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CDKL5 and ARX mutations in males with early-onset epilepsy

Ghayda M. Mirzaa, M.D.^{*}, Alex R. Paciorkowski, M.D.[†], Eric D. Marsh, M.D.^{‡,||}, Elizabeth M. Berry-Kravis, M.D., Ph.D.[§], Livija Medne, M.S.[‡], Art Grix, M.D.[¶], Elaine C. Wirrell, M.D.[#], Berkley R. Powell, M.D.^{**}, Katherine C. Nickels, M.D.[#], Barbara Burton, M.D.^{††}, Andrea Paras, M.S.^{††}, Katherine Kim, M.S.^{††}, Wendy Chung, M.D.^{‡‡}, William B. Dobyns, M.D.^{*}, and Soma Das, Ph.D.^{‡‡}

^{*}Division of Genetic Medicine, Department of Pediatrics, University of Washington and Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, Washington, USA

[†]Departments of Neurology, Pediatrics and Biomedical Genetics, Center for Neural Development and Disease, University of Rochester Medical Center, Rochester, New York, USA

[‡]Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

^{||}Departments of Neurology and Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[§]Departments of Pediatrics, Neurological Sciences, Biochemistry, Rush University Medical Center, Chicago, Illinois, USA

[¶]Department of Genetics, Permanente Medical Group, Roseville, California, USA

[#]Department of Neurology, Divisions of Epilepsy and Child and Adolescent Neurology, Mayo Clinic, Rochester, Minnesota, USA

^{**}Children's Hospital Central California, Madera, California, USA

^{††}Children's Memorial Hospital and Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

^{‡‡}Division of Molecular Genetics, Department of Pediatrics, Columbia University, New York, USA

^{‡‡}Department of Human Genetics, University of Chicago, Chicago, Illinois, USA

Abstract

Mutations in *CDKL5* and *ARX* are known causes of early-onset epilepsy and severe developmental delay in males and females. While numerous males with *ARX* mutations associated with various phenotypes have been reported in the literature, the majority of *CDKL5* mutations have been identified in females with a phenotype characterized by early-onset epilepsy, severe global developmental delay, absent speech, and stereotypic hand movements. To date, only ten males with *CDKL5* mutations have been reported. Our retrospective study reports on the clinical, neuroimaging and molecular findings of 18 males with early-onset epilepsy caused by either *CDKL5* or *ARX* mutations. The 18 patients include eight new males with *CDKL5* mutations and ten with *ARX* mutations identified through sequence analysis of 266 and 346 males, respectively, at our molecular diagnostic laboratory. Our large data set therefore expands on the number of reported males with *CDKL5* mutations and highlights that aberrations of *CDKL5* and *ARX* combined are an important consideration in the genetic forms of early-onset epilepsy.

INTRODUCTION

Early infantile epileptic encephalopathy (EIEE or Ohtahara syndrome), early myoclonic epilepsy (EME), infantile spasms (ISS) and Lennox-Gastaut syndrome (LGS) are age-dependent epilepsy syndromes that show overlap in phenotype and, increasingly, in genotype [1]. These disorders are categorized as “developmental epilepsy” and even though this latter term is not a part of the International League Against Epilepsy (ILAE) classification, we feel it best captures the demonstrated biological kinship among these disorders of early-onset epilepsy [1, 2]. Moreover, “developmental epilepsy” is an improvement over the term “epileptic encephalopathy” since although the epilepsy is a key feature, it is more likely that the genetic lesion, when present, is the primary driver of developmental outcome in these children [3]. The causes of these early-onset epilepsies include specific cortical malformations, metabolic disorders, and several genetic etiologies without co-existent metabolic or structural brain abnormalities such as *ARX*, *CDKL5*, *SLC25A22*, *STXBPI*, and *SPTAN1*, among others [4–12].

Mutations in *CDKL5* have been predominantly reported in females with a phenotype characterized by early-onset refractory epilepsy, severe developmental delay, absent or very limited speech, and additional features such as hand stereotypies and postnatal deceleration of head growth in some patients [13–18]. In a large study, *CDKL5* mutations were estimated to occur in approximately 8% of females with early-onset seizures (before nine months of age) and up to 28% of females with early-onset seizures and ISS [19]. Mutations in males, on the other hand, appear less common, having been reported in ten boys to date with a more severe phenotype characterized by early-onset tonic and myoclonic seizures, intractable ISS, severe global developmental delay, cortical visual impairment, sleep disturbances, and hand stereotypies in some patients [13; 20–25].

ARX is the first gene implicated in non-syndromic early-onset epilepsy and is associated with numerous other malformation and non-malformation developmental brain disorders. The former group includes X-linked lissencephaly with abnormal genitalia (XLAG), severe hydrocephalus, Proud syndrome (agenesis of the corpus callosum with abnormal genitalia), and polymicrogyria with periventricular nodular heterotopia; whereas the latter includes X-linked infantile spasms (ISSX), Partington syndrome (intellectual disability and dystonia), and non-specific X-linked intellectual disability [8; 26–30]. Previous studies of *ARX* mutations in 115 boys with ISS of unknown cause identified expansions of the first polyalanine (polyA) tract in six boys (5.2%). All had ISS by six months of age, developmental delay, and generalized dystonia leading to a severe movement disorder [28].

We studied two cohorts of 266 and 346 males referred to our clinical molecular laboratory for *CDKL5* and *ARX* mutation analyses, respectively. The indications for testing included early-onset, medically-refractory epilepsy (ISS, EIEE, EME), global developmental delay (DD) or both. We identified *CDKL5* mutations in eight males and *ARX* mutations in ten, allowing us to estimate the prevalence of *ARX* and *CDKL5* mutations in boys presenting with developmental epilepsy. Here we report these 18 boys and discuss their phenotypic features. Analysis of these genes in these two large cohorts of boys provides further evidence that mutations in *CDKL5* and *ARX* combined account for a significant fraction of the genetic causes underlying early-onset epilepsy in males.

MATERIALS AND METHODS

Patient ascertainment

Between 2002 and 2011, 266 males were referred for mutation analysis of *CDKL5* and 346 for mutation analysis of *ARX* to the clinical molecular diagnostic laboratory at the University of Chicago, for the above-mentioned indications. Patients with congenital brain malformations were excluded from our study. Retrospective clinical, neuroimaging and electroencephalographic (EEG) data were obtained on patients in whom a mutation was identified. This study was approved by the Institutional Review Board at The University of Chicago.

Molecular Methods

Genomic DNA was extracted from peripheral blood samples using either the Puregene kit®, Magnapure® or Autogen® systems following the manufacturers' recommendations. PCR amplification was performed with 50ng of genomic DNA using Taq DNA polymerase (Applied Biosystems®). Primers were used to amplify the coding and flanking noncoding regions of *CDKL5* and *ARX*. Optimized conditions were used for the GC-rich exon 2 of the *ARX* gene, as previously described [27]. The coding regions of the *CDKL5* (exons 2–21) and *ARX* (exons 1–5) genes were targeted for mutation analysis performed by Sanger sequencing using standard methods.

Magnetic Resonance Imaging (MRI) and electroencephalogram (EEG) studies

Brain MRI imaging was performed on all subjects and images were evaluated for structural and maturational abnormalities by the investigators. Pre-treatment EEG studies were performed in all subjects, and were evaluated for epileptiform activity and abnormalities in background rhythm. A consensus definition of hypsarrhythmia (voltage >200 uV, interhemispheric asymmetry, lack of anterior-posterior gradient, multifocal sharp waves) was used [31]. The investigators were not blinded to the mutation status of the individuals at the time of review.

RESULTS

The clinical, molecular and neuroimaging findings in the eight *CDKL5* mutation-positive and ten *ARX* mutation-positive males are summarized in tables 1 and 2.

Molecular Findings

CDKL5—The *CDKL5* mutations identified in our cohort include four nonsense mutations (p.Y171X, p.Q805X, p.R59X, p.Q865X), two missense mutations (p.E21G and p.D193G) and two intragenic deletions (of exon 3 and exons 10–15). Maternal testing of six patients revealed that mutations were *de novo* in all six. Patient LR03-031 and his maternal half-sister had the same missense mutation (p.D193G) whereas maternal testing was negative, suggesting maternal germline mosaicism. Figure 1A illustrates these identified *CDKL5* mutations.

ARX—Seven of the ten identified *ARX* mutations were repeat expansions of the first polyalanine tract in exon 2, six of which were the smallest alanine-repeat expansions (+7) and one was an eight alanine-repeat expansion (+8) [(c.333_335dup(GGC)₇ and c.333_335dup(GGC)₈, respectively]. The other three mutations include an expansion of the second polyalanine tract (c.429_452dup24), a missense mutation (p.T333S), and a 103 bp insertion at the intron 4-exon 5 junction (IVS4-82_ex5 1469ins103) resulting in frameshift of amino acid sequence. Maternal analysis of four patients revealed that mothers were

carriers in two patients (LR11-423 and LR04-427), while the other two patients (LR03-309 and LR02-457) had *de novo* mutations. Figure 1B illustrates these identified *ARX* mutations.

Frequency of *CDKL5* and *ARX* mutations in males with early-onset epilepsy—

Analysis of our two cohorts reveals a mutation frequency of 3% and 2.8% in the *CDKL5* and *ARX* genes, respectively, in boys with early-onset intractable epilepsy referred to our laboratory for genetic testing. 205 boys were analyzed for *both* genes, and include the 18 mutation-positive boys. This results in a combined mutation frequency of approximately 8.7% in either *CDKL5* or *ARX* in this large group of males.

Clinical Findings

***CDKL5* cohort—**The age of the boys with *CDKL5* mutations ranged from 2 months to 14 years at the time of evaluation. Seven are Caucasian and one Somali in ethnicity. Infantile spasms, defined as epileptic spasms with ictal electrodecrement and interictal hypsarrhythmia, occurred at some point in all eight patients [31]. Other seizure types include tonic, myoclonic and partial seizures. Seizure onset was from two weeks to six months of age. The characteristic 3-stage evolution of epilepsy, as described in *CDKL5* girls but also a general pattern of seizures with EIEE, occurred in all eight patients [17]. The seizure evolution consists of onset of epilepsy before three months of age (stage 1), progression into infantile spasms (stage 2) and resulting in a multifocal refractory epilepsy (stage 3) [17]. These eight patients also had early-onset profound developmental delay with minimal/no language and motor skills, severe tone abnormalities, and cortical visual impairment. Abnormal involuntary movements were seen in four patients and included hand stereotypies (seen in two patients), dystonic movements and head stereotypies resembling spasmus nutans (in one patient each). Borderline postnatal microcephaly developed in one patient whose occipitofrontal circumference (OFC) at nine months of age was 2.5 standard deviations (SDs) below the mean. At least seven patients had feeding difficulties with severe gastro-esophageal reflux with secondary respiratory insufficiency requiring gastrostomy (G-tube) placement. These clinical data are summarized in table 1.

Patient LR03-031 had a maternal half sister with the same missense mutation in *CDKL5*. She had severe global developmental delay, hand stereotypies, hypotonia, cortical-visual impairment and breath-holding spells, yet her epilepsy phenotype was milder than that of her brother. She did not have ISS or refractory epilepsy at any point during her epilepsy course. Further details regarding her epilepsy phenotype are in table 1.

***ARX* cohort—**The ten boys with *ARX* mutations were 13 months to 19 years of age at the time of assessment. All had refractory epilepsy, with onset within the first six months of life. Seizure types include ISS (in seven patients), tonic, myoclonic and partial seizures. Of note, five had early-onset seizures that evolved to ISS and a pattern similar to the 3-stage seizure evolution seen with *CDKL5*. All ten patients had severe global DD. Eight boys on whom we have detailed clinical data had severe tone abnormalities consisting of hypotonia or mixed tone but one patient had significant spasticity. Abnormal movements were seen in six patients and consisted predominantly of chorea and/or dystonia. Interestingly, patient LR11-423 had paroxysmal autonomic instability with dystonia (PAID) syndrome characterized by severe dystonia and choreoathetosis of the face and extremities in association with fever, tachycardia tachypnea and pupillary dilatation. He was treated with baclofen and clonazepam. Cortical visual impairment and postnatal microcephaly were observed in one patient each. These clinical findings are summarized in table 2.

Neuroimaging Findings

CDKL5 cohort—Neuroimaging studies were performed on all patients. While early brain MRIs were normal in some, later imaging in at least three boys showed progressive cerebral and cerebellar atrophy with increased extra-axial space, widening of the sulci, ventriculomegaly and thinning of the white matter. Cerebral and cerebellar atrophy were evident on brain MRI as early as six weeks of age in patient LR05-277. Representative brain MRI findings are illustrated in figure 2.

ARX cohort—Brain MRI studies in the males with *ARX* mutations showed the characteristic T2 signal intensities in the globus pallidus and putamen in four patients [27, 28]. Serial imaging demonstrated progressive diffuse cerebral atrophy with ventriculomegaly as early as one year of age in at least seven patients. These neuroimaging findings are illustrated in figure 3.

Epilepsy and EEG Findings

CDKL5 cohort—All patients had EEGs or EEG reports reviewed. All eight patients with *CDKL5* mutations in our cohort developed ISS at some point in their epilepsy course, with some patients exhibiting very early onset of this form of epilepsy. Subject LR03-031a1 had onset of epileptic spasms in clusters at one month of age with hypsarrhythmia on EEG. There was partial response to phenobarbital, carbamazepine, and vigabatrin, but the child was never seizure-free. At last follow-up at seven years of age he continues to have up to eight seizures a day characterized by tonic stiffening of the extremities with eye deviation. Atonic and myoclonic seizures also occur. EEGs continue to show periodic burst suppression and discontinuous background. Subject LR04-421 had tonic seizures at 5.5 weeks of life that evolved into ISS. The seizures were refractory to adrenocorticotropin (ACTH), multiple anti-epileptics, and the ketogenic diet. EEG at five months showed hypsarrhythmia. Subject LR03-121 began to have extensor posturing and apnea at six weeks of life with normal EEG. By eight weeks, clinical extensor epileptic spasms were observed. At four months the EEG showed continuous bihemispheric epileptiform discharges but characteristics of hypsarrhythmia were not noted. Subsequent EEG at 23 months showed high voltage and frequent focal sharp discharges. Epilepsy semiology evolved from epileptic spasms that responded briefly to treatment with ACTH at four months, until tonic and myoclonic seizures emerged at eight months. These were refractory to 17 anti-epileptic medications in succession as well as the ketogenic diet. At age two years, he began to have atonic seizures as well as tonic and myoclonic seizures. EEG at three years showed continuous bioccipital epileptiform activity, very slow background, and lack of electrographic response to stimulation. Figure 4 illustrates representative EEG abnormalities.

The ARX cohort—The boys identified with *ARX* mutations similarly had early onset of epilepsy. Subject LR04-272 exhibited flexor epileptic spasms at six weeks of age combined with right-sided tonic seizures and behavioral arrest. EEG initially demonstrated burst-suppression that evolved into hypsarrhythmia with frequent electrodecrement with and without epileptic spasms by four months of age. Subject LR04-427 began having tonic seizures at 21 days of age, increasing in frequency to every 15 minutes. There was initial response to pyridoxyl phosphate and then to carbamazepine. By three months, seizures were tonic spasms with burst suppression and generalized polyspikes on EEG. Subject LR02-457 had seizures in the first days of life with multifocal spikes and polyspike and wave with frequent electrodecrement. By 6 weeks, EEG showed high-voltage (100–150 microVolts) with bilateral spikes and waves with electrodecrement. Clear infantile spasms emerged at six months. Epilepsy was ultimately lifelong and intractable, with EEG and clinical features meeting criteria for Lennox-Gastaut syndrome. Subject LR11-423 had his first seizures at

ten days of life, with recurrent rhythmic movements of the upper extremities that were initially thought to be benign myoclonic movements of infancy. These evolved into flexor epileptic spasms with leading to a diagnosis of ISS by three months that were subsequently unresponsive to ACTH, vigabatrin, and the ketogenic diet. EEGs showed hypsarrhythmia. ISS continued at last assessment at 20 months.

DISCUSSION

Common clinical features of the developmental epilepsies (EIEE, EME, ISS and LGS) include drug-resistant seizures, profound neurodevelopmental delay and, for most affected children, poor neurological outcome. Although developmental epilepsies are often associated with structural brain abnormalities, inborn errors of metabolism and other genetic disorders, the underlying etiology for many remains to be identified.

CDKL5—Mutations in *CDKL5* have been reported extensively in females with epileptic encephalopathy and a severe developmental syndrome where multiple neurofunctional domains are affected [13–18]. Some of the phenotypic features observed in females with *CDKL5* mutations, particularly hand stereotypies, limited hand use, deceleration of head growth and absent or limited speech, resemble those seen in Rett syndrome, and have thus been collectively termed “atypical Rett syndrome.”

Reports of males with *CDKL5* mutations have been infrequent, suggesting possibly early male lethality and X-linked inheritance. The ten reported males had a more severe phenotype characterized by early-onset intractable epilepsy, predominantly ISS, and profound developmental delay with lack of motor skills, stereotypic movements, and cortical visual impairment [18]. The *CDKL5* mutations identified to date include five missense mutations, two frameshift mutations, one splicing mutation (in a somatic mosaic form), one nonsense mutation in a male with Klinefelter syndrome (47,XXY), and a deletion of exon 1 [13, 20–23, 25, 32]. Larger aberrations involving the *CDKL5* gene have been reported in two patients with microdeletions of the X chromosome and expanded syndromes: (1) a large 2.8 Mb deletion of Xp22.2-Xp22.13 including exons 1–11 of *CDKL5* and the *NHS* (Nance-Horan syndrome) gene in a boy with tetralogy of Fallot, microphthalmia, microcornea, congenital cataracts and dysmorphic features [33] and (2) a mosaic 128kb deletion that includes exons 1–3 of *CDKL5* in a male with severe refractory epileptic encephalopathy [24].

Here, we report eight males with *CDKL5* mutations, two of whom have been included in a separate publication [32]. These patients share many phenotypic features of those previously reported in the literature and demonstrate that males with *CDKL5* mutations have a severe neurodevelopmental profile characterized by refractory neonatal or infantile-onset epilepsy (with EIEE or ISS occurring in most), severe global developmental delay with minimal acquisition of skills, cortical visual impairment, tone abnormalities, and hand stereotypies. As expected with most developmental epilepsies, serial neuroimaging shows nonspecific features such as progressive cerebral and cerebellar atrophy with progressively enlarging extra-axial space and ventriculomegaly, as shown in figures 2 and 3. EEGs were all poorly organized and had multifocal epileptiform abnormalities in addition to many having hypsarrhythmia. Patients LR05-277 and LR11-420 of our cohort had intragenic deletions of *CDKL5* and are phenotypically similar to males with point mutations.

All boys reported to date, including our cohort, have intractable epilepsy, severe developmental delay and poor neurocognitive outcome. The brother-sister pair reported here illustrates milder epilepsy severity in females. In addition to these siblings, germline mosaicism for a *CDKL5* mutation has been identified in at least one other family [13]. This

previously reported family included a severely-affected brother and his identical twin sisters, one of whom had mild developmental delay with autistic features, but no seizures. This also highlights the difference in the severity of phenotypes between males and females with *CDKL5* mutations and both families illustrate the importance of considering germline mosaicism in recurrence risk counseling.

ARX—Mutations in *ARX* have been reported in a series of overlapping phenotypes, divided broadly into those with or without brain malformations, with a clear distinction in phenotypes between affected males and females and strong-genotype-phenotype correlation [27]. The mildest phenotypes of non-syndromic developmental delay generally occur with expansions of the first and second polyA tracts and missense mutations, whereas severe brain malformation phenotypes occur with more severe mutations predicted to lead to complete loss of *ARX* function such as splicing, nonsense, insertion and deletion mutations [27]. Moreover, single base pair substitutions leading to missense changes in the homeodomain region and the region prior to the aristaless domain also lead to very severe phenotypes, suggesting these residues are critical to *ARX* function [26–30].

It is estimated that aberrations in *ARX* may account for up to 5.2% of the genetic etiologies in males with early epileptic encephalopathy, or X-linked ISS [27]. Expansions within the first or second polyalanine tracts are the most common events, with expansions of the second polyA more commonly reported than expansions of the first. No reported mutations or expansions of the third and fourth tracts exist to date. The severity of the clinical presentation generally increases with increasing length of the expansion [27]. Consistent with these data, seven of our patients had polyalanine repeat expansions – yet six of these had expansions of the first rather than the second tract. Additionally, patient LR11-423 had an expansion of eight GGC trinucleotides instead of the more commonly reported seven trinucleotides. To date this is the first patient identified with this c.333_335dup(GGC)₈ mutation. Notably, this patient had the earliest onset of seizures (at ten days of age), suggesting a more severe phenotype compared to patients with the common c.333_335dup(GGC)₇ mutation. Previously, two males presenting with EIEE were reported with *de novo* 33 bp duplications, c.298_300dup(GCG)₉, expanding the first polyA tract and suggesting that longer expansions are consistent with earlier onset and more severe phenotypes [34].

Several of our *ARX* mutations are novel and include a missense mutation within the homeodomain (p.T333S), a 103 bp insertion at the intron 4-exon 5 junction (IVS4-82_ex5 1469ins103), and a missense mutation within the octapeptide domain of the *ARX* region (p.D30Y). The IVS4-82_ex5 1469ins103 mutation at the intron 4-exon 5 region is predicted to affect normal splicing, similar to one other mutation reported in this region [34]. Of our two maternally inherited mutations, one was an expansion of the first polyalanine tract (c.333_335dup(GGC)₈) and the other was of the splicing mutation. Studies have shown that carrier females of expansions are asymptomatic, whereas carrier females of exon 1–4 truncations and homeodomain mutations are often symptomatic. This is suggestive of loss of *ARX* function secondary to these mutations [35].

Movement disorders have been reported with several *ARX* mutations. Examples include hand dystonia in Partington syndrome due to expansion of the second polyA tract, status dystonicus in 3 of 115 males with ISS and expansions of the first polyA tract, and mild dystonia in familial cases of syndromic and nonsyndromic X-linked developmental delay in association with the common 24-bp duplication of the second polyA tract [26, 27, 36, 37]. Four patients with expansions of the first polyA tract in our cohort had abnormal movements. Patients LR02-457 and LR11-422 had choreoathetosis and dyskinesias, and patient LR04-386 had spontaneous hand movements that were not further characterized.

Additionally, patient LR11-423 with the c.333_335dup(GGC)₈ mutation had paroxysmal autonomic instability with dystonia (or PAID syndrome), a poorly-understood phenomenon of episodic central dysautonomia consisting of hyperthermia, hypertension, tachycardia, intense hyperhidrosis, and dystonia typically seen in association with various cerebral insults [38, 39]. To our knowledge, this is the first report of PAID syndrome with an *ARX* mutation.

The clinical phenotypes of *ARX*- and *CDKL5*-positive males appear similar with a severe neurodevelopmental course characterized by early-onset and refractory epilepsy (ISS being the most common seizure type), severe global developmental delay, severe tone abnormalities and a poor neurocognitive outcome. As would be expected with the seizure types these patients presented, the EEG abnormalities frequently observed was a burst suppression pattern evolving into hypsarrhythmia.

In conclusion, our data allow an estimation of the prevalence of *ARX* and *CDKL5* mutations in boys presenting with developmental epilepsy. These findings add to that of two recent reports underlying the critical role these two genes in the pathogenesis of developmental epilepsy in infancy [25, 40]. We suggest that aberrations within these two genes may collectively account for up to 8% of males with early-onset epilepsy with an underlying genetic basis. Given our ascertainment bias, these findings may not be representative of the true frequencies of *CDKL5* and *ARX* mutations in all males with early-onset epilepsy, yet future large-scale studies similar to ours will help substantiate these data. We also describe the clinical findings of eight males with *CDKL5* mutations, and report several novel *ARX* mutations. Taken together, these data further our understanding of the important roles on *ARX* and *CDKL5* in developmental epilepsies.

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(A) *CDKL5* (B) *ARX* **Figure 1.**

The identified mutations in *CDKL5* (A) and *ARX* (B). (A) The mutations indicated above the gene are the previously-reported *CDKL5* mutations, whereas those indicated below the gene are the identified mutations in our cohort that include four nonsense mutations, two missense mutations, and two intragenic deletions. (B) The identified *ARX* mutations including eight polyalanine repeat expansions, one insertion mutation in an intron/exon boundary and one missense mutation. The p.D30Y mutation was identified in our laboratory in the carrier state in a mother whose son was tested elsewhere.

Figure 2.

Brain MRI images of three males with *CDKL5* mutations. T1-weighted mid-sagittal, parasagittal and axial and T2-weighted coronal images of patient LR04-421 (A–D), T1-weighted mid-sagittal, parasagittal and coronal and T2-weighted axial images of patient LR03-121 (E–H) and patient LR11-048 (I–L). Note widening of the cortical and cerebellar sulci consistent with atrophy, mildly increased extra-axial space and thin corpus callosum.

Figure 3.

Brain MRI images of males with *ARX* mutations. T1-weighted mid-sagittal, and two T2-weighted axial images of patient LR11-423 (A–C), and patient LR02-457 at 5 years (D–F) and 19 years of age (G–I). Note the characteristic T2 signal intensities in the basal ganglia bilaterally (arrows in images E and H), progressive cortical and cerebellar atrophy, ventriculomegaly as well as thinning of the corpus callosum.

Figure 4.

Representative EEG tracings of males with *CDKL5* mutations. (A) shows patient LR04-421 at 2 months with high voltage slow waves. (B) shows the same patient at 3 years 11 months with left-sided slowing. A 4–5 Hz posterior dominant rhythm is present on the right. (C) shows patient LR11-048 at 12 months with high voltage slow wave followed by electrodecrement. (D) also shows patient LR11-048 at 12 months with high voltage and multifocal sharp waves typical of hypsarrhythmia.

Table 1

Summary of the clinical, neuroimaging, and molecular findings in males with *CDKL5* mutations (n=8)

Patient No.	1	2	3	4	5**	6**	7	8
ID#	LR03-031a1	LR04-421	LR04-409	LR03-121	LR11-421	LR11-048	LR05-277	LR11-420
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Somali	Caucasian	Caucasian	Caucasian
Age at last assessment	7.5y	6y	4y	14y	10m	2m	6y	4y
Seizure onset	4w	5.5w	8–10w	6w	6m	2m	2w	5.5w
Seizure types	ISS, T, MC, atonic (head drop)	T, ISS, GTC	ISS	ISS, MC, T	ISS, PS, multifocal	PS, ISS	T, TC, ISS, MC	T, ISS
Intractable epilepsy	+	+	+	+	+	+	+	+
EEG findings	HYPs, multifocal bursts of polyspike activity	HYPs	HYPs	Continuous bihemispheric epileptiform discharges	Multifocal epileptiform discharges, recorded generalized seizures	Slow background (5 Hz), multifocal discharges (max. posteriorly)	Slow, R sharp waves (1 st EEG); slow, freq. sharp waves, multifocal discharges (last EEG)	Localization - related epilepsy, likely arising from the R frontocentral area
CBL atrophy	–	+	–	++	–	–	++	+
Last MRI	9y	4y	<4y	9y	nd	2m	6w	2y
Severe GDD	+	+	+	+	+	+	+	+
Tone	Mixed (appendicular spasticity, axial hypotonia)	Mixed (appendicular spasticity, axial hypotonia)	Hypotonia	Mixed (appendicular spasticity, axial hypotonia)	Hypotonia	Severe hypotonia	Severe hypotonia	Severe hypotonia
CVI	+	+	+	+	+	+	+	+
Earliest OFC –SD (age)	nd	0–1 (4m)	+2 (birth)	+2 (2m)	nd	1–2 (birth)	nd	nd
Last OFC – SD (age)	0–1 (7y)	–2.5 (9m)	nd	–1–2 (14y)	+1 (10m)	0–1 (2m)	–1–2 (10y)	0–1 (4y)
Postnatal MIC	–	+	nd	–	–	–	–	–
Abnormal movements	+	–	–	+	+	–	+	–
Other medical problems	GER (G-J tube), constipation, mild optic atrophy, mild scoliosis, mild facial DYSM	G-tube, sleep apnea (due to hypoventilation), mild facial dysmorphism, hypoplastic scrotum	Neutropenia, G-tube	GER, growth failure, recurrent aspiration pneumonia, choking and difficulties	Dysphagia with aspiration pneumonia, on thick liquid diet	GER	Neurogenic bladder, G-tube fed, constipation	G tube-fed, chronic respiratory insufficiency, osteopenia

Patient No.	1	2	3	4	5**	6**	7	8
Outcome	Deceased	Alive	Alive	Alive	Alive	Alive	Alive	Alive
Mutation	c. 578A>G (exon 9) p.D193G	c. 513C>A (exon 8) p.Y171X	c.2413C>T (exon 17) p.Q805X	c.175C>T (exon 5) p.R59X	c.2593C>T (exon 18) p.Q865X	Ex 10-15del	Ex 3del	c.62A>G (exon 2) p.E21G
Type	Missense	Nonsense	Nonsense	Nonsense	Nonsense	Deletion	Deletion	Missense
Inheritance	Germline mosaicism	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	nd	<i>De novo</i>	<i>De novo</i>
Family history	Affected maternal half-sister*	2 maternal SAB	2 maternal SAB	No known neurologic problems	No known neurologic problems	No known neurologic problems	No known neurologic problems	No known neurologic problems

Abbreviations: CBR: cerebellar; CBL: cerebellar; CPK: creatine phosphokinase; CPS: complex partial seizures; CVI: cortical visual impairment; d: days; DD: developmental delay; DYSM: dysmorphic features; EEG: electroencephalographic; GDD: global developmental delay; GER: gastro-esophageal reflux; GJ: gastro-jejunoscopy tube; HYPS: hypsarrhythmia; ISS: infantile spasms; LG: Lennox-Gastaut; m: months; M: myoclonic; MIC: microcephaly; nd: no data; OFC: occipito-frontal circumference; PS: partial seizures; T: tonic; SAB: spontaneous abortion; SD: standard deviations; VNS: vagal nerve stimulator; w: weeks; y: years

* Affected maternal half sister (assessed at 5.5 years) had seizures with onset at 6–7 months of age consisting of eye fluttering and jerky movements of the arms. Initial EEG showed pre-hypsarrhythmic changes. She subsequently had brief complex partial seizures every 1–3 months that were well-controlled on one medication. She had severe developmental delay. She rolled over at one year of age and at 5.5 years had minor head control but was unable to sit independently and remained non-ambulatory. She vocalized without distinct words. She also has abnormal movements, teeth-grinding and breath-holding spells. The mother of these two siblings is asymptomatic.

** These two patients were also included in a recent report by our co-authors [32]

Table 2

Summary of the clinical, neuroimaging, and molecular findings in males with *ARX* mutations (n=10)

Patient No.	1	2	3	4	5	6	7	8	9	10
ID#1	LR04-386	LR04-272	LR03-309	LR02-457	LR11-422	LR11-425	LR11-423 10.2790	LR02-310 02.2838	LR04-427 04.1087	LR03-413 03.3113
Ethnicity	Caucasian	Caucasian	Asian	nd	nd	Mixed European	German/Native American/Polish	Asian	Caucasian/African-American	Eastern European (Jewish)
Age at last assessment	17y	6y	21m	19y	3y	16y	20m	nd	nd	13m
Seizure onset	Neonatal	6w	13w	Neonatal	4m	2m	10d	3m	24d	6m
Seizure types	T	ISS, CPS	ISS, MC, CPS	ISS, PS	ISS	ISS, CPS	ISS, CPS, PS	T, tonic spasms	T, tonic spasms	ISS, MC
Intractable epilepsy	+	+	+	+	+	+ s/p VNS placement at 12y	+	–	+	+
EEG findings	Generalized atypical spike, polyspike, slow wave discharges, multifocal characteristics	HYPs, frequent electrodecereental responses	Multifocal epileptiform discharges, disorganized background	HYPs (6w), Lennox-Gastaut-like pattern (17y)	HYPs	Focal sharps at frontoparietal areas, slow background (16y)	HYPs	HYPs	Burst suppression, generalized polyspikes	HYPs
CBR-CBL atrophy	+	+	+	++	+	–	+	–	–	+
Last MRI	4y	nd	nd	19y	nd	12y	14m	2y	Infancy	1y
Severe GDD	+	+	+	+	+	+	+	+	+	+
Tone	Spasticity (on baclofen)	Hypotonia	Severe hypotonia	Mixed (appendicular spasticity and axial hypotonia)	Axial hypotonia	Hypotonia	Axial hypotonia with opisthotonic posturing	Hypotonia	nd	Hypotonia
Earliest OFC – SD	nd	nd	nd	nd	nd	nd	1–2 (11d)	nd	nd	nd
Last OFC – SD (age)	–0.5 (17y)	nd	–0.5–0 (3y)	nd	–1–0 (3y)	0 (16y)	–0.5–0 (20m)	nd	nd	0–1 (13m)
Postnatal MIC	–	nd	–	nd	–	–	–	nd	+	–
Abnormal movements	+	+	–	+	+	+	+ ^a	–	–	–
Other medical problems	Tracheostomy, pneumonia	–	–	G-tube, constipation, choking (esp. at night, treated with Botox & scopolamine)	nd	–	CVI, irritability due to GER, constipation, aspiration with PO feeds at 13m (supplemental NG feeds), ↑ CPK (due to underlying movement disorder), intrauterine hiccups	CVI	Scrotal hypoplasia, ear dimples	–
Outcome	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Alive

Patient No.	1	2	3	4	5	6	7	8	9	10
Mutation	c.333_335dup (GGC) ₇ (exon 2)	c. 333_335dup (GGC) ₇ (exon 2)	c. 333_335dup (GGC) ₇ (exon 2)	c. 333_335dup (GGC) ₇ (exon 2)	c. 333_335dup (GGC) ₇ (exon 2)	c. 333_335dup (GGC) ₇ (exon 2)	c..333_335dup (GGC) ₈ (exon 2)	c.429_452dup24 (exon 2)	IVS4-82_ex5_1469ins103 (intron 4/exon 5)	c. 998C>G (p.T333S) (exon 2)
Type	In-frame; insertion 7 polyalanine repeats	In-frame; insertion 7 polyalanine repeats	In-frame; insertion 7 polyalanine repeats	In-frame; insertion 7 polyalanine repeats	In-frame; insertion 7 polyalanine repeats	In-frame; insertion 7 polyalanine repeats	In-frame; insertion 8 polyalanine repeats	In-frame; insertion 8 polyalanine repeats	Insertion 103 bp	Missense
Inheritance	nd	nd	<i>De novo</i>	<i>De novo</i>	nd	nd	Maternally-inherited ^b	nd	Maternally-inherited	<i>De novo</i>
Family history	nd	nd	Febrile seizures	No known neurologic problems	nd	No known neurologic problems	See below	nd	nd	2 healthy sibs, 1 SAB

^aThis patient had PAID syndrome (paroxysmal autonomic instability with dystonia), dystonic posturing and choreoathetosis, and bilateral clonus

^bThis patient's mother is asymptomatic and has never had a brain MRI. A maternal cousin has a history of autism-spectrum disorder, seizures and DD. A maternal half-aunt had seizures at 13–14 yrs and a maternal uncle had seizures secondary to head trauma during a motor vehicle accident.

For abbreviations, see table 1

Table 3

Summary of the clinical and molecular data of published males with *CDKL5* mutations (n=10)

Reference	[13] (pt III.3)*	[20] (pt 1/8)	[20] (pt 2/8)	[20]	[21] (pt 3/8)	[22]	[23]	[25] (pt 1)	[25] (pt 6)	[25] (pt 9)
Ethnicity	nd	Italian	Italian	Italian	nd	nd	nd	nd	nd	nd
Age at assessment	16y	13y	9y	3y6m	4y	4y	2y8m	6m	1y9m	2y
Seizure onset	Neonatal	3m	8m	2m	2m	15d	2m	1m	2w	3m
Seizure types	ISS, LG	ISS, MC, T, CPS	CPS, ISS, T, MC	T, ISS, CPS	ISS, GTC, T, MC	ISS, T, GTC, MC	ISS, T, PS	ISS	Spasms	ISS
Intractable epilepsy	+	+	+	+	+	+	+	+	+	+
CBR-CBL atrophy	nd	+	-	-	+	+	+	+	+	+
Abnormal movement s	+	+	+	-	-	+	-	nd	-	nd
Poor eye contact	-	+	+	-	+	+	-	nd	nd	nd
Tone	Spastic quadriparesis	Hypotonia	nd	Hypotonia	Hypotonia	Hypotonia	Hypotonia	No hypotonia	Hypotonia	Hypotonia
Earliest OFC-SD (age)	Normal (birth)	Normal (birth)	Normal (birth.)	nd	Normal (birth)	-1-2 (birth)	Normal (birth)	Normal (birth)	-1 (birth)	Normal (birth)
Last OFC- SD (age)	-2 (14y)	nd	nd	nd	nd	< 2 (2y)	0 (2y8m)	nd	nd	nd
Postnatal MIC	+	nd	nd	nd	nd	+	-	-	-	-
Severe GDD	+	+	+	+	+	+	+	+	+	+
Other neurologic findings	Cortical blindness, hyperventilation	-	Pyramidal signs, ataxia, echolalia	-	-	breath- holding spells	-	nd	nd	nd
Other medical problems	GER, constipation, kyphoscoliosis	Mild facial DYSM		Mild facial DYSM	-	Mild facial DYSM	-	nd	nd	nd
Outcome	Deceased at 16y (aspiration pneumonia)	Alive	Alive	Alive	Alive	Alive	Alive	nd	nd	nd
Mutation/Deletion	c.183del T (exon 5)	c.872G> A (p.C291 Y)	c.863C> T (p.T288I)	c.872G> A (p.C291 Y)	c.191T> C (p.L64P)	c.1675C > T (p.R559 X) (47,XXY) Balanced Xi (50%-50%)	c.99+5G > A (somatic mosaicism)	Exon1 deletion	c.533G> A (p.R178 Q)	c.1079de IT (p.L36 0F>367 X)
Type	Frameshift	Missense	Missense	Missense	Missense	Nonsense	Splicing; frameshift	Deletion	Missense	Frameshift
Inheritance	De novo	De novo	De novo	De novo	nd	nd	Somatic mosaicism	nd	De novo	nd
Family history	2 affected sisters (twins)	Noncons anguinous parents; 1 healthy sib	Noncons anguinous parents; first child	Noncons anguinous parents; 2 SAB	Noncons anguinous parents; 1 healthy sib	Noncons anguinous parents; 1st child	Noncons anguinous parents	nd	nd	nd

* This patient's two sisters (twins; 19 y) had the same mutation. III.1 had profound DD, decreased OFC (50-98% at birth; 2-50% at 19y), epileptic encephalopathy (seizure onset 9 wks; ISS, later had GTC, myoclonic, and absence seizures), generalized spasticity, scoliosis, constipation. III.2 had mild DD (IQ ~ 70), autistic features. She had no seizures, or tone abnormalities. Family history is also remarkable for two still-births and one miscarriage.