Maternal origin of a novel C-terminal truncation mutation in CDKL5 causing a severe atypical form of Rett syndrome


The CDKL5 gene has been implicated in infantile spasms and more recently in a Rett syndrome-like phenotype. We report a case of a young girl presenting generalized convulsions at 10 days of life. Subsequent mutation analysis by denaturing high-performance liquid chromatography of MECP2 and CDKL5 genes revealed heterozygosity for a c.47_48insAGG insertion in exon 1 of MECP2 and heterozygosity for a new nonsense mutation p.Q834X and a new missense variant p.V999M in the CDKL5 gene. Co-segregation analysis showed that the nonsense mutation was a de novo mutation and that the insertion and the missense variant were also found in the asymptomatic mother. In the absence of skewed X inactivation in the mother, it is likely that these last two variants are not pathogenic. Reverse transcription-polymerase chain reaction from lymphoblastoid cells of the patient showed only the transcript without the nonsense and missense variations suggesting decreased stability of mature mRNA by nonsense-mediated decay. These data also suggest an occurrence of the de novo mutation in maternal germ line cells. Moreover, this report reinforces the observation that the CDKL5 phenotype overlaps with Rett syndrome and that CDKL5 gene analysis is recommended in females with a seizure disorder commencing in the first weeks of life.
younger brother. Her paternal aunt and two cousins had congenital cataract. She was born after a normal pregnancy and at-term delivery with normal parameters (birth weight: 3455 g, height: 52.5 cm, cranial circumference: 35 cm). The neonatal period was normal except for diarrhea. She presented generalized convulsions at 10 days of life, well controlled by vigabatrin treatment. Her psychomotor development was delayed: she was able to hold her head at 4 months and could sit unaided only at 18 months. The parents noticed an arrest of progression between 18 and 24 months. She also had persistent diarrhea leading to a failure to thrive. She was referred to one of our hospitals at 2 years of age. She was unable to stand and walk alone and had no language. She presented behavioral problems such as hyper-excitability, stereotypic activities of the hands and inappropriate laughter. Her head circumference remained within the normal range. Clinical examination showed hypotonia, amyotrophy, and strabismus. At the age of 3 years, she no longer suffered from convulsions and the previous 18 months had been free of antiepileptic treatment, but she could not speak and was hypotonic with stereotypic movements of the hands. She had a normal weight and height (25th percentile), and her head circumference still remained within normal parameters (50th percentile). She had morphologic, electrophysiological and metabolic tests which were all normal. The cytogenetic examination results were also normal. On the basis of a recent consensus panel review, this girl would fit the criteria for atypical RTT, with early-onset seizures, hand apraxia, generalized hypotonus, stereotypic hand activities, and absence of language (6).

DNA samples were also tested for deletions using a MECP2 multiplex ligation-dependent probe amplification (MLPA) kit (MRC Holland, Amsterdam, The Netherlands) according to manufacturer’s instructions.

X chromosome inactivation
X-inactivation studies were performed using the method of Allen (7) as previously described. Briefly, aliquots of DNA obtained from peripheral blood lymphocytes were digested overnight using the methylation-sensitive restriction enzyme HpaII (New England Biolabs, Ipswich, MA, USA). PCR was used to amplify 100 ng of either digested or undigested DNA using fluorescent PCR primers. The amplified PCR fragment contains an HpaII site, as well as the highly polymorphic trinucleotide repeat of the androgen receptor gene. As the HpaII sites on the inactive X chromosome are methylated, only this allele is amplified following genomic restriction enzyme digestion with HpaII. The allele peak areas were analyzed using an ABI 3100 automated sequencer and genotyper software (Applied Biosystems).

mRNA analysis
RNA isolation from lymphoblastoid cells from the patient and cDNA synthesis were performed according to standard protocols. We used primers designed to form cDNA products spanning exons 17–21 of CDKL5 gene (17RTF: 5'-TAGCACTCCAAAGCAGCAGAC-3' and 21RTR: 5'-TCTCATGGTATGTCAGGAGC-3'). Amplification was performed at an annealing temperature of 55 °C to yield a 638-bp product. Reverse transcription (RT)-PCR products were separated by electrophoresis through a 2% agarose gel. RT-PCR products were sequenced on both strands by using PCR primers with fluorescent dye terminators on an ABI 3100 genetic analyser (PE Applied Biosystems).

Results
The screening of the nucleotide sequence of the MECP2 gene was initially performed by dHPLC as previously described (including the recently identified exon 1 (8, 9). In addition, we performed MLPA analysis to search for deletions. DHPLC analysis of MECP2 revealed an abnormal chromatographic pattern in exon 1 from the affected girl. Direct sequencing of this exon showed an insertion of three nucleotides AGG, thus giving rise to a
c.47_48insAGG mutation. This sequence variation was also found in her unaffected mother. Skewed X-inactivation can sometimes mask the pathogenic effect of a MECP2 mutation, but in this case, X-inactivation appeared random in lymphocyte DNA from both the girl and her unaffected mother. The proportion of the X-inactivated allele was 55% in the mother and 53% in the girl. However, although the mother has random X-chromosome inactivation (XCI) in the blood, a protective skewing in the brain cannot be excluded. Moreover, this insertion of three nucleotides is located in a trinucleotide repeat consisting of six glycine residues in human, cattle, dog, and mouse. However, this repeat is not conserved and is reduced to five in rat and possum and to one in Xenopus and Zebrafish. Finally, this insertion has been reported in the RettBase database by Bradford Coffee as a polymorphism (named c.45_47dupAGG; http://mecp2.chw.edu.au). It thus seems unlikely that this rare nucleotide change, not seen in 600 RTT X chromosomes and in 100 non-RTT X chromosomes (mother and sister of RTT patients), is pathogenic. No other MECP2 changes, including large genomic rearrangements screened using MLPA, were identified in the patient.

Secondly, we screened the whole coding sequence of the CDKL5 gene by DHPLC and identified an abnormal chromatographic pattern in exon 18 of the patient. Direct sequencing of the PCR product identified a CDKL5 nonsense mutation p.Q834X (CAG>TAG; c.2500C>T) (Fig. 1). Analysis of parental DNA revealed that it is a de novo mutation, not present in either parent. To test the consequences of this mutation on mRNA expression, we performed RT-PCR using lymphoblastoid cell-derived RNA from the patient. No amplified fragments of a different size that could have resulted from aberrant splitting were present (data not shown). On the contrary, we found by direct sequencing of RT-PCR products that only the expected amplified fragment from the normal transcript was present. These results suggest that the mutated transcript is likely to be highly unstable, probably because of nonsense-mediated mRNA decay. Moreover, we identified a c.2995 G>A nucleotide change in exon 21, not previously described, that results in a valine-to-methionine substitution at position 999 (p.V999M) (Fig. 1). This nucleotide variation appears to be a rare variant as it was seen in only one affected case (1/100 affected girls) and not at all in 100 controls (healthy adults). This variant is

Fig. 1. (a) Denaturing high-performance liquid chromatography (DHPLC) patterns of polymerase chain reaction (PCR) products corresponding to exon 18 and exon 21 of CDKL5: DHPLC profile corresponding to normal exon sequence (gray lane); altered DHPLC profile due to a mutation (black lane). (b) Fluorescence sequence analysis of exon 18 and exon 21 of the CDKL5 gene using the reverse primers. The arrow indicates the position of the p.Q834X mutation in exon 18 and the position of the p.V999M variant in exon 21. (c) Pedigree of the family showing the segregation of the CDKL5 mutations.
located in a non-conserved region of CDKL5. The lack of conservation of this residue and region between different species thus suggests that this is a polymorphism. Moreover, the asymptomatic mother of the patient also had the p.V999M variant, further suggesting that this change is not pathogenic (Fig. 1). Sequencing of RT-PCR products from the affected girl showed that the normal allele (without the p.Q834X mutation) also did not have the p.V999M variant, suggesting that the de novo p.Q834X mutation had a maternal origin.

Discussion

CDKL5 is a member of a proline-directed kinase subfamily that has homology to both Mitogen–activated protein (MAP) and cell cycle-dependent kinases known as the cyclin-dependent kinase-like (CDKLs) kinases (10). Other members of this family include p56KKIAMRE (CDKL1), p42KKIALRE (CDKL2), and NKIAMRE (CDKL3). In our report, we identified the first nonsense mutation to date identified in exon 18 of the CDKL5 gene. Interestingly, this new mutation was associated in cis with a novel non-pathogenic missense variant, p.V999M (c.2995G>A). Other non-pathogenic missense variants have previously been identified in this gene (such as p.H467R, p.R444C, and p.Q791P) (2–4).

We investigated whether mutated and normal alleles were expressed in the affected girl. We first studied X inactivation in DNA from lymphocytes from both the girl and her unaffected mother and were able to show balanced X inactivation in both cells. We then performed RT-PCR to test whether mutated mRNA alleles were degraded probably by nonsense-mediated RNA decay, the mechanism by which nonsense mutations reduce cellular mRNA levels by causing instability of mature mRNA (11). We were not able to detect the expression of the mutated nonsense allele in the girl. Severe reduction of mRNA has previously been observed with other nonsense mutations in different genes (12–14). The absence of the detection of the p.V999M variant in the normal CDKL5 transcript suggests that the de novo nonsense mutation has a maternal origin. Up to now, CDKL5-linked disorders were associated with an excess of affected female to male patients (13 females, one male) (4). Our observation suggests that the discrepancy in the numbers of affected males and females could be attributed to gestational lethality in males.

The phenotypes associated with CDKL5 mutations range from X-linked infantile spasms (ISSX) and infantile epileptic encephalopathy to atypical RTT and one case of autism (Table 1). At the most severe end of the spectrum, two female patients have been described who did not express any normal CDKL5 because one copy of the gene was disrupted by a translocation and the normal copy was inactive because of completely skewed X-inactivation. These girls were very severely affected (infantile spasms, global developmental arrest, and profound mental retardation) and died at the age of 3 and 16 years (1). At the other end of the spectrum is mild mental retardation with autistic features, present in one female twin with a frameshift mutation in CDKL5 (3). Many of female patients with CDKL5 mutations have intermediate phenotypes (2, 4–5, this study). It seems that the severity is at least (i) associated with the proportion of functional or partially functional CDKL5 produced by the normal or mutated alleles, respectively, and (ii) associated with the variability in XCI. In our case, the nonsense mutation was

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Location</th>
<th>Phenotype</th>
<th>References</th>
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<tbody>
<tr>
<td>t(X;6)(p22.3;q14)</td>
<td>Intron 1</td>
<td>Severe ISSX and MR</td>
<td>(1)</td>
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<td>c.163_166delGAAA</td>
<td>Exon 5</td>
<td>Atypical RTT (early-onset RTT variant)</td>
<td>(2)</td>
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<tr>
<td>c.183delT</td>
<td>Exon 5</td>
<td>Atypical RTT, autistic disorder with MR, profound MR with seizures</td>
<td>(5)</td>
</tr>
<tr>
<td>c.215T&gt;A (p.I72N)</td>
<td>Exon 5</td>
<td>Atypical RTT (early seizure variant)</td>
<td>(4)</td>
</tr>
<tr>
<td>c.455G&gt;T (p.C152F)</td>
<td>Exon 7</td>
<td>Atypical RTT (early-onset RTT variant)</td>
<td>(3)</td>
</tr>
<tr>
<td>c.464-2A&gt;G</td>
<td>Intron 7</td>
<td>Atypical RTT, severe epileptic encephalopathy</td>
<td>(4)</td>
</tr>
<tr>
<td>c.525A&gt;T (p.R175S)</td>
<td>Exon 8</td>
<td>Atypical RTT (early-onset RTT variant)</td>
<td>(3)</td>
</tr>
<tr>
<td>t(X;7)(p22.3;p15)</td>
<td>Intron 10</td>
<td>Severe ISSX and MR</td>
<td>(1)</td>
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<tr>
<td>c.2047-1G&gt;A</td>
<td>Intron 13</td>
<td>Atypical RTT (early-onset RTT variant)</td>
<td>(5)</td>
</tr>
<tr>
<td>c.2376+1G&gt;C</td>
<td>Intron 16</td>
<td>ISSX</td>
<td>(4)</td>
</tr>
<tr>
<td>c.2500C&gt;T (p.Q834X)</td>
<td>Exon 18</td>
<td>Atypical RTT (early-onset RTT variant)</td>
<td>This study</td>
</tr>
<tr>
<td>c.2635_2636delCT</td>
<td>Exon 18</td>
<td>Atypical RTT (early-onset RTT variant)</td>
<td>(2)</td>
</tr>
<tr>
<td>136-kb deletion</td>
<td>Intron 19</td>
<td>X-linked juvenile retinoschisis (with epilepsy)</td>
<td>(15)</td>
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</table>

ISSX, X-linked infantile spasm syndrome; MR, mental retardation; RTT, Rett syndrome.
associated with an mRNA instability suggesting the absence of production of a truncated protein. That could explain the severe phenotype in our patient: seizures at 10 days, with no speech, and an inability to walk. Interestingly, a C-terminal deletion of CDKL5 has already been reported by Huopaniemi et al. (15) in a family with X-linked juvenile retinoschisis. No additional phenotypes were reported in these patients with the exception of epilepsy in one. This deletion, spanning from the 5′ region of the RS1 gene to intron 3, disrupts two other genes, PPEF-1 and CDKL5 (exon 20) (15). It is possible that this truncation, more 3′ than the deletion in exon 18 (c.2635_2636delCT) (2), generates a milder phenotype, because the truncated protein is stable, although this has not yet been demonstrated.

In conclusion, we have identified a patient with a novel pathogenic mutation in the CDKL5 gene, and this brings the total number of published mutations in this large gene to 13. As previously observed, our report reinforces the fact that mutations in CDKL5 are relatively common in patients whose seizures begin before the age of 2 months (10 days for our proband). The phenotype did not correlate with the nature or position of the mutation but is more probably affected by the transcriptional and translational consequences of the pathogenic nucleotide change. Hence, different truncating mutations in CDKL5 can cause different but overlapping phenotypes. Our report also suggests an occurrence of the de novo mutation in maternal germ line cells, suggesting that the discrepancy in the numbers of affected males and females could be attributed to gestational lethality in males.

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References


