

Pyridoxine-dependent epilepsy in two Turkish patients in Turkey and review of the literature

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Received: 18 August 2014, Revised: 7 October 2015, Accepted: 17 November 2015

SUMMARY: Gül-Mert G, İncecik F, Hergüner MÖ, Ceylaner S, Altunbaşak Ş. Pyridoxine-dependent epilepsy in two Turkish patients in Turkey and review of the literature. *Turk J Pediatr* 2015; 57: 394-397.

Pyridoxine-dependent epilepsy (PDE) is a rare autosomal recessive enzyme defect in the vitamin B6 metabolism characterized by intractable seizures which are usually resistant to all antiepileptic drugs but respond to pharmacological doses of pyridoxine. We present the clinical and molecular genetic findings of two patients with c.1597_1597delG mutations in ALDH7A1 gene. There are different clinical phenotypes in PDE: patients with complete seizure control with pyridoxine and normal development (group 1), patients with complete seizure control with pyridoxine and development delay (group 2), and patients with persistent seizures despite pyridoxine treatment and with development delay (group 3). Our two patients have persistent seizure despite pyridoxine treatment and with development delay. Pyridoxine-dependent epilepsy can be identified in any neonate with signs of encephalopathy and refractory seizures, with no evidence of hypoxic-ischemic damage or other underlying metabolic disturbance. Neurodevelopmental outcomes of patients with PDE is multifactorial; early diagnosis and treatment of these patients is vital.

Key words: pyridoxine dependent seizures, ALDH7A1, vitamin B6.

Pyridoxine dependent epilepsy (PDE) is an autosomal-recessive (PDS; OMIM 266100) disorder characterized by recurrent seizures mainly during newborn period or infantile periods and is resistant to antiepileptic drugs (AEDs) but responsive to pharmacologic amounts of pyridoxine.^{1,2} The prevalence is approximately between 1 per 276,000 and 1 per 700,000 births³. Clinical features have been described in patients with PDE, including abnormal fetal movements, features suggestive of perinatal hypoxic-ischemic injury, irritability, muscle tone alterations, respiratory distress, abdominal distension, hepatomegaly, hypothermia, shock, and acidosis.¹ Conventionally, four clinical criteria are required for the diagnosis: seizures resistant to AED, good response to pyridoxine, complete seizure control on pyridoxine monotherapy, and seizure recurrence after pyridoxine withdrawal.³ The responsible gene antiquitin (ALDH7A1) encoding alpha-aminoacidic semialdehyde dehydrogenase (α -AASA dehydrogenase) in

the pipercolic acid pathway of lysine catabolism is located on chromosome 5q31.⁴ Antiquitin (ATQ) deficiency causes seizures, because it leads to severe secondary deficiency of pyridoxal 5-phosphate (PLP) which is essential enzyme cofactor in the metabolism of several amino acids and neurotransmitters⁵. We present the clinical and molecular genetic findings of two patients with pyridoxine dependent epilepsy carrying the same mutation in the ALDH7A1 gene.

Case Reports

We present the clinical and molecular genetic findings of two unrelated patients with PDE carrying the same mutation in the ALDH7A1 gene.

The 18 exons and flanking intron regions of the ALDH7A1 gene were tested for mutation in the PDE patients by sequence analysis. Following DNA extraction, the coding regions and intron-exon boundaries of the ALDH7A1 gene were

amplified and sequenced as previously described. A homozygous mutations c.1597_1597delG in ALDH7A1 gene was identified in our patients.

Case 1

The 6-year-old girl was born after uncomplicated pregnancy at 39 weeks of gestation (birth weight 3250 g, length 41 cm). The Apgar score was 10, 10 and 10 after 1, 5, and 10 minutes without sign of any infection or anoxic-ischemic encephalopathy. The parents were consanguineous and no family history of seizure reported. At 6 hours of life a first focal clonic seizure occurred, resistant to phenobarbital (20 mg/kg first and then 5 mg/kg per day). Laboratory investigation showed no signs of infection and normal serum biochemistry. The results of metabolic screening of blood and urine were normal. Electroencephalography (EEG) showed epileptic activity on the right temporooccipital region. Cranial magnetic resonance imaging shows thin corpus callosum. Cerebral ultrasound was normal. Immediately 100 mg intravenous pyridoxine was introduced, seizure control was achieved. At the age of 11 months, pyridoxine withdrawal was spontaneously challenged by the parents and seizure recurred within 48 hours. Therefore, pyridoxine was reintroduced. Carbamazepine was added because of the recurrent seizures. Physical and neurological examinations were normal.

In the following years, the girl was continuously treated with pyridoxine, 100 mg twice/day, carbamazepine and phenobarbital. She had seizures three or four times in a year. Psychomotor evaluation at age 6 with Stanford-Binet test showed a full scale IQ of 65 points. She is in the range of mild mental retardation.

Case 2

The 5-year-old boy was born at 40 weeks gestation with a birth weight of 2800 g p25, length of 47 cm (P3-10), and head circumference of 35 cm (50P). The Apgar score was 9 and 10 at 1 and 5 minutes. The parents were consanguineous and no family history of seizures reported. The neonatal period was uncomplicated until he started complex partial seizures on day 10. Phenobarbital (20 mg/kg first and then 5 mg/kg per day) and pyridoxine (100 mg twice/day) were started for the seizures. At the age of 13 months,

pyridoxine discontinuation was spontaneously challenged and seizure occurred within 72 h. Phenobarbital was switched to valproic acid (30 mg/kg/day) at the age 3 years because of the side effects.

Results of laboratory examinations including complete blood count, biochemical analyses were within normal limits. His cerebrospinal fluid examination, and the results of metabolic screening of blood and urine were normal. Physical examination was unremarkable. His neurologic examination was shown significant hypotonia, ataxia and hyperactive deep tendon reflexes. The remainder of the neurological examination was unremarkable.

Cranial magnetic resonance imaging showed asymmetrical left lateral ventricle dilatation. Electroencephalography showed epileptic activity on the left temporooccipital region.

Currently, he takes pyridoxine, 100 mg twice/day and valproic acid (30 mg/kg/day). The complex partial seizures persist two-three times in a year and most of these seizures are associated with febrile events. He can walk independently. Psychomotor evaluating at the age 5 years with Stanford-Binet test showed a full scale IQ of 45 points. He was in the range of moderate mental retardation.

Discussion

Pyridoxine dependent epilepsy was first described in 1954 in an infant with treatment-resistant seizures who showed seizure control after the administration of a multivitamin cocktail including vitamin B6⁶. Until now, over 200 patients have been described in the literature. Because of the lack of a biological diagnostic marker, diagnosis may have been missed in many cases⁷. Moreover, the diagnosis of PDE was confirmed by pyridoxine withdrawal followed by seizure recurrence. Currently, pyridoxine withdrawal is no longer needed to prove the diagnosis of PDE due to availability of genetic testing and of reliable diagnostic biomarkers, namely elevated levels of pipercolic acid in serum and more specifically, alpha-aminoadipic acid in urine⁸. A same homozygous mutation was identified in our two patients. We could not search the other biomarkers such as pipercolic acid, alpha-aminoadipic acid. These tests are unavailable in our laboratory.

The main clinical features of PDE are seizures intractable to anticonvulsants but responding well to administration of pyridoxine. The classical presentation is usually at birth or in neonatal period as our patients. Affected neonates mostly show signs of severe encephalopathy (e.g. hypothermia, jitteriness, irritability, muscle tone alterations and poor feeding), and misdiagnosis of hypoxic-ischemic encephalopathy is not uncommon⁹.

In this disorder, seizure types are quite variable even in the individual patient, ranging from myoclonic to clonic and bilateral tonic seizures and partial seizures, and a tendency to develop status epilepticus. Afterwards, complex partial seizures, infantile spasms and other myoclonic seizures as well as a mixed seizure pattern may come out¹⁰⁻¹². EEG (electroencephalogram) patterns may vary from normal to high voltage delta activity, focal spike wave discharges, burst suppression patterns and rarely hypsarrhythmia. Both of our patients presented with partial seizures¹¹. In accordance with, no specific pattern of EEG abnormalities has been documented.

Cranial magnetic resonance imaging shows a spectrum of changes from normal to hypoplasia of the corpus callosum, mega cisterna magna, enlarged ventricles and diffuse cerebral hemispheric gray and white matter atrophy¹³. One of our patients' cranial magnetic resonance imaging showed thinning of the corpus callosum, the other one's showed asymmetrical left lateral ventricle dilatation.

The clinical spectrum of PDE patients was not limited to seizures; many patients showed associated neurologic dysfunctions such as muscle tone alterations, irritability, and psychomotor retardation. These symptoms may be attributed to a delay in the onset of treatment with pyridoxine. However, in some patients, neurologic signs were already present at the onset of seizures¹⁴. Further research is needed to investigate genotype-phenotype correlations and clarify whether specific ALDH7A1 mutations can explain specific or atypical clinical features in these patients.

Until now, more than 70 different pathogenic mutations within the 18 exons of the ALDH7A1 gene have been published in the literature associated with PDE phenotypic spectrum

in patients from diverse ethnic backgrounds. ALDH7A1 mutations include missense, nonsense, and splice site mutations¹⁵. We detailed the molecular analysis of PDE in two Turkish patients belonging to two unrelated consanguineous families from the same city. They present a homozygous mutation (c.1597_1597delG) in ALDH7A1 gene. This mutation is present in Human Genome Mutation Database – Public Version (HGMD public) with CD061355 code. The mutation was firstly described by Mills et al⁵. This mutation causes a frameshift and loss of the stop codon. Protein becomes prolonged rather than normal. Last 9 amino acid sequence in original protein is PLAQQIKFQ* while PLPKESSFSK, GVLDEHPLI* in mutant form.

Pyridoxine dependent epilepsy patients require life-long pyridoxine treatment to prevent recurrent seizures. The optimal dose of pyridoxine for the patients has not been found. Currently, the daily administration of 50 to 200 mg (given once daily or in two divided doses) is generally effective in preventing seizures in most patients⁹. Prophylactic intrauterine treatment of an at-risk or confirmed PDE fetus with supplemental pyridoxine given during pregnancy can prevent intrauterine seizures and improve neurodevelopmental outcome; however, treatment exposure should be limited to the shortest possible interval so as to prevent any potential problems related to toxicity¹⁶.

Recently developed novel therapies based on the pathophysiology of PDE also provide potential treatment options. Standard treatment for inborn errors of metabolism affecting catabolic pathways of essential amino acids consists of substrate reduction for the deficient enzyme through dietary modification. While treatment with pyridoxine compensates for chemical PLP inactivation, the accumulation of substrates from lysine degradation is not sufficiently reduced. The presence of these potentially neurotoxic compounds could explain the partial efficacy of pyridoxine, as 75–80 % of patients suffer from developmental delay or intellectual disability (IQ < 70) despite excellent seizure control in the majority of patients¹⁷. Thus, for ATQ deficiency, dietary lysine restriction can reduce the accumulation of lysine-derived substrates and possibly contribute to the improvement of cerebral function (neurodevelopment, cognition,

behavior, and seizure control). An open-label observational study was conducted to test the effectiveness and safety of dietary lysine restriction as an adjunct to pyridoxine therapy on chemical biomarkers, seizure control, and developmental outcome¹⁸. Cerebral lysine influx and oxidation can be modulated by arginine, which competes with lysine for transport at the blood–brain barrier and the inner mitochondrial membrane¹⁹. It is hypothesized that arginine supplementation will compete with lysine, thereby reducing the excess lysine influx into the brain, which can lead to accumulation of the neurotoxic substrates caused by ATQ deficiency. Arginine fortification has proven safe as an add-on treatment to lysine restriction. Following 12 months of arginine supplementation at 400 mg/kg/day, together with pyridoxine 200 mg/kg/day, a decrease in CSF α -AASA and improvement in general abilities, as well as verbal and motor functions, were reported²⁰.

Pyridoxine-dependent epilepsy should be considered if there is a parental consanguinity, seizures of unknown etiology in previously normal infant without an abnormal gestational or perinatal history, long-lasting focal and unilateral seizures and a history of severe epilepsy in a sibling. While the neurodevelopmental outcome of PDE patients is multifactorial, still the early diagnosis and the treatment of these patients is vital.

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