To date, 43 patients have been described with mutations in or involving the CDKL5 gene. The typical phenotype includes early-onset, often intractable epileptic seizures and severe mental retardation with very limited progress in psychomotor development. Most patients also show impaired social interaction with avoidance of eye-to-eye contact, and some clinical features reminiscent of Rett syndrome (RTT), including stereotypic hand movements, lack of purposeful hand use, acquired microcephaly, and generalized hypotonia. We report on the case of a 5-year-old girl with a de novo CDKL5 gene mutation who developed early puberty, which has not been described before.

**How to Cite this Article:**

Most of the patients carrying CDKL5 mutations had often been diagnosed initially with a severe form of atypical Rett syndrome or the early-seizure, or Hanefeld, variant of RTT [Tao et al., 2004; Weaving et al., 2004; Evans et al., 2005; Mari et al., 2005; Scala et al., 2005; Pintaudi et al., 2008; Rosas-Vargas et al., 2008], in which the seizures begin at a few months old, often in the form of spasms with or without hypsarrhythmic EEG, accompanied by psychomotor developmental delay and preceding the clinical signs of RTT [Hanefeld, 1985; Goutieres and Aicardi, 1986].

It is worth noting, however, that this variant of RTT was described before mutations in the methyl-CpG-binding protein 2 (MECP2) gene were discovered in most RTT cases. So far, no MECP2 mutations have been reported in the Hanefeld variant, and no CDKL5 mutations have been found in typical RTT cases. Moreover, seizures in the first 6 months or year of life have been described in very few RTT patients with MECP2 mutations, while infantile spasms have never been reported [Steffenburg et al., 2001; Weaving et al., 2004; Evans et al., 2005; Archer et al., 2006].

Taken together, the evidences suggest that CDKL5 mutations are responsible for a specific phenotype of a severe early-onset seizure disorder distinct from RTT, but resembling the clinical picture previously described by Hanefeld; on the other hand, the early seizure variant described by Hanefeld is probably almost

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**INTRODUCTION**

Involvement of the cyclin-dependent kinase-like 5 (CDKL5) gene, located in the Xp22 region [Montini et al., 1998], has so far been reported in 43 patients with neurodevelopmental disorders.

The gene was originally found disrupted in a balanced X-autosomal translocation in two unrelated female patients with a phenotype of severe infantile spasms syndrome X-linked (ISSX) [Kalscheuer et al., 2003].

Different point mutations have since been detected in another 39 patients with various clinical phenotypes including: (1) infantile spasms and/or early-onset, often intractable, epileptic seizures, and severe developmental delay, with or without clinical features reminiscent of Rett syndrome (RTT) [Tao et al., 2004; Weaving et al., 2004; Evans et al., 2005; Mari et al., 2005; Scala et al., 2005; Archer et al., 2006; Buoni et al., 2006; Nectoux et al., 2006; Grosso et al., 2007; Li et al., 2007; Bahl-Buisson et al., 2008; Pintaudi et al., 2008; Rosas-Vargas et al., 2008]; (2) autistic disorder with mild-to-moderate intellectual impairment in a female twin [Weaving et al., 2004]; and (3) severe epileptic encephalopathy with spastic quadriaparesis and cortical blindness in a boy [Weaving et al., 2004].

More complex neurological pictures have also been described in two male patients, correlating with different deletions of the Xp22 region, also involving the CDKL5 gene [Huopaniemi et al., 2000; Van Esch et al., 2007].
always not RTT, but a disorder with some features reminiscent of RTT due to other genes, such as CDKL5.

This is a case report on another patient with a de novo CDKL5 gene mutation responsible for a clinically severe phenotype associated with precocious puberty.

**CLINICAL REPORT**

The patient is a girl 5 years 4 months old. She was born to unrelated and healthy parents, at term by scheduled cesarean due to a podalic presentation. Birth weight, length, and head circumference were 2,690 g (10th centile; −1.23 standard deviations (SD) below the mean), 46 cm (<10th centile; −1.68 SD), and 33 cm (10th centile; −0.63 SD), respectively, and the Apgar scores at 1 and 5 min were 5 and 8. She initially had no sucking or swallowing difficulties. At 6 weeks old, moderate-to-severe gastroesophageal reflux was diagnosed and adequately treated. The girl subsequently developed severe feeding problems that were often difficult to manage.

The child’s psychomotor development was severely delayed and characterized by very limited progress: she could remain seated with support at 3 years, but she never became able to stand or walk; she never pronounced any words or used her hands to reach for objects. From her second year onwards, she developed a stereotypic activity with her hands.

She was 2 months old when the epileptic episodes started, characterized by isolated and serial flexing spasms followed by tonic extensions of the upper and lower limbs, both awake and asleep, several times a day. Repeated EEGs did not show a hypersynchronous pattern. The seizures persisted over the following 2 years, with no change in their characteristics, despite antiepileptic treatment with numerous drugs, alone or in association. A cycle of ACTH was administered at 2 years old, with good results, but only for 2 months, after which the fits began again. By 2 years 5 months old, however, the introduction of felbamate succeeded in reducing the number of seizures to 1 a day and this situation remained stable for approximately 2 years.

With time, various diagnostic investigations were performed, including repeated brain MRI (revealing a mild, non-progressive diffuse enlargement of the ventricle and subarachnoid spaces, and thinning of the corpus callosum). Routine investigations including MECP2 sequencing were normal.

The patient had numerous seizures a day (up to 15), characterized by a sudden flexing hypertonia of the trunk and limbs, sometimes followed by diffuse hyperextension, isolated or in irregular clusters and of variable duration (up to about 10 min). The seizures were sometimes associated with guttural sounds, vomiting, clonic eyelid movements, masticatory movements, and tonic gaze deviation. The EEG background activity included rhythmic theta activity, prevalent on the fronto-central EEG derivations, frequently comprising prolonged sequences of diffuse high-voltage (150–350 μV) spike, and wave discharges, better defined on both anterior regions, at irregular low frequencies. In drowsiness, minimal myoclonic seizures were associated with diffuse spike and wave discharges, followed by brief EEG flattening. More evident seizures, characterized by sustained tonic contractions, were associated with initial spike and wave discharges, followed by diffuse flattening and rhythmic alpha (9–12 Hz) activity. A second cycle of ACTH and an increase in the daily dose of felbamate was associated with a reduction in the frequency and intensity of the seizures.

Screening for mutations of the CDKL5 gene (using the DHPLC/ direct sequencing method according to Scala et al. [2005]) evidenced a novel missense mutation in exon 5: c.215T > C, p.I72T. Parental analyses confirmed the “de novo” origin of the mutation. The pathogenic role of the mutation can be inferred from the highly conserved position of the amino acid isoleucine at position 72. As reported by Evans et al. [2005], it appears conserved in Homo sapiens, Mus musculus, Gallus gallus, Fugu rubripes.

At the age of 5 years 3 months, the child was assessed for suspected precocious pubarche. Pubertal development was rated B1P2 according to the Tanner stages [Tanner and Whitehouse, 1976]. Her height and weight were 102 cm (10th centile; −1.78 SD) and 14.9 kg (3rd centile; −1.54 SD) and her rate of growth in height was 11 cm a year (+4.16 SD, normal range for age 4.3–8.3 cm a year). Her head circumference was 47.5 cm (<3rd centile; −2.78 SD). The gonadotropin-releasing hormone (GnRH) test showed high luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responses (by ICMA), that is, 10.4 mU/ml and 9.59, respectively, and an LH/FSH ratio of 1.09 (normal prepubertal values of LH are <6.9 mU/ml and the LH/FSH ratio is <0.7) [Oerter et al., 1990; Neely et al., 1995]. Plasma estradiol (by ICMA) was 15 pg/ml (normal values <13.6 pg/ml) [Brito et al., 1999]. Ultrasound showed ovaries of normal volume with numerous small follicles and a uterus of prepubertal size but with a midline endometrial echo and an increased fundal-cervical ratio, the first signs of the onset of puberty [Buzzi et al., 1998]. The child’s bone age was estimated at 4.5 years using the Greulich and Pyle [1959] method. These findings are consistent with the diagnosis of precocious puberty [Lebrethon and Bourguignon, 2000].

**DISCUSSION**

The common CDKL5 phenotype includes early-onset epileptic seizures and severe developmental delay.

The early onset of seizures, usually by 3 months of life [Grosso et al., 2007; Bahi-Buisson et al., 2008], is the most consistent clinical feature and represents the key for identifying patients likely to have CDKL5 mutations [Archer et al., 2006; Bahi-Buisson et al., 2008].
et al., 2008]. Initial seizures are usually tonic, not associated with hysparrhythmia; infantile spasms with hysparrhythmia commonly follow [Bahi-Buisson et al., 2008]. In most patients, generalized, frequent and often severely drug-resistant epileptic seizures subsequently develop, mainly tonic and myoclonic [Buoni et al., 2006; Grosso et al., 2007; Bahi-Buisson et al., 2008]. In a group of patients, however, epilepsy has a favorable outcome with a transient “honeymoon period” of several months that may even become permanent [Bahi-Buisson et al., 2008]. There is sometimes evidence of partial seizures with a localized paroxysmal activity [Mari et al., 2005; Archer et al., 2006; Buoni et al., 2006]. In the case of our patient, spasmy-like and tonic seizures began at 2 months and were followed by myoclonic and tonic seizures unresponsive to drugs.

Lctal EEGs were consistent with the picture previously reported in patients carrying CDKL5 gene mutations [Buoni et al., 2006; Bahi-Buisson et al., 2008], while interictal EEGs, characterized by frontal-central rhythmic theta activity, were similar to those described in a small number of patients with CDKL5 mutations [Tao et al., 2004; Grosso et al., 2007; Bahi-Buisson et al., 2008], but were also reminiscent of the situation described in stages III and IV of classical RTT [Glaze et al., 1987], and in many other genetically determined conditions causing epilepsy and mental retardation [Doose and Gundel, 1986].

Another characteristic common to patients with CDKL5 gene mutations is severe mental retardation, which emerges early, with very limited subsequent progress in psychomotor development and language. With the exception of two less severe cases with a clinical phenotype involving autistic features, good gross motor abilities, and some verbal skills [Weaving et al., 2004; Archer et al., 2006], the majority of patients with CDKL5 gene mutations, like ours, fail to achieve a sitting posture unaided or to pronounce any words.

The typical CDKL5 phenotype also exhibits some Rett-like features, such as acquired microcephaly, absence of speech and of purposeful hand use, intense stereotypic hand activity, and generalized hypotonia. These clinical features were also seen in our patient and, as in others, could have met the criteria for a clinical diagnosis of atypical RTT. We observed other characteristics, however, that are commonly reported in CDKL5-mutated patients, but not in RTT cases, including a lack of valid eye-to-eye contact and poor autonomic features [Kalscheuer et al., 2003; Tao et al., 2004; Evans et al., 2005; Archer et al., 2006; Rosas-Vargas et al., 2008]. The avoidance of valid eye fixation has often been seen as a feature distinguishing the CDKL5 phenotype from RTT, which is characterized by a very marked, intense eye gaze [Tao et al., 2004; Evans et al., 2005; Archer et al., 2006]. Autonomic function disturbances are also inconsistent in people with CDKL5 mutations, but are almost invariably seen in RTT [Archer et al., 2006; Rosas-Vargas et al., 2008].

The human CDKL5 gene encodes a protein 1,030 amino acids (aa) long that contains an N-terminal catalytic domain (aa 13–297) including the ATP-binding region (aa 14–47) and the serine–threonine kinase active site (aa 127–144), and a large C terminal tail that seems to be involved both in regulating the subcellular distribution of the protein from the nucleus to the cytoplasm, and in negatively influencing the catalytic activity of the protein [Rusconi et al., 2008]. To date, 35 different pathogenic mutations have been identified, including missense mutations within the catalytic domain, deletions, insertions, nonsense mutations causing the premature termination of the protein distributed over the entire open reading frame, and deletions of genomic regions including the entire CDKL5 locus. No clear genotype–phenotype correlation has been established, but recent data suggest that the electro–clinical severity of the epilepsy may be related to the type and location of the mutations: patients with missense mutations within the catalytic domain or truncating mutations upstream from the catalytic domain of the protein apparently tend to develop epilepsy earlier and with a more severe outcome than patients with truncating mutations downstream from the catalytic domain in the C terminal region [Bahi-Buisson et al., 2008]. The more severe phenotype in patients with proximal mutations seems to be attributable to the fact that the absence or reduction of CDKL5 catalytic activity is more deleterious to the nervous system than a gain of function due to hyperactivity of the kinase activity [Rusconi et al., 2008].

A missense mutation affecting the catalytic domain has been identified in our patient. This raises the total number of reported pathogenic missense mutations to 6 in 10 cases, including our patient [Tao et al., 2004; Evans et al., 2005; Archer et al., 2006; Li et al., 2007; Rosas-Vargas et al., 2008]. All the missense mutations are located in the N-terminal end of the protein and result in changes to highly conserved residues in the kinase domain. A clinical summary of the 10 patients is given in Table I. All but one of the patients developed a similar clinical picture with refractory epilepsy; by contrast, the neurological impairment was not homogeneous because the gross motor abilities varied, walking being possible with support in six cases. The mutation identified in our patient affects the same codon, albeit with a different amino acid substitution, as in two patients showing a less severe clinical phenotype—they could both walk and, in the case described by Li, the epilepsy developed later on [Evans et al., 2005; Li et al., 2007]. The different phenotype in these three cases carrying mutations of the same type and location can presumably be explained by the different properties of the protein and the variable X-chromosome inactivation.

Precocious puberty had so far not been reported in RTT syndrome or CDKL5 patients. In girls suffering from RTT, puberty usually takes place at the normal time, with the onset of menarche at a mean age of 11 years 2 months [Holm, 1986]. In our patient, brain MRI revealed no hypothalamus–pituitary changes potentially responsible for her early sexual development. The question is whether her early, frequent, and severely drug-resistant epileptic episodes could have had a causal role in her early puberty. The relationship between epilepsy and reproductive hormones is a much-debated issue because there is evidence of a mutual influence [Herzog and Fowler, 2005; Herzog, 2006]. Hormone changes during puberty can induce or exacerbate epilepsy [Klein et al., 2003] and estrogens play a part in facilitating seizures, while progesterone has an antiepileptic effect [Herzog et al., 1997; Herzog, 1999]. Ictal and interictal paroxysmal discharges can also induce sex hormone changes, influencing the functionality of the hypothalamus–pituitary axis [Herzog et al., 2003], but there is no evidence of epilepsy affecting the age of menarche [Herzog, 2006], despite a few case series of epileptic female patients reaching puberty early by comparison with the healthy population [Nalin et al., 1988; El-Khayat et al., 2004]. A
### TABLE I. Clinical Features of the Patients With CDKL5 Missense Mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Refs</th>
<th>Sex/age</th>
<th>CDKL5 mutation</th>
<th>Acquired microcephaly</th>
<th>Growth retardation</th>
<th>Poor eye fixation</th>
<th>Hypotonia</th>
<th>Hand stereotypes</th>
<th>Hand apraxia</th>
<th>Absence of speech</th>
<th>Seizure onset</th>
<th>Seizure at onset</th>
<th>Late seizures</th>
<th>Refractory epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tao et al. [2004]</td>
<td>Female; 5 yrs</td>
<td>c.455G &gt; T p.L152F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5 w</td>
<td>IS-like</td>
<td>AS</td>
<td>Improvement with vagus-nerve stimulator at 4.5 yrs old</td>
</tr>
<tr>
<td>2</td>
<td>Tao et al. [2004]</td>
<td>Female; 41 yrs</td>
<td>c.525A &gt; T p.R175S</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2 mo</td>
<td>IS</td>
<td>IS</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tao et al. [2004]</td>
<td>Female; 41 yrs</td>
<td>c.525A &gt; T p.R175S</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10 w</td>
<td>IS</td>
<td>IS</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Evans et al. [2005]</td>
<td>Female; 11 yrs</td>
<td>c.215T &gt; A p.I72N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6 w</td>
<td>IS</td>
<td>IS</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Archer et al. [2006]</td>
<td>Female; 13 yrs</td>
<td>c.539C &gt; T p.P180L</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6 mo</td>
<td>IS</td>
<td>IS</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Li et al. [2007]</td>
<td>NK</td>
<td>c.216T &gt; A p.I72I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2 mo</td>
<td>IS</td>
<td>IS</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Rosas-Vargas et al. [2008]</td>
<td>Female; 3 yrs</td>
<td>c.119C &gt; T p.A40V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3 yrs</td>
<td>IS</td>
<td>IS</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Rosas-Vargas et al. [2008]</td>
<td>Female; 6.5 yrs</td>
<td>c.119C &gt; T p.A40V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8 yrs</td>
<td>IS</td>
<td>IS</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Rosas-Vargas et al. [2008]</td>
<td>Female; 3 yrs</td>
<td>c.659T &gt; C p.I272T</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6 yrs</td>
<td>IS</td>
<td>IS</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>This work</td>
<td>Female; 5.4 yrs</td>
<td>c.215T &gt; A p.I72N</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>With support</td>
<td>With support</td>
<td>With support</td>
<td>With support</td>
</tr>
</tbody>
</table>

Yrs, years; mo, months; w, weeks; NK, not known; IS, infantile spasms; TS, tonic seizures; AA, atypical absences; AS, absence seizures; MS, myoclonic seizures; GTCS, generalized tonic clonic seizures; +, presence of the sign; -, absence of the sign.
recent epidemiological study comparing age at menarche in a large sample of girls with epilepsy whose seizures developed in infancy versus those whose seizures developed in adolescence and adulthood also seems to rule out any correlation between the age of onset of epilepsy and any anticipation of pubertal development [Svalheim et al., 2006]. Moreover, there are no reports in the literature of girls with severe epileptic encephalopathy and precocious puberty. Antiepileptic therapy, particularly valproic acid and/or polytherapy, also seems to be capable of interfering with the reproductive hormones, disrupting the menstrual cycle, or causing polycystic ovary syndrome and hyperandrogenism [Isojarvi et al., 1993; Betts et al., 2003; Verrroelli et al., 2005], but for the time being there have been no reports of precocious puberty correlating with any antiepileptic drugs.

On the other hand, it is not unusual to find precocious puberty associated with genetic syndromes with mental retardation: this has been reported, for instance, in Kabuki Make-up [Kasuya et al., 1998], Cohen [North et al., 1995], Angelman [Katzos et al., 2004], Williams [Partsch et al., 2002], and Klinefelter [Fryns and Devriendt, 1997] syndromes, and in patients with chromosomal disorders involving chromosome 15 [Grosso et al., 2001]. The etiopathogenic mechanisms behind the association between early puberty and genetic syndromes nonetheless remain to be explained [Katzos et al., 2004].

In conclusion, this report reinforces the observation that the CDKL5 phenotype represents a distinct early-onset seizure disorder, which overlaps to some degree with the early-seizure variant of Rett syndrome.

The association of precocious puberty and CDKL5 mutation has not been reported before and further experience is needed for its clarification.

REFERENCES


Betts T, Yarrow H, Dutton N, Greenhill L, Rolfe T. 2003. A study of anticonvulsant medication on ovarian function in a group of women with epilepsy who have only ever taken one anticonvulsant compared with a group of women without epilepsy. Seizure 12:329–329.


