mutation in exon 4 of the ALDH7A1 gene resulting in the substitution of arginine with stop codon at amino acid position 110 (p.Arg110\*) and a heterozygous single transversion of G > T at a highly conserved donor splice site in intron 17 (c.1566-1 G > T). Both mutations have been reported previously in patients with PDE<sup>3,4</sup>.

After 3 weeks, the baby was discharged from the hospital in a good clinical condition with oral pyridoxine (50 mg/day). Five months after starting pyridoxine, the patient's EEG was normal in drowsy and sleep states. His neurological examination showed normal head growth, truncal hypotonia, and mildly decreased peripheral tone in the extremities. At 12 months of age the patient's neuropsychological testing was done. According to Brunet Lézin-scale development, the overall index was 83 units, i.e. at the level of a 10-month-old infant.

### Discussion

Clinical course of events in our patient suggests a classic form of PDE. Seizures occurred in the first days of life. After admission at our Neonatal Unit anticonvulsants were administered, but the attacks were repeated. After 24 hours, pyridoxine was applied, and the seizures ceased completely. During the 12 months of life on pyridoxine treatment, neither seizures have been reported nor was EEG recorded. To make sure that late and masked response is not missed, treatment with oral/enteral pyridoxine should be continued until ALDH7A1 or ATQ deficiency is excluded by negative biochemical and/or genetic testing.

For diagnostic workup of neonates and infants with therapy-resistant seizures, many previous studies recommended serum or/and urine samples for the determination of  $\alpha$ -AASA and PA regardless of a therapeutic trial with pyridoxine<sup>3,5,7,8</sup>. While  $\alpha$ -AASA is a pathognomonic marker of defects in the ALDH7A1 or ATQ gene, as illustrated by our pa-

tient, PA has the disadvantage of also being elevated secondarily in liver disease and peroxisomal defects like Zellweger syndrome<sup>8,9</sup>. In contrast,  $\alpha$ -AASA is the primary substrate of the affected enzyme, ALDH7A1 or ATQ, and remains more markedly elevated than PA in plasma and urine in all patients while on pyridoxine. Levels of urinary  $\alpha$ -AASA in normal individuals decline during the first year of life: < 6 months: < 2.0 mmol/mol creatinine; 6–12 months: < 1.0 mmol/mol creatinine, > 1 year: < 0.5 mmol/mol creatinine. Pathological values for  $\alpha$ -AASA are several folds above the upper limit of the appropriate reference range and seem to depend on the nature of the mutation, and the child's age, treatment with pyridoxine, and possibly on nutritional lysine intake. In our patient urinary  $\alpha$ -AASA level was markedly elevated. It is important to note that PA levels in urine normalize under treatment, and patients with mild missense mutations may have near normal PA levels in plasma while on pyridoxine<sup>3,4</sup>.

Mutation analysis of the ALDH7A or ATQ gene is recommended to confirm the diagnosis.  $\alpha$ -AASA and/or PA are reliable markers to select PDE patients for molecular analysis of the ALDH7A or ATQ gene. To date more than 60 different mutations within the 18 exons of the ALDH7A or ATQ gene at chromosome 5q31 have been published<sup>10–12</sup>. Of these, 50–60% are missense mutations resulting in an altered amino acid in the protein sequence. A sequence analysis has shown that our patient is an apparent compound heterozygote for two mutations both of which are highly likely to be pathogenic. Furthermore, an intronic mutation, c.1566-1G > T, as that of our patient, may have an increased frequency among European patients<sup>3</sup>. Prenatal diagnosis by molecular analysis is feasible in forthcoming pregnancies<sup>3,13</sup>.

If seizures subside after the administration of pyridoxine, instead of PDE, the patients may have: folinic acid-responsive

seizures (genetically identical to ATQ deficiency but not biochemically), pyridox(am)ine phosphate oxidase deficiency, neonatal/infantile hypophosphatasia (tissue-nonspecific alkaline phosphatase deficiency), familial hyperphosphatasia (phosphatidylinositol glycan anchor biosynthesis type V deficiency) and hyperprolinemia type II<sup>2,3</sup>. Importantly, these affected patients with pyridoxine responsive seizures haven't elevations in  $\alpha$ -AASA in plasma and/or urine. Detection of elevated levels of this organic acid is a sensitive marker for PDE. While  $\alpha$ -AASA was first thought to be a specific biomarker for PDE, recent research has demonstrated that  $\alpha$ -AASA is also elevated in patients with molybdenum cofactor deficiency (MoCoFa) and isolated sulfite oxidase deficiency (SOX)<sup>14</sup>. In patients with elevated levels of  $\alpha$ -AASA, these latter two conditions may be differentiated from PDE by measuring urinary sulfite/sulfo-cysteine levels. MoCoFa and SOX deficiency are serious and often fatal diseases for which no effective therapy has been available until cyclic pyranopterin was used successfully in a patient with MoCoFa<sup>14,15</sup>.

### Conclusion

The diagnosis of PDE is not routine and requires significant involvement of medical personnel, so that the patient could receive appropriate treatment. Clinical diagnostic approach to PDE can now be replaced by measurement of reliable diagnostic marker. Our patient with possible PDE was diagnosed according to the elevated concentrations of urinary  $\alpha$ -AASA and did not undergo the risk of a diagnostic pyridoxine withdrawal. So far all published PDE patients with mutations of *ATQ* gene have had neonatal onset of seizures. Future studies are needed to investigate genotype-phenotype correlation of PDE.

### R E F E R E N C E S

1. *Genetic Testing Registry*. Pyridoxine-dependent epilepsy. Available from: <http://ghr.nlm.gov/condition/pyridoxine-dependent-epilepsy>
2. Stockler S, Plecko B, Gospe SM, Coulter-Mackie M, Connolly M, van Karnebeek C, et al. Pyridoxine dependent epilepsy and antiquitin deficiency: Clinical and molecular characteristics and recommendations for diagnosis, treatment and follow-up. *Mol Genet Metab* 2011; 104(1–2): 48–60.
3. Plecko B, Paul K, Paschke E, Stoekler-Ipsiriglu S, Struys E, Jakobs C, et al. Biochemical and molecular characterization of 18 patients with pyridoxine-dependent epilepsy and mutations of the antiquitin (ALDH7A1) gene. *Hum Mutat* 2007; 28(1): 19–26.
4. Gospe SM Jr, Hecht ST. Longitudinal MRI findings in pyridoxine-dependent seizures. *Neurology* 1998; 51(1): 74–8.
5. Gospe SM. Pyridoxine-dependent seizures: Findings from recent studies pose new questions. *Pediatr Neurol* 2002; 26(3): 181–5.
6. Baxter P. Pyridoxine-dependent and pyridoxine-responsive seizures. *Dev Med Child Neurol* 2001; 43(6): 416–20.
7. Basura GJ, Hagland SP, Wiltse AM, Gospe SM. Clinical features and the management of pyridoxine-dependent and pyridoxine-responsive seizures: Review of 63 North American cases submitted to a patient registry. *Eur J Pediatr* 2009; 168(6): 697–704.
8. Gospe SM. Pyridoxine-Dependent Epilepsy and Related Conditions. In: Pearl PL, editor. *Inherited Metabolic Epilepsies*. New York: Demos Medical Publishing; 2013. p. 145–76.
9. Peduto A, Baumgartner MR, Verhoeven NM, Rabier D, Spada M, Nassogne M, et al. Hyperpepcolic acidemia: A diagnostic tool for peroxisomal disorders. *Mol Genet Metab* 2004; 82(3): 224–30.
10. Mills PB, Footitt EJ, Mills KA, Tuschl K, Aylett S, Varadkar S, et al. Genotypic and phenotypic spectrum of pyridoxine-dependent epilepsy (ALDH7A1 deficiency). *Brain* 2010; 133(Pt 7): 2148–59.
11. Millet A, Salomons GS, Cneude F, Corne C, Debillon T, Jakobs C, et al. Novel mutations in pyridoxine-dependent epilepsy. *Eur J Paediatr Neurol* 2011; 15(1): 74–7.
12. Mills PB, Struys E, Jakobs C, Plecko B, Baxter P, Baumgartner M, Clayton PT. Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nat Med* 2006; 12(3): 307–9.
13. Bok LA, Halbertsma FJ, Houterman S, Wevers RA, Vreeswijk C, Jakobs C, et al. Long-term outcome in pyridoxine-dependent epilepsy. *Dev Med Child Neurol* 2012; 54(9): 849–54.

14. Mills PB, Footitt EJ, Ceyhan S, Waters PJ, Jakobs C, Clayton PT, et al. Urinary AASA excretion is elevated in patients with molybdenum cofactor deficiency and isolated sulphite oxidase deficiency. *J Inherit Metab Dis* 2012; 35(6): 1031–6.
15. Veldman A, Santamaria-Aranjo JA, Sollazzo S, Pitt J, Gianello R, Yaplito-Lee J, et al. Successful treatment of molybdenum cofactor deficiency type A with cPMP. *Pediatrics* 2010; 125(5): e1249–54.

Received on March 11, 2015.  
Revised on November 15, 2015.  
Accepted on February 18, 2016.  
Online First September, 2016.