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CDKL5 in different atypical Rett syndrome variants: Description of the first eight patients from Spain

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Abstract. Mutations in cyclin-dependent kinase-like 5 (*CDKL5*) have been observed in patients with epileptic encephalopathies and atypical variants of Rett syndrome (RTT) associated with early epilepsy. Determination of the type and location of *CDKL5* mutations may provide molecular diagnosis and prognostic information and aid in genetic counseling for the family. Molecular analysis of *CDKL5* and X-chromosome inactivation pattern in 53 Spanish RTT girls (without identifiable methyl-CpG-binding-protein 2) and nine boys with epileptic encephalopathy was performed. *De novo CDKL5* mutations were identified in eight atypical RTT patients: one late regression; one preserved speech, one congenital variant with epilepsy onset at 3 years of age, and five patients. An additional five patients with early-onset epilepsy. Seizure types and *CDKL5* mutations were identified. Seizures types included infantile spasms or tonic seizures, and developing polymorphic seizures that were resistant to antiepileptic drugs. Electroencephalography records were abnormal, without characteristic pathologic pattern. Epilepsy control (total or partial) was achieved with valproate (four patients) or carbamazepine (three patients). Long-term outcome was variable, depending on type of mutation and epilepsy control. These are the first eight girls from Spain with *CDKL5* mutations. Mutations were identified in early epilepsy variants with early hypotonia, but also in other atypical variants without a molecular diagnosis. This study highlights the importance of *CDKL5* analysis in all atypical RTT patients without an identifiable methyl-CpG-binding-protein 2 mutation. Type and location of *CDKL5* mutation and the resultant effect on protein appear to determine the severity of epilepsy.

Keywords: Cyclin-dependent kinase-like 5, drug-resistant epileptic encephalopathy, early epilepsy RTT variant, phenotype-genotype, Rett syndrome

1. Introduction

Rett syndrome (RTT) is a neurodevelopment disorder affecting mostly girls and is the second most common molecular cause of mental retardation in females. Classical RTT consists of normal development during the first 6 to 12 mo of life, followed by global deterioration (loss of acquired language, purposeful use of hands, onset of hand stereotypes, loss of social interest). In 95% of cases, there are associated mutations in methyl-CpG-binding protein 2 gene (*MECP2*) can be found. Atypical RTT variants show a milder phenotype (preserved speech and late regression variants), or a more severe presentation (early epilepsy and congenital forms); *MECP2* mutations in this group are congenital forms in only 40% of cases [2,3].

Since the discovery of new RTT-related genes (cyclin-dependent kinase-like 5 [*CDKL5*] and forkhead

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box G1 [*FOXG1*]), previously molecularly undiagnosed patients with RTT variants have been genetically characterized. *CDKL5* mutations in girls with the early seizure variant (more than 50% have infantile spasms [IS] starting in the first 6 mo of life) are especially relevant, for these patients frequently develop polymorphic seizures and drug-resistant epilepsy. Mutations in this gene have also been observed in drug-resistant early epilepsy encephalopathy [3,9] and, less frequently, in autism without epilepsy [10,11].

CDKL5 encodes a protein with a kinase function dependent on cyclin, and up to 48 deleterious sequence variants have been reported [12,13]. *CDKL5* and *MECP2* are located on different arms of the X-chromosome (*CDKL5* in Xp22 and *MECP2* in Xq28). Experimental studies [14] show that *MECP2* acts as a transcriptional repressor of *CDKL5* (modulating the transcription of different genes, as a genome stabilizer); *CDKL5* is also able to phosphorylate itself and to mediate *MECP2* phosphorylation [1], and participates in neuronal migration and maturation [12–17]. This suggests common biological pathways and a regulatory interaction between these two genes. Mutations in these genes may generate overlapping neurological symptoms.

Brain derived neurotrophic factor (BDNF) has been implicated in adult neural plasticity, including learning and memory [18]. The Val66Met polymorphism in BDNF has formerly been described as a "seizure protector", although recent studies show that common BDNF polymorphisms do not have a mitigating effect on seizures; however, they may modify seizure severity in patients with particular *MECP2* mutations [19]. X-chromosome inactivation (XCI) pattern may influence the genetically determined phenotype in children with early epilepsy if more than 70% of one X allele is active [12,20,21].

The first aim of this study was to identify *CDKL5* mutations amongst our patients with a clinical diagnosis of early epileptic encephalopathy, classical RTT or RTT variants without identifiable *MECP2* mutations (using sequencing and multiplex ligation-dependent probe amplification [MLPA]). Secondly, potential genotype-phenotype relationships were examined. A further aim was to evaluate XCI and BDNF polymorphisms and determine if these influenced clinical severity.

2. Materials and methods

According to the established protocol to access clinical data of Sant Joan de Déu Children's Hospital, clinical charts of 408 patients with classical RTT or RTT variant diagnosis over the last 26 yr were reviewed. Genetic testing of *MECP2* (sequencing of entire coding region and MLPA) was performed in all patients: 258/408 children showed some *MECP2* pathologic mutation and 150/408 patients remained genetically undiagnosed. Parents' consent for the analysis of the genetic material held at the hospital's DNA bank was obtained according to the current ethics protocols in order to study other RTT-related genes. Fifty-three of the 150 *MECP2*-negative samples were selected for *CDKL5* molecular testing, with priority given to children with early epilepsy RTT variant and RTT with polymorphic seizures or drug-resistant epilepsy. Also, samples of nine boys with drug-resistant epileptic encephalopathy in the first year of life were analyzed. Exons 1–22 of the *CDKL5* gene were amplified using the polymerase chain reaction with primers as described elsewhere and sequenced using 3130 Genetic Analyzer (ABI Prism, Applied Biosystems [22]). Detection of large rearrangements of *CDKL5* was performed by MLPA with the Salsa MLPA kit P185 (MRC-Holland). Both parents of *CDKL5* mutation carriers were screened to study the parental origin of the change. All new changes were studied in 200 control chromosomes.

XCI pattern was estimated at the human androgen receptor locus as described elsewhere [21] on DNA extracted from lymphocytes. Skewing XCI was defined as inactivation of more than 70% for one X allele (rate 70:30), and random XCI when inactivation was 50 to 69% (rate50:50 to 69:31).

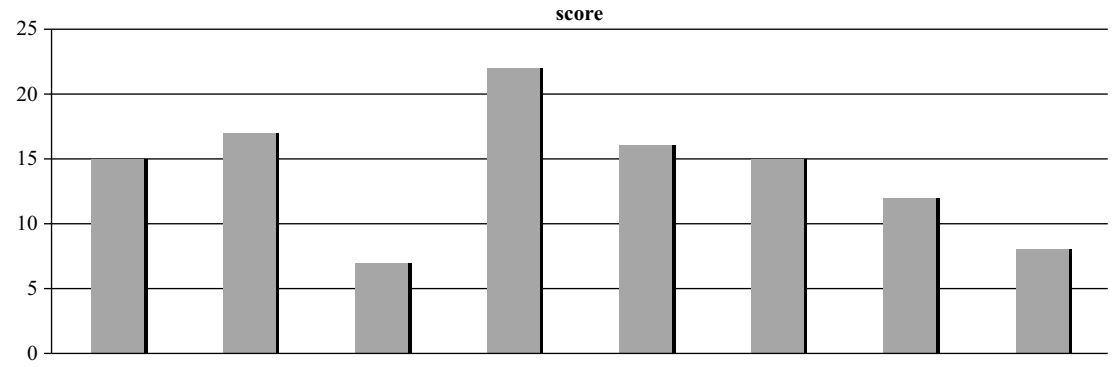
The Val66Met polymorphism in BDNF gene was studied in all samples using single nucleotide polymorphisms-real time polymerase chain reaction technique.

CDKL5-mutated patients' phenotype is described, including current age, RTT form (classical or variant), epilepsy onset and outcome. RTT criteria and related pathology other than epilepsy (breathing disorders, autonomic dysfunction, motor disabilities, etc) are summarized, together with genetic and neuroimaging findings.

3. Results

Novel pathogenic mutations in *CDKL5* were identified in 8/53 (15%) girls of the RTT group and in none of the nine boys with early epilepsy (Fig. 1). All mutations were *de novo* and not present in controls.

A



B

Patient	p.3	p.4	p.2	p.1	p.7	p.8	p.5	p.6
Score	15	17	7	22	16	15	12	8
Mutation	Deletion exons 1 and 2	c.527G > T (p.Trp176Cys)	c.1266C > A (p.Asp422Glu)	c.1341delC (p. Phe447 LeufsX45)	c.1415insA (p. Ile463 AsnfsX5)	c.1549delT (p. Phe516 PhefsX5)	c.2704C > T (p.Q902X)	c.2927C > T (p.Pro976Leu)
BDNF	V66V	V66M	V66V	V66V	V66V	M66M	V66V	V66V
IXC	74:26	54:46	68:32	61:39	homozigote	74:26	70:30	54:46

C

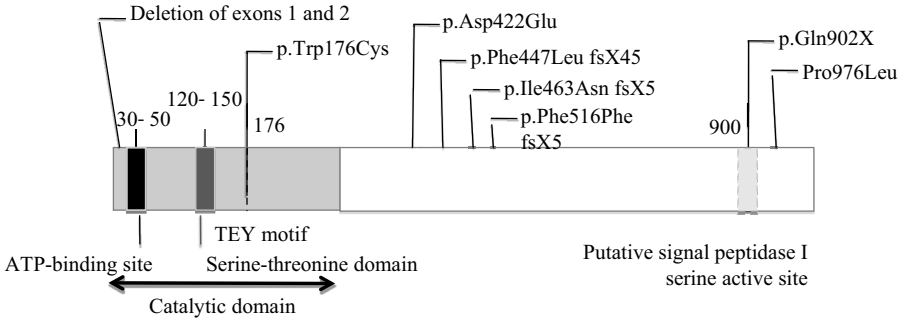


Fig. 1. Correlation between severity score, type and location of cyclin-dependent kinase-like 5 mutations. (A). Representation of severity score (higher for more severe phenotype) of our patients. (B). Table summarizing severity score, mutation, brain derived neurotrophic factor polymorphism and X-chromosome inactivation. A and B, patients were sorted by mutation location in the protein, in order to correlate the clinical presentation with the gene mutation. (C). Functional domain representation with patients mutation found in our series (modified of Bertani et al. 2006).

Sequencing of the coding region and MLPA of *MECP2* was negative for all patients tested for *CDKL5*. Karyotype and metabolic screening both in blood and urine were normal.

BDNF polymorphism analysis showed homozygosity for Val66Val in patients 1, 2, 3, 5, 6 and 7; the previously described "epilepsy protector factor" Val66Met was present in patient 4 and Met66Met was present in patient 8. XCI pattern was determined in all patients but one, who was homozygous for the androgen receptor gene polymorphism. Random XCI (50:50 to 69:31) was present in four patients and skewed XCI (over 70:30) in three.

Polymorphisms in *CDKL5* were found in 14 patients: c.64+26G>A (maternal origin), c.145+17A>G (maternal origin), 145+27delTA (*de novo* in 3 patients but found in 2/200 control chromosomes), c.2372A>C (p.Gln791Pro) in 6 cases (3 of maternal origin, 3 of unknown origin), c.2389G>A (p.Asp797Asn) in one patient and in one control chromosome, c.2714-47G>T in one patient. Three silent mutations of maternal origin were found in two patients: c.2673A>G (p.Gln891Gln), c.3003C>T (p.Hys1001Hys) and c.3084G>A (p.Thr1028Thr).

All patients are Caucasian, from Spain, born of non-related healthy parents after a normal pregnancy (except for patient 3, who was born after in vitro fertilization; the twin sibling died in utero during pregnancy). Clinical phenotype and course of the eight girls with *CDKL5* mutations are summarized in Table 1. All were atypical variants of RTT: five had early epilepsy, one was a

Table 1
Phenotype of the patients with pathogenic *CDKL5* mutations

Features	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Actual age (yr)	11	22	14	3.5	7	22	13	26
Clinical form	Rett syndrome, early epilepsy	Rett syndrome, preserved speech	Rett syndrome, early epilepsy	Rett syndrome, early epilepsy	Rett syndrome, early epilepsy	Rett syndrome, late regression	Rett syndrome, congenital form	Rett syndrome, early epilepsy
Alert symptom	Hypotonia	Unknown	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia
Age of head circumference stagnation	14 mo	Unknown (< 3 yr)	2 yr	6 mo	14 mo	No	No	No
Acquired microcephaly	−3SD	−2SD	−2SD	No	−2SD	No	No	No
Neurodevelopment delay age	14 mo	Unknown up to 3 yr	12 mo	25 days	5 mo	2 mo	< 3 mo	11 mo
Language	No	Partially lost at 3, regained at 5	Lost	No	No	Lost at 15 mo, regain at 10 yr, 2-word phrases at 13 yr	No	Lost, not regained
Stereotypes	Hands washing 15 mo	Hands washing 4 yr	Medium line 15 mo	Hands washing 2 yr	Hands washing 14 mo	Counting coins, clapping, 18 mo	Hands washing 3 yr	hands washing before 3 yr
Purposeful hands use	No	Since 12 mo, clumsy	No	No	Yes	Yes	Yes, at 2 yr, lost at 3 yr	Yes
Unaided ambulation	No	Yes	No	No	Yes	Yes	No	No
Autonomic dysfunction	Yes	No	N o	Yes	No	Yes	No	Yes
Breathing dysfunction	No	No	No	No	No	No	Yes, apnea	Yes
Sphincter control	No	Yes	No	No	Yes	Yes	No	No
Sleep disorder	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Brain magnetic resonance imaging	Normal	Normal	Cortical and subcortical atrophy	Cortico-frontal atrophy	Normal	Normal	Not done	Normal
Epilepsy onset	47 days	Supposed at 2 yr	11 mo	25 days	30 days	No	3 yr	3 mo
Type of first seizures	Infantile spasms	Generalized tonic-clonic	Infantile spasms	Infantile spasms	Partial tonic	No	Generalized tonic-clonic	Myoclonic, tonic-clonic, generalized
Initial electroencephalogram	Normal	Unknown	Occipital spikes, frontal discharges, subclinic	Activation of medium line rolandic paroxysms	Left hemisphere epileptic activity	Paroxysmal activation on sleep	Unstructured slow background, multifocal discharges	Unstructured slow background, multifocal discharges
Polymorphic seizures	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Drug resistance	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Age at epilepsy control (yr)	Partial control (10)	Controlled (13)	Partial control (12.5)	Controlled (2.5)	Controlled (4.3)	Never epilepsy	Controlled	Partial control
Best antiepileptic association	Valproate + Clobazam	Valproate	Carbamazepine + Phenobarbitone	Carbamazepine	Carbamazepine	Not needed	Valproate	Valproate

Table 1. Phenotype of the patients with pathogenic *CDKL5* mutations (Continued)

Features	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Pineda score (age, yr)	22 (10)	7 (10)	15 (9,12)	17 (2)	12 (5)	8 (8,22)	16 (3)	15 (10)
Mutation *CDKL5*	c.1341delC (p.Leu447Phe fsX45)	c.1266C>A (p.Asp422Glu)	Del ex1-2	c.527G>T (p.Trp176Cys)	c.2704C>T (p.Gln902Stop)	c.2927 C>T (p.Pri976Leu)	c.1415insA (p.Ile463Asn fsX5)	c.1549delT, p.Phe517Ser fsX5
Brain derived neurotrophic factor	V66V	V66V	V66V	V66M	V66V	V66V	V66V	M66M
Inactivation X	61:39	32:68	74:26	46:54	70:30	46:54	Homozygote	26:74

congenital variant (epilepsy onset at 3-year-old), one had preserved speech (epilepsy onset before 3-year-old), and one had late regression without epilepsy. None of the patients had completely normal neurodevelopment within the first 3 mo of life: generalized hypotonia was the first alert sign, and neurodevelopment delay was diagnosed between the neonatal period and the first year of life, with delayed head control and sitting position.

Head circumference growth stagnation appeared between 6 and 24 mo in five patients (patients 1, 2, 3, 4 and 5, though only patient 1 achieved-3SD values); patients 6, 7 and 8 did not show head circumference growth stagnation.

Patients 2, 6 and 8 acquired some language, which was lost between 15 mo and 3 yr, but two of them regained simple phrases (patient 2, preserved speech and patient 6, late regression). Five patients did not develop any language.

Hand stereotypies were present in all patients, and began between 14 mo and 3 years of age (including hand washing, hands-to-mouth, clapping, fingers twisting, hands winging). These persist on follow up, albeit less intense and frequent after adolescence.

All eight patients showed impaired hand skills manipulation difficulties, but three (patients 5, 6 and 8) conserve partially purposeful hand use and are able to hold objects or eat with cutlery; patients 2 and 7 lost their ability at 3-year-old. Patients 1, 3 and 4 never acquired a purposeful hand use, unsupported sitting position nor unaided ambulation (although patient 4 is only 3.5-year-old at the time of this report); patients 1 and 3 developed different degrees of scoliosis; patient 7 was able to sit at 2-year-old, but not to walk unaided, and patient 8 acquired sitting position at 2-year-old, aided ambulation at 5, and lost it at 6-year-old. Patient 2 conserves her partially independent apraxic gait, and patients 5 and 6 are completely autonomous for walking, with a clumsy gait.

Autonomic dysfunction (cold feet) was present in four patients, and only two had a breathing dysfunction. Two patients showed feeding difficulties: patient 3 required gastrostomy at 11-year-old, with subsequent improvement of her general health; patient 1 had a hiatus hernia which contraindicated gastrostomy.

All the patients have suffered sleep disorders (frequent night waking) inconstantly along follow up. All epileptic patients developed polymorphic seizures, without a characteristic electroencephalography (EEG) pattern. In four of seven patients, epilepsy onset occurred by 3 mo of life.

3.1. Patient 1

This patient presented with IS at 47 days of life (interictal EEG reported as normal at 3 mo), which led to left upper limb clonic seizures at 4 mo of age; she developed daily myoclonic jerks of upper limbs at 7 yo, with multifocal paroxysms over a partially structured background activity on EEG. Night polysomnography (NPSG) at 8-year-old showed eight seizures of heterogeneous morphology and variable duration (5 sec to 4 min) with clinical (tonic-clonic limbs movements) and subclinical expression; left and bilateral high voltage slow spike-wave complexes were present. At 10 yo, after several drug combinations (vigabatrin, diazepam, levetiracetam, pregabalin), partial control of epilepsy (reduction of seizure frequency from 4–6/day to 1/day, and shortening of duration) was achieved with valproate and clobazam, though multiple fronto-central paroxysms persisted on the latest EEG record. Molecular studies revealed a *CDKL5* c.1341delC coding for protein p.Phe447LeufsX45.

3.2. Patient 2

This patient was adopted at 3 yo (clinical records unknown up to that age, but epilepsy was believed to have started at 2 yo with generalized tonic-clonic seizures), which initially responded to valproate. She developed polymorphic seizures (generalized, tonic-clonic, myoclonic, partial tonic), without clear response to antiepileptic drugs. Follow up was pursued in her local area. Genetic testing showed *CDKL5* c.1266C>A, coding for p.Asp422Glu.

3.3. Patient 3

This patient presented with IS at 11 mo with occipital sharp waves and right frontal subclinical seizures on the initial interictal EEG; she was treated with valproate and vigabatrin but epilepsy worsened by 20 mo, not responding to different drugs (carbamazepine, clobazam, lamotrigine, primidone, topiramate, phenobarbitone, levetiracetam, ethosuximide, pregabalin and ketogenic diet). At 7 yo, seizure frequency increased and social contact, attention and spontaneous mobility decreased. At 9 yo, scoliosis, breathing and swallowing dysfunction appeared and button feed gastrostomy was set up at 11 yo. Since the combination of carbamazepine and phenobarbitone at 12 yo, seizures decreased in frequency, intensity and duration, with improvement of her social contact and breathing dysfunction, although not recovering ambulation. Molecular analysis identified a deletion of exons 1–2 of *CDKL5*, and 5`UTR.

3.4. Patient 4

This patient presented with IS in the first month of life, and had paroxysmal, low intensity, medium line rolandic activity in the initial interictal EEG record; different combinations of antiepileptic drugs (pyridoxine, phenobarbitone, valproate, clonazepam, levetiracetam) did not stop the progression towards polymorphic seizures (sucking movements, tonic gaze deviation, IS with predominance of lower limbs); a normal EEG record was obtained in the following month under carbamazepine treatment and the patient remained clinically seizure-free since 20 mo of age; control NPSG showed parietal-occipital and multifocal paroxysms activated during sleep at 27 mo and clobazam was added. Seizures reappeared at 2.5 yr, and did not respond to lacosamide treatment, but disappeared with higher doses of carbamazepine and clobazam. Genetic testing revelaed a *CDKL5* c.527G>T, p.Trp176Cys.

3.5. Patient 5

This patient presented with partial tonic seizures of right upper limb at 30 days of life, with left hemisphere epileptic activity on the initial interictal EEG; seizures did not improve with valproate, and severe neurodevelopment delay was established by 5 mo; at 2 yr and 2 mo, she had polymorphic seizures (absence seizures, partial clonic, generalized tonic and others), unresponsive to different combinations of pregabalin, topiramate, oxcarbazepine, levetiracetam, valproate, lamotrigine or clobazam. At 3 yr 5 mo, seizures were controlled with carbamazepine and clobazam. Continuous EEG monitoring for four days at 4 yo did not show paroxysms, and the patient remains seizure-free with carbamazepine only. Molecular studies showed a *CDKL5* c.2704 C>T, p.Gln902Stop.

3.6. Patient 6

This patient never had clinical seizures. NPSG performed at the age of 12 yo because of frequent night awakenings showed no epileptic activity, but only activation of few paroxysms during sleep. A *CDKL5*

c.2927C>T mutation, coding for protein p.Pro976Leu was identified.

3.7. Patient 7

This patient presented with generalized tonic-clonic seizures at 3 yo; interictal EEG background activity was unstructured at onset, but polymorphic seizures rapidly appeared, and were partially controlled with valproate. Formal follow up was discontinued in Neurology department of our hospital and at her local area. She maintains seizure control under supervision of her local physician, where epilepsy is apparently controlled. Genetic studies revealed a *CDKL5* c.1415insA, p.Ile463Asn fsX5.

3.8. Patient 8

This patient presented with polymorphic seizures (myoclonic, tonic-clonic, generalized) at 3 mo, resistant to antiepileptic therapy, but achieving partial control with valproic acid in combination with other antiepileptics. Follow up was pursued in her local area. Molecular testing showed a *CDKL5* c.1549delT, p.Phe517Ser fsX5.

4. Discussion

Since the discovery of *MECP2* mutations in children with RTT in 1999, up to 95% of patients with classical RTT have been molecularly characterized. This molecular diagnosis may establish a genotype-phenotype correlation and may provide information related to different clinical aspects of the syndrome, such as ambulation abilities, scoliosis, breathing dysfunction, hands and language skills or epilepsy [13–19,23]. However, molecular confirmation in Rett patients who do not fulfill inclusion criteria for the classical form is only achieved in 40% of cases [1–4].

Patients in this study were selected for *CDKL5* analysis following certain inclusion criteria, which influenced the mutation frequency in our population (15%), whereas other groups found mutations in only three to 6% of the screened patients [11,13,22].

Epilepsy onset occurred in the first yr of life in five of seven patients with epilepsy, and in the first 3 months of life in four of them (including IS or partial tonic seizures). Despite this early onset, these data show a slight discrepancy with other groups' experience [23–25], with an onset in the first 3 mo of life in more than 90%. One patient never suffered seizures.

As in the English, French and Italian series [23–25], all eight girls with mutations in *CDKL5* presented with severe hypotonia in the first mo of life and neurodevelopmental delay, as well as early onset epilepsy in five patients. The three "stages of epilepsy" (early epilepsy onset followed by epileptic encephalopathy and drug-resistant epilepsy further on) previously described by Bahi-Buisson et al. [13] were not constantly observed in our series, and hypsarrhythmic pattern was not recorded in any. In contrast with other studies [23–25], epilepsy remained controlled or partially controlled in most patients and, most importantly, a *CDKL5* mutation was identified in a late regression RTT patient who has never presented seizures, including subclinical epileptic discharges during sleep (only activation of few paroxysms). Variable evolution in these girls, with mutations in the same gene, is influenced by the type and location of mutation in the *CDKL5* gene [15]: patients with better autonomy, ambulation, hand skills and epilepsy control have mutations in distal regions of *CDKL5* (patient 5 was predicted to have a truncated protein product and patient 6, a missense mutation) (Fig. 1). Girls with a more severe clinical expression (patient 3, a truncated protein and patient 4, a missense mutation) have mutations in more proximal regions of the gene. Patient 6 (21 yo), the least severe phenotype of the series, has a missense mutation in the most distal region of the gene, not affecting the functional domains of the protein: she presented with a late regression form. Her Pineda severity score [26] at 10-year-old was eight (mild), and she is able to walk, use her hands and speak simple phrases, and has not developed epilepsy during follow up. Patients 1, 2, 7 and 8, present different types of mutations in different locations of the same exon 12 and show variable grades of clinical severity: patients 1, 7 and 8 carry frameshift mutations causing premature stop codons in all cases, whereas patient 2 carries a missense mutation in a non functional domain of the protein and presents a milder phenotype: patients 1 and 8 presented with severe early epilepsy and patient 7 had a congenital variant, while patient 2 had a preserved speech variant and epilepsy onset before 3-year-old; however, all of them had polymorphic seizures and drug-resistant epilepsy during follow up.

Based on the reports describing BDNF and X-inactivation influence on the clinical phenotype [17,20], these molecular studies were performed. Val66Met polymorphism was not present in any

these four patients and XCI showed random pattern in patients 1 and 2, not detectable in patient 7 and skewed pattern in patient 8, which could explain the milder clinical expression in the three frameshift mutations. Patient 4 (mutation located in exon eight, coding for missense protein) with Val66Met BDNF polymorphism and random XCI, presented early epilepsy (onset at 25 days), with polymorphic seizures but a good clinical control with carbamazepine initially; although epilepsy relapsed 8 mo later, it responded to carbamazepine in combination with clobazam better than other combinations; however, she is still too young (3.5 yo) to establish a prognosis. Patient 8, with Met66-Met BDNF polymorphism and skewed XCI, presented epilepsy onset at 3 mo, with partial control on follow up. Although this is a small group of patients, the Val66-Met polymorphism does not seem to be a protective factor against epilepsy and brain damage in patients with *CDKL5* mutations, but larger samples and longer follow up will provide important information for future studies. Absence of *CDKL5* mutations in the nine boys with epileptic encephalopathy should be understood in the context of the small size of this sample.

Genetic analysis of *CDKL5* in a larger sample of patients with either severe epilepsy in the first yr of life, classic RTT, or a RTT variant without a molecular diagnosis would facilitate the molecular diagnosis of a higher number of patients and potentially expand the phenotype of *CDKL5*.

In conclusion, this study reports the first eight patients from Spain with RTT variants (congenital onset, early epilepsy, preserved speech and late regression) and mutations in *CDKL5*. As a "RTT-related" gene, *CDKL5* must be a mandatory test in patients with RTT and early epilepsy, as well as in those with epileptic encephalopathy with onset within the first yr of life. Furthermore, our study has shown that it is also fundamental in other RTT variants and classical RTT without an identifiable *MECP2* mutation. As the number of patients with *CDKL5* mutations increases, it may be possible in a future to elucidate clearer genotype-phenotype correlations that may guide management and genetic counseling.

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