

Clinical Report: CNVs (Deletions of 3p24.1, 6p12.2; 12q24.22) Detected by Array CGH in Patient with Microcephaly and Early Epileptic Encephalopathy with Infantile Spasms

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Abstract: *Introduction:* Infantile spasms (ISs) are an age-dependent epileptic seizures that are associated with mental retardation, autism, and cerebral palsy. They are part of epileptic encephalopathies such as West and Ohtahara syndromes and early myoclonic encephalopathy. There is growing evidence that ISs result from disturbances in important genetic pathways of brain development. Recent studies show that patients with ISs may have mutations in several genes including *ARX*, *CDKL5*, *FOXG1*, etc. as well as other candidate genes identified from pathogenic copy number variants (CNVs). *Case study:* The male child was born at term by normal delivery after non-complicated pregnancy. At 3 months of age infantile spasms (ISs) started alongside with deterioration of psychomotor development, affecting head control, reaching for objects and eye tracking. At time of hospitalization ISs were numerous (hundreds per day), they were combined with myoclonic seizures and episodes of motor arrest with staring. Child had axial hypotonia, lack of hand grasping and eye contact. An electrodecremental event during spasms, generalized discharges of polyspykes during myoclonias and focal spikes during motor arrest were recorded. Brain MRI showed non-specific brain atrophy. Metabolic screening, including urine and serum amino acids, organic acids, lactate, pyruvate and liver function tests, was normal. Treatment with vigabatrin with doses up to 192 mg/kg/day was ineffective and injections of synthetic analog of ACTH were started. But the child deteriorated progressively, the burst-suppression pattern was recorded on EEG and an epileptic status developed. IV benzodiazepine and valproic acid were only partly efficacious. Refractory epileptic status was stopped by general anaesthesia. The child survived but his developmental prognosis is poor. Since the child lacked features specific for any aetiological diagnosis an array comparative genome hybridization (array CGH) was performed. It's revealed several copy number variants (CNVs): deletion of 3p24.1 with involvement of gene *EOMES* that encodes the protein-regulator of neurogenesis; deletion of 6p12.2 with involvement of gene *TMEM14A* (inhibitor of apoptosis) and deletion of DNA of gene *FBXO21*, which is highly expressed in prefrontal cerebral cortex. *Discussion:* Epileptic encephalopathies (EE) are severe brain disorders in which the epileptic electrical discharges may contribute to progressive psychomotor dysfunction. There are single gene disorders among them (with well described phenotype) but majority of cases remained unexplained, sometimes because of the fact that clinical and EEG features of different EE are overlapping. Molecular karyotyping can help us in defining their etiology. In our patient with EE with ISs the rare combination of copy number variations (CNVs) was found. We speculate that these CNVs (including genes playing important role in brain development) may be responsible for severe epileptic encephalopathy with ISs.

Keywords: Infantile spasms, microcephaly, CNVs, *EOMES*, *TMEM14A*.

INTRODUCTION

Infantile spasms (ISs) are characterized by clusters of sudden bilateral brief tonic contractions of the axial and limb muscles, usually occurring before the age of 1 year. The incidence of ISs is 0.25–0.4 per 1,000 live births, and they are important because of their frequent association with severe developmental outcome, including mental retardation and autism [10]. Commonly IS are part of the triad of West syndrome symptoms including also such inter-ictal EEG pattern as hypsarrhythmia and mental retardation or regression. They can be part of other epileptic syndromes – early myoclonic encephalopathy and Ohtahara syndrome [11,12]. It is important to know the aetiology of ISs because it determines the prognosis of psychomotor development. Aetiology of ISs is multiple

and diverse [11]. Approximately 30% of children with ISs will have no identifiable cause (such as hypoxic-ischemic encephalopathy, intrauterine insults and etc) after completion of the history, physical, neurological and ophthalmological examination, EEG, and magnetic resonance imaging. Of these children, a concrete metabolic or genetic aetiology will likely be established for fewer than 50% [10], in other 50% aetiology is unknown. Recent studies show that patients with ISs may have mutations in several genes including *ARX*, *CDKL5*, *FOXG1*, *GRIN1*, *GRIN2A*, *MAGI2*, *MEF2C*, *SLC25A22*, *SPTAN1*, and *STXBP1* [10]. It's supposed that ISs are a genetically heterogeneous condition involving abnormalities in key developmental pathways in the ventral forebrain and synaptic functional pathways [14]. Alongside with monogenic mutations copy number changes (copy number variants or CNVs) can play an important role in development of ISs. We report a case of patient with microcephaly and early epileptic encephalopathy with infantile spasms due to the rare CNVs.

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CASE REPORT

The male child was born at term by normal delivery after non-complicated pregnancy. Parents were not consanguineous. Family history was negative for epilepsy and mental retardation. Neonatal period was uneventful. At 3 months of age myoclonic seizures started and two weeks later ISs followed. There was deterioration of psychomotor development, affecting head control, reaching for objects and eye tracking. Inter-ictal EEG at age of 3 months was described as atypical hypsarrhythmia with ictal electrodecrement during ISs and short generalized spikes-and slow waves during myoclonias. High doses of pyridoxine (100 mg per day) and pyridoxal phosphate (30 mg/kg/day) intravenously for 5 days were tried to exclude pyridoxine-dependant epilepsy and related conditions but they were ineffective. We didn't use valproic acid because mitochondrial diseases were not excluded at that time. Vigabatrin was started and for two months the child was receiving medium doses of medication (40 - 60 mg/kg/day) without any positive effect.

At time of hospitalization (at age of 6 months) clusters of symmetric flexor ISs (abrupt flexion of the neck and the trunk, the arms raised sideways with flexion at the elbows) were numerous (hundreds per day). ISs were combined with myoclonic seizures and episodes of motor arrest with staring. Myoclonias were erratic: they shifted from one part of the body to another in a random and asynchronous fashion that affected face and limbs. They were brief, single and very frequent. Episodes of motor arrest with staring were associated with apnoea. Child had microcephaly (head circumference 40 sm), marked truncal hypotonia, lack of hand grasping, disconjugate eye movements. There was no trace of intelligent activity, eye contact and patient was incapable of following moving object with his eyes. He didn't have any lethargy, vomiting, failure to thrive or any peculiar odours.

Inter-ictal EEG consists of repetitive bursts of high amplitude spikes and sharp-and slow waves last for 1-2 sec that alternate with periods of almost flat EEG, lasting for 3-5 sec (the so called "burst-suppression")

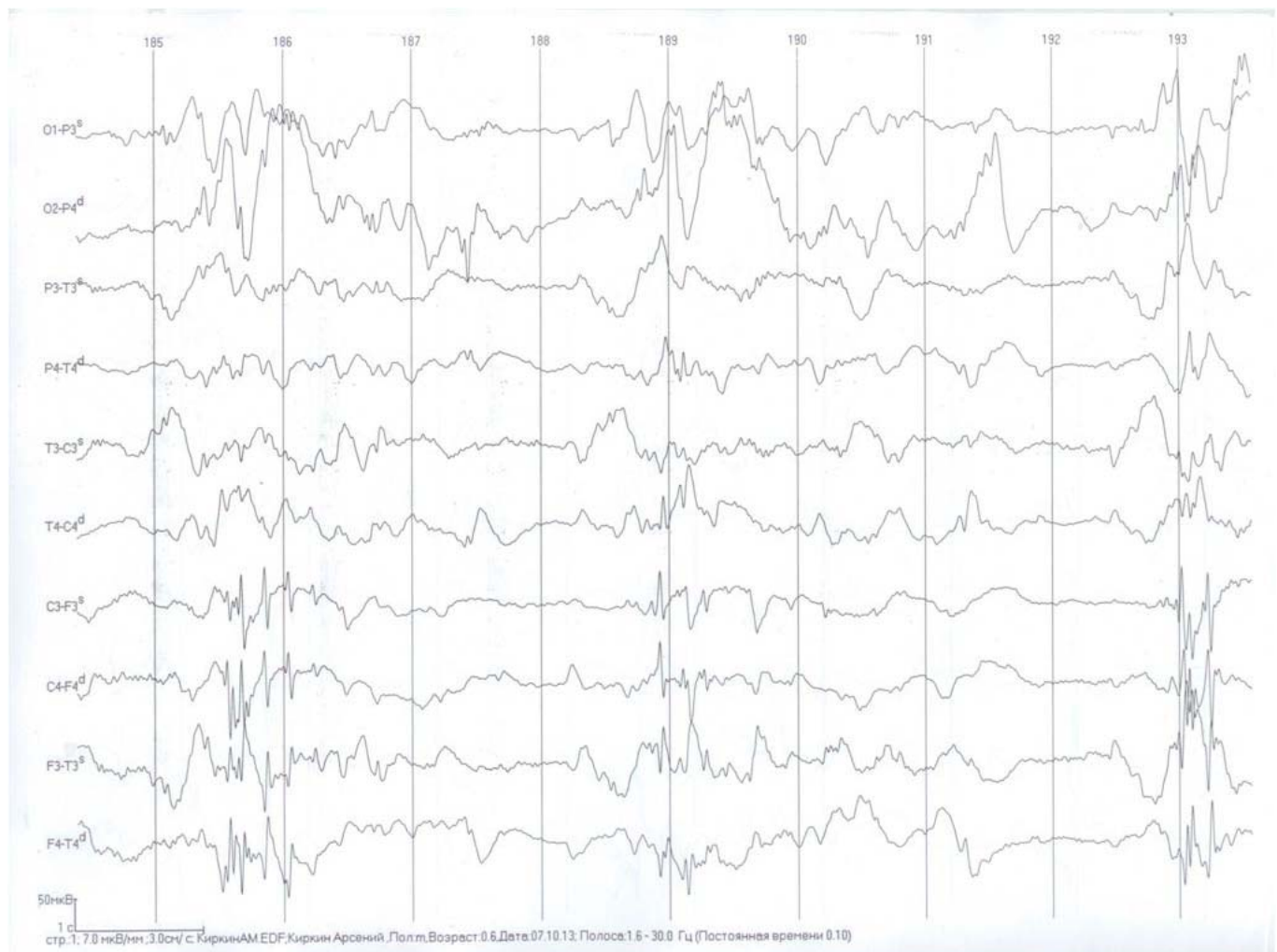


Figure 1: Interictal EEG: burst-suppression pattern.

pattern) – Figure 1. *Ictal EEG* recorded marked diffuse attenuation of electrical activity during IS, generalized discharges of spikes-polyspykes during myoclonias and focal spikes during motor arrest. *Brain MRI* showed non-specific cortical atrophy and absence of evident cortical displasias – Figure 2. *Metabolic screening*, including urine and serum amino acids, organic acids, lactate, pyruvate and liver function tests, was normal. Infantile form of neuronal ceroid lipofuscinosis was excluded.



Figure 2: Brain MRI: non-specific brain atrophy.

The child was diagnosed as early encephalopathy with IS. As the dominating type of at time of hospitalization seizures were ISs daily dose of vigabatrin was increased up to 192 mg/kg, but it was ineffective. Injections of synthetic analog of ACTH were started and levetiracetam was titrated up. But the child deteriorated progressively, and an epileptic status developed. Intravenous diazepam (0.5 mg/kg) and valproic acid (36 mg/kg/day) were only partly efficacious (they interrupted myoclonic and clonic seizures only for short time). Patient was transferred to emergency department and full range of intensive therapy was applied. Refractory epileptic status developed and thiopental was used, but seizures recurred after the repeated attempts of thiopental withdrawal. Gradually over several weeks the number of seizures decreased, the child survived but developed permanent severe mental and neurological deficits. There is no trace of intelligent activity and ISs are preserved.

Since the child lacked specific features for any definable aetiological diagnosis an array comparative genome hybridization (array CGH) was performed. His molecular karyotype (according to ISCN, 2013) [5] is arr3p24.1 (27, 752, 705-27, 762, 076) x 1, 6p12.2 (52, 549, 321-52, 560, 788) x 1, 12q24.22 (117, 591, 588-117, 595, 986) x 1.

Three microchromosomal copy number variants were recorded: deletion of 3p24.1 (localization 27752705-27762076, size 9371pn); deletion of 6p12.2 (localization 52549321-525560788, size 11467pn) and deletion of two exons (10 and 11) of gene FBXO21 (localization 12q24.22/11759188-117595986, size 4398 pn); [OMIM:609095]- [9].

DISCUSSION

It was difficult to diagnose the precise epileptic syndrome in our patient. Clinical picture resembled a certain epileptic syndrome - early myoclonic encephalopathy, but it starts usually earlier – during the first month of life; cases with onset of seizures after the second month are very rare [11]. Dominating type of seizures at time of hospitalization were ISs. The child could have Ohtahara syndrome, but myoclonic seizures in it are rare and erratic myoclonias are not featured [11]. Both early myoclonic encephalopathy and Ohtahara syndrome are one of the most dreadful diseases. More than half of the patients die within weeks or months of onset and there is no efficient treatment [11]. It was evident that our child had early epileptic encephalopathy with ISs, and maybe it was borderline with two syndromes mentioned and West syndrome. In spite of difficulties with syndromic diagnosis the key question was the aetiology of this encephalopathy.

Three microchromosomal CNVs in our patient were recorded. The first one - deletion of 3p24.1 - includes gene EOMES [OMIM:604615]- [9], which is encoding the protein-regulator of neurogenesis in subventricular zone of the brain, participating in migration of neurons [1]. Disturbances in expression of this gene during early brain development cause microcephaly and severe behavioral deficits in animals. One case of microcephaly with polymicrogyria and corpus callosum agenesis due to silencing of T-box transcription factor EOMES was described by Baala L. et al. in 2007 [2]. The second CNV is deletion of 6p12.2, including the 5th exon of gene TMEM14A and gene LOC100420627.

Transmembrane protein 14A (TMEM14A) is a novel mitochondria-associated membrane protein containing a putative transmembrane domain. It is supposed that TMEM14A inhibits apoptosis by blocking the mitochondrial permeability transition and stabilizing mitochondrial membrane potential [15]. It's interesting that Spreafico R *et al.* (1993) proposed a common neuropathological basis for early epileptic encephalopathies. According to this author their pathogenesis is closely connected with disruption of apoptosis at the end of gestation or soon after birth [13]. The high expression of LOC100420627 is seen in different parts of the brain (OMIM). The third CNV is deletion of two exons (10 and 11) of gene FBXO21, which is highly expressed in the brain especially in prefrontal cortex.

Due to the fact that these CNVs are not mentioned in nonpathogenic CNV data base and that at least two genes (EOMES and TMEM14A) are playing important role in brain development we consider them to be pathogenic for our patient. We speculate that this unique combination of CNVs is responsible for the uncommon phenotype and devastating course of the disease.

Microchromosomal copy number variants (CNVs) have been known as causative for epilepsy for a long time [8]. However, the efficient and routine recognition of them is relatively recent. It's well known that recurrent deletions at 15q13.3 (1.5 Mb, seven genes), at 16p13.11 (1.2 Mb, eight genes) and at 15q11.2 (1.3 Mb, four genes) are closely connected with intellectual disability, autism, and genetic generalized or focal epilepsy [3,6,7]. Apart from the recurrent CNVs, the rare non-recurrent CNVs are also likely to play some role in the genetic aetiology of epilepsy. H.C. Mefford *et al.* evaluated 517 individuals with various types of epilepsy and found that nearly 10% carried one or more rare CNVs that had not been previously found in controls [8]. Rare or unique non-recurrent CNVs are more common than the recurrent ones. For the majority of them there is insufficient data to decide if they are pathogenic. But in our patient we've found the combination of the CNVs including genes that are closely engaged in brain ontogenesis – in process of neuronal migration and in process of apoptosis. So it is reasonable to conclude that the presence of these CNVs is a major genetic factor contributing to the patient's disease. We don't know yet are this CNVs *de novo* variant in our patient or there are other carriers in the family. Overall genetic counselling with respect to CNVs remains a challenge. Genetic profiles of susceptibility genes for each individual are likely to be

unique and fit the polygenic heterogeneity concept [4,7].

Alongside with other authors [7,10] we suggest array CGH as the first-line analysis in the ISs with "unknown aetiology".

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