Pediatr Pol 2022; 97 (3): 264-268
DOI: https://doi.org/10.5114/polp.2022.120119
Submitted: 04.04.2022, Accepted: 30.05.2022, Published: 30.09.2022

PEDIATRIA Polska
Polish Journal of PAEDIATRICS

CASE REPORT

Deletion of the *RNF6* gene in a patient with epileptic encephalopathy – a case report and literature review

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ABSTRACT

Epilepsy is one of the most common neurological diseases of developmental ages, and epileptic encephalopathy is one of its most severe forms. The *RNF6* gene encodes a protein that is a ubiquitin ligase. It has been associated so far mainly with the development of neoplastic diseases. We present a case of a 3.5-year-old girl with epileptic encephalopathy and deletion 13q. The patient has been suffering from seizures since infancy. Therapy with 3 antiepileptic drugs and the ketogenic diet reduced the frequency of seizures, but they remained at 2–3 per day. Testing with the next-generation sequencing technique – gene panel and whole-exome sequencing – did not show any pathogenic variants in single genes (single nucleotide polymorphism). The array-based comparative genomic hybridization detected a 75 kbp deletion in the 13q12.13 region containing the *RNF6* gene. The RNF6 protein is involved in the development of axonal projections, which suggests that the presumable deletion may affect the presented patient's clinical features.

KEY WORDS:
epilepsy, brain diseases, epileptic encephalopathy, deletion.

INTRODUCTION

Epilepsy is one of the most common neurological diseases of the developmental ages [1]. It is also one of the most widespread diseases generally – it is estimated that about 50 million people worldwide have epilepsy [2]. Epileptic encephalopathy is a severe epileptic disorder characterized by frequent recurrent discharges due to a specific central nervous system (CNS) dysfunction, consisting of a permanent predisposition to seizure disorders due to abnormal excessive or synchronous bioelectrical activity of the brain. It can be caused by (a) external factors, such as physical – causing, e.g., structural changes; chemical – resulting from acute and chronic poisoning; and biological, e.g., CNS infections, or (b) genetically determined – including congenital developmental defects, e.g., neuromigration disorders or an enzyme defect defined as inborn errors of metabolism [3].

The *RNF6* (Ring Finger Protein 6) gene, located on 13q12.13, encodes a zinc finger domain protein that acts as an E3 ubiquitin ligase. So far, abnormalities in this gene have been associated mainly with the development of tumours [4]. The aim of the study is to draw attention to the chromosomal abnormality in a 3.5-year-old girl, ascertain its possible impact on the clinical features, and consider the expression of the *RNF6* gene as a possible aetiological factor of seizure disorders in children.

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CASE REPORT

A 3.5-year-old girl, the first offspring of healthy unrelated parents, was born following the unremarkable pregnancy by uncomplicated delivery at 41 weeks of gestation, in good general condition – 9 points by the Apgar scale. Birth parameters of physical development were as follows: body weight – 2900 g (11th percentile – [–1.253 SD]), length 98th percentile [2.154 SD], head circumference 45th percentile [–0.125 SD], according to the RCPCH UK-WHO Growth Chart NICM for Girls. The first epileptic seizure occurred in the first month of life. Initially, the seizures were exclusions; later, they evolved and assumed the character of inclinations with eye rotation, and then they were generalized. The girl's condition showed a significant delay in psychomotor development despite the implemented multi-profile rehabilitation techniques from an early period. Currently, she shows poor spontaneous motor activity with an abnormal distribution of muscle tension in the form of spastic limbs and laxity in the axial skeleton. She does not raise her head yet, has no development of verbalization, and does not babble. Lack of normal reflexes typical of her life period, including sucking coordination and swallowing, resulting from which she has a feeding tube. In the proband's morphological phenotype, noticeable frontal bumps and single transverse furrows on the hands are present. Due to drug resistance to seizures, antiepileptic add-on therapy was applied according to the adopted scheme recommended by the Polish Society of Epileptology (Depakine 2 × 100 mg, levetiracetam 2 × 750–1000 mg, vigabatrin 2 × 500 mg). At 11 months of age, a ketogenic diet was introduced, which reduced the number of seizures. Currently, episodes of variable character occur with a frequency of up to 3–4 times a day.

Due to the unknown cause of epileptic encephalopathy and the suspicion of metabolic disease, the patient was hospitalized several times in the Department of Paediatric Neurology. During the diagnostics, irregularities in additional tests were found. Analysis of the aminogram of the cerebrospinal fluid using the high-performance liquid chromatography method showed an increased concentration of tyrosine – 27.1 μmol/l (norm 8–14 μmol/l). The video-EEG monitoring test showed an incorrect record of generalized changes in the form of a continuous series of spike and wave complexes of 2 Hz, amplitude up to 600 μV, with maximum amplitude in frontal and pre-temporal regions. Magnetic resonance imaging of the CNS showed astrocytic gliosis lesions related to possible perinatal hypoxia or metabolic damage, which corresponds to periventricular leukoencephalopathy; moreover, there was a narrowing of the corpus callosum and a slight dilatation of the ventricular system (Figure 1).

The analysis performed with the next-generation sequencing (NGS) method with the 49-gene panel for epileptic encephalopathy did not identify any pathogenic variants. Subsequently, the whole-exome sequencing (WES) test was performed using the NGS method (on the Illumina HiSeq 1500 platform, Illumina, CA), which also did not show a pathogenic variant in any of the known genes conditioning epileptic encephalopathy. Additionally, an array-based comparative genomic hybridization (aCGH) test was performed using probes with an average resolution of 30 kbp (CytoSure Epilepsy Research [4 × 180 k], GRCh37/hg19, Oxford Gene Technology, UK), which revealed 2 deletions: one size of 75 kbp in the region 13q12.13 and the second of 71.5 kbps in the region Xq27.2 – arr [GRCh37] 13q12.13 (26655766_26731034) x1, Xq27.2 (140691477_140762932) x1.

The deletion within the X chromosome does not include genes encoding proteins, while the deletion in the long arm of chromosome 13 includes exon 6 of the *RNF6* gene. The entire *RNF6* gene consists of 9 exons.

The patient continues to take antiepileptic drugs, and the ketogenic diet regimen is maintained. She is continuously controlled by several specialist clinics, including neurological and genetic clinics, and general development therapy is carried out.

DISCUSSION

Epileptic encephalopathies encompass a broad group of seizure disorders characterized by different types of seizures, abnormal EEG activity, and abnormal development. The spectrum of these disorders includes well-defined disease entities, such as Dravet, West, or Lennox-Gaustat syndrome, as well as hypoxic-ischemic conditions [5]. Epileptic encephalopathy can also occur in patients with inborn errors of metabolism [6, 7]. The tests performed on our patient using the NGS technique – gene panel and WES – did not show pathogenic variants, which indicates that this condition's cause is different from the previously known monogenic diseases with seizure disorders.

Cases of deletions in the 13q12.13 region, manifested by a wide range of symptoms, have been reported in the medical literature. These variable symptoms in the deletions in the above-mentioned 13q12 region depend on the size of the deletion and the number and meaning of genes involved. Ritvo *et al.* reported a 19-year-old woman with autism, retinoblastoma of one eyeball, decreased esterase D activity, and deletion in chromosome 13q12–q14 [8]. A similar description was presented by Smith *et al.*, who described the case of a 3.5-year-old boy with a deletion of approximately 30 Mbp at loci 13q12–q13. The patient did not have any congenital abnormalities or dysmorphic features but presented features of autism and auditory processing deficits [9]. A different clinical profile was shown by Pavone *et al.*, who described a 2.5-year-old girl with multiple congenital abnormalities, including choanal atresia, micrognathia, retrognathia, aplasia of the skin on the head, syndactyly, and clinodactyly. The authors also found the following CNS anomalies: narrowing of the

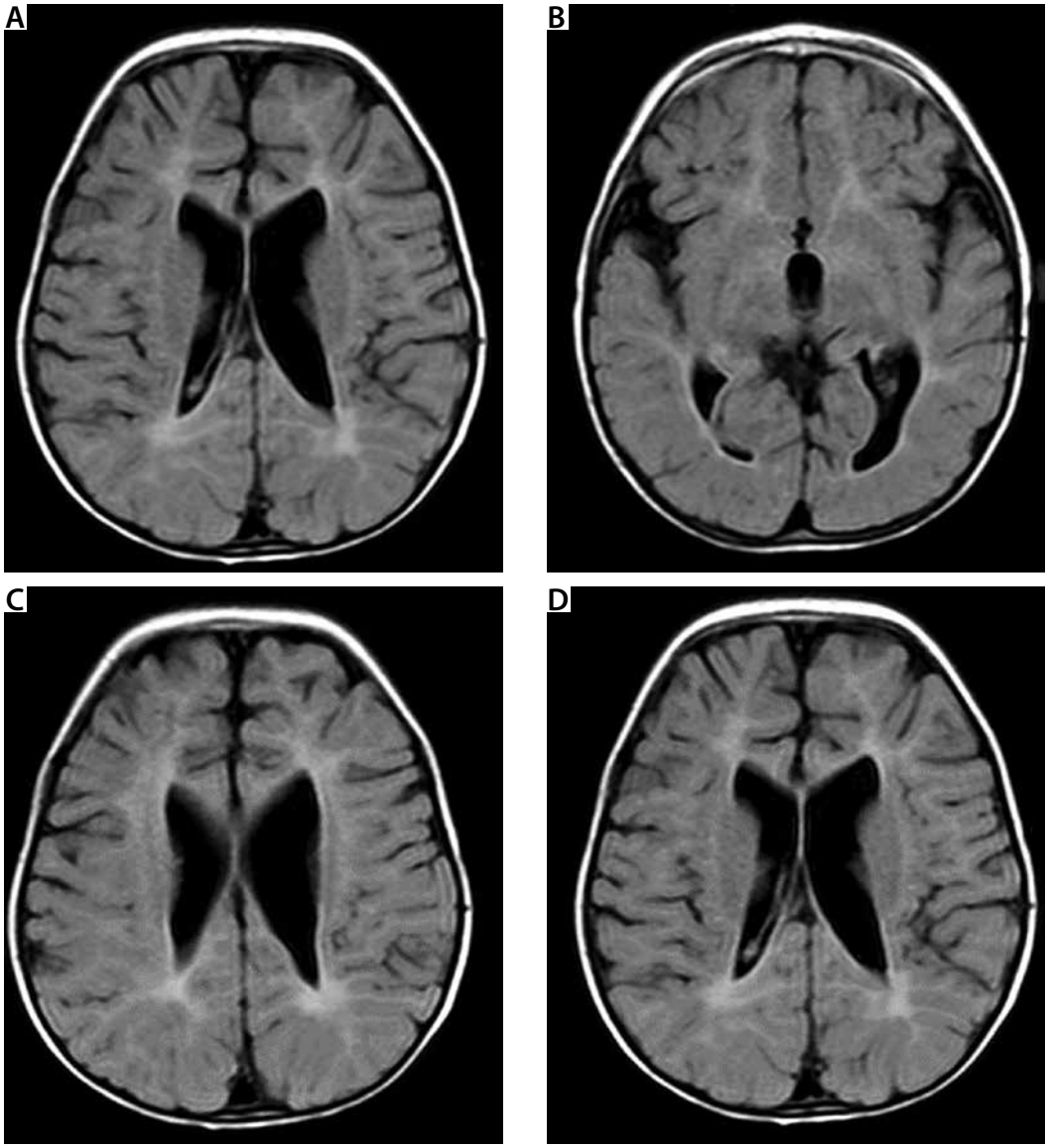

FIGURE 1. Magnetic resonance imaging of the central nervous system in the transverse plane in the T1-weighted sequence

corpus callosum, widening of the ventricular system, and reduction of the hippocampus area. A video-EEG examination revealed sporadic seizure activity in the right occipital region, but it did not confirm an epilepsy diagnosis. In this case, there was a 5.6 Mbp deletion in the 13q12.11–q12.13 region [10]. A similar clinical picture has been described by Friedman *et al.* in a 6-year-old girl with microcephaly, growth retardation, haematological abnormalities, and moderate intellectual disability. The authors identified a 5.7 Mpz deletion at loci 13q12.11–q12.13 [11].

Variable symptoms and congenital disabilities characterize the above cases. A patient described by Pavone *et al.* [10] had changes in the v-EEG recording and structural abnormalities of the CNS, in which he seems to be the closest to our proband. However, he had numerous congenital defects, probably related to the greater size of the deletion and loss of other gene functions. According to the literature review, this is the first case of coexisting epileptic encephalopathy and 13q12 deletion, to the best of our knowledge.

Our patient's deletion includes the *RNF6* gene. It encodes the 685-amino acid Ring Finger Protein 6 (RNF6) containing the RING-H2 finger domain at the C-terminus, responsible for ubiquitin ligase activity [12]. As a ubiquitin ligase, RNF6 is primarily associated with polyubiquitination and degradation of other proteins [13, 14]. So far, research on this protein has focused mainly on its role in cancer [4, 15–18].

In the context of the presented case, we draw attention to the work of Tursun *et al.* [19] on the role of RNF6 protein in axon growth regulation. The authors performed studies on mouse hippocampal cell cultures and CHO, Cos7, HEK293T, and HeLa cell lines. It has been shown that RNF6 partially collocates in hippocampal cells with LIMK1 kinase, which is involved in the activation of the actin polymerization pathway [20]. RNF6 leads to the degradation of LIMK1, which in turn inhibits axon

growth. On the other hand, inhibiting RNF6 by a specific siRNA leads to a significant neurite extension [19]. A schematic representation of the interaction of RNF6 and LIMK1 is presented in Figure 2.

RNF6 probably locally regulates the activity of LIMK1, which is associated with changes in the dynamics of actin polymerization and the correct development of the axonal growth cone [19]. Presumably, a deletion of 75 kb in the *RNF6* gene could lead to a change in the activity of the RNF6 protein and a dysregulation of the LIMK1 kinase, which could disrupt the process of neuronal migration and synaptic formation in the early stages of the CNS development. The hemizygous mutation involving the critical region for Williams-Beuren syndrome – 7q11.23, where the *LIMK1* gene is located, is linked to the resulting cellular cytoskeleton changes that may be responsible for the neurodevelopmental disorders observed in this syndrome. The authors consider whether the identified variant could have had a critical impact on the development of epileptic encephalopathy in the proband.

On the other hand, the suggested perinatal hypoxia abnormalities found in CNS imaging may also lead to changes of this nature. Still, a relatively good perinatal assessment (9 points on the Apgar scale) indicates that this is not the only reason for our patient's clinical condition. The not fully understood nature of the RNF6 protein and its interactions in the CNS development pathways makes it difficult to determine its role in the pathogenesis of changes in the presented case. More research on the function of this protein is needed.

CONCLUSIONS

Epileptic encephalopathy is a complex condition with variable clinical symptoms, often associated with drug resistance. The plausible complex aetiology of the disease in the presented case makes it difficult to select the appropriate treatment. The detected deletion in chromosome 13q, involving the *RNF6*, is likely to impact the patient's condition due to the involvement of the RNF6 protein in essential pathways in the development of the CNS. In light of the prospect of targeted therapy and personalized medicine, further research into the role of *RNF6* seems appropriate. Studies based on exome sequencing did not reveal any pathology in the presented case, while aCGH pointed to a possible aetiological factor. In undiagnosed developmental delay cases, it is indicated that the diagnostic recommendations of the American Society of Human Genetics should be followed [21].

ACKNOWLEDGMENT

We would like to thank Małgorzata Bocheńska, MD for her help in preparing this work.

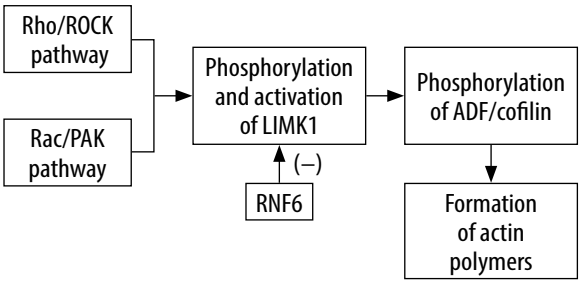

FIGURE 2. A linkage between RNF6 and LIMK1 kinase and the actin polymerization pathway

ADF – actin depolymerizing factor, PAK – p21-activated serine-threonine kinases, ROCK – Rhoassociated coiled-coil containing protein kinase

DISCLOSURE

The authors declare no conflict of interest.

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