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Pyridoxine-dependent epilepsy due to antiquitin deficiency: achieving a favourable outcome

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ABSTRACT – We report 4 pyridoxine-dependent epilepsy patients in which good outcome was determined in three. The 4 patients were male and aged from 7 to 24 years old (from three unrelated Caucasian families). A clinical diagnosis of neonatal pyridoxine-dependent epilepsy was confirmed by biochemical and genetic studies. Clinical evaluation was performed and medical records were reviewed for therapy implementation and management, neurodevelopment outcome, magnetic resonance imaging, and electroencephalography. All were taking pyridoxine treatment and were seizure-free. Elevated urinary alpha-aminoadipic semialdehyde excretion was found in all patients. Antiquitin gene analysis identified a large homozygous deletion in one patient and two heterozygous mutations in the others. Treatment with pyridoxine should be attempted for all cases of infantile and childhood refractory epilepsy, as has been the case over the last 20 years. Currently, urinary alpha-aminoadipic semialdehyde is a reliable biomarker of pyridoxine-dependent epilepsy, even under pyridoxine treatment. Detection of mutations in the antiquitin gene, encoding alpha-aminoadipic semialdehyde dehydrogenase, establishes the diagnosis and allows for adequate genetic counselling.

Key words: α-AASA, ALDH7A1, pyridoxine-dependent epilepsy

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Pyridoxine-dependent epilepsy (PDE) (MIM#266100) is a rare autosomal recessive disorder that usually presents with neonatal intractable seizures. Its prevalence is around 1/400,000 children, but is probably underestimated (Been *et al*., 2005). It was first described by Hunt in 1954 as a severe infantile seizure disorder which responded radically to pyridoxine treatment (Hunt *et al*., 1954).

The classic presentation of PDE is during the neonatal period (first 48 hours of life) with polymorphic seizures (frequent and brief) that may exhibit individual variability. Several additional symptoms/features have been described, including abnormal foetal movements, signs of birth asphyxia, and other systemic manifestations such as hyperalertness, irritability, and tremulousness and abnormal cry, and a misdiagnosis of hypoxic-ischaemic encephalopathy may be made (Baxter, 2001; Mills *et al*., 2010).

Seizures are typically refractory to common antiepileptic drugs (AEDs) and are usually controlled with pharmacological doses of pyridoxine.

The atypical presentation (in about a third of PDE cases) can occur later, up to 2 years old. In these cases, seizures may initially respond to AEDs but become later intractable, or may not respond to pyridoxine early in life but become controlled by pyridoxine months later; prolonged seizure-free intervals may occur even after pyridoxine discontinuation (Baxter, 2001; Stockler *et al*., 2011).

The EEG pattern is not specific. It may vary and include normal to high-voltage delta activity, focal spike-wave discharges, burst suppression patterns, and, rarely, hypsarrhythmia, with no pathognomonic features, it may also be normal (Nabbout *et al*., 1999; Nassan *et al*., 2009; Schmitt *et al*., 2010). Most patients are described as having mild development delay, with difficulties in expressive language.

The most frequent cause of PDE is alpha-aminoadipic semialdehyde dehydrogenase (ALDH7A1) deficiency due to mutations in the *ALDH7A1*/antiquitin (*ATQ)* gene. ALDH7A1 plays a role in cerebral lysine catabolism through the pipecolic acid pathway. Its deficiency causes α-AASA and Δ1-piperideine-6-carboxylate (P6C) accumulation. This interferes with pyridoxal phosphate (PLP) activation in the brain, as P6C undergoes chemical condensation with PLP resulting in its deficiency. Pyridoxine intake compensates the loss of PLP, but does not correct the primary deficiency, as demonstrated by the persistent excretion of metabolites in urine (Stockler *et al*., 2011). The accumulated alpha-aminoadipic semialdehyde (α-AASA) has become a biomarker for PDE (Mills *et al*., 2006).

PDE diagnosis has classically relied on clinical response to pyridoxine and the recurrence of seizures upon pyridoxine discontinuation. The recent availability of biomarkers for *ALDH7A1* deficiency, along with genetic studies, allows for confirmation of diagnosis, even under pyridoxine treatment.

The purpose of this report was to present a series of patients with typical neonatal PDE, whose diagnosis was confirmed by biochemical and genetic analysis, and discuss long-term epileptic and cognitive data, which was favourable in three patients. All patients are presently taking pyridoxine treatment and are seizure-free.

Patients and methods

The 4 patients were male, aged 7 to 24 years old, and from three unrelated Caucasian families. Their medical records were reviewed for therapy implementation and management, neurodevelopment outcome, magnetic resonance imaging, and electroencephalography. Clinical re-evaluation and neurocognitive assessment with the Wechsler Intelligence Scale for Children (third edition, WISC-III) and Wechsler Adult Intelligence Scale (third edition, WAIS-III), both adapted for the Portuguese population, were performed for the younger and older patients, respectively. Urinary alpha-aminoadipic semialdehyde excretion was measured and antiquitin gene molecular analysis performed in all patients. Informed consent was obtained.

Case studies

Patient 1

A male (DOB 21.10.1988) was born to non-consanguineous parents, with normal birth parameters. Abnormal foetal movements during the last weeks of gestation were recorded. Epileptic spasms began during the first 12 hours of life, responding to phenobarbital and pyridoxine (100 mg, IV). The EEG at 1 month of age showed slow basal rhythm and all drugs were suspended. At 3 and 4 months of age, a few tremors were noted and after the seventh month there was a resumption of seizures of various types (tonic, tonic-clonic, and atonic), associated with fever leading to ICU admission. The EEG revealed slow basal rhythm. He maintained sporadic seizures, which at 10 months of age progressively increased in frequency, leading to status epilepticus which prompted a second admission to the ICU. Seizures stopped with AEDs (phenobarbital and phenytoin) plus pyridoxine (100 mg, IV). The EEG pattern normalised and psychomotor development improved under valproic acid and pyridoxine. Brain CT showed general atrophy with hypoplasia of corpus callosum. After 12 months, under only pyridoxine (80 mg/day,

Table 1. Clinical and genetic data from the four patients.

Subject	Patient 1	Patient 2	Patient 3	Patient 4
Age (years) / gender	23 / male	22 / male	8 / male	6 / male
Clinical presentation	12H - Seizures with epileptic spasms	1H - myoclonic seizures	D8 - recurrent myoclonic seizures, vertical nystagmus, hypertonia and apnoea	D6 - myoclonic seizures, vertical nystagmus
Family History	Irrelevant	Irrelevant	Irrelevant	Brother with PDE (patient 3)
EEG evaluation	1m-delay of bioelectric rhythm 12m - 16y - no paroxysmal features	3-7m - Normal pattern 12m - delay of bioelectric rhythm 4y-15y - generalized paroxysmal activity, predominantly bifrontal - no clinical correlation	D23 - global depressed amplitude with left paroxymal activity 13m - paroxysmal centro-temporal activity 15m - multifocal convulsive activity 20m - posterior paroxysmal activity	Normal pattern
Evolution / Treatment	12H - Phenobarbital plus Pyridoxine 1m - Pyridoxine suspended 4-7m - tonic, tonic-clonic and atonic seizures with fever - Valproic acid 9m- Status Epilepticus: phenobarbital +phenytoin + pyridoxine 12m - Phenobarbital stopped Adolescence - myoclonic movements	D1 - seizures recurred only after pyridoxine plus phenobarbital D12 - suspended pyridoxine 12m - pyridoxine reintroduction 4y - episodes of seizures with fever - pyridoxine plus carbamazepine	D8 - Phénobarbital; trial with pyridoxine was inconclusive 13m - status epilepticus; anticonvulsant drugs‡ resistance; pyridoxine reintroduction - seizures resolved	D6 – pyridoxine – never interrupted treatment
CNS image	9m - Brain CT: general atrophy with hypoplasia of corpus callosum	11y- Cerebral MRI - thinning of the posterior part of corpus callous	D28 and 15m - Cerebral MRI normal	Not done
Development	9m-DD 3y-adequate development	Normal psychomotor development	5y - difficulties in expressive language	Normal psychomotor development
Urinary α-AASA	3,5 mmol/mol creat. (age 22 y)	4,2 mmol/mol creat. (age 22 y)	7,8 mmol/mol creat. (age 6 y)	3,9 mmol/mol creat. (age 5 y)
Genotype (*ALDH7A1 gene mutations*)	Compound heterozygosity • *c.505C>, p.(Pro169Ser)* • *c. 1217_I218delAT.p.(Tyr406CysfsX3)*	Homozygous deletions comprising *exons* 17 and 18	Compound heterozygosity: • *c.505C>, p.(Pro169Ser)* • *c.920G>A,p.(Arg307Gln)*	Compound heterozygosity • *c.505C>, p.(Pro169Ser)* • *c.920G>A, p.(Arg307Gln)*
Current Status	Pyridoxine monotherapy - free of seizures; College in economics	Pyridoxine monotherapy - free of seizures; Informatics course	Pyridoxine monotherapy - free of seizures; Third grade - normal classroom	Pyridoxine monotherapy - free of seizures; First grade - normal classroom

DD: developmental delay; ‡: phenobarbital, sodium valproate, topiramate, and levetiracetam; H: hours; D: days; m: months; y: years old. The normal range for urinary αAASA was considered 0.5-1 mmol/mol creatinine.

PO), no more seizures were recognised, EEGs showed normal pattern, and psychomotor development was adequate. A pyridoxine dose was gradually increased, from 100 mg/day PO at 3 years old to 600 mg/day PO at present, and he continues to remain seizure-free. Urinary α-AASA levels were elevated and sequence analysis of *ALDH7A1* gene revealed two pathogenic mutations. Parental studies confirmed compound heterozygous status (*table 1*). Recent neuropsychological evaluation with WAIS-III, at age 24, revealed normal cognitive development, with a Full Scale IQ of 107, a verbal IQ of 109, and a performance IQ of 103. He attends college for economics.

Patient 2

A male (DOB 31.01.1989) was born to unrelated parents, with normal birth parameters. The mother noticed unusual intrauterine movements in the third trimester. He started with myoclonic seizures in the first hour of life, which responded to phenobarbital and pyridoxine (100 mg, IV) treatment. At Day 12, drugs were suspended. At 7 months, myoclonic seizures relapsed and pyridoxine was reintroduced at 80 mg/day PO. The EEG at 3, 7, and 12 months revealed slow basal rhythm with no paroxysmal activity. He had expressive language problems. At age 4, after a seizure-free period, seizures relapsed during fever episodes and pyridoxine was increased from 150 mg/day PO to 300 mg/day PO. Interictal EEG showed bifrontal paroxysmal activity. Since he still had frequent fits without clonic movements, pyridoxine (600 mg/day PO) and carbamazepine (600 mg/day PO) were administered. Seizure frequency was reduced to 1-2/year. Cerebral MRI showed thinning of the posterior part of the corpus callosum. Since the age of 11, he has been free of seizures, with normal EEG. Until 19 years of age, he was under pyridoxine (600 mg/day PO) and carbamazepine (600 mg/day PO). Currently, he is on pyridoxine, 600 mg/day PO, and is seizure-free.
Urinary α-AASA levels were elevated and genetic analysis of *ALDH7A1* gene showed that exons 17 and 18 could not be amplified by PCR, indicating a large homozygous deletion comprising these exons (*table 1*). Recent neuropsychological evaluation with WAIS-III, at age 24, revealed low-average cognitive development, with a Full Scale IQ of 85, a verbal IQ of 92, and a performance IQ of 78. He is attending law college.

Patients 3 and 4

Two male brothers were born to non-consanguineous parents, after uneventful pregnancy and delivery.
Patient 3 (DOB 17.04.2004) started to have seizures (tonic-clonic seizures) at Day 8 of life which responded to phenobarbital. Ventilation support was needed. At the end of the neonatal period (Day 28), phenobarbital was stopped. The EEG showed a slow basal rhythm with left paroxysmal activity. Brain MRI was performed without any evidence of anomaly. After six months, seizures reappeared during fever episodes and the patient was treated with valproic acid (EEG revealed centrotemporal paroxysms with a normal base rhythm). He was readmitted twice to ICU at age 13 and 15 months in status epilepticus, again after a febrile event. EEG at 13 months revealed a global slowing-down with encephalopatic characteristics. Since there was no response to AEDs (phenobarbital, topiramate, and levetiracetam), trials with pyridoxine at 100 mg IV and 300 mg IV, were proposed, respectively (*figure 1*), with remission of symptoms with the latter. Subsequent brain MRI was performed with no abnormal findings. He was discharged with pyridoxine (100 mg/day PO) and progressive withdrawal of AEDs. Before 3 years of age, he was admitted to the emergency unit a few more times with seizures during febrile episodes. Since then, he has been receiving pyridoxine monotherapy (100 mg/day PO). Currently, at 8 years old, he is free of seizures; he attends school in third grade and is part of a special education program. Recent neuropsychological evaluation using the WISC-III, at age 8, revealed moderately delayed cognitive development with a Full Scale IQ of 62, a verbal IQ of 73, and a performance IQ of 59 (the other composite scores were: verbal Comprehension index: 76; Perceptual Reasoning Index: 58; and Processing Speed Index: 74).
Patient 4 (DOB 09.09.2005) presented with myoclonic generalised seizures at Day 6 of life. He was immediately given pyridoxine (150 mg IV) because of the family history and seizures promptly ended. He was discharged under pyridoxine (100 mg/day PO), which he continues to take. Currently, at age 7, he is seizure-free, has a normal cognitive outcome, and attends normal school. The neuropsychological assessment, evaluated using the WISC-III, showed a Full Scale IQ of 109, a verbal IQ of 113, and a performance IQ of 102 (the other composite scores were: verbal Comprehension index: 111; Perceptual Reasoning Index: 103; and Processing Speed Index: 106).
The urinary α-AASA level of both patients was elevated and genetic analysis of the *ALDH7A1* gene identified two pathogenic mutations. Parental studies confirmed compound heterozygous status (*table 1*).

Discussion

These four cases presented with symptoms during the early neonatal period (Days 1-8). For all, diagnosis was based on response to pyridoxine treatment. Diagnosis was later confirmed by elevated urinary

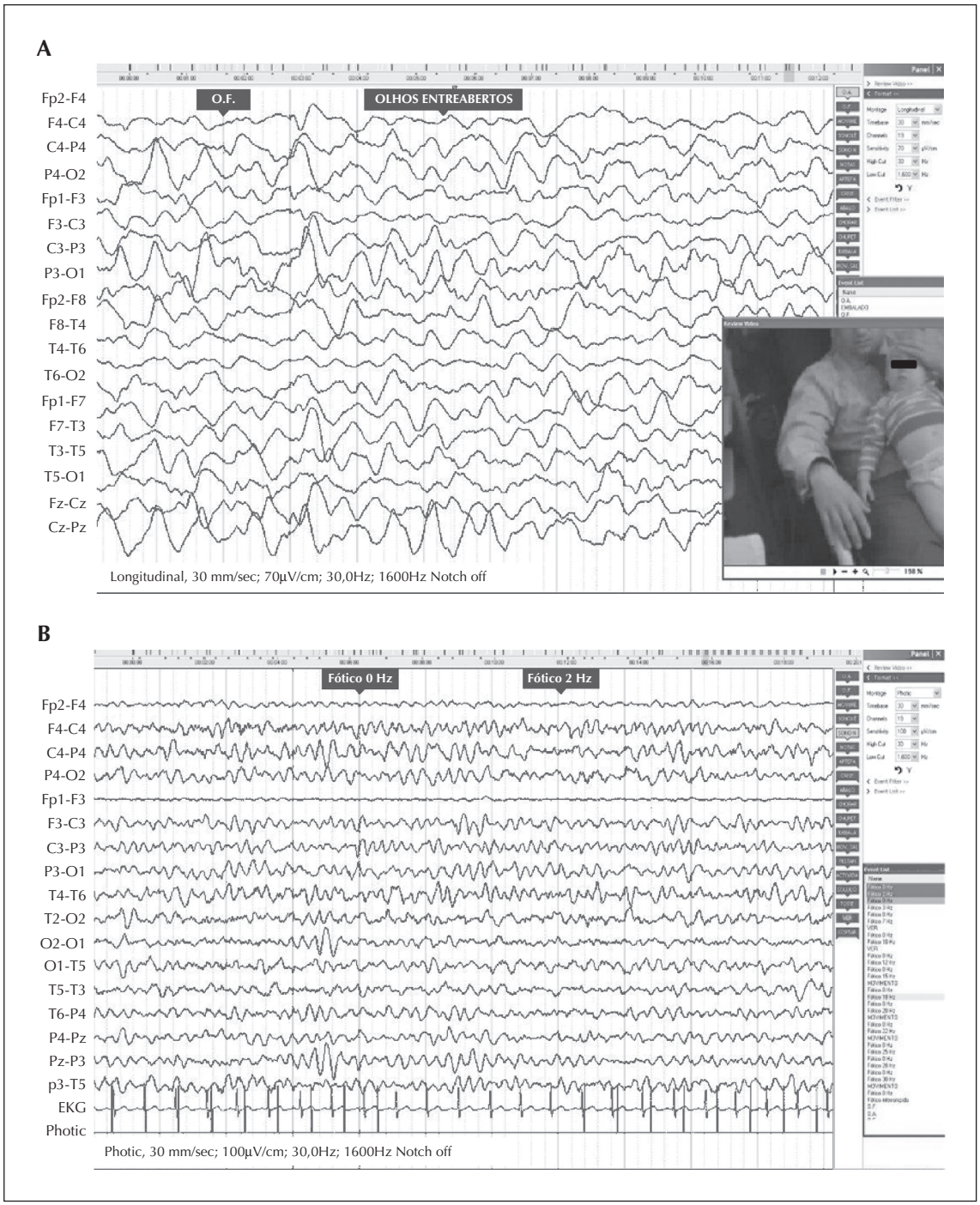

Figure 1. EEG of Patient 3.
A) at 13 months, before pyridoxine trial, showing bilateral slow-wave activity; B) at 19 months, four months after the second trial, with pyridoxine, showing a well-structured pattern.

α-AASA and the presence of mutations/deletions in the *ALDH7A1* gene[1]. As previously explained, ALDH7A1 deficiency results in α-AASA and P6C accumulation which interfere with cerebral lysine catabolism due to the convergence of its metabolic pathways: the saccharopine and pipecolic acid pathways. In the latter, the pipecolic acid (PA) is believed to accumulate due to backpressure from the enzymatic block and some authors also speculate that accumulating P6C can be converted to PA *via* Δ1-pyrroline-5-carboxylate

[1] Tests available since 2006.

reductase. PA acts as a modulator of GABA and its accumulation is believed to contribute to seizure pathophysiology. Nevertheless, its impact on brain dysfunction requires further elucidation (Stockler *et al.*, 2011).

It has been suggested that early onset of clinical seizures and delay in diagnosis and pyridoxine treatment is associated with a poor prognosis with regards to cognitive function (Baxter, 2001; Bok *et al*., 2012). Intellectual disability, mostly regarding expressive language, is a common feature in individuals with PDE, but there are reports of normal intellectual function (Basura *et al.,* 2009). The language expression disorder in Patient 2 was minor and improved with speech therapy, and his outcome was favourable. Furthermore, his cognitive evaluation revealed a lower score for verbal IQ. Patients 1 and 3 had severe clinical presentation, although Patient 1 had good neuro-developmental outcome. For Patient 4, the knowledge of family history was of benefit; he began pyridoxine therapy earlier and had a good outcome. Such an outcome is unusually reported (Bok *et al*., 2012; Gospe, 2012). Patient 3 had the worse outcome, with moderate intellectual disability. Such a poor outcome probably results from a more serious and severe clinical situation, a later diagnosis, and thus implementation of pyridoxine therapy.

For Patients 1 and 2, a good outcome was probably due to higher doses of pyridoxine given, compared to other studies (Bok *et al*., 2012). As for Patient 4, good outcome was probably related to an earlier initiation of therapy and less episodes of seizure.

Regarding verbal and performance IQ data, the majority of patients reported in the literature had a performance IQ which was higher than verbal IQ. However, in all our four patients, as well as some reported in the study of Bok *et al*. (2012), verbal IQ was higher than performance IQ. We cannot explain such findings.

As previously reported, and as observed in our patients, seizure morphology may vary, from generalised, partial, tonic-clonic, atonic, myoclonic, and spasms to status epilepticus, even in the same patient (Baxter, 2001). EEG patterns are non-specific and do not always normalise with pyridoxine treatment (Nabbout *et al*., 1999).

Patients 1 and 2, who had intrauterine seizures, exhibited the earliest postnatal manifestations. First seizures were dissimilar except for the two brothers, Patients 1 and 3, who presented with partial status epilepticus and both had more severe and diverse fits.

Similar to other reports, brain images of our patients revealed different patterns, from general atrophy plus hypoplasia of corpus callosum (Patients 1 and 2) to normal brain (Patient 3) (*table 1*). These differences did not reflect neurocognitive outcome, as Patient 3 demonstrated developmental delay and his brain MRI did not show any anomaly.

As was the case in our patients, and according to other reports, the time interval between withdrawal of pyridoxine and recurrence of seizures is variable and exacerbation of seizures and/or encephalopathy during a febrile illness is frequent. In such circumstances, the dose of pyridoxine may be doubled for several days until the acute illness resolves (Stockler *et al.,* 2011), as was performed for Patient 3.

There are around 64 different reported mutations associated with ATQ deficiency which comprise missense, splice site, and nonsense mutations as well as insertions and single base deletions (Mills *et al*., 2010). Compound heterozygous status, as we found in Patients 1, 3 and 4, is frequent. The missense mutation c.505C>T, p.(Pro169Ser) and the small deletion, c.1217_1218delAT, p.(Tyr406CysfsX3), were first described in three patients with PDE by Mills *et al*. (2010), and Scharer *et al*. (2010). The deletion found in Patient 2 has not previously been reported; this was a large deletion and due to its homozygous nature, is highly suspected to be pathogenic, however the significance of the mutation is still under investigation. Genotype-phenotype correlations have not been established, although such correlations have been suggested (Mills *et al*., 2010; Scharer *et al*., 2010).

As this is an autosomal recessive disorder, biochemical and molecular confirmation of diagnosis provides an option for first-time expectant parents to choose to begin therapy with pyridoxine for the neonate at risk, or even during the prenatal period, as early as possible, during which time biochemical analyses are underway.

For several years, treatment with pyridoxine has been based on empirical evidence, and whether or not PDE patients should be treated for life is still under discussion. For Patient 1 and Patient 2, the confirmation of diagnosis, based on objective data such as biochemical and genetic studies, was only possible in adulthood, justifying lifelong pyridoxine maintenance, based on persistent "biochemical disease".

PDE requires lifelong pharmacological doses of pyridoxine but the optimal dose has not yet been well defined. It is accepted, for most patients, that maintenance doses should be between 15 and 30 mg/kg/day PO in children or up to 200 mg/day in neonates, and 500 mg/day in adult patients (Stockler *et al.,* 2011). In our patients, therapy was individually tailored according to age, weight, clinical situation, and family history, as can be inferred from the clinical descriptions above. Maintenance dosage varied between 80 mg/day and 150 mg/day in infancy (10-35 mg/kg/day) and 80 mg/day and 600 mg/day PO in adulthood. Most probably, a lower dose may have been sufficient for our adult patients, since the increase

was empirically determined based on age, and not on symptoms. We believe that empirically-based treatment with pyridoxine should be performed in all situations of early-onset epilepsy, with a maintenance dose as recommended (between 15 and 30 mg/kg/day PO for children and 15 and 500 mg/day for adults).

Conclusion

In conclusion, a therapeutic trial with pyridoxine should be performed in all cases of neonatal, infantile, and childhood refractory epilepsy. There should no longer be any clinical uncertainty regarding cases of suspected PDE and pyridoxine withdrawal to demonstrate recurrence of seizures is no longer needed since urinary α-AASA is a reliable biomarker of ATQ deficiency (the most frequent cause of PDE) and is elevated in biological fluids even under pyridoxine treatment. The molecular confirmation of diagnosis is of utmost importance for adequate genetic counselling and implementing early therapy. PDE patients should maintain lifelong pyridoxine therapy with a maintenance dose as recommended, as well as adequate clinical surveillance for eventual toxicity. □

Disclosures.
None of the authors has any conflict of interest to disclose.

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