Gene 512 (2013) 70–75

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

# Validation of high-resolution DNA melting analysis for mutation scanning of the *CDKL5* gene: Identification of novel mutations

Laure Raymond [a], Bertrand Diebold [a], Céline Leroux [a], Hélène Maurey [b], Valérie Drouin-Garraud [c], Andre Delahaye [d], Olivier Dulac [e], Julia Metreau [b], Gia Melikishvili [f], Annick Toutain [g], François Rivier [h], Nadia Bahi-Buisson [e,i,j], Thierry Bienvenu [a,i,j,*]

[a] *Laboratoire de Biochimie et Génétique Moléculaire, Hôpital Cochin, Assistance Publique, Hôpitaux de Paris, Paris, France*
[b] *Neurologie pédiatrie, Hôpital Bicêtre, Assistance Publique, Hôpitaux de Paris, Le Kremlin-Bicêtre, France*
[c] *Génétique clinique, Hôpital Charles Nicolle, Rouen, France*
[d] *Laboratoire de cytogénétique, Hôpital Jean Verdier, Bondy, France*
[e] *Neurologie pédiatrique, Hôpital Necker-Enfants Malades, Assistance Publique, Hôpitaux de Paris, Paris, France*
[f] *Mrcheveli laboratories, Tbilisi, Georgia*
[g] *Génétique, Hôpital Le Bretonneau, Tours, France*
[h] *Neuropédiatrie et épileptologie infantile, Hôpital Gui de Chauliac, and U1046, Inserm, Université Montpellier 1, Montpellier 2, Montpellier, France*
[i] *Inserm, U1016, Paris, France*
[j] *Institut Cochin, Université Paris Descartes, CNRS (UMR 8104), Paris, France*

ARTICLE INFO

*Article history:*
Accepted 14 September 2012
Available online 11 October 2012

*Keywords:*
High-resolution DNA melting analysis
Mutation scanning
CDKL5
Rett syndrome

ABSTRACT

Mutations in the cyclin-dependent kinase-like 5 gene (*CDKL5*) have been predominantly described in epileptic encephalopathies of female, including infantile spasms with Rett-like features. Up to now, detection of mutations in this gene was made by laborious, expensive and/or time consuming methods. Here, we decided to validate high-resolution melting analysis (HRMA) for mutation scanning of the *CDKL5* gene. Firstly, using a large DNA bank consisting to 34 samples carrying different mutations and polymorphisms, we validated our analytical conditions to analyse the different exons and flanking intronic sequences of the *CDKL5* gene by HRMA. Secondly, we screened *CDKL5* by both HRMA and denaturing high performance liquid chromatography (dHPLC) in a cohort of 135 patients with early-onset seizures. Our results showed that point mutations and small insertions and deletions can be reliably detected by HRMA. Compared to dHPLC, HRMA profiles are more discriminated, thereby decreasing unnecessary sequencing. In this study, we identified eleven novel sequence variations including four pathogenic mutations (2.96% prevalence). HRMA appears cost-effective, easy to set up, highly sensitive, non-toxic and rapid for mutation screening, ideally suited for large genes with heterogeneous mutations located along the whole coding sequence, such as the *CDKL5* gene.

© 2012 Elsevier B.V. All rights reserved.

## 1. Introduction

Rett syndrome (RTT; MIM#312750) is an X-linked devastating neurodevelopmental disorder characterized by a wide spectrum of clinical manifestations. Beside the classic RTT form, several RTT variants have been described, including the early-seizure variant (seizure onset before regression). The early seizure variant was initially described by Hanefeld in 1985, who reported a girl with infantile spasms with hypsarrhythmia in her early development (Hanefeld, 1985). This Hanefeld variant of RTT presents a phenotypic overlap with West syndrome, also called infantile spasm syndrome, X linked (ISSX; OMIM 308350). ISSX is characterized by the triad of infantile spasms, hypsarrhythmia, and severe to profound mental retardation. In 2003, Kalscheuer et al. (2003) characterized two unrelated female patients with an apparently balanced translocation, 46, X,t(X;7)(p22.3;p15) in one case and 46,X,t(X;6)(p22.3;q14) in the other (Kalscheuer et al., 2003). The two patients presented a similar phenotype, comprised of severe early onset infantile spasms with hypsarrhythmia and profound global developmental arrest. In both patients, the X-chromosomal breakpoints disrupted *CDKL5* (cyclin dependent kinase-like 5/serine threonine kinase 9) (GenBank accession number Y15057). In 2004, as there is phenotypic overlap between the Hanefeld variant and ISSX, two independent groups tested *CDKL5* for mutations in patients who had been diagnosed with RTT or a variant of RTT and in whom no

*Abbreviations:* CDKL5, cyclin-dependent kinase-like 5; HRMA, high resolution melting analysis; DNA, deoxyribonucleic acid; dHPLC, denaturing high performance liquid chromatography; RTT, Rett syndrome; ISSX, infantile spasm syndrome X-linked; MECP2, methyl CpG-binding protein 2; PCR, polymerase chain reaction; EE, epileptic encephalopathy; MLPA, multiplex ligation-dependent probe amplification; dNTP, deoxyribonucleotide triphosphates; DGGE, denaturing gradient gel electrophoresis; EEG, electroencephalogram; CGH, comparative genomic hybridization.

* Corresponding author at: Université Paris Descartes, Institut Cochin, 24 rue du Faubourg St Jacques, 75014 Paris, France. Tel.: +33 1 44 41 24 79; fax: +33 1 58 41 15 80.
*E-mail address:* thierry.bienvenu@inserm.fr (T. Bienvenu).

0378-1119/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2012.09.056

*MECP2* mutation had been identified. They identified point mutations in the *CDKL5* gene in a subset of patients with a clinical picture resembling the early-onset RTT variant or with a history of early-onset seizures (Tao et al. 2004; Weaving et al. 2004). The *CDKL5* gene was subsequently analysed in patients with both classic and atypical variants of RTT by several groups but mutations were identified only in patients with seizure onset before six months of age (Archer et al. 2006; Evans et al. 2005; Scala et al. 2005). To date, more than 70 different point sequence variations have been described in the *CDKL5* gene resulting in missense, nonsense, splice and frameshift mutations.

Initially, *CDKL5* was found to be composed of 23 exons. The first three exons (1, 1a, and 1b) are untranslated and probably represent two transcription start sites of the *CDKL5* gene (Kalscheuer et al. 2003). The initiation codon is located within exon 2. Recently, Fichou et al. (2011) reported the identification of an additional 123-bp (base pairs) exon between exons 16 and 17 of *CDKL5* gene, referred to as *CDKL5* exon 16b (Fichou et al. 2011; Rademacher et al. 2011). Moreover, by aligning both the human and mouse CDKL5 proteins to the orthologs of other species, expression of another CDKL5 isoform with an alternative C-terminus that terminates in intron 18 has been demonstrated, suggesting that the first nucleotides of intron 18 can be translated to produce the $CDKL5_{107}$ isoform (Williamson et al. 2012).

That makes the detection of mutations by sequencing laborious, expensive and time-consuming. To simplify the analysis of such broad mutation spectrum, a rapid and reliable method is required. There are many available scanning methods such as denaturing high-performance liquid chromatography (dHPLC) which are time-consuming and the sensitivity depends on the experience of the operator. On the other hand, HRMA presents a rapid, high-throughput, closed-tube method for mutation scanning (Wittwer et al. 2003). The sample preparation consists of a standard PCR reaction with a saturant dsDNA intercalation fluorescent dye and does not require any post-PCR handling. Products can be analysed directly after PCR amplification using specially designed instruments for HRMA. The heterozygous and wild-type samples are differentiated according to their melting profile, which is represented by plotting fluorescence over the temperature range, and are distinguished by different melting temperatures (Tm) and the shape of the melting curve. Many publications have documented the successful use of HRMA for mutation scanning. It has been reported to have near 100% sensitivity and specificity when the analysed PCR products were up to 400 bp in length (Wittwer, 2009).

Because this method seems rapid, robust, and less expensive than other scanning techniques, we firstly decided to analyse the *CDKL5* gene by HRMA, to define our conditions according to the melting curve profiles of the 22 coding exons of the gene and to compare the results with our dHPLC technique that has been routinely used for 7 years in our laboratory. Secondly we decided to screen a cohort of 135 patients with idiopathic epileptic encephalopathy (EE) and found four novel pathogenic *CDKL5* mutations.

## 2. Materials and methods

### 2.1. DNA

Genomic DNA was extracted from leukocytes of peripheral blood using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). DNA samples carrying 27 mutations and 7 polymorphisms (previously identified by direct sequencing), 30 control samples free of *CDKL5* sequence variations (confirmed by dHPLC analysis) (HRMA set-up), and 135 samples of patients with idiopathic EE (HRMA screening) were used in this study. All eligible patients had their first seizure before the age of 12 months with developmental delay, and had to have normal mutation screening results of *MECP2* using multiplex ligation-dependent probe amplification (MLPA) kit (MRC-Holland) analysis and DNA sequencing exons 1–4. A written consent was obtained from each patient included in this study. DNA samples were quantified using Nanodrop® technology and diluted to 20 ng/μL using the DNA rehydration solution (Promega). All samples were analysed by both HRMA and dHPLC. The list of the known *CDKL5* mutations and polymorphisms tested in this study is given in Table 1.

### 2.2. Primer design

Each exon was analysed in a single PCR product, except exons 12 and 18, which were, in this study, split into 4 and 2 amplicons, respectively. For the other exons (smaller than 400 bp), primers previously described to amplify the *CDKL5* gene were used (Table 2) (Bahi-Buisson et al. 2008). Each primer was previously analysed by primer3 and Blast to select specific and efficient primers.

### 2.3. DNA amplification

DNA amplification was performed in a 50 μL final volume containing 100 ng gDNA, 1.25U AmpliTaq Gold DNA polymerase (Applied Biosystems, Courtaboeuf, France), 0.30 μM each primer, 0.25 mM dNTP, 5 μL 10× buffer, and 3.5 mM $MgCl_2$ (2.5 mM for exon 14). Cycling conditions were 94 °C for 7 min, followed by 40 cycles at 94 °C for 30 s, annealing for 30 s (see Table 2), 72 °C for 30 s, and a last step of 7 min at 72 °C for elongation. All PCR products were checked on ethidium bromide stained gels. For male samples, PCR product was mixed in a 1:1 proportion with a wild type amplified DNA to create conditions of artificial heterozygosity.

**Table 1**
*CDKL5* genotypes in the 34 DNA samples used to set up the HRMA assay.

| Fragment | Sequence variation (NM_003159) | Mutation (NP_003150) | Gender |
|---|---|---|---|
| Exon 2 | *c.58G>C | p.G20R | F |
| | *c.59G>A | p.G20D | F |
| Exon 3 | *c.99+1G>T | – | F |
| Exon 4 | *c.119C>T | p.A40V | F |
| | *c.145+2T>C | – | F |
| Exon 5 | *c.175C>T | p.R59X | F |
| | *c.191T>C | p.L64P | M |
| | *c.214_216del | p.I72del | F |
| | *c.229_232del | p.E77HfsX111 | F |
| Exon 6 | *c.380A>G | p.H127R | F |
| | *c.400C>T | p.R134X | F |
| Exon 7 | *c.404-1G>T | – | F |
| | *c.425T>A | p.L142X | F |
| Exon 8 | *c.532C>T | p.R178W | F |
| Exon 9 | #c.555-19C>G | – | F/M |
| | *c.659T>C | p.L220P | F |
| Exon 11 | *c.865insA | p.E285EfsX325 | F |
| Exon 12 (fragment 1) | *c.1071delC | p.D357EfsX367 | F |
| Exon 12 (fragment 2) | #c.1431T>C | p.S477S | F/M |
| | *c.1432insT | p.R478MfsX493 | F |
| | *c.1247_1248del | p.E416VfsX417 | F |
| Exon 12 (fragment 3) | *c.1648C>T | p.R550X | F |
| | *c.1675C>T | p.R559X | F |
| Exon 13 | *c.2016delC | p.T672TX783 | F |
| Exon 14 | *c.2152G>A | p.V718M | F |
| Exon 16 | *2323_2324del | p.E775EfsX799 | F |
| | #c.2372A>C | p.Q791P | F/M |
| Exon 18 (fragment 1) | *c.2500C>T | p.Q834X | F |
| | *c.2530insA | p.H844QfsX909 | F |
| | *c.2635_2636del | p.L879EfsX908 | F |
| Exon 20 | #c.2928G>A | p.P976P | F |
| Exon 21 | #c.2995G>A | p.V999M | F/M |
| | #c.3003C>T | p.H1001H | F |
| | #c.3084G>A | p.T1028T | F |

*mutations, # polymorphisms; F: female, M: male.

Download English Version:

https://daneshyari.com/en/article/5907061

Download Persian Version:

https://daneshyari.com/article/5907061

Daneshyari.com