

A Novel Mutation in the X-Linked Cyclin-Dependent Kinase-Like 5 (*CDKL5*) Gene Associated With a Severe Rett Phenotype

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Mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene have recently been reported in patients with severe neurodevelopmental disorder characterized by early-onset seizures, infantile spasms, severe psychomotor impairment and very recently, in patients with Rett syndrome (RTT)-like phenotype. Although the involvement of *CDKL5* in specific biological pathways and its neurodevelopmental role have not been completely elucidated, the *CDKL5* appears to be physiologically related to the *MECP2* gene. Here we report on the clinical and *CDKL5* molecular investigation in a very unusual RTT case, with severe, early-neurological involvement in which we have shown in a previous report, a novel P388S *MECP2* mutation [Conforti et al. (2003); Am J Med Genet A 117A: 184–187]. The patient has had severe psychomotor delay since the first month of life and infantile spasms since age 5 months. Moreover, at age 5 years the patient suddenly presented with renal failure. The severe pattern of symptoms in our patient, similar to a *CDKL5* phenotype, prompted us to perform an analysis of the *CDKL5*, which revealed a novel missense mutation never previously described. The X-inactivation assay was non-informative. In conclusion, this report reinforces the observation that the *CDKL5* phenotype overlaps with RTT and that *CDKL5* analysis is recommended in patients with a seizure disorder commencing during the first months of life. © 2009 Wiley-Liss, Inc.

Key words: *CDKL5* gene; *CDKL5* phenotype; mutational screening; rett syndrome

INTRODUCTION

Rett syndrome (RTT; OMIM 312750) is a progressive neurological disorder almost exclusively affecting females with an incidence of 1:15,000 [Hagberg, 1985]. This disorder is characterized by a wide spectrum of clinical manifestations.

Amir et al. [1999] found that RTT is caused by mutations in the X-linked methyl-CpG-binding protein 2 (*MECP2*; OMIM 300005) gene on chromosome Xq28.

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To date, mutations have been identified in the *MECP2* sequences of ~90% of classical RTT and 40–50% of RTT variants, suggesting that this last group is both clinically and genetically more heterogeneous [Weaving et al., 2005].

Recently, a second gene has been connected to X-linked neurological developmental disorders involving severe mental retardation and seizures that appear in the first few postnatal months. This gene is the cyclin-dependent kinase-like 5 (*CDKL5*; OMIM 300203), previously known as serine–threonine kinase 9 (*STK9*). The first described mutations of the *CDKL5* were found in two unrelated female patients with apparently balanced translocations: 46,X,t(X;7) (p22.3;p15) and 46,X,t(X;6) (p22.3;p14), respectively [Kalscheuer et al., 2003].

Because of the potential importance of *CDKL5* in RTT syndrome, the existence of overlapping phenotypes and, in particular,

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an intriguing phenotype in our patient, we performed a *CDKL5* mutation analysis. Within this analysis we gave a detailed clinical description of the case, which was already investigated on the molecular level for the *MECP2* gene [Conforti et al., 2003].

METHODS

Patient

We investigated a 13-year-old girl affected by early-onset convulsions who fulfilled the revised criteria for RTT variant phenotypes [Hagberg et al., 2002]. Informed consent and blood samples were obtained from the patient's family.

Molecular Analysis

A *MECP2* analysis (exons 2–4) was performed in 2001 and we screened the whole coding sequence of the *CDKL5* using the transgenomic WAVE denaturing high-performance liquid chromatography (DHPLC). The *CDKL5* coding portion (exon 2–21) and neighboring intronic regions were entirely analyzed using primers as previously described [Scala et al., 2005]. PCR products resulting in abnormal DHPLC profiles were sequenced on both strands using PCR primers with fluorescent dye terminators on an ABI 3130XL genetic analyzer (PE Applied Biosystems, Foster City, CA).

The sequencing primers were the same as those used for PCR amplification. Mutation analyses were performed using the normal *CDKL5* sequence as a reference (GenBank accession No. Y15057).

To confirm that the alteration found in this work was a novel mutation and not a polymorphism, more than 300 normal control alleles were examined directly using PCR and DHPLC. The variation was verified by sequencing with forward and reverse primers.

To better shed light on whether the *MeCP2* or the *CDKL5* mutations, or both, are likely to be pathogenic, we examined the potential impact an amino acid variant may have on the function of the encoded protein with the use of an innovative sequence homology-based program, Polimorphisms Phenotype (PolyPhen; <http://www.bork.embl-heidelberg.de/PolyPhen/>). For each gene of interest (*MeCP2* and *CDKL5*), we analyzed the protein sequence identified using the PolyPhen program. The PolyPhen algorithm uses sequence alignments and it utilizes protein structure databases, such as Protein Data Bank (PDB) or Protein Quaternary Structure (PQS) and three-dimensional structure databases to determine if a variant may have an effect on the protein's secondary structure, interchain contacts, functional sites and binding sites. This program gives results as "benign," "possibly damaging," "probably damaging," or "unknown."

RESULTS

Our patient's phenotype is described below.

Patient

The patient, at the time of writing, is a 13-year-old girl, with unrelated parents. The father died at age 45 from kidney failure caused by amyloidosis. Pregnancy was complicated due to gestational diabetes and at birth the baby weighed 4,200 g. She presented

no asphyxia or hyperbilirubinemia, but a first adaptation phenomenon was impaired and she needed naso-gastric tube feeding for the first 10 days after her birth. Her neurological development was abnormal from the beginning: she was hypotonic, poorly reactive to the environment, and cried all day long.

We first observed the patient at age 10 months for psychomotor retardation and hypotonia (she was able to lift and maintain her head in an upward position at age 10 months). At this time she was not able to maintain a sitting position; moreover, her head circumference was below the 50th centile (43.5 cm) and her speech was made by monotone babbling with frequent laryngeal stridors. Hand stereotypies, such as hand washing and mouthing were sporadically observed. During hospitalization, she presented with infantile spasms (both flexion and extension spasms). A CT scan demonstrated brain atrophy with subarachnoidal and perisylvian space enlargement. EEG showed spike/waves and slow waves in all derivations. VPA therapy needed to be integrated with lamotrigine to better control the patient's seizures. The infant's psychomotor development failed to progress and at follow-up visit, at age 1 year; she was unable to sit or manipulate objects. She continued to perform persistent hand stereotypies, drooling, and unmotivated crying. At age 20 months, her head circumference was 44 cm (below the 3rd centile).

She appeared extremely hyperactive with frequent hyperventilation crises, drooling, and stereotypies. She had no eye contact, did not interact with the environment and used no forms of verbal or gestural communication. She showed severe trunk hypotonia and ataxia.

Brain MRI demonstrated marked cortex atrophy with main involvement of the frontotemporal area and aspecific alterations of the white matter pattern. Psychomotor evaluation took place at age 3 months. At age 6 years, the patient suddenly developed acute kidney failure: she had hypertension, mild eyelid edema, but normal diuresis. Laboratory exams revealed hyperazotemia (143 mg/dl), low creatinine (8.6 mg/dl), metabolic acidosis and anemia (RBC 2,212,000/mm³ and Hb 12.2 g/dl). An abdomen ultrasound showed hyperlucent and large kidneys and an end stage glomerular sclerosis was diagnosed by kidney biopsy. She was rapidly treated with albumin, furosemide and RBC transfusion, and a hypertension therapy was started.

At the time of writing, at age 13, the patient's psychomotor retardation is severe; she makes frequent hand mouthing and screaming but no eye contact. She has progressively become dystrophic, with thin dry skin and muscle mass reduction. Thanks to VPA treatment, seizures are only sporadically observed but eyelid myoclonus and startles are frequent. She is daily treated with peritoneal dialysis and weekly erythropoietin treatment cannot be stopped without leading to severe anemia.

Molecular Analysis

The screening of the entire coding sequence of the *CDKL5* by DHPLC revealed an abnormal chromatographic pattern in exon 12 of the patient. Direct sequencing of the amplicon identified a *CDKL5* missense mutation AAC > ACC; c.1417 A > C. This substitution causes the amino acid change of asparagine for threonine (p.N399T), and the sequence variation was not found in the patient's mother or in her unaffected brother.

The previously described *MECP2* mutation, found in the same patient, determines the amino acid substitution of proline for serine (p.P388S) at the C-terminus of the gene. This substitution was not found in 100 normal chromosomes and in the patient's family members [Conforti et al., 2003].

The results of the PolyPhen program for each gene of interest (*MECP2* and *CDKL5*) was assigned as “benign” for the P388S *MECP2* mutation with a Position Specific Independent Counts (PSIC) value of 1.42 and as “possibly damaging” for the N399T *CDKL5* mutation with a PSIC value of 1.75, suggesting a structural effect, buried site or cavity creation.

DISCUSSION

Mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene have recently been reported in patients with severe neuro-developmental disorder characterized by early-onset seizures, infantile spasms and severe psychomotor impairments and, more recently, in RTT-like phenotypes. In this study we have screened the *CDKL5* in a patient carrying the previously described novel *MECP2* mutation [Conforti et al., 2003], suffering from a severe form of variant RTT. Indeed, our patient manifests the Hanefeld variant RTT [Hanefeld 1985] and had early-onset seizures, hand stereotypies, congenital psychomotor delay, and hypotonia. The clinical features of patients with *CDKL5* mutations are summarized in Table I (supporting information Table I which may be found in the online version of this article summarizes electroclinical data on literature cases [Weaving et al., 2004; Evans et al., 2005; Archer et al., 2006; Buoni et al., 2006; Grosso et al., 2007; Bahi-Buisson et al., 2008; Pintaudi et al., 2008; Rosas-Vargas et al., 2008]). The molecular investigation of this case has revealed a previously unreported *CDKL5* missense mutation (N399T). Unfortunately, the father's sample was not available and analysis of parental DNA was made only of the patient's mother and brother. This analysis showed that the variation found is, probably, a de novo mutation because it is not present in other family members. In fact, the majority of patients with the *CDKL5* mutation are female and only rare occurrences are in males suffering from severe mental retardation,

infantile spasms, or early-onset epilepsy [Chahrour and Zoghby, 2007].

The phenotypes associated with *CDKL5* mutations range from X-linked infantile spasms (ISSX) and infantile epileptic encephalopathy to atypical RTT, plus one case of autism. At the most severe end of the spectrum, we find patients affected by severe intractable early-onset seizures often accompanied by RTT-like features; at the other end of the spectrum, we find patients affected by mild mental retardation with autistic features [Tao et al., 2004]. Many female patients with *CDKL5* mutations have intermediate phenotypes. It seems that severity is associated with the proportion of functional or partially functional *CDKL5* produced by the normal or mutated alleles [Nectoux et al., 2006].

As mutations in *MECP2* and *CDKL5* genes lead to similar phenotypes, their involvement in a common pathway is suspected. Interestingly, recent data have shown that the spatio-temporal expression of the *CDKL5* during mouse development significantly overlaps with that of the *MECP2*, and that the two proteins interact in vitro and in vivo [Mari et al., 2005]. In our case, the mutation is located in a conserved region of the *CDKL5* (www.ensembl.org; ENSG00000008086 genomic sequence alignment), closest to the region in the middle of the protein. The substitution of an asparagine with a threonine residue would likely alter or decrease binding with the *MECP2* and presumably lead to decreased *MECP2* phosphorylation.

Taken together, several lines of evidence argue the pathogenic nature of this missense mutation. First, the mutation most likely occurred de novo. Second, the mutation lies within the critical region of the interacting domain and, therefore, most likely has deleterious effects on *CDKL5* activity. Third, the mutated amino acid is conserved in different species. Finally, this mutation has not been previously described and was not found in more than 300 normal chromosomes; therefore, we cannot exclude that this mutation is related to RTT.

Even if we could make the same type of remarks for the *MECP2* mutation (except for the number of tested controls: 50 vs. 150), the results of the “in silico” analyses and above all the clinical phenotype of our patient prompt us to believe that the previously described *MECP2* mutation could be considered a very rare poly-

TABLE I. Clinical Features of Patients With CDKL5 Mutations

	Phenotype	Patients age (yrs) (mean; median; upper/lower values)	Age at seizure onset (mo) (mean; median; upper/lower values)	EEG features	MRI
Present study 1/F	A RTT (Hanefeld variant)	13	10	Abnormal	Cortical generalized atrophy with deepening of insular and temporal gyri
Other studies 47/F and 1/M	ISSX/autism, EE, A RTT, ME, autistic disorder, MR (for details see ^a)	9.7; 6.6; 1.5/41	1.5; 1.3; 1 d/12	See ^a	16 N and for other see ^a

A RTT, atypical Rett syndrome; ISSX, X-linked infantile spasms; EE, epileptic encephalopathy; ME, myoclonic encephalopathy; MR, mental retardation; N, normal; d, days; mo, months; yrs, years.
^aSupporting information Table I.

morphism that does not contribute to the pathogenesis of this unusual variant RTT phenotype.

In conclusion, we have identified a novel *CDKL5* pathogenic mutation N399T that contributes to enlarge the *CDKL5* gene variation database. Nonetheless, this finding reinforces the idea that altered *CDKL5*-regulation of *MECP2* is responsible for a specific RTT phenotype and suggests that *CDKL5* mutation screening should be performed in RTT patients, mainly females, presenting a history of early onset of a severe intractable seizure disorder.

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