

Turkish cases of early infantile epileptic encephalopathy: two novel mutations in the cyclin-dependent kinase-like 5 (CDKL5) gene

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Cyclin-dependent kinase-like 5 gene-related epileptic encephalopathy is gradually becoming better known in child neurology practice. The related gene mutations cause early infantile epileptic encephalopathy characterized by intractable epilepsy, severe mental retardation and, later, the development of Rett syndrome-like features. Herein, we report the first two Turkish cases of cyclin-dependent kinase-like 5 gene-related epileptic encephalopathy with novel mutations in exon 8, which is located in the catalytic domain of the gene.

Key words: Rett syndrome-like features, cyclin-dependent kinase-like 5 gene, infantile spasm, epileptic encephalopathy.

Epileptic encephalopathy (EE), described as a form of epileptic activity, may contribute to severe cognitive and behavioral impairment above and beyond what might be expected from the underlying pathology alone; and this impairment can become worse over time. The condition was only formally recognized in 2006, in an International League Against Epilepsy (ILAE) report, and then not specifically defined until 2010^{1,2}. Due to developments in genetic technology, the number of EE cases with an identifiable genetic cause has been gradually increasing. Mutations in the cyclin-dependent kinase-like 5 (CDKL5) gene (previously known as the serine/threonine kinase 9 gene, *STK9*), located on chromosome Xp22 (CDKL5, OMIM 300203), are considered responsible for early infantile EE. It is characterized by intractable epilepsy and severe mental retardation, followed later by the development of Rett syndrome (RTT)-like features. Here, the first two Turkish cases of early infantile epileptic encephalopathy due to novel mutations in the CDKL5 gene are presented.

Case Report

Case 1

The patient was born at full-term via normal vaginal delivery. Perinatal history was normal. Her parents were healthy and nonconsanguineous. Her first display of recurrent afebrile generalized seizures occurred at the age of 6 weeks. Seizure control was achieved with immediate phenobarbital treatment. However, the seizures recurred after 15 days; valproate was added to the phenobarbital. Her psychomotor development was normal; she had head control and eye contact, and was able to follow large, bright objects and to smile at her mother. At that point her EEG was normal (Fig. 1a). Extensor spasms began at three months of age; they were easily controlled by vigabatrin. Her head circumference (39 cm, 50%), neurological examination and psychomotor development were all normal. Her EEG showed bilateral slow waves over the parietooccipital regions (Fig. 1b). Recurring spasms continued until six months of age, but they were easily controlled with increased



Fig. 1. Serial sleep electroencephalography recordings of case 1. 1a: Normal electroencephalography with asynchronous sleep spindles. 1b: High-amplitude, sharp waves over the parietooccipital regions. 1c: Slowing of background activity with scarce sleep spindles. High-amplitude, multifocal sharp waves, especially over the parietooccipital regions. 1d: Diffuse slowing of background activity. High-amplitude, focal sharp waves over the centrotemporal and posterior regions.

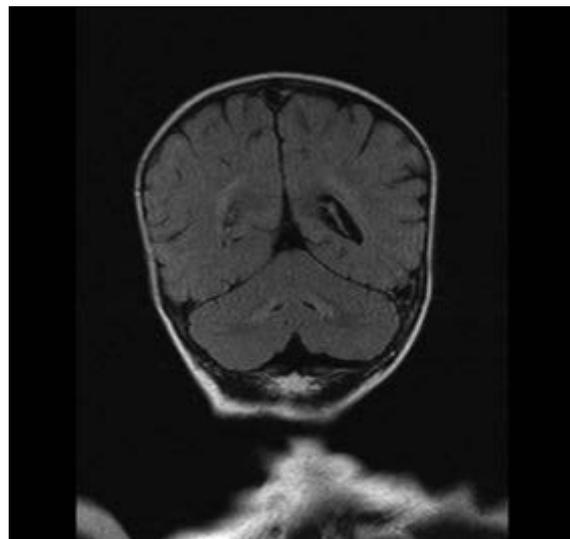


Fig. 3. Hyperintensities in periventricular white matter and cerebellar dentate nuclei on coronal fluid attenuated inversion recovery image of case 1.

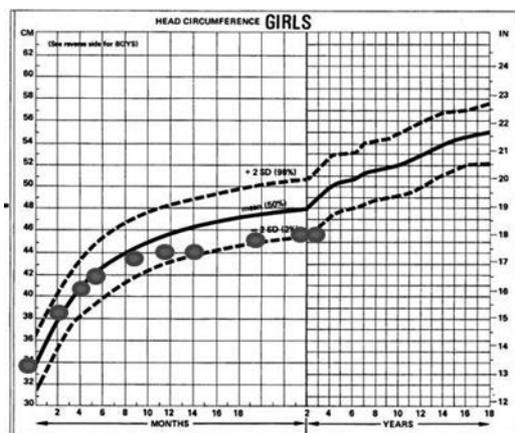


Fig. 2. Serial head circumference values of the first case.

doses of vigabatrin. By six months of age she had become hypotonic; she could sit with support, but had poor eye contact and did not notice objects. She had a broad, prominent forehead and deep-set eyes. At the age of 18 months, she had recurrent spasms controlled by adrenocorticotrophic hormone (ACTH). Her EEG displayed high-amplitude background slowing with interspersed multifocal sharp waves (Fig. 1c). She was very hypotonic and had lost head control and eye contact. At the same time, she began to display some abnormal movements, such as rapid rolling, neck hyperextension and extension of the extremities and hands. At the 18th month of



Fig. 4. Dysmorphic facial features of patient 2 (prominent forehead, deep-set eyes, well-defined philtrum).

age, her head circumference was 44 cm (2nd percentile). She had acquired microcephaly (Fig. 2). Her cranial magnetic resonance imaging (MRI) showed hyperintensities in the periventricular white matter and cerebellar

dentate nuclei on T2 weighted and fluid attenuated inversion recovery (FLAIR) images (Fig. 3). Screening of the entirety of the coding sequences of the *CDKL5* gene using denaturing high pressure liquid chromatography (DHPLC) revealed a sequence variation in exon 8. Direct sequencing was performed to identify that variation. A novel frameshift mutation, c.536ins4(AAGA)del119 (p.S179X), was found in a heterozygous state. The mutation, which results in a premature stop codon and truncated protein, was not observed in DNA samples of the parents and was considered to be *de novo*. Due to the severe damage it causes to protein structure, this mutation may explain the EE phenotype of our case.

This patient is now four years old; she has few spasms and is able to sit with support. She has poor visual tracking but has minimally gained some purposeful hand movement. Her EEG displayed high-amplitude slow waves over the posterior regions and focal sharp waves over the centrottemporal regions (Fig. 1d).

Case 2

A 13-month-old girl was referred with spasms and myoclonias of the eyelids and hands. She was delivered via cesarean section following a normal pregnancy, and had an uneventful perinatal history. She was the first child of nonconsanguineous parents. She experienced recurrent, afebrile generalized tonic and generalized clonic seizures on her 35th day and was given phenobarbital. The seizures ceased for two months. Spasms began at four months of age, and trials of vigabatrin, clonazepam, topiramate and levetiracetam all failed. She had a broad, prominent forehead, large, deep-set eyes and a well-defined philtrum (Fig. 4). On neurological examination her head circumference was normal; she had severe hypotonia with no head control and poor eye contact. She had 50-80 spasms per day and myoclonias of her eyelids and hands. Her EEG was indicative of hypsarrhythmia. After treatment with clobazam, valproate and ACTH, her seizures stopped. After discontinuation of ACTH, spasms recurred 5-10 times daily, but without myoclonias. At 29 months of age, she was having 10-15 spasms/day; she had no motor achievement and was also displaying dyskinetic movement of her hands.

She was receiving vigabatrin, valproate and phenobarbital. Serial EEG recordings showed persistence of hypsarrhythmia. At the age of two, cranial MRI demonstrated cerebral atrophy with normal spectroscopic findings. Her head circumference was 48 cm, which is within the normal range. Sequence analysis of the entire coding region of the *CDKL5* gene revealed a heterozygous mutation in exon 8 (c.459C>G, p.D153E). This mutation was not present in the public version of the Human Genome Mutation Database (HGMD). A disease-causing mutation at the c.458 position was given in HGMD, with the code CS095939. Results of in silico evaluation of this mutation with the SIFT, Mutation Taster, Polyphen2 and Mutation Assessor programs strongly suggest its disease-causing effect. Functional analysis of the mutation is the only way to arrive at safe clinical conclusions, but all these data indicate that this is most likely a disease-causing mutation. This variation most probably causes loss of the protein kinase domain between amino acids 13 and 297. The mutation was not observed in DNA samples of the parents and thus considered to be *de novo*.

Discussion

Knowledge concerning *CDKL5*-related EE is gradually increasing in child neurology practice. To date, 141 individuals (127 females and 14 males) with a *CDKL5* mutation have been described, generally within small case series from different countries³.

Our cases are the first reported Turkish cases with novel mutations. Both cases had similar seizure histories, beginning with afebrile motor seizures within the first two months followed by infantile spasms. The course of the epilepsy was compatible with that described in a previous report by Bahi-Buisson et al.⁴ Following anticonvulsant treatment, the seizures were controlled for a short period (15 and 60 days, respectively). Our first case had normal EEG findings at the time the spasms started. That is an important clue from the viewpoint of the clinical diagnosis, and one that has been noted by many other authors⁴⁻⁷. Although our first case displayed fairly controlled seizures, the second case had intractable seizures despite receiving polytherapy. The first case also displayed a deceleration of head growth

from 6 months of age, resulting in acquired microcephaly. Both cases were unable to achieve purposeful hand skills and speech. They both exhibited dyskinetic movements, sometimes dystonic, sometimes choreathetic, in the extremities from two years of age; the movements completely disappeared during sleep. The diagnostic criteria for both typical and atypical Rett syndrome were revised and published in 2010⁸. A regression period followed by recovery or stabilization is a prerequisite for both groups. Atypical Rett cases are also expected to have at least two of the four main diagnostic criteria for Rett syndrome. Hand stereotype, one of the main criteria for Rett syndrome, was not observed in our cases. Atypical Rett cases often share some features of Rett syndrome. Various subtle dysmorphic features have also been described in patients with *CDKL5* mutation^{6,9}. Recently, Fehr et al.³ evaluated the clinical findings of Rett syndrome in comparison with those of *CDKL5*-related cases. They reported that subtle dysmorphic features, including three or more of the following—broad/prominent forehead, large, deep-set eyes, full lips, tapered fingers and, in males, anteverted nares—suggest a likely diagnosis of a *CDKL5*-related disorder. Both of our cases fit this criterion, with dysmorphic features such as prominent forehead and deep-set eyes.

Different cranial MRI findings have been reported in *CDKL5*-related EE cases. In addition to normal MRI, cerebral-cerebellar atrophy and white matter changes have been described in the literature^{6,10}. In a study of 20 cases, Bahi-Buisson et al.¹⁰ reported cortical atrophy and posterior white matter hyperintensities on T2/FLAIR images in 65% and 45% of cases respectively. Cerebellar atrophy and hyperintensity on dentate nuclei were also described in four of 20 cases. However, Artuso et al.⁶ reported that cranial MRI findings were normal in a small group consisting of five cases. White matter changes on T2 weighted images in the parietooccipital region and cerebellar dentate nuclei were observed in the first case, while the second case displayed cortical atrophy.

Both cases had *de novo* mutations in exon 8, located in the catalytic domain of *CDKL5*. It has been reported that mutations in the catalytic region generally impair the kinase activity of

CDKL5 protein¹¹. Bahi-Buisson et al.⁴ observed that patients with missense mutations in the ATP binding site had a milder phenotype than that of patients with mutations in the kinase domain, who later displayed a more severe phenotype. Some authors have also reported that patients with stop codon mutations had a milder phenotype than did those with missense or splicing mutations. In accordance with the literature, our first case, who had a stop codon mutation, had milder phenotype than our second case, who had a missense mutation in the catalytic region, and hence more severe clinical findings.

Studies have suggested that *CDKL5* and *MeCP2* proteins share a common signaling pathway, and that *CDKL5* plays a critical role in neuronal morphogenesis⁵. Since *MECP2* and *CDKL5* mutations result in a fairly similar phenotype, it has been suggested that both genes play a role in common pathogenic processes. Mari et al.¹² compared the expression patterns of *MeCP2* and *CDKL5* in embryonic and postnatal mouse brains, demonstrating that their expression increases with neuronal maturation and synaptogenesis. They noted that the two genes generally display overlapping expression, both spatially and temporally, and suggested that their results indicate a possible involvement of the two proteins in the same developmental pathway.

Because understanding of *CDKL5*-related EE is still limited, an increase in the awareness of the syndrome will be helpful in the clarification of its natural history and the development of new treatment options.

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