The Genetics of Rett Syndrome

John Christodoulou

Head, NSW Rett Syndrome Research Unit
Western Sydney Genetics Program, Children’s Hospital at Westmead
Discipline of Paediatrics & Child Health, University of Sydney
Clinical Diagnosis

- specific developmental profile based on a consistent constellation of clinical features

- diagnostic criteria developed

- classical and variant RTT phenotypes
  - atypical Rett syndrome
  - “speech preserved” variant
  - congenital onset variant
  - male Rett syndrome equivalent
**Genetics of Rett Syndrome**

**X: autosome translocations:**
- t (X; 22) - Xp11.22
- t (X; 3) - Xp21.3

**Deletions:**
- del (3) (3p25.1 - p25.2)
- del (13) (13q12.1 - q21.2)

**mtDNA mutation screening:**
- 16S rRNA - A2706G (1 patient & mother)

**Exclusion mapping in familial cases:**
(incl. Brazilian family with 3 affected sisters)
- gene likely to be in Xq28 or Xpter
"Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG binding protein 2"

(Amir et al, Nature Genet 1999: 23; 185 - 188)

<table>
<thead>
<tr>
<th>patients</th>
<th>mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>sporadic RTT-39</td>
<td>sporadic RTT-24</td>
</tr>
<tr>
<td>sporadic RTT-6</td>
<td>sporadic RTT-22</td>
</tr>
<tr>
<td>sporadic RTT-29</td>
<td></td>
</tr>
</tbody>
</table>

6 mutations identified in 21 sporadic classical cases
- 4 *de novo* missense mutations in methyl-binding domain (MBD)
- 1 *de novo* frame-shift mutation in transcription repression domain (TRD)
- 1 *de novo* nonsense mutation in TRD
MECP2 Mutations Identified

> 200 to date

RettBASE: http://mecp2.chw.edu.au
Large Deletions in RTT Patients

-~15 kb~
- 4.609 kb ~47 kb
- ~37 kb
- ~40 kb
- ~65 kb
Gene Silencing by Chromatin Condensation

Methylated DNA

MeCP2 binds to methyl-CpGs

mSin3a recruitment of mSin3a & histone deacetylase (HDAC)

MeCP2 binds to methyl-CpGs

mSin3a

recruitment of mSin3a & histone deacetylase (HDAC)

HDAC

MeCP2
mSin3a
SWI/SNF
HDAC

chromatin accessible & active

chromatin condensed & inactive
Factors Contributing to Phenotypic Variability

- **type of mutation**
  - truncation mutations worse than missense mutations

- **location of mutation**
  - MBD mutations worse than TRD mutations

- **skewing of X-inactivation**
  - favourable or unfavourable effect depending on which X is preferentially inactivated

- **other epigenetic factors?**
“Non-Rett” Clinical Phenotypes

- **X-linked mental retardation:**
  - severe non-specific XLMR
  - mild non-specific X-linked mental retardation
  - XLMR with progressive spasticity
  - PPM-X; psychosis, pyramidal signs, macro-orchidism

- **severe neonatal encephalopathy:**
  - esp. if unexplained central hypoventilation, severe seizures & abnormal tone

- **Angelman-like syndrome:** (no abn involving chromosome 15)
  - ~8% (10/125) had \textit{MECP2} mutations
  - most (but not all) retrospectively found to have regressed
Who Should have MECP2 Mutation Screening?

**Definitely:**

- all patients with a clinical diagnosis of RTT
  - follow-up specific mutation testing in first degree female relatives
  - prenatal testing where requested

- male sibs of RTT who show MR &/or neurological abn

- Angelman syndrome with no abnormality of chr 15
  - especially if there is an evolving regressive clinical picture
Who Should have *MECP2* Mutation Screening?

**Maybe:**

- XLMR, FraX(A) negative?
- MR + autism???
- Isolated MR???

yield seems very low so far (decision on an individual basis)
Summary

Our MECP2 Studies to Date

- 75% have missense, nonsense, small frame-shifts
- 15% have large deletions
- exon 1 mutations rare
- promoter sequence variations of uncertain significance
- some phenotype-genotype correlations
- 5 – 10% - no apparent MECP2 mutation
Family with no *MECP2* mutation

**III:1**
- atypical (milder RTT)
- infantile spasms from 9 weeks

- **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)

**III:4**
- clinically normal brother

**III:5**
- clinically normal sister

**II:1**
- clinically normal mother
CDKL5 Mutation Screening

II:1

II:2

c.183delT (L75X)

III:1

III:2

III:3

III:5

183T/183delT

183T/183delT

183T/183T
Summary of currently known CDKL5 mutations

t(X;6) (severe ISSX)
c.163_166delGAA

t(X;7) (severe ISSX)
c.2635_2636delCT

136kb deletion (retinoschisis and epilepsy)

(X;7) (severe ISSX)

136kb deletion (retinoschisis and epilepsy)

IVS13-1G>A

c.183delT

c.215T>A (I72N)

IVS7-2A>G

IVS16+1G>C

c.676G>T(C152F)

c.746A>T(R175S)

c.163_166delGAAA

c.2635_2636delCT

XLRS1 gene

STK9 gene
CDKL5 (aka STK9)

• novel, conserved serine/threonine kinase - function unknown, substrate unknown

• large gene of 23 exons with 2 alternative transcription start sites

• CDKL5 protein localisation - cytoplasm/nucleus?

• wide tissue expression, including fetal and adult brain

• participates in the regulation of expression of other genes (upstream of or parallel to MeCP2?)
Summary

✓ **MECP2** - major RTT gene
  (80-90% classical RTT, 60-70% atypical RTT)
  ? mutations involving the promoter
  ? mutations outside MECP2 ORF?

✓ **CDKL5** - new RTT/atypical RTT gene
  ✓ 12 patients with STK9 mutations identified
  ? ISSX
  ? autism spectrum disorder
  ? Aicardi syndrome
  ? other
Netrin-G1: a 3rd RTT gene?

• single case report of a female with atypical RTT and early onset seizures

• de novo translocation 46XX, t(1;7) (p13.3; q31.33)
  – disrupts the NTNG1 (Netrin-G1) gene on chromosome 1
  – involved in axonal guidance & signalling & in NMDA receptor functioning

• but no mutations in 115 patients with RTT (females - 25 classic and 84 atypical; males - 6)
Conclusions

- most cases of RTT are due to mutations in the X-linked gene *MECP2*

- subset of RTT patients have mutations in the *CDKL5* gene
  - responsible for other clinical phenotypes

- role of *NTNG1* in RTT uncertain

- pathogenesis of RTT remains largely unknown
Funding Acknowledgements

NHMRC
Apex Foundation for Research into Intellectual Disability
International Rett Syndrome Association
Rett Syndrome Research Foundation
Rotary Club of Narellan
CWA of NSW
Rett Syndrome Australian Research Fund
THE GP'S KID INvariably DOMINATED SHOW-AND-TELL
Collaborators

Children’s Hospital at Westmead Group

Current team
- Angela Beaton
- Bruce Bennetts
- Carolyn Ellaway
- Andrew Grimm
- Hooshang Lahooti
- Vidya Vasudevan
- Rose White
- Sarah Williamson

Past team
- Linda Weaving
- Joanne Gibson
- Vince Repaci
- Alexandra Bezler
- Kirsten Reuter
- Lauren Curphy
- Abid Mohamedali

Children’s Medical Research Institute
- Patrick Tam
- Catherine Watson
- Gregory Pelka
- Phil Robinson

Westmead Millennium Institute
- Barry Slobedman, Chris Bye & Josh Stern

Institute of Medical Genetics,
University College of Medicine, Cardiff
- Angus Clarke, Hayley Archer & Julie Evans

Women’s & Children’s Hospital, Adelaide
- Jozef Gécz, Kathie Friend & Olivia McKenzie

Baylor College of Medicine, Houston
- Huda Zoghbi

TVW Telethon Research Institute, Perth
- Helen Leonard & her APSU team

West Australian Institute for Medical Research
- David Ravine & Alka Saxena
Male Lethality or Male Sparing?

- **X-linked dominant disorders**
  - increased male lethality
  - increased spontaneous miscarriage rate

- **Rett Syndrome**
  - 85% of single base mutations involve CpG “hotspots”
  - sperm highly methylated; X completely methylated

```
 methytransferases  5-methyl-cytosine  deamination
 cytosine              uracil
```

- 3 studies reviewing parental origin of *de novo* mutations
  (Kondo, AJHG 2000; Trappe, AJHG 2001; Girard, EJHG 2001)

- 90% (54/60) - mutation arose on the paternal X
  - many but not all at CpGs
“Non-Rett” Clinical Phenotypes

• X-linked mental retardation:
  – severe male congenital encephalopathy (Wan, AJHG 1999; Villard, Neurology 2000)
  – severe non-specific XLMR (Orrico, FEBS Lett 2000)
  – XLMR with progressive spasticity (Meloni, AJHG 2001)
  – MR in isolated male cases (2-3%?) (Couvert, Hum Mol Gen 2001)

• male neonatal encephalopathy:
  – no reports of mutations in isolated cases yet

• Angelman syndrome: (no abn involving chromosome 15)
  – (Imessaoudene, JMG 2001; Watson, JMG 2001)
  – ~9% (11/127) had MECP2 mutations
    » most (but not all) retrospectively found to have regressed
Our **MECP2 Mutation Studies**

  - pathogenic mutations in 74% of 234 patients
    (80% classical RTT patients, 70% atypical RTT patients)
  - truncation mutations clinically more severe than missense mutations
  - TRD mutations clinically more severe than MBD mutations
  - higher proportion with skewing of X-inactivation Vs normal controls


- **development of clinical and mutation databases** (J Child Neurol, 2003; Hum Mut, 2003)
Welcome

We welcome you to the IRSA database, which contains information on mutations and polymorphism data from hundreds of published reports. Our database includes a comprehensive data set that has been extracted from various studies and publications and is being sent to us.

A search engine has been developed to facilitate the retrieval of information.

We invite you to:
- Browse mutation and polymorphism data.
- Perform simple or complex searches.
- Submit your unpublished data to us.
- Alert us to publications.
- Offer suggestions and comments.

Acknowledgments

Initial construction and maintenance were funded by The Children's Hospital of Westmead, Sydney, Australia.

<table>
<thead>
<tr>
<th>Directly submitted</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Mutation/polymorphism</th>
<th>Phenotype</th>
<th>Sex</th>
<th>X-inactivation ratio</th>
<th>Detection method</th>
<th>Extent of coding region screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.473C&gt;T</td>
<td>p.T158M</td>
<td></td>
<td>Mutation associated with disease</td>
<td>Rett syndrome - Classical</td>
<td>F</td>
<td>81% : 19%</td>
<td>direct</td>
<td>Part of coding exons 3</td>
</tr>
<tr>
<td>c.473C&gt;T</td>
<td>p.T158M</td>
<td></td>
<td>Mutation associated with disease</td>
<td>Rett syndrome - Classical</td>
<td>F</td>
<td>83% : 17%</td>
<td>direct</td>
<td>Coding exons 1-3</td>
</tr>
<tr>
<td>c.473C&gt;T</td>
<td>p.T158M</td>
<td></td>
<td>Mutation associated with disease</td>
<td>Rett syndrome - Not certain</td>
<td>F</td>
<td>Not known</td>
<td>SSCP</td>
<td>Coding exons 1-3</td>
</tr>
<tr>
<td>c.473C&gt;T</td>
<td>p.T158M</td>
<td></td>
<td>Mutation associated with disease</td>
<td>Rett syndrome - Classical</td>
<td>F</td>
<td></td>
<td>homologous markers on both chromosomes</td>
<td>SSCP</td>
</tr>
<tr>
<td>c.473C&gt;T</td>
<td>p.T158M</td>
<td></td>
<td>Mutation associated with disease</td>
<td>Not Rett synd. - Unaffected family member</td>
<td>F</td>
<td>99 : 1</td>
<td>SSCP</td>
<td>Coding exons 1-3</td>
</tr>
</tbody>
</table>
InterRett

- international study to examine clinical features of RTT
- data are collected from 2 sources
  - Families
  - Clinicians
- data are stored and compiled to produce an output database
  - this will be a searchable form in the future
- funded by IRSA - International Rett Syndrome Association