# Finding of compound heterozygous mutations in the *ALDH7A1* gene. Clinical case.

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Epilepsy is a neurological disorder affecting approximately 1-2% of the worldwide population. The reasons of epilepsy are different; in case of genetic-based epilepsy the finding of mutations could prove any clinical diagnosis and even affect on treatment strategy. Here we present a clinical case of the pyridoxine-dependent epilepsy (PDE), the result of compound heterozygous at *ALDH7A1* gene detected by high-throughput sequencing. The main feature of the described clinical case was weak response to admission of pyridoxine that significantly complicated the disease diagnostic and delayed the medical treatment.

Pyridoxine-dependent epilepsy (PDE) (MIM: 266100) - autosomal recessive disease caused by mutations in the gene *ALDH7A1* (MIM: 107323). The main criteria of PDE is the response to admission of pyridoxine that could be fixed by the electroencephalography (EEG) and resistance to treatment by anti-epileptic drugs (AED). The incidence varies widely (depending from local screening policy) from 1:700,000 (United Kingdom) [1] to 1:20,000 (Germany) [2]. A several dozen cases of PDE, confirmed by genetic studies, was described at the scientific medical literature, however no one case has not yet been described in Russia.

Proband, female, 9 months of age was admitted to our Children Medical Center for studying the daily epileptic seizures. Initial testing on response to pyridoxine under EEG was negative. During several hospitalizations anticonvulsant therapy with different AED did not give the desired or any effect, polymorphism seizures (focal clonic epileptic spasms, myoclonic seizures) was observed. In the health dynamics the frequency and severity of seizures was growing up giving the status epilepticus. At the age of 1 year the proband was moved to the intensive care unit, the state of health was heavy and getting worse.

The initial search of genes, responsible for disease was conducted among genes associated with epileptic encephalopathies (34 genes). Among findings the rare variant

(Gly257Arg, NP_950238.1) at gene *SCN1B* (encodes ß-subunit of voltage-dependent sodium ion channel, Nav1.1) was found. The studied unaffected parent of the proband has the same allele variant; moreover the clinical picture of proband's seizures was uncharacteristic for mutations at the sodium ion channel. So we decided to make a search for mutations among all genes which are involved at the pathogenesis of epilepsy on literature data. Karyotype and chromosomal microarray analysis did not reveal any abnormalities. High-throughput sequencing (Illumina, PE reads, SureSelect target enrichment) revealed a number of mutations involved in pathogenesis of epilepsy (on the basis of ClinVar database), however all of them (except mutations at *ALDH7A1* gene, see below) are the similar for the proband and his unaffected parent. All stages of sample preparation and sequencing we have performed in accordance with standard protocols of manufacturers. Basic data processing and variant calling was performed by commonly used software; for further variant data analysis we used in-house soft. During analysis we have considered all found pathogenic or potentially pathogenic variants taking into account the clinical picture of the disease.

As a result of study we have found compound heterozygous in the gene *ALDH7A1* (acetaldehyde dehydrogenase): c.328C>T (NM_001182.4), p.Arg82Term (NP_001188306.1) (exon 4) and c.1279G>C (NM_001182. 4), p.Glu399Gln (NP_001188306.1) (exon 14). An unaffected parent had only heterozygous c.1279G>C (p.Glu399Gln), the another unaffected parent was out of our genetic research. Glu399Gln and Arg82Term are important for enzyme functioning: Glu399Gln affects on the binding of the cofactor, Arg82Term prevents the interaction between enzyme subunits violating the destruction of the salt bridge between Arg82 and Ser499 aminoacids. Compound heterozygous finding followed by admission of pyridoxine allowed us to stop and control the seizures. In the medical literature only one case of a similar compound heterozygous (p.Arg82Term and p.Glu399Gln) was described, for the Dutch boy 6 years of age [3]. We suppose other clinically significant mutations at genes associated with epilepsy gave the significant contribution to severity of the proband condition and complicated the early PDE recognition.

To malfunctioning of *ALDH7A1* gene lead a missense and nonsense mutations, splice site mutations, deletions (maximum length of the described deletions was 1937 bp [6]). Summary, to date was described 26 missense mutations, 13 of which are located in exons 14-

16; 5 nonsense mutations; 11 small deletions or insertions; and 10 mutations at splice sites. In 60% of all cases are detected 9 mutations (arranged in descending order): Glu399Gln, Arg82Term, c.750G>A, Pro403Leu, Gly477Arg, Ser430Asn, del(Thr495-Ser499), Cys450Ser, Gly83Glu. Also to date are described several tens cases of PDE confirmed by genetic studies [3-6]. In addition to homozygous or compounding heterozygous cases, there are interesting cases of PDE even without missense/nonsense mutations: compound heterozygosities: (c.1348T> A; p.Cys450Ser) and (c.750G>A; splice errors) [5], (c.1405+5G>A; IVS16+5G>A) and (c.244C>T; p.Arg82Term) [4]; "silent" homozygous (c.433+5G>A; IVS5+5G>A) [4] and even it was described the case of PDE in the absence of mutations in the gene *ALDH7A1* [6].

Conclusions. We have studied an uncharacteristic PDE case, unfixed by EEG response at pyridoxine admission. The next high-throughput sequencing and data analysis of about four hundreds genes associated with epilepsy revealed compound heterozygous at the gene *ALDH7A1*; other clinically significant mutations at genes associated with epilepsy gave the significant contribution to severity of the proband condition and complicate the PDE recognition. Currently, using pyridoxine-based therapy we stopped and control the epileptic seizures. The research was supported by the Department of Health of Moscow.

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