

CDKL5 Gene-Related Epileptic Encephalopathy in Estonia: Four Cases, One Novel Mutation Causing Severe Phenotype in a Boy, and Overview of the Literature

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Abstract

Cyclin-dependent kinase-like 5 (CDKL5) gene mutations have mainly been found in females with early infantile epileptic encephalopathy (EIEE), severe intellectual disability, and Rett-like features. To date, only 22 boys have been reported, presenting with far more severe phenotypic features. We report the first cases of CDKL5 gene-related EIEE in Estonia diagnosed using panels of epilepsy-associated genes and describe the phenotype–genotype correlations in three male and one female patient. One of the mutations, identified in a male patient, was a novel de novo hemizygous frameshift mutation (NM_003159.2:c.2225_2228del (p.Glu742Afs*41)) in exon 15 of CDKL5. All boys have a more severe phenotype than the female patient. In boys with early onset of seizures and poor development with absent or poor eye contact, CDKL5 gene-related EIEE can be suspected and epilepsy-associated genes should be analyzed for early etiological diagnosis. Early genetic diagnosis would be the cornerstone in personalized treatment in the future.

Keywords

► CDKL5 gene-related early infantile epileptic encephalopathy
► cyclin-dependent kinase-like 5 gene
► Phenotype–genotype correlation

Introduction

Mutations in the cyclin-dependent kinase-like 5 (CDKL5) gene are responsible for an early-onset epileptic encephalopathy.1 CDKL5 gene encodes a 1,030 amino acid long protein with a highly conserved serine/threonine kinase domain in the N-terminal region and a large C-terminal region involved in either the catalytic activity or the subcellular localization.2 Mutations in the CDKL5 gene have mainly been reported in female patients with an early infantile epileptic encephalopathy (EIEE) phenotype including infantile spasms, severe intellectual disability, and Rett-like features with absent or limited speech, stereotypic hand movements, and deceleration of head growth.2–5 As the CDKL5 gene is located on the X chromosome (Xp22.13), genetic traits of CDKL5 alterations have been
considered to be X-linked dominant. CDKL5 mutations appear to be less common in males and only 22 boys have been reported so far according to our best knowledge. In most of the cases, the phenotype is more severe in boys. We report the phenotype of three male and one female patient with CDKL5 mutations.

Patients

**Patient 1**
A 3-year-old boy was born from normal pregnancy and delivery. His early development was delayed; no eye contact developed before seizure onset. First seizures at the age of 2.5 months were focal onset secondary generalized clonic followed by epileptic spasms at 3.5 months. Neurological examination at 3.5 months showed poor psychomotor development; lack of eye contact, no head control, and central hypotonia. He had drug-resistant epilepsy with severe psychomotor development delay. At the age of 2 years, he had epileptic spasms and myoclonias and the highest psychomotor skill was short-term eye contact (Table 1).

**Electroencephalography (EEG)** at the age of 2.5 months showed bilateral and unilateral interictal epileptiform discharges (IED) and atypical hypsarrhythmia at 3.5 months (Supplementary Fig. S1, online-only). At the age of 2 years 1 month, EEG still revealed hypsarrhythmia awake (Supplementary Fig. S2, online-only) and burst-suppression (BS) pattern in sleep (Supplementary Fig. S3, online-only).

Magnetic resonance imaging (MRI) at the age of 2.5 months showed signs of mild frontal and temporal lobe brain atrophy and thin corpus callosum. By the age of 5 months, progression of the frontotemporal atrophy, mild cerebellar atrophy, and thin corpus callosum were seen (Fig. 1A–F).

**Patient 2**
Our second patient is a 14-year-old boy with uneventful perinatal history. At the age of 3 weeks, he had his first seizures described as epileptic spasms. At 2 months, he had generalized tonic-clonic seizures and epileptic spasms, neurological examination showed poor psychomotor development; no eye contact...
and central hypotonia. He had drug-resistant epilepsy with persisting tonic-clonic seizures and severe psychomotor developmental arrest. At the age of 4 years, he developed nystagmus and hyperkinesis. At 13 years, he had occasional tonic seizures and myoclonias, and the highest psychomotor skill was short-term eye contact (►Table 1).

First EEGs at the age of 1 and 2 months were within the normal range. At 4 months, generalized IED were seen. By the age of 7 months, IED were solved, but there was slowing of EEG background activity. From the age of 1 year 8 months, his EEG showed general progressive slowing (1.5–2.5 Hz at the age of 13 years) with multifocal IED. MRI showed progressive brain atrophy, mainly in frontal and temporal lobes and cerebellar vermis, and thin corpus callosum at the age of 4 and 13 years (►Fig. 1G–L).

Patient 3

A 14-year-old boy was born from uneventful pregnancy and delivery. The first seizures (focal) were documented at the age of 5 months and he had delay in psychomotor development; no eye contact with presence of periodic horizontal nystagmus, no head control, and central hypotonia was also present. At 8 months, epileptic spasms occurred and at 3 years of age, he had myoclonias and generalized tonic–clonic seizures. At 13 years of age, he had epileptic spasms and generalized tonic–clonic seizures. He had drug-resistant epilepsy with severe psychomotor development arrest, the highest psychomotor skill was short-term eye contact (►Table 1).

EEG at the age of 5 months showed spike-wave complexes in posterior areas. At 8 months, hypsarrhythmia appeared. From the age of 3 years, multifocal IED in awake and BS pattern in sleep were present. BS pattern persisted until the age of 10 years. Last EEG at 13 years of age showed moderate slowing of background activity with multifocal IED.

MRI showed progressive brain atrophy, mainly in frontal and temporal lobes and cerebellar vermis, and corpus callosum at the age of 2 years 10 months and 11 years (►Fig. 1M–R).

Patient 4

A 4-year-old girl was born from uneventful pregnancy and delivery. The first seizures at the age of 2 months were focal and neurological examination showed poor psychomotor development; brief eye contact and central hypotonia. At the age of 4 months, she presented with generalized tonic and focal onset with secondary generalized tonic–clonic seizures. At the age of 6 months, myoclonic seizures developed.

At the age of 3 years, she had several focal seizures every day, and the highest skill was walking without support. She is severely autistic, has no speech, and her epilepsy was drug resistant (►Table 1).

EEG at the age of 3 months showed occasional focal IED over right hemisphere. At the age of 6 to 12 months, ictal and interictal myoclonias were present. From 1 year 5 months, multifocal IEDs could be detected only in sleep recording. Her EEGs background activity had been with mild general slowing.

MRI at the age of 2.5 months was normal. At the age of 6 months, signs of mild brain atrophy of frontal and mainly temporal lobe was already present. By the age of 1 year 7 months, further asymmetrical progression of temporal and frontal lobe atrophy occurred. Atrophy of cerebellum or corpus callosum was not noted.

Molecular Analysis

Patient 1, 2, and 3 were investigated using next generation sequencing panels of epilepsy-associated genes (►Supplementary Table S1, online-only) and in patient 4, sequencing of CDKL5 gene was performed. A novel hemizygous frameshift mutation in exon 15 of the CDKL5 gene (NM_003159.2:c.2225_2228del (p.Glu742Asfs’41)) was found, and EIEE caused by a mutation in the CDKL5 gene was diagnosed in patient 1. The (p.Glu742Asfs’41) variant of the CDKL5 gene was also not present in the Broad’s ExAc database. The mutation creates a shift in the reading frame starting at codon 742. The new reading frame ends in a stop codon 40 positions downstream, which is very likely to result in a truncated protein or loss of protein production. A hemizygous missense mutation in exon 14 of CDKL5 gene (NM_003159.2:c.2152G > A (p.Val718Met)), previously reported in a female patient,2 was found in male patient 2, and a hemizygous splice-site variant in exon 14 of CDKL5 gene (NM_003159.2:c.2152 + 1G > A) that is next to the mutation found in our second male patient, was found in patient 3. A previously reported17 heterozygous nonsense mutation in exon 12 (NM_003159.2: c.1648C > T (p.Arg550*)) was found in patient 4 (►Table 2). All CDKL5 mutations in this study were confirmed by Sanger sequencing (►Supplementary Fig. S4, online-only). All identified mutations were not observed in all parents, indicating de novo origins (►Table 1).

Discussion

We report four cases of CDKL5 gene-related EIEE in Estonia including a novel CDKL5 mutation in patient 1. To our best knowledge, the mutation in patient 1 has not been reported before in correlation with EIEE phenotype. The persistence of hypsarrhythmia, early development arrest (before seizure onset), and early progressive brain atrophy are characteristic to the newly described mutation (NM_003159.2:c.2225_2228del (p.Glu742Asfs’41)).

Boys present with more severe phenotype with early-onset intractable epilepsy, exclusively infantile spasms, severe global developmental delay, cortical visual impairment, muscle tone abnormalities, mostly axial hypotonia, and hand stereotypes.5,12,18 Our boys show an extension of the clinical spectrum of the previously reported male patients (►Table 3).6–15 We also found that our three male patients presented with a more severe phenotype than the female patient; they showed the onset of epileptic encephalopathy at a younger age, presented with more severe developmental arrest and more profound brain atrophy in contrast to the female patient. The highest skill in all boys was short-term eye contact, whereas the girl could walk without support (►Table 1).

The milder clinical spectrum of CDKL5 mutations in female patients is hypothesized to be due to variable X-chromosome inactivation that also affects CDKL5.19 The dominance of boys

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Table 1 Summary of the clinical, neuroimaging, and molecular findings in three male and one female patient with CDKL5 mutation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age at the moment (y)</th>
<th>Seizure onset (mo)</th>
<th>Seizure types</th>
<th>Drug-resistant epilepsy</th>
<th>EEG findings</th>
<th>Cerebral atrophy</th>
<th>Global developmental delay</th>
<th>Highest skill</th>
<th>Muscle tone</th>
<th>Poor eye contact</th>
<th>Postnatal microcephaly</th>
<th>Abnormal movement</th>
<th>Outcome</th>
<th>Mutation</th>
<th>Mutation type</th>
<th>Family History</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Male</td>
<td>3</td>
<td>2.5</td>
<td>Focal onset secondary generalized tonic-clonic, epileptic spasms, myoclonic</td>
<td>+</td>
<td>Bilateral and unilateral IEDs, atypical hypersynchrony, multifocal IEDs, burst-suppression pattern</td>
<td>+ (last MRI at 5 mo)</td>
<td>Severe</td>
<td>Short-term eye contact</td>
<td>Central hypotonia</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>Alive</td>
<td>c.2252C &gt; A</td>
<td>Missense De novo</td>
<td>Nonconsanguineous, no known neurologic problems</td>
</tr>
<tr>
<td>Patient 2</td>
<td>Male</td>
<td>14</td>
<td>3</td>
<td>Epileptic spasms, generalized tonic-clonic, tonic, myoclonic</td>
<td>+</td>
<td>Generalized IEDs, multifocal IEDs Progressive slowing in background activity (1.5–2.5 Hz at the age of 13 y)</td>
<td>+ (last MRI at 13 y)</td>
<td>Severe</td>
<td>Short-term eye contact</td>
<td>Central hypotonia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Alive</td>
<td>c.2152G &gt; A</td>
<td>Exon 14</td>
<td>p.Val718Met</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Male</td>
<td>14</td>
<td>5</td>
<td>Focal, epileptic spasms, generalized tonic-clonic, myoclonic</td>
<td>+</td>
<td>Multifocal IEDs, hypersynchrony, burst-suppression pattern, moderate slowing of background activity</td>
<td>+ (last MRI at 11 y)</td>
<td>Severe</td>
<td>Short-term eye contact</td>
<td>Central hypotonia</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>Alive</td>
<td>c.2152G &gt; A</td>
<td>Exon 14</td>
<td>p.Val718Met</td>
</tr>
<tr>
<td>Patient 4</td>
<td>Female</td>
<td>4</td>
<td>2</td>
<td>Focal, tonic, focal onset tonic-clonic, myoclonic</td>
<td>+</td>
<td>Focal IEDs, generalized and focal onset with secondary generalization epileptiform discharges, multifocal IEDs, mild general slowing in background activity</td>
<td>+ (last MRI at 1 y 7 mo)</td>
<td>Moderate</td>
<td>Walking without aid</td>
<td>Central hypotonia</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>Alive</td>
<td>c.1648C &gt; T</td>
<td>Exon 12</td>
<td>p.Arg550*</td>
</tr>
</tbody>
</table>

Abbreviations: EEG, electroencephalography, IED, interictal epileptiform discharges; MRI, magnetic resonance imaging.
in our study is difficult to explain. The girls could have been diagnosed before by direct sequencing of the CDKL5 gene and therefore we identified more male patients with the panels of epilepsy-associated genes for male patients where CDKL5 gene as an etiological factor was not suspected. Another explanation is that CDKL5 gene-related EIEE may be under-diagnosed in boys in other countries as the common opinion is that it occurs mainly in females.

We would like to underline that the seizure semiology and EEG findings in presented patients are changing over the time. In 3 out of 4 of our patients, the first seizures were focal onset and during the course of the disease, all boys have had epileptic spasms, followed by multiple seizure types in the course of the disease. Slowing of background activity in EEG in boys has not been reported earlier, except for Mirzaa et al in 2013, but it seems that progressive slowing is an important and specific symptom. We also observed the same variation in clinical symptoms at different age with changing seizure type and EEG findings in a patient with epileptic encephalopathy caused by GNAO1 mutation, who had the classical clinical picture of Ohtahara syndrome at the age of 3 months, but later the seizure types and EEG findings changed.

Brain atrophy, especially dominant in frontal region, is an additional clinical finding in patients with CDKL5 mutations. This correlates with our findings, as all our male patients with CDKL5 mutation had progressive brain atrophy, mainly in frontal and temporal lobes. Our first male patient had signs of mild frontotemporal brain atrophy as early as at 2.5 months of age with progression of the disease by the age of 5 months. Cerebellar, mainly vermis and corpus callosum atrophy, develops later, but may be present as early as 5 months as was noted in the first patient. Thus, it underlines the importance of follow-up in cases of epileptic

Table 2 The phenotypic characteristics of all reported patients with (NM_003159.2: c.1648C > T (p.Arg550*)) mutations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Our patient (patient 4)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Present in literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset</td>
<td>2 mo</td>
<td>1.5 mo</td>
<td>4 mo</td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td>Focal, tonic, focal onset tonic-clonic, myoclonic</td>
<td>Fixed gaze, generalized tonic-clonic, myoclonic</td>
<td>Infantile spasms, myoclonic, tonic, generalized tonic-clonic</td>
<td></td>
</tr>
<tr>
<td>Hypsarrhythmia</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Severe intellectual difficulties</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Regression</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Expressive speech</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hand stereotypies</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motor function</td>
<td>Walking</td>
<td>Sitting</td>
<td>Walking</td>
<td></td>
</tr>
<tr>
<td>Brain atrophy</td>
<td>+ (at 1 year 7 mo)</td>
<td>– (at 3 y)</td>
<td>+ (at 7 y)</td>
<td></td>
</tr>
<tr>
<td>Deceleration of head growth</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Effective treatment</td>
<td>± Phenobarbital and topiramate</td>
<td>Topiramate</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 3 Clinical features of our male patients with CDKL5 mutation compared with all male patients reported in the literature (22 patients)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Present in literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizure onset &lt; 3 mo</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>86%</td>
</tr>
<tr>
<td>Infantile spasms</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>95%</td>
</tr>
<tr>
<td>Drug-resistant epilepsy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100%</td>
</tr>
<tr>
<td>Cerebral atrophy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>50%</td>
</tr>
<tr>
<td>Severe global developmental delay</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>100%</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>64%</td>
</tr>
<tr>
<td>Cortical visual impairment/poor eye contact</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>73%</td>
</tr>
<tr>
<td>Postnatal microcephaly/ deceleration of head growth</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>18%</td>
</tr>
</tbody>
</table>
encephalopathies, as clinical symptoms change during disease course, including development of cerebral atrophy. In our female patient, the clinical picture was milder and the progression of frontotemporal atrophy was slower. Cerbellar and corpus callosum atrophy was not found at all in the last brain MRI performed at 1 year and 7 months, but repeated MRI is needed to estimate the course of the disease. However, it also may suggest that brain atrophy is more pronounced in male patients. The absent or poor eye contact is an early notable risk factor for poor prognosis. Epilepsy-associated genes should be analyzed in case of absent or poor eye contact and early-onset multiple seizures in both sexes for early etiologic diagnosis. We would like to underline the importance of gene panels being the best tool for early diagnosis of epileptic encephalopathies. Early etiologic diagnosis is extremely important, avoiding additional unnecessary expensive testing and being a cornerstone for future personalized treatment. It is also of utmost importance to have the exact diagnosis to inform families and offer genetic counseling.

In conclusion, we like to underline that CDKL5 gene-related EIEE should be suspected in cases of early onset of seizures, neurodevelopmental arrest, or severe delay. In most of the patients, epilepsy starts with focal onset seizures followed by epileptic spasms. Very characteristic is presence of multiple seizure types in early disease course and severely affected background activity on EEG in male patients. The absent or poor eye contact is an early notable risk factor for poor prognosis. Epilepsy-associated genes should be analyzed in case of absent or poor eye contact and early-onset multiple seizures in both sexes for early etiologic diagnosis. We would like to underline the importance of gene panels being the best tool for early diagnosis of epileptic encephalopathies. Early etiologic diagnosis is extremely important, avoiding additional unnecessary expensive testing and being a cornerstone for future personalized treatment. It is also of utmost importance to have the exact diagnosis to inform families and offer genetic counseling.

Author Contributions
S. L., I. T., and K. N. are the first authors who equally contributed to this work. T. T. is the mentor of this work. I. T. and T. T. conceived the study. S. L., I. T., K. N., and T. T. participated in its design and coordination. S. L. drafted the manuscript. I. T., K. N., and T. T. critically revised the manuscript for important intellectual content. S. L., I. T., K. N., and V. S. cared for the patients. U. V. conducted and interpreted the EEG studies. T. R. and K. Õ. were responsible of the genetic testing and interpretation of the findings. P. I. was responsible of the neuroradiologic investigations and the interpretation of the findings. All authors critically read the final manuscript and gave final approval before submitting the article.

Conflict of Interest
The authors declare that there is no conflict of interest.

Ethical Approval
The Research Ethics Committee of the University of Tartu approved this study and informed consent was obtained from the patient’s parents.

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