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Novel homozygous missense mutation in ALDH7A1 causes neonatal pyridoxine dependent epilepsy

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ABSTRACT

Pyridoxine dependent epilepsy (PDE) (OMIM#266100) is a neonatal form of epilepsy, caused by dysfunction of the enzyme α-aminoadipic semialdehyde dehydrogenase (ALDH7A1 or Antiquitin). This enzyme converts α-aminoadipic semialdehyde (α-AASA) into α-aminoadipate (AAA), a critical step in the lysine metabolism of the brain. ALDH7A1 dysfunction causes an accumulation of α-AASA and δ^1-piperideine-6-carboxylic acid (P6C), which are in equilibrium with each other. P6C binds and inactivates pyridoxal 5′-phosphate (PLP), the active form of pyridoxine. Individuals affected by *ALDH7A1* deficiency show pre-natal and post-natal seizures, which respond to oral pyridoxine but not to other pediatric anti-epileptic drugs. We discovered a novel missense mutation (c.566G > A, p.Gly189Glu) in homozygous state residing in the NAD+ binding domain coding region of exon 6 and affecting an highly conserved amino acid residue. The seizures stopped under post-natal pyridoxine therapy, nevertheless a longer follow-up is needed to evaluate the intellectual development of the child, who is additionally treated with oral L-arginine since the 13th month of life.

Developmental delay with or without structural cortex abnormalities were reported in several patients. A brain MRI scan revealed hyperintense white matter in the right cerebellum compatible with cerebellar gliosis. Taken together, our studies enlarge the group of missense pathogenic mutations of *ALDH7A1* gene and reveal a novel cerebellar finding within the PDE patients cohort.

1. Introduction

Post-natal epilepsy responsive to pyridoxine (PDE) is a very rare neonatal epilepsy form (OMIM 266100) with an estimated prevalence of 1:100.000 [1–4]. The seizures start usually within the first hours after birth (even if pre-natal seizures have been also described), do not respond to usual pediatric anti-epileptic drugs and the electroencephalogram (EEG) does not present a characteristic pattern. Increased levels of α-aminoadipic semialdehyde (α-AASA) in urine is patognomonic for this disease and increased levels of pipecolic acid (PA) in plasma and cerebrospinal fluid were reported in some, but not in all patients [1,3]. In 2006, dysfunction of *ALDH7A1* gene was demonstrated to cause PDE [2]. The gene maps on chromosome 5, contains 18 exons and spans over 50 Kb. The encoded protein antiquitin has a key role in the catabolic pathway of amino acid lysine in the brain and converts α-AASA into α-aminoadipate (AAA). Antiquitin dysfunction causes accumulation of alpha-aminoadipic-semialdehyde (α-AASA), which is in equilibrium with P6C. The letter one binds and deactivates pyridoxal 5′-phosphate (PLP), the active form of pyridoxine. Pyridoxine at 30 mg/kg/day stops the seizures, but does not support the intellectual out-come of the patients.

Abbreviations: α-AASA, α-aminoadipic semialdehyde; ALDH7A1, α-aminoadipic semialdehyde dehydrogenase; NAD+, nicotinamide adenine dinucleotide (oxidized); PA, pipecolic acid; PDE, pyridoxine dependent epilepsy; P6C, L-Δ^1-piperideine-6-carboxylate; PLP, pyridoxal 5′-phosphate.

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During the last 10 years, missense mutations were described throughout several exons and corresponding pathogenic amino acid exchanges were revealed in NAD+ binding, oligomerization and catalytic domains of Antiquitin [2,4–12]. In a male newborn with seizures since the 4th day of life, we defined a novel homozygous missense mutation (c.566G > A, p.Gly189Glu) in *ALDH7A1* gene, which causes the exchange of a highly conserved glycine into glutamic acid within the NAD+ binding domain of the protein (Fig. 1).

2. Materials and methods

2.1. Gene sequencing and schematic representation of mutated amino acid

Sanger sequencing was done using standard protocols and the data was evaluated using SeqPilot Software (JSI medical systems). Primer sequences are available upon request.

The schematic representation of the positioning of mutated amino acid Gly189 was produced by using PyMOL (Schrodinger), based on PDB entry 4ZUK [13].

2.2. Magnetic resonance imaging

Cerebral MRI scan was performed on clinical 1.5T MRI systems (Magnetom Avanto and Aera, Siemens Medical, Germany) using standardized MRI protocols including multiplanar T1 and T2-weighted MR-sequences.

2.3. Web resources

The GenBank, Ensembl and OMIM, PDB browser accession numbers of ALDH7A1 are, respectively, NM001182.4, ENST00000409134.7, OMIM 266100 and 4ZUK.

3. Results

3.1. Clinical, neuro-physiological and neuro-radiological features

The index-patient was born as fourth child of consanguineous healthy parents (Fig. 2). The oldest sister of index-patient died at 4 weeks of age due to an unclear disease characterized by untreatable seizures. In the consanguineous family, 4 other male cousins of the index-patient's parents suffered from a neonatal epilepsy.

The patient was born at the 40th pregnancy week with a weight of 3685 g (50th centile), length of 52 cm (40th centile) and head circumference of 36.5 cm (75th centile). No dysmorphic features were observed. During the first 2 days, the adaptation was uncomplicated. Starting from the 3rd day after birth, the clinical scenario was characterized by increasing tremor, muscle hypotony, weak sucking reflex and respiratory insufficiency. On the 4th day, generalized clonic seizures and repeated apneas were observed. Bihemispheric spike-waves were measured by EEG. Although the epileptic discharges were resistant to high-dose Phenobarbital, the oral administration of pyridoxine (30 mg/kg/d) stopped the seizures and the respiratory insufficiency and normalized the muscle tonus. The clinical suspect of PDE was finally demonstrated by highly increased levels of α-AASA in urine (43.1 mmol/mol Creatinine; normal value 0–0.19 mmol/mol Creatinine). Although not patognomonic for PDE, PA was also measured and was found elevated in urine (119 mmol/mol Creatinine; normal value 0.55–24.1 mmol/mol Creatinine), plasma (41.3 μmol/l; normal value 3.8–10.8 μmol/l) and cerebrospinal fluid (11.8 μmol/l; normal value 0–0.12 μmol/l).

At the age of 4 weeks, the patient had normal muscle tonus and deep tendon reflex; Moro and Galant reflex, asymmetrical tonical neck reflex (ATNR), palmar grasp reflex and plantar reflex were present. He actively turned the head to both sides following visual stimuli and had normal spontaneous movements of upper and lower extremities. He was fed by the mother and his sucking reflex was strong.

Since the 13th month of life, oral L-arginine (150 mg/kg/d) was administered additionally to oral pyridoxine. Currently, the toddler is 15 months old, seats unassisted, crawls and starts to walk supported at both hands. Muscle tonus and deep tendon reflex are normal. He produces different sounds but no clear words, interacts with the persons around him by pointing, takes small objects and brings them from one to the other hand.

Cortex abnormalities and developmental delay being symptoms of PDE [8,9,14], the patient underwent brain MRI scan at the age of 9 months, which surprisingly revealed a hyperintense white matter region in the right cerebellar hemisphere compatible with gliosis, surrounded by edema (Fig. 3).

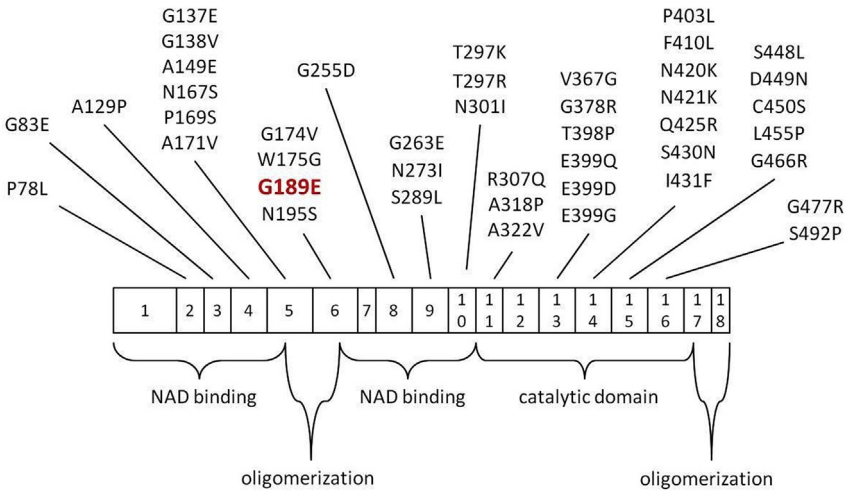

Fig. 1. Schematic representation of the most frequently reported missense mutations in *ALDH7A1* gene. Missense mutation – dependent exchanges of amino acids are depicted above the corresponding exons of *ALDH7A1* cDNA. The novel mutated amino acid discovered in the patient is depicted in red. The 3 domains are depicted below the cDNA schema. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)