CDKL5 Deficiency
Overview of clinical, molecular and biological aspects

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There are no conflicts of interest I wish to declare.

Funding support:

Current:

Previous:

National Health & Medical Research Council of Australia

Rett Syndrome Research Foundation

Rett Syndrome Australian Research Foundation
CDKL5 Defects – First Reports

- L Huopaniemi et al., Hum Mutat, 2000
  - large deletion involving the C-terminal CDKL5 and RS1
  - patient with retinoschisis and epilepsy
CDKL5 Defects – First Reports

- VM Kalscheuer et al., Am J Hum Genet, 2003
  - X:autosome translocations disrupting CDKL5 – (X;7), (X;6)
    » infantile spasms (onset < 2 months), MR, dysmorphism
  - severe X-linked infantile spasms and mental retardation

\[46X,t(X;7)(p22.3;p15) \text{ (CDKL5 – intron 10)}\]
\[46X,t(X;6)(p22.3;q14) \text{ (intron 1)}\]

Am J Hum Genet 2003: 72; 1401-1411
III:1
- infantile spasms from 9 weeks
- normal development until 10 m
- head growth deceleration
- lost babble + social withdrawal @ 10-15 m
- loss of pincer grip
- development of hand stereotypies
- hyperventilation & breathholding
- small hands & feet
- scoliosis
- constipation
- vasomotor disturbance
- never walked
- currently has mixed clonic, myoclonic & absence seizures
- severe mental retardation
- compatible with atypical RTT

III:2
- autism & mild MR
- never had seizures

III:3
- infantile spasms in the newborn period
- poor head control
- severe psychomotor retardation
- died age 16 yrs (vegetative, frequent myoclonic jerks)
atypical Rett syndrome

autism/MR

neonatal onset seizures and profound MR

MECP2 - c.C426T  (p.F142F)
CDKL5 Mutation Screening

c.183delT (p.L75X)
Screening of 44 RTT patients

II:1:
- born at term
- infantile spasms from 7 weeks (intractable)
- very slow progress
  - sat 17 months, stood 4 yrs
  - single words, but lost speech
  - walked 7 yrs (unsteady)
- @ 28 yrs - ht, wt, OFC all < 3rd %ile
  - no verbal skills
  - severe mental retardation
  - hand stereotypies, small feet
  - scoliosis, hyperventilation
  - peripheral autonomic disturbance
  - disturbed sleep

II:2: - classical RTT

Classical RTT
- XCI = 85%:15%
- IVS13-1G

Atypical RTT-like phenotype

II:1 & II:2 had different maternal & paternal haplotypes at the CDKL5 & MECP2 loci

IVS13-1G>A
Mutations of CDKL5 Cause a Severe Neurodevelopmental Disorder with Infantile Spasms and Mental Retardation

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Report

Mutations in the X-Linked Cyclin-Dependent Kinase–Like 5 (CDKL5/STK9) Gene Are Associated with Severe Neurodevelopmental Retardation

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Tao et al. Am J Hum Genet
(CDKL5 screening of 32 patients with a clinical diagnosis of RTT)

c.455G>T(p.C152F) - de novo
c.525A>T(p.R175S) - de novo

- neonatal period normal
- at 5w salaam-like seizures
- at 5 y no speech, problems to sit
- Angelman/RTT considered
- severe psychomotor retardation
- XI not known

- neonatal period normal
- IS at 2 mo, disappeared at 5 mo, absences.
- severe psychomotor retardation, sit at 3 y, walk few steps at 10 y
- no speech, mild ataxia, hand movements
- no X-inactivation skewing
Further *CDKL5* Screening of Patient Cohorts

- **Scala et al., J Med Genet 2005: 42; 103-107**
  - mutations in 2 females with early onset seizure severe (Hanefeld) RTT
  - no mutations in 19 classical RTT or PSV RTT patients

- **Evans et al., Eur J Hum Genet 2005: 13; 1113-1120**
  - screening of 94 patients (13 classical RTT, 25 atypical RTT, 40 RTT-like ♀, 16 RTT-like ♂)
  - mutations in 2 early onset seizure RTT variant and 1 early onset seizure RTT-like – all ♀

- **Archer et al., J Med Genet 2006: 43; 729-734**
  - 7 of 42 (17%) ♀ with seizures commencing < 6 months of age (mostly of myoclonic or infantile spasm type)
  - all with poor developmental progress but few clinical signs suggestive of RTT
  - males rarely show *CDKL5* mutations
Further CDKL5 Screening of Patient Cohorts

- **Elia et al., Neurology 2008: 71; 997-999**
  - 3 of 8 ♂ with severe-profound intellectual disability & early-onset (2 – 8 months) intractable seizures had missense mutations

- **Bahi-Buisson et al., Brain 2008 (online September 12)**
  - 183 ♀ with early onset seizures (<3 months) & encephalopathy
  - mutations identified in 8 with RTT-like features, 5 with infantile spasms and 7 with encephalopathy and refractory seizures

- **our studies – 282 patients screened for mutations in the CDKL5 gene, incl. 101 RTT, 52 ISSX/West, 59 autism, 7 XLMR, 54 others (incl. 7 with Aicardi syndrome)**
  - 1 de novo missense mutation - c.586C>T (p.S196L)
  - variant RTT with infantile spasms (onset 5 months)
### Catalogued CDKL5 Mutations

(RettBASE: http://mecp2.chw.edu.au)

<table>
<thead>
<tr>
<th>Short Citation</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Type of sequence change</th>
<th>Domain change location</th>
<th>Phenotype</th>
<th>Mutation/polymorphism</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans, ...</td>
<td>CDKL5 c. -440G&gt;T</td>
<td>5'UTR variation</td>
<td>5'UTR variation</td>
<td>CDKL5</td>
<td>Not known</td>
<td>Unknown</td>
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<td>5'UTR variation</td>
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<td>Polymorphism not causing disease</td>
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<td>5'UTR variation</td>
<td>5'UTR variation</td>
<td>CDKL5</td>
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<td>Polymorphism not causing disease</td>
<td>F</td>
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<tr>
<td>Evans, ...</td>
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<td>5'UTR variation</td>
<td>5'UTR variation</td>
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<td>Unknown</td>
<td>F</td>
</tr>
<tr>
<td>Jumon, ...</td>
<td>CDKL5: 3'UTR c.145+17A&gt;G; 3003C&gt;G, 3084G&gt;A</td>
<td>5'UTR variation</td>
<td>Intrinsic variation, silent</td>
<td>CDKL5</td>
<td>Rett syndrome - Not certain</td>
<td>Polymorphism not causing disease</td>
<td>U</td>
</tr>
<tr>
<td>Nettun, ...</td>
<td>CDKL5: c. [2500C&gt;T, 2995G&gt;A], MECF2_e1.c.45_47delAGG</td>
<td>5'UTR variation</td>
<td>Nonsense, missense, frameshift insertion or deletion</td>
<td>N-term, CDKL5</td>
<td>Rett syndrome - atypical</td>
<td>Mutation associated with disease</td>
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<tr>
<td>Rosas, Vargas, ...</td>
<td>CDKL5: c. 119C&gt;T</td>
<td>5'UTR variation</td>
<td>Missense</td>
<td>CDKL5</td>
<td>Rett syndrome - early seizure</td>
<td>Mutation associated with disease</td>
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<td>Mutation associated with disease</td>
<td>F</td>
</tr>
</tbody>
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34 pathogenic mutations; 12 non-pathogenic; 3 silent polymorphisms; 14 of unknown significance
Is there a **CDKL5** Facial Phenotype?

...Note the deep set eyes, straight eyebrows, slightly short upturned nose, relatively large ears with large earlobes and high forehead...
“…Note the deep set eyes, straight eyebrows, slightly short upturned nose, relatively large ears with large earlobes and high forehead…”
Emerging CDKL5 Deficiency Phenotype

- early seizure onset severe RTT (Hanefeld variant)
  - typically seizures have onset < 6 months
  - seizures often very resistant to therapies

- severe early onset seizures with poor development, poor eye contact and no RTT-like features

- no characteristic seizure type – pattern may evolve over time
  - includes infantile spasms, generalised tonic-clonic seizures, myoclonic seizures, focal seizures, atypical absence seizures

- facial gestalt?
  - prominent and broad forehead, straight well-defined eye brows, deep set eyes, short upturned nose, midface hypoplasia, large down-turned mouth

- males with CDKL5 mutations
  - seem to be rare (under-ascertained?)
  - early onset seizures and severe-profound intellectual disability
Cyclin dependent kinase like 5

- encodes a Serine Threonine Kinase
- 23 exons - two transcripts (alternate splicing)
Cdkl5 Expression in mouse brain

- Widespread expression of Cdkl5 (esp. olf. bulb, cer. cortex, cerebellum, hippocampus)
- Similar distribution to Mecp2
- Cdkl5 expression unchanged in Mecp2 KO (deleted for TRD and C-terminus)
- Cdkl5 acts in parallel or upstream of Mecp2?

Weaving et al., Am J Hum Genet 2004
lower expression of Mecp2 and Cdkl5 in late embryogenesis vs postnatal brain

strongest expression in neocortex, piriform cortex, hippocampus, amygdala complex, dorsal geniculate nucleus

Different levels of expression in specific cerebellar regions
- ↑ Mecp2, ↓ Cdkl5 in Purkinje cells
- ↓ Mecp2, ↑ Cdkl5 in granular cells

ie Cdkl5 and Mecp2 co-exist in the same cells and may be independently regulated
confirmation that Cdkl5 expression is induced in postnatal brain

confirmation that Cdkl5 & Mecp2 are expressed widely in brain (albeit to different levels)
Cdkl5 is expressed in neuronal but not glial cells.

Cdkl5 expression appears to vary across brain regions and cell types & may be both nuclear and cytoplasmic.
Subcellular Localisation of CDKL5

Lin et al Hum Molec Genet 2005: 14; 3775-3786

Transiently expressed (A) & endogenous (B) full length CDKL5 are predominantly nuclear

CDKL5 with a C-terminal truncation is perinuclear & cytoplasmic
Importance of the C-terminal region of CDKL5 for nuclear localisation confirmed by Bertani et al, J Biol Chem, 2006)
However, N-terminal mutations also lead to loss of nuclear localisation;
- p.A40V punctate cytoplasmic staining
- p.L220P homogenous staining

*Rosas-Vargas et al., J Med Genet 2008: 45; 172-178.*
MeCP2-CDKL5 Interactions

- in pull down assays MeCP2 and CDKL5 directly interact
  (Mari, Hum Molec Genet 2005; Lin, Hum Molec Genet, 2005)
  – TRD→C term of MeCP2 interacts with C term half of CDKL5

- CDKL5 is a kinase – able to autophosphorylate itself
  – deletion of the C-terminus results in ↑ expression and autophosphorylation

- Does CDKL5 phosphorylate MeCP2?
  – Lin et al (Hum Molec Genet 2005) say no
• Cdkl5 expression increases dramatically postnatally
• Cdkl5 is neuronal not glial in location
• Cdkl5 and Mecp2 are found in same brain regions and cell types, but may be at differing levels
• Cdkl5 may be nuclear or cytoplasmic in its subcellular location
• Cdkl5 and Mecp2 directly interact
• Cdkl5 is a kinase
• Mecp2 is a specific Cdkl5 phosphorylation target?
Unanswered Questions - CDKL5

- Do specific MECP2 mutations affect its interaction with CDKL5?

- How does the MeCP2-CDKL5 interaction affect brain function?

- Which are the important CDKL5 phosphorylation targets?

- What is the functional significance of the regional and cellular variation in Cdkl5 expression?

- What regulates CDKL5 expression?

- Will mouse models for Cdkl5 deficiency help us understand the biology of Rett syndrome?
Collaborators

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