CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms

E Scala, F Ariani, F Mari, R Caselli, C Pescucci, I Longo, I Meloni, D Giachino, M Bruttini, G Hayek, M Zappella, A Renieri

Background: Rett syndrome (RTT; OMIM 312750) is a neurodevelopmental disorder characterised by a wide spectrum of clinical manifestations. In the classic form, after a period of normal development, patients show growth retardation and regression of speech, along with purposeful hand movements and appearance of stereotyped hand movements. RTT variants have been described, including the preserved speech variant (PSV), characterised by the recovery of some degree of speech; the congenital variant (recognised from birth); the “early seizure variant” (seizure onset before regression); and the “forme fruste”, with a milder, incomplete clinical course (regression between 1 and 3 years). These variants present some symptoms of RTT, but show considerable variation in type and age of onset, severity of impairment, and clinical course. Among these, the “early seizure variant” was initially described by Hanefeld in 1985, who reported a girl with infantile spasms with hypsarrhythmia in her early development.

Approximately 80% of patients with classic RTT have a mutation in the methyl CpG binding protein 2 gene (MECP2; OMIM #300005). MECP2 mutations have also been identified in about 50% of PSV cases and in a lower percentage of other variants. In the variant with early development of convulsions described by Hanefeld, MECP2 mutations have not been published.

The Hanefeld variant of RTT presents a phenotypic overlap with West syndrome, also called infantile spasm syndrome, X linked (ISSX). ISSX is characterised by the triad of infantile spasms, hypsarrhythmia, and severe to profound mental retardation. Some families with ISSX carry mutations in the methyl CpG binding protein 2 gene (MECP2). Several other variants of Rett syndrome have been described. In 1985, Hanefeld described a variant with the early appearance of convulsions. In this variant, the normal perinatal period is soon followed by the appearance of seizures, usually infantile spasms. We have observed two patients with signs of Rett syndrome showing acquired microcephaly and stereotyped midline hand movements. The disease started with generalised convulsions and myoclonic fits at 1.5 months in the first patient and with spasms at 10 days in the other, suggesting a diagnosis of the Hanefeld variant. In these patients, MECP2 point mutations and gross rearrangements were excluded by denaturing high performance liquid chromatography and real time quantitative PCR.

Methods: Based on the clinical overlap between the Hanefeld variant and West syndrome, we analysed ARX and CDKL5 in the two girls.

Results: We found frameshift deletions in CDKL5 in both patients; one in exon 5 (c.163_166delGAAAA) and the other in exon 18 (c.2635_2636delCT). CDKL5 was then analysed in 19 classic Rett and 15 preserved speech variant patients, all MECP2 negative, but no mutations were found.

Conclusion: Our results show that CDKL5 is responsible for a rare variant of Rett syndrome characterised by early development of convulsions, usually of the spasms type.

METHODS

Patients
We investigated two patients, aged 9 and 8 years respectively, with early development of convulsions, who later developed many characteristics of RTT. They both fulfilled the criteria for the early seizure variant of RTT.

We then investigated 19 classic RTT and 15 PSV patients. The girls with classic RTT were diagnosed according to the international criteria. The PSV girls fulfilled the criteria of Hagberg and Skjedal for RTT variants. In particular, the PSV

Abbreviations: DHPLC, denaturing high performance liquid chromatography; ISSX, infantile spasm syndrome, X linked; PSV, preserved speech variant; RS, retinoschisis; RTT, Rett syndrome.

See end of article for authors’ affiliations

Correspondence to: Dr A Renieri, Associate Professor, Medical Genetics, University of Siena, Policlínico Le Scotte, viale Bracci 2, 53100, Siena, Italy; renieri@unisi.it

Received 7 August 2004
Revised 4 October 2004
Accepted 5 October 2004

ORIGINAL ARTICLE

CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms

E Scala, F Ariani, F Mari, R Caselli, C Pescucci, I Longo, I Meloni, D Giachino, M Bruttini, G Hayek, M Zappella, A Renieri

Background: Rett syndrome is a severe neurodevelopmental disorder, almost exclusively affecting females and characterised by a wide spectrum of clinical manifestations. Both the classic form and preserved speech variant of Rett syndrome are due to mutations in the MECP2 gene. Several other variants of Rett syndrome have been described. In 1985, Hanefeld described a variant with the early appearance of convulsions. In this variant, the normal perinatal period is soon followed by the appearance of seizures, usually infantile spasms. We have observed two patients with signs of Rett syndrome showing acquired microcephaly and stereotyped midline hand movements. The disease started with generalised convulsions and myoclonic fits at 1.5 months in the first patient and with spasms at 10 days in the other, suggesting a diagnosis of the Hanefeld variant. In these patients, MECP2 point mutations and gross rearrangements were excluded by denaturing high performance liquid chromatography and real time quantitative PCR.

Methods: Based on the clinical overlap between the Hanefeld variant and West syndrome, we analysed ARX and CDKL5 in the two girls.

Results: We found frameshift deletions in CDKL5 in both patients; one in exon 5 (c.163_166delGAAAA) and the other in exon 18 (c.2635_2636delCT). CDKL5 was then analysed in 19 classic Rett and 15 preserved speech variant patients, all MECP2 negative, but no mutations were found.

Conclusion: Our results show that CDKL5 is responsible for a rare variant of Rett syndrome characterised by early development of convulsions, usually of the spasms type.

METHODS

Patients
We investigated two patients, aged 9 and 8 years respectively, with early development of convulsions, who later developed many characteristics of RTT. They both fulfilled the criteria for the early seizure variant of RTT.

We then investigated 19 classic RTT and 15 PSV patients. The girls with classic RTT were diagnosed according to the international criteria. The PSV girls fulfilled the criteria of Hagberg and Skjedal for RTT variants. In particular, the PSV

Abbreviations: DHPLC, denaturing high performance liquid chromatography; ISSX, infantile spasm syndrome, X linked; PSV, preserved speech variant; RS, retinoschisis; RTT, Rett syndrome.
cases show the same clinical features and stages of RTT in the first years of life, but they subsequently improve in fine motor ability and start to speak with an increasing number of words and phrases.

**Molecular analysis**

Blood samples were obtained after informed consent. DNA was extracted from peripheral blood using a QIAamp DNA blood kit (Qiagen). DNA samples were screened for mutations in ARX and CDKL5 using transgenicomic WAVE denaturing high performance liquid chromatography (DHPLC). CDKL5 coding portion was entirely analysed using the primers and conditions given in table 1. PCR products resulting in abnormal DHPLC profiles were sequenced on both strands by use of PCR primers with fluorescent dye terminators on an ABI Prism 310 genetic analyser (PE Applied Biosystems, Foster City, CA, USA). PCR products of exon 5 and exon 18 were separated on 6% polyacrylamide gel to define exactly the deleted bases. Normal and mutant alleles were cut from the gel and sequenced individually. X inactivation studies were performed using the assay of Pegoraro et al. Intensity of silver stained bands was measured using the Diversity Database program (Biorad) and the values were corrected for preferential allele amplification. RNA isolation from lymphoblasts and cDNA synthesis were performed according to standard protocols. We used primers designed to form cDNA products spanning exons 4–6 (4RTf: 5'-GAAACA CATGAAATTGTGGCCTG-3'; 6RTr: 5'-GTGAATAGCCTTGATTAG CTG-3') and 17–18 (17RTf: GAGAAGATCTCAGATCTGCAG; 18RTr: AGCTGGAGGGCTGGCGTCTG). RT-PCR products were separated by electrophoresis through a 6% polyacrylamide gel and silver stained.

**Table 1**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product length (bp)</th>
<th>PCR annealing temp (°C)</th>
<th>DHPLC analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>AGGTAAGATGGTATCTAGAG</td>
<td>AATAAATACGTTCATCTGTC</td>
<td>350</td>
<td>58</td>
<td>55 57</td>
</tr>
<tr>
<td>3</td>
<td>TGGAGCAATGCTGGTTATGAG</td>
<td>CCGTTCATGCTCCACACC</td>
<td>201</td>
<td>58</td>
<td>55 53</td>
</tr>
<tr>
<td>4</td>
<td>CTTGGTCTTCCCTCTCTCACTCT</td>
<td>TCCCACTCTCCACACTC</td>
<td>242</td>
<td>58</td>
<td>54 53</td>
</tr>
<tr>
<td>5</td>
<td>AGGTGTCGTTGAGATCTTGG</td>
<td>GGCACATTGTGACACTGGCC</td>
<td>244</td>
<td>54</td>
<td>57</td>
</tr>
<tr>
<td>6</td>
<td>CTTGGTAGTGGATGAAATTAC</td>
<td>TTCTTAAAGACGTTAACATGTG</td>
<td>303</td>
<td>59</td>
<td>54 55</td>
</tr>
<tr>
<td>7</td>
<td>TTTCTGTTGACACCATCATCA</td>
<td>AACTTCTCCAGCACAATT</td>
<td>237</td>
<td>56</td>
<td>53 53</td>
</tr>
<tr>
<td>8</td>
<td>GCCCACTCGAGAAGACCTCATC</td>
<td>GCAAATGACAATAGAATCAGCAG</td>
<td>280</td>
<td>55</td>
<td>56 54</td>
</tr>
<tr>
<td>9</td>
<td>TTTATTTGCTGGTCTGATGTAG</td>
<td>CAAATGCAGTTGATTCGTC</td>
<td>410</td>
<td>54</td>
<td>58 58</td>
</tr>
<tr>
<td>10</td>
<td>TATGAAATTTGACCTGGATTTGG</td>
<td>CTATGTCGACATGAGACAC</td>
<td>275</td>
<td>59</td>
<td>57 54</td>
</tr>
<tr>
<td>11</td>
<td>TTGAATTTGCATGTGGGATTTGG</td>
<td>AGCCACCTTCCTCCACCTAC</td>
<td>333</td>
<td>62</td>
<td>55 56</td>
</tr>
<tr>
<td>12 <em>a</em></td>
<td>TTGGTGTGCAGCTTATTGAGG</td>
<td>GGTGGTCGAGCTGTCGTCG</td>
<td>406</td>
<td>60</td>
<td>56 60</td>
</tr>
<tr>
<td>12 <em>b</em></td>
<td>CAAACACATCACACACACT</td>
<td>TTTCTGTCGTCAGCTGTCG</td>
<td>422</td>
<td>60</td>
<td>57 60</td>
</tr>
<tr>
<td>12 <em>c</em></td>
<td>ACTCCAAGTCGTGTCAAGCAAC</td>
<td>AGATGAGCCTCCATCATCAC</td>
<td>541</td>
<td>60</td>
<td>57 63</td>
</tr>
<tr>
<td>13</td>
<td>GTTAGTCTGCTTCTTCATC</td>
<td>CACTTCACTTTATTTGTTGCG</td>
<td>298</td>
<td>60</td>
<td>57 55</td>
</tr>
<tr>
<td>14</td>
<td>CAATAGTTAGGAGACCTGCTC</td>
<td>CTGAGTCTGGTGAGAACATG</td>
<td>279</td>
<td>65</td>
<td>57 55</td>
</tr>
<tr>
<td>15</td>
<td>AAAAGTCCAATGCTCTACTAC</td>
<td>CCTAGCAGGAAAGGACAGCAC</td>
<td>262</td>
<td>60</td>
<td>55 54</td>
</tr>
<tr>
<td>16</td>
<td>TATAGGACCACTAGTCAGCT</td>
<td>CAACTTGGATGCGAACTGTC</td>
<td>293</td>
<td>59</td>
<td>53 57</td>
</tr>
<tr>
<td>17</td>
<td>CTGAGTGTGGTGGATCTACATC</td>
<td>CTGTAACATGAGGCTAGT</td>
<td>296</td>
<td>60</td>
<td>59 55</td>
</tr>
<tr>
<td>18</td>
<td>CTTGACAATGCTGCTGTTC</td>
<td>CACCAGCAGCTGACGATG</td>
<td>418</td>
<td>62</td>
<td>61 58</td>
</tr>
<tr>
<td>19</td>
<td>ACTCTGTCATGGGGGACAT</td>
<td>CATCAGTACGTCAGGGGTCG</td>
<td>249</td>
<td>60</td>
<td>59 53</td>
</tr>
<tr>
<td>20</td>
<td>TGGGCTTGAGTGCTGCTC</td>
<td>CATCAGCTGTCTCACACTC</td>
<td>345</td>
<td>61</td>
<td>61 58</td>
</tr>
<tr>
<td>21</td>
<td>CATTACCAGAGTGCCGCTC</td>
<td>AGGAAGACATCACTTCAGGCC</td>
<td>290</td>
<td>60</td>
<td>59 55</td>
</tr>
</tbody>
</table>

**RESULTS**

We observed two patients with early development of convulsions who later showed characteristics of RTT. The phenotype of each case is described below.

**Patient 1**

This patient is a girl, now aged 9 years. Her pedigree is represented in fig 1A (left). The mother had a normal pregnancy and delivery. The child was affected with slight cyanosis after birth. Birth weight was 3600 g and head circumference was 34 cm (50th centile). Generalised convulsions appeared at 1.5 months of age and were barely controlled by various antiepileptic drugs. In the following months she was examined in hospital, and myoclonic fits resembling infantile spasms were noted, although her EEG was not typical of hypsarrhythmia. Her developmental milestones were delayed and she was able to sit unaided at 1 year and to walk unaided at 6.5 years. She was examined again in hospital at the age of 2 years. MRI of the head, evaluations for aminoacidemia, karyotype, search for Fragile X syndrome, methylation pattern for Angelman syndrome, and UBE3A gene sequencing were all negative. The patient was initially examined in our unit at the age of 8.5 years. She was able to briefly hold an object in her hands, dropping it shortly afterwards. She brought her hands frequently to her mouth, or beat them together. She had stereotypic hand-washing activities, reported as occurring since the age of 1 year. Her facial expression varied and she was able to interact at a pre-verbal level. She was able to utter one word and had occasional bruxism and hyperventilation. Her head circumference was 48.5 (3rd centile). Scoliosis, kyphosis, cold extremities, and constipation were not present. The EEG showed sharp waves in the central and occipital regions.
CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms

Myoclonic fits and generalised convulsions were still present. Her head circumference was 50 cm (tenth centile). Hands were still present. She occasionally uttered one or two words. Her head circumference was 49 cm (tenth centile), she had a moderate degree of generalised hypotonus, and was unable to hold an object in her hands, which were brought frequently to her mouth and occasionally twisted together. She was able to sit alone at 1.5 years of age. She was delayed; in the first years of life she did not respond to interactions, she had been found hypotonic by medical professionals, and she was unable to hold an object in her hands. She was able to sit alone at 1.5 years of age. She was examined in our unit when 4 years 10 months of age. Her personality was still present: she was able to sit unaided but unable to walk without support. The stereotypic activities of her hands were still present. She occasionally uttered one or two words. Her head circumference was 50 cm (tenth centile). Scoliosis, kyphosis, cold extremities, and constipation were absent. Myoclonic fits and generalised convulsions were still present in spite of various treatments, including phenobarbital, valproate, lamotrigine, and carbamazepine. An EEG showed the persistence of generalised sharp and slow waves and the presence of additional multifocal abnormalities.

Molecular analysis

MECP2 point mutations, including the recently identified exon 1, and gross rearrangements were excluded by DHPLC and quantitative PCR, respectively.\(^{20-22}\) Based on the partial clinical overlap between the Hanefeld variant and ISSX, we screened the two RTT variant patients for point mutations in ARX and CDKL5. ARX analysis did not identify any mutation. DHPLC analysis of CDKL5 revealed a different frameshift mutation in the two patients, absent in the DNA of the parents in both cases (fig 1B).

In case 1 we identified a 4 bp deletion in exon 5 (c.163_166delGAAA) (fig 1C; left) leading to loss of most of the CDKL5 protein (NP_003150). The frameshift mutation creates a stop codon in position 74, after a short stretch of abnormal amino acids (fig 2A, 2B; top). This deletion interrupts the catalytic domain covering the first 300 amino acids, and creates a non-functional CDKL5 product.

Case 2 showed a 2 bp deletion in exon 18 (c.2635_2636delCT) (fig 1C; right) leading to protein truncation in position 908, after a short stretch of incorrect amino acids (fig 2A, 2B; bottom). This deletion eliminates a putative signal peptidase I serine active site, as predicted by the ScanProsite program (http://ca.expasy.org/prosite) (fig 2A).

We then investigated whether mutated and normal alleles were expressed in the two patients. We studied X inactivation in DNA from blood cells of both patients. The assay showed the persistence of generalised sharp and slow waves and the presence of additional multifocal abnormalities.

Patient 2

This patient is a girl, now aged 8 years. Her pedigree is represented in fig 1A (right). The mother had a normal pregnancy and delivery, and the child was affected with slight cyanosis at birth. Her birth weight was 3800 g but no data concerning her head circumference are available. At 10 days, generalised convulsions, lasting only a few seconds, appeared, which were difficult to control with various antiepileptic drugs and persisted during the following years. Myoclonic seizures, when her arms suddenly opened, possibly infantile spasms, were also reported by her parents, although clinical records concerning these details were not available. Her MRI was normal. Her development was delayed; in the first years of life she did not respond to interactions, she had been found hypotonic by medical professionals, and she was unable to hold an object in her hands.

An EEG showed generalised discharges of sharp and slow waves and the presence of additional multifocal abnormalities. Furthermore, she had myoclonic epileptic fits occurring two or three times a day, and occasional generalised convulsions. An EEG showed generalised discharges of sharp and slow waves and focal sharp waves in the right centrontemporal region. She was evaluated at the age of 7 years 10 months, when a slight improvement in the use of her hands was noted. She could hold a biscuit and some bread in her hands, although clinical records concerning these details were not available. Her MRI was normal. Her development was delayed; in the first years of life she did not respond to interactions, she had been found hypotonic by medical professionals, and she was unable to hold an object in her hands.
The first case described by Hanefeld (personal communication) interacted in a manner adequate to their reduced general abilities at ages close to 8 and 5 years, they were expressive and had had the typical stage sequence of RTT. When examined system. It was difficult to retrospectively assess whether they stereotypic hand activities. Hyperventilation was present microcephaly, hand apraxia, generalised hypotonus, and otherwise fulfilled the criteria for RTT, including acquired convulsions very early in life, respectively at 15 PSV cases, all MECP2 negative; no mutation was found. We then extended the CDKL5 analysis to 19 classic RTT and 15 PSV cases, all MECP2 negative; no mutation was found.

**DISCUSSION**

The two girls reported above had a similar clinical course: they showed convulsions very early in life, respectively at 1.5 months and at 10 days, drug resistant in both cases. They otherwise fulfilled the criteria for RTT, including acquired microcephaly, hand apraxia, generalised hypertonus, and stereotypic hand activities. Hyperventilation was present in one girl. Scoliosis, constipation, and cold feet were absent in both, suggesting a possibly better preserved autonomic system. It was difficult to retrospectively assess whether they had the typical stage sequence of RTT. When examined at ages close to 8 and 5 years, they were expressive and interacted in a manner adequate to their reduced general abilities, and they fulfilled the criteria for the early seizure variant of RTT. It should be noted that only a few cases of this disorder have been reported and, with the exception of the first case described by Hanefeld (personal communication), no MECP2 mutations have been described. In the two RTT variant patients, MECP2 point mutations and gross rearrangements were excluded by DHP LC and qPCR.

Our results indicate that RTT variant with infantile spasms may be due to inactivating CDKL5 mutations. CDKL5 is a member of the serine-threonine kinase gene family. Kinase proteins are a large superfamily of homologous proteins, characterised by a highly conserved kinase domain (250–300 amino acids). The CDKL5 kinase domain is most closely related to human KKIALRE and KKIAMRE and their orthologues. The sequence alignment of the CDKL5 protein with these homologues showed two kinase signatures in the catalytic domain: an ATP binding region (amino acids 14 to 47) and a serine–threonine protein kinase active site (amino acids 127 to 144). In addition, a Thr-Xaa-Tyr motif was identified, and the dual phosphorylation of these Thr and Tyr residues has been shown to be essential for activation of the MAP kinase group.

The frameshift mutation found in patient 1 is located in the conserved kinase domain of the CDKL5 protein, causing loss of both the serine threonine active site and the Thr-Xaa-Tyr motif. Thus, the deletion abolishes the catalytic function of the protein. Furthermore, it has been reported that lack of functional CDKL5 protein causes severe ISSX. This finding was derived from a study of two severely affected ISSX female patients with apparently de novo balanced Xautosome translocations, both disrupting the CDKL5 gene in the kinase domain. Additional studies are needed to further elucidate why different truncating mutations in CDKL5 cause different but overlapping phenotypes.

Except for the kinase domain, the function of the CDKL5 protein is unknown. The region between positions 300 and 1030 is not conserved in different species and does not share a homology with other human proteins, making it difficult to predict its function. The deletion found in case 2 falls in the C terminus of the protein. We could speculate that this region contains a functional domain specific for human CDKL5. Using the ScanProsite program we identified a signal peptidase I serine active site (GTSMCPTL), located between amino acids 127 to 144. In addition, a Thr-Xaa-Tyr motif was identified, and the dual phosphorylation of these Thr and Tyr residues has been shown to be essential for activation of the MAP kinase group.

The frameshift mutation found in patient 1 is located in the conserved kinase domain of the CDKL5 protein, causing loss of both the serine threonine active site and the Thr-Xaa-Tyr motif. Thus, the deletion abolishes the catalytic function of the protein. Furthermore, it has been reported that lack of functional CDKL5 protein causes severe ISSX. This finding was derived from a study of two severely affected ISSX female patients with apparently de novo balanced Xautosome translocations, both disrupting the CDKL5 gene in the kinase domain. Additional studies are needed to further elucidate why different truncating mutations in CDKL5 cause different but overlapping phenotypes.

Except for the kinase domain, the function of the CDKL5 protein is unknown. The region between positions 300 and 1030 is not conserved in different species and does not share a homology with other human proteins, making it difficult to predict its function. The deletion found in case 2 falls in the C terminus of the protein. We could speculate that this region contains a functional domain specific for human CDKL5. Using the ScanProsite program we identified a signal peptidase I serine active site (GTSMCPTL), located between amino acids 127 to 144. In addition, a Thr-Xaa-Tyr motif was identified, and the dual phosphorylation of these Thr and Tyr residues has been shown to be essential for activation of the MAP kinase group.

The frameshift mutation found in patient 1 is located in the conserved kinase domain of the CDKL5 protein, causing loss of both the serine threonine active site and the Thr-Xaa-Tyr motif. Thus, the deletion abolishes the catalytic function of the protein. Furthermore, it has been reported that lack of functional CDKL5 protein causes severe ISSX. This finding was derived from a study of two severely affected ISSX female patients with apparently de novo balanced Xautosome translocations, both disrupting the CDKL5 gene in the kinase domain. Additional studies are needed to further elucidate why different truncating mutations in CDKL5 cause different but overlapping phenotypes.

Except for the kinase domain, the function of the CDKL5 protein is unknown. The region between positions 300 and 1030 is not conserved in different species and does not share a homology with other human proteins, making it difficult to predict its function. The deletion found in case 2 falls in the C terminus of the protein. We could speculate that this region contains a functional domain specific for human CDKL5. Using the ScanProsite program we identified a signal peptidase I serine active site (GTSMCPTL), located between amino acids 127 to 144. In addition, a Thr-Xaa-Tyr motif was identified, and the dual phosphorylation of these Thr and Tyr residues has been shown to be essential for activation of the MAP kinase group.

The frameshift mutation found in patient 1 is located in the conserved kinase domain of the CDKL5 protein, causing loss of both the serine threonine active site and the Thr-Xaa-Tyr motif. Thus, the deletion abolishes the catalytic function of the protein. Furthermore, it has been reported that lack of functional CDKL5 protein causes severe ISSX. This finding was derived from a study of two severely affected ISSX female patients with apparently de novo balanced Xautosome translocations, both disrupting the CDKL5 gene in the kinase domain. Additional studies are needed to further elucidate why different truncating mutations in CDKL5 cause different but overlapping phenotypes.

Except for the kinase domain, the function of the CDKL5 protein is unknown. The region between positions 300 and 1030 is not conserved in different species and does not share a homology with other human proteins, making it difficult to predict its function. The deletion found in case 2 falls in the C terminus of the protein. We could speculate that this region contains a functional domain specific for human CDKL5. Using the ScanProsite program we identified a signal peptidase I serine active site (GTSMCPTL), located between amino acids 127 to 144. In addition, a Thr-Xaa-Tyr motif was identified, and the dual phosphorylation of these Thr and Tyr residues has been shown to be essential for activation of the MAP kinase group.

The frameshift mutation found in patient 1 is located in the conserved kinase domain of the CDKL5 protein, causing loss of both the serine threonine active site and the Thr-Xaa-Tyr motif. Thus, the deletion abolishes the catalytic function of the protein. Furthermore, it has been reported that lack of functional CDKL5 protein causes severe ISSX. This finding was derived from a study of two severely affected ISSX female patients with apparently de novo balanced Xautosome translocations, both disrupting the CDKL5 gene in the kinase domain. Additional studies are needed to further elucidate why different truncating mutations in CDKL5 cause different but overlapping phenotypes.

Except for the kinase domain, the function of the CDKL5 protein is unknown. The region between positions 300 and 1030 is not conserved in different species and does not share a homology with other human proteins, making it difficult to predict its function. The deletion found in case 2 falls in the C terminus of the protein. We could speculate that this region contains a functional domain specific for human CDKL5. Using the ScanProsite program we identified a signal peptidase I serine active site (GTSMCPTL), located between amino acids 127 to 144. In addition, a Thr-Xaa-Tyr motif was identified, and the dual phosphorylation of these Thr and Tyr residues has been shown to be essential for activation of the MAP kinase group.
ACKNOWLEDGEMENTS

This study was supported by Telethon grants GGP02372A and GTFO2006, by the Emma and Ernesto Rulfo Foundation, by the Ministry of Health (Progetti di Ricerca Finalizzata, D.L. 502/92-2003), by MIUR (FIRB 01), and by the University of Siena (PAR 2001 and PAR 2002) to A Renieri. We thank F Hanefeld for a critical reading of the manuscript.

Authors’ affiliations

E Scala, F Ariani, F Mari, R Caselli, C Pescucci, I Longo, I Meloni, D Giachino, M Bruttini, A Renieri, Medical Genetics, Department of Molecular Biology, University of Siena
G Hayek, M Zappella, Child Neuropsychiatry, University of Siena

Competing interests: none declared

REFERENCES


CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms

E Scala, F Ariani, F Mari, R Caselli, C Pescucci, I Longo, I Meloni, D Giachino, M Bruttini, G Hayek, M Zappella and A Renieri

doi: 10.1136/jmg.2004.026237