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)

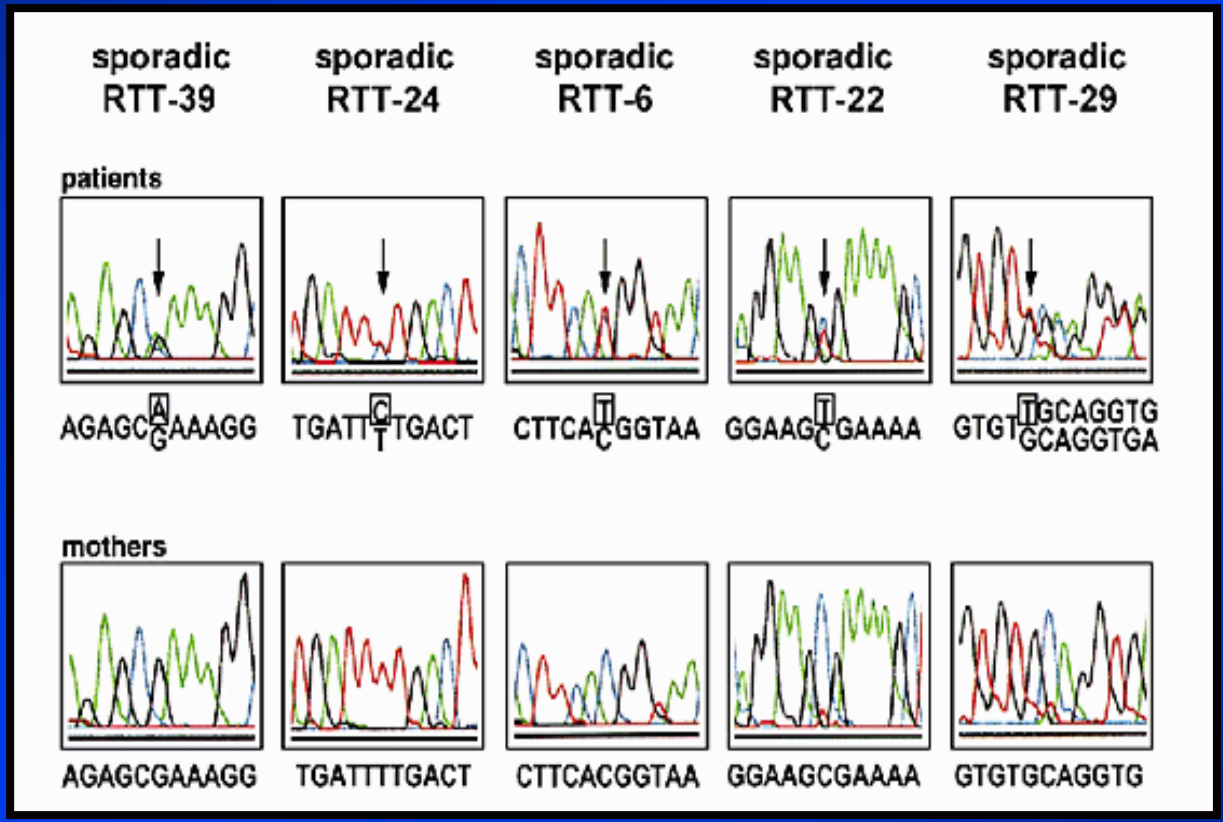
Exclusion mapping in familial cases:

(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"

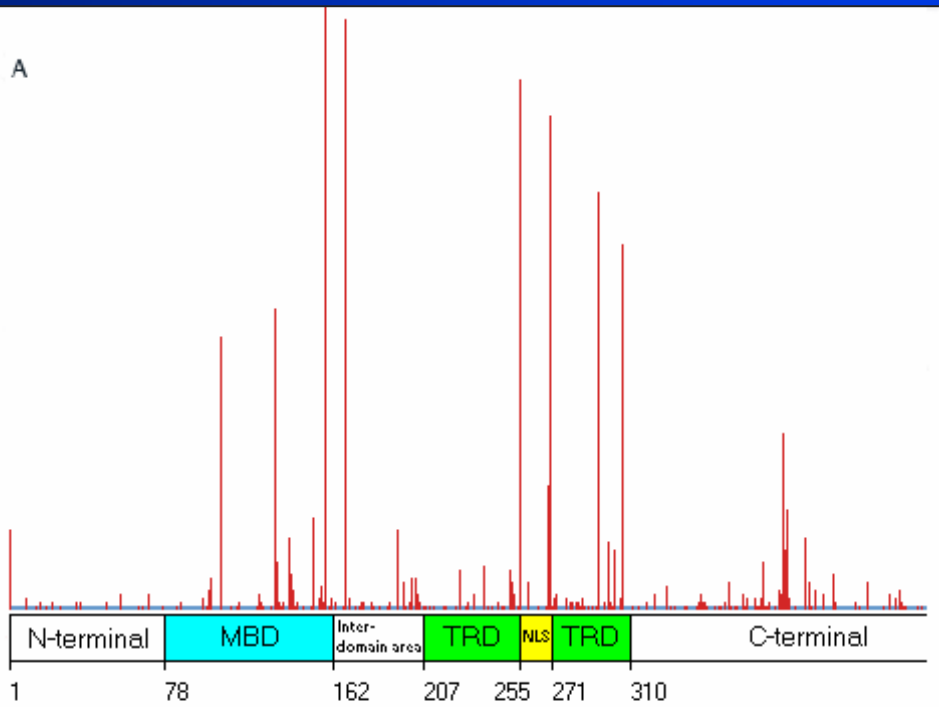
(Amir et al, Nature Genet 1999: 23; 185 - 188)



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



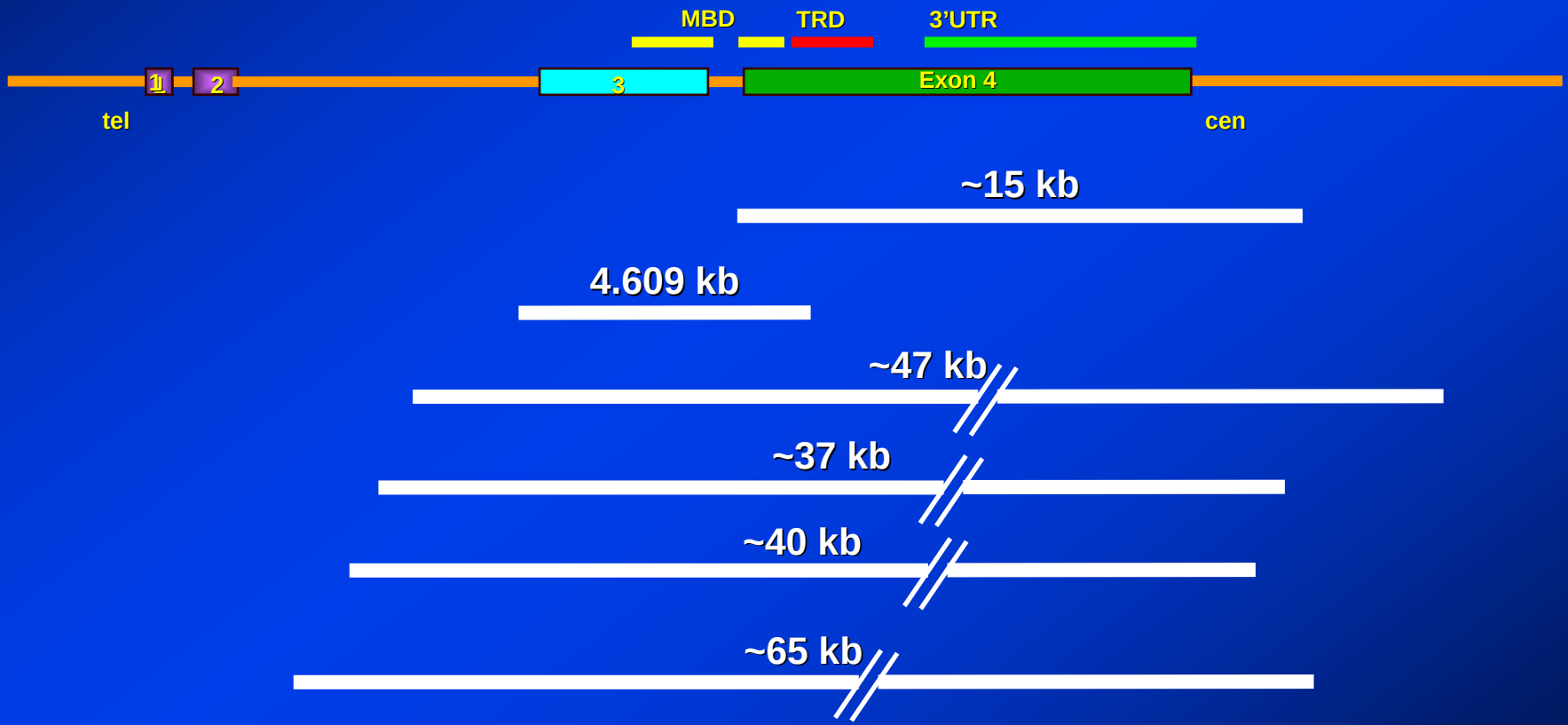
B

Nucleotide change	Amino acid change	Cases reported	Percentage
316C>T	R106W	59	4.80%
397C>T	R133C	67	5.40%
473C>T	T158M	150	12.10%
502C>T	R168X	147	11.90%
763C>T	R255X	124	10.00%
808C>T	R270X	118	9.50%
880C>T	R294X	101	8.20%
916C>T	R306C	79	6.40%
20-100bp deletions		112	9.10%
Total		1236	77.40%

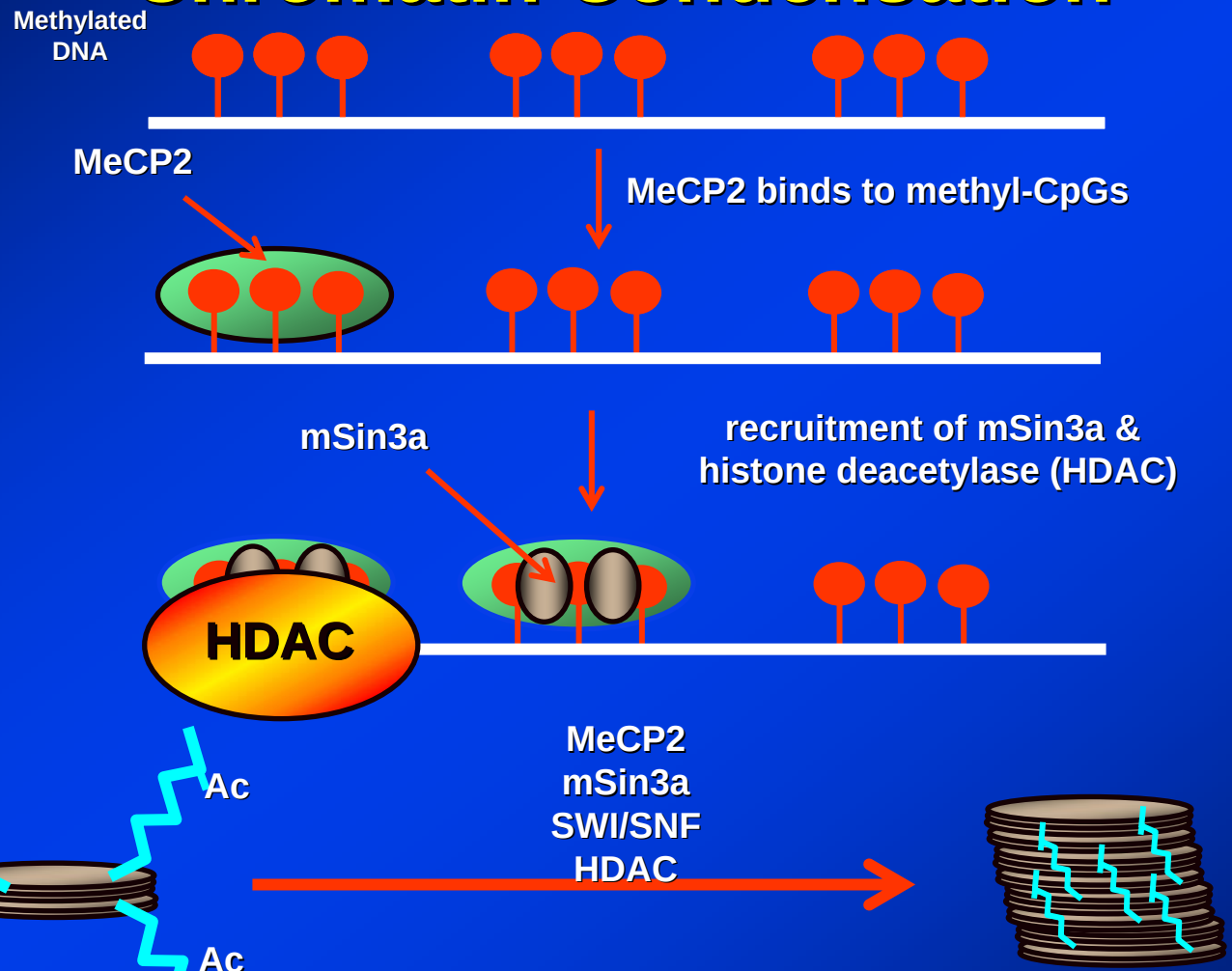
> 200 to date

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

Large Deletions in RTT Patients



Gene Silencing by Chromatin Condensation



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 *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???**

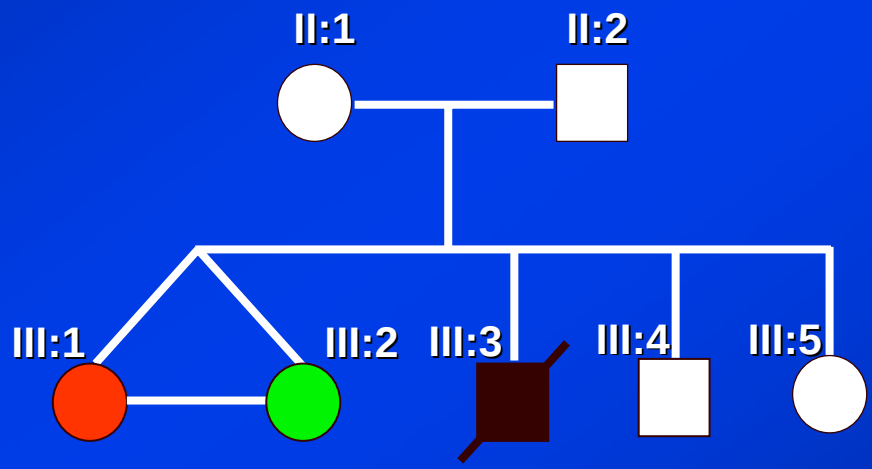
yield seems very low so far

(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

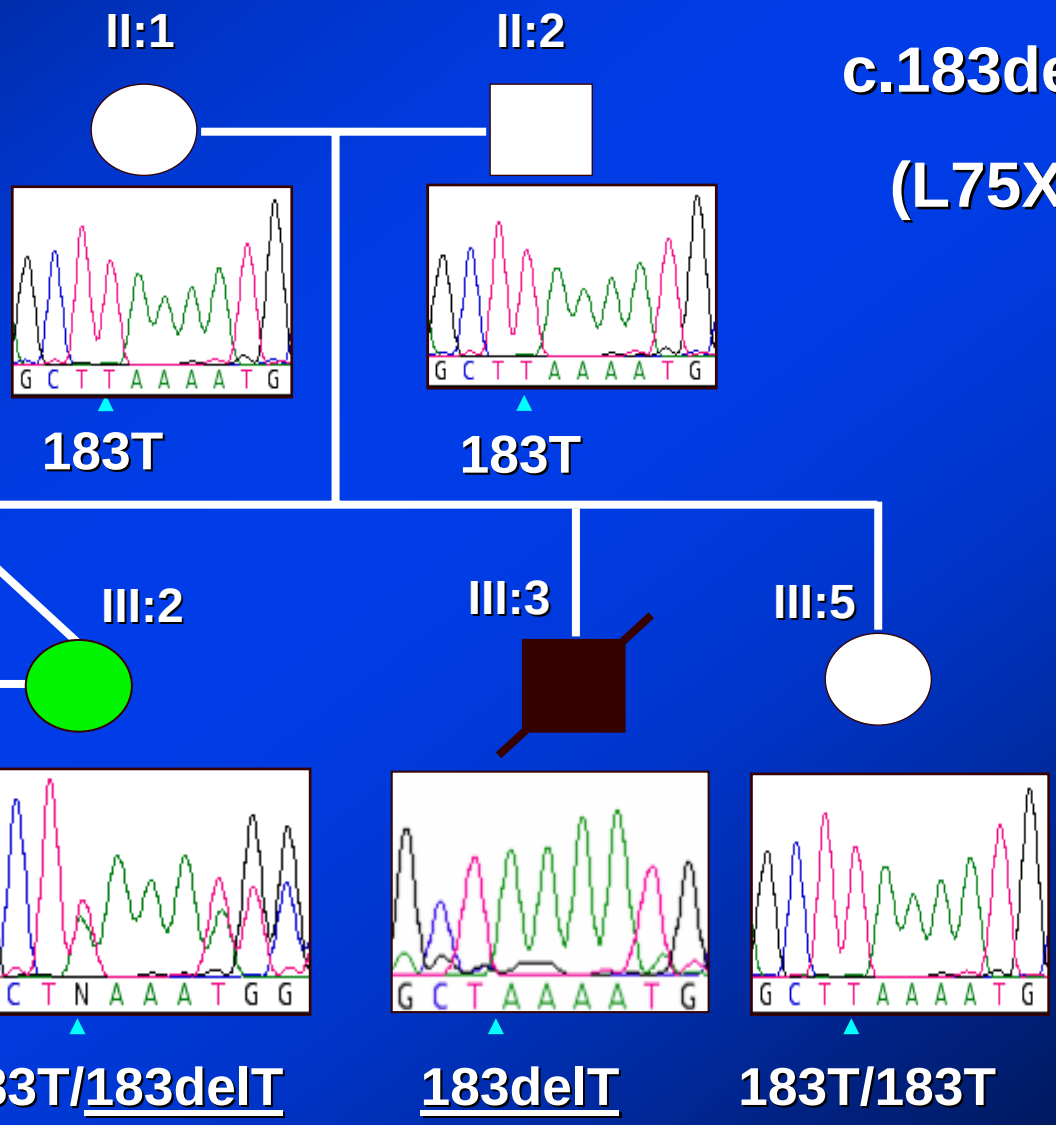
III:3
- infantile spasms in the newborn period
- poor head control
- severe psychomotor retardation
- died age 16 yrs (vegetative, frequent myoclonic jerks)

III:4
- clinically normal brother

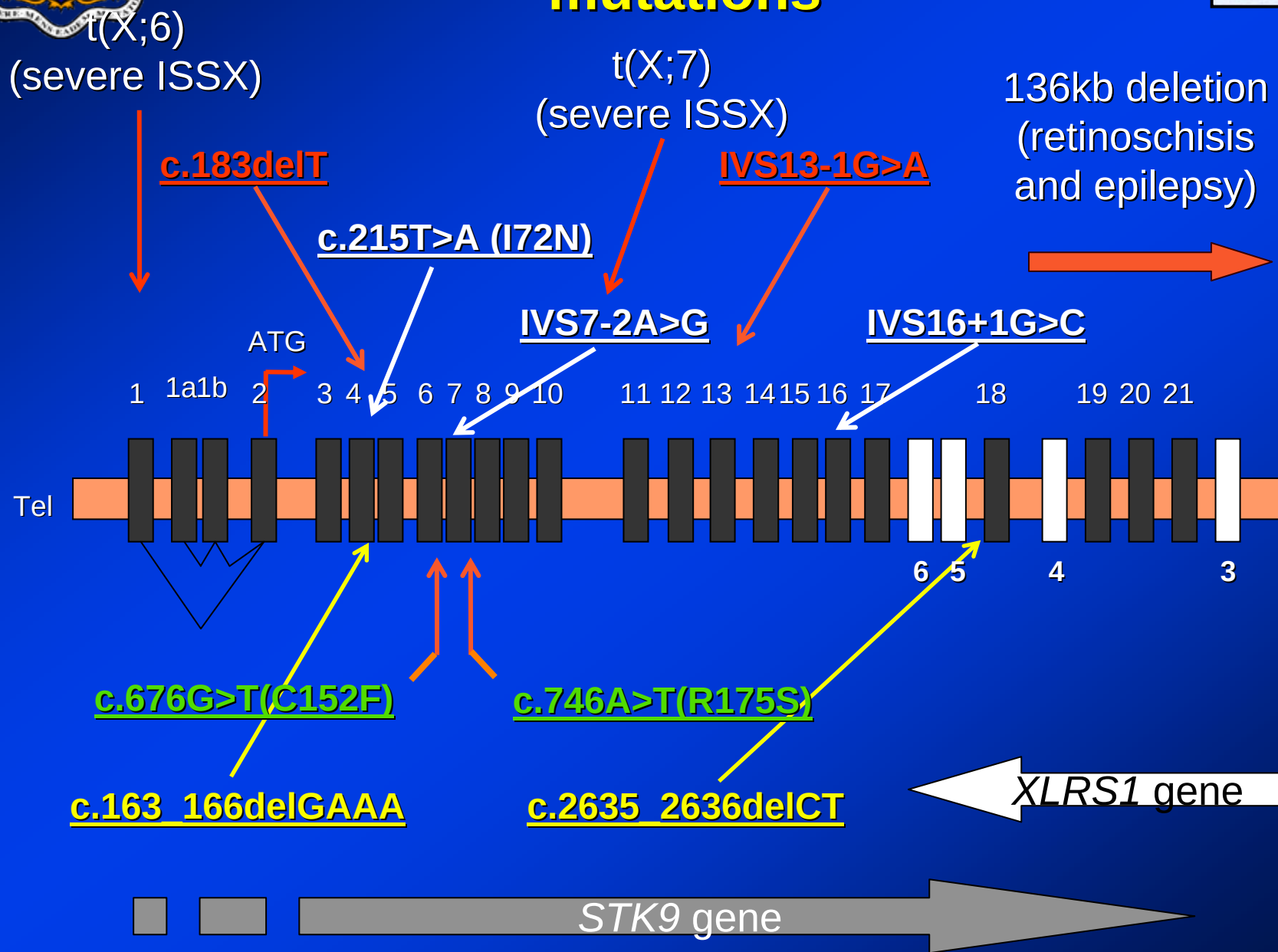
III:5
- clinically normal sister

II:1
- clinically normal mother

CDKL5 Mutation Screening



Summary of currently known *CDKL5* mutations



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


OVARIAN CYST
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

TVW Telethon Research Institute, Perth

Helen Leonard & her APSU team

Baylor College of Medicine, Houston

Huda Zoghbi

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

cytosine —(methyltransferases)→ 5-methyl-cytosine —(spontaneous deamination)→ 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

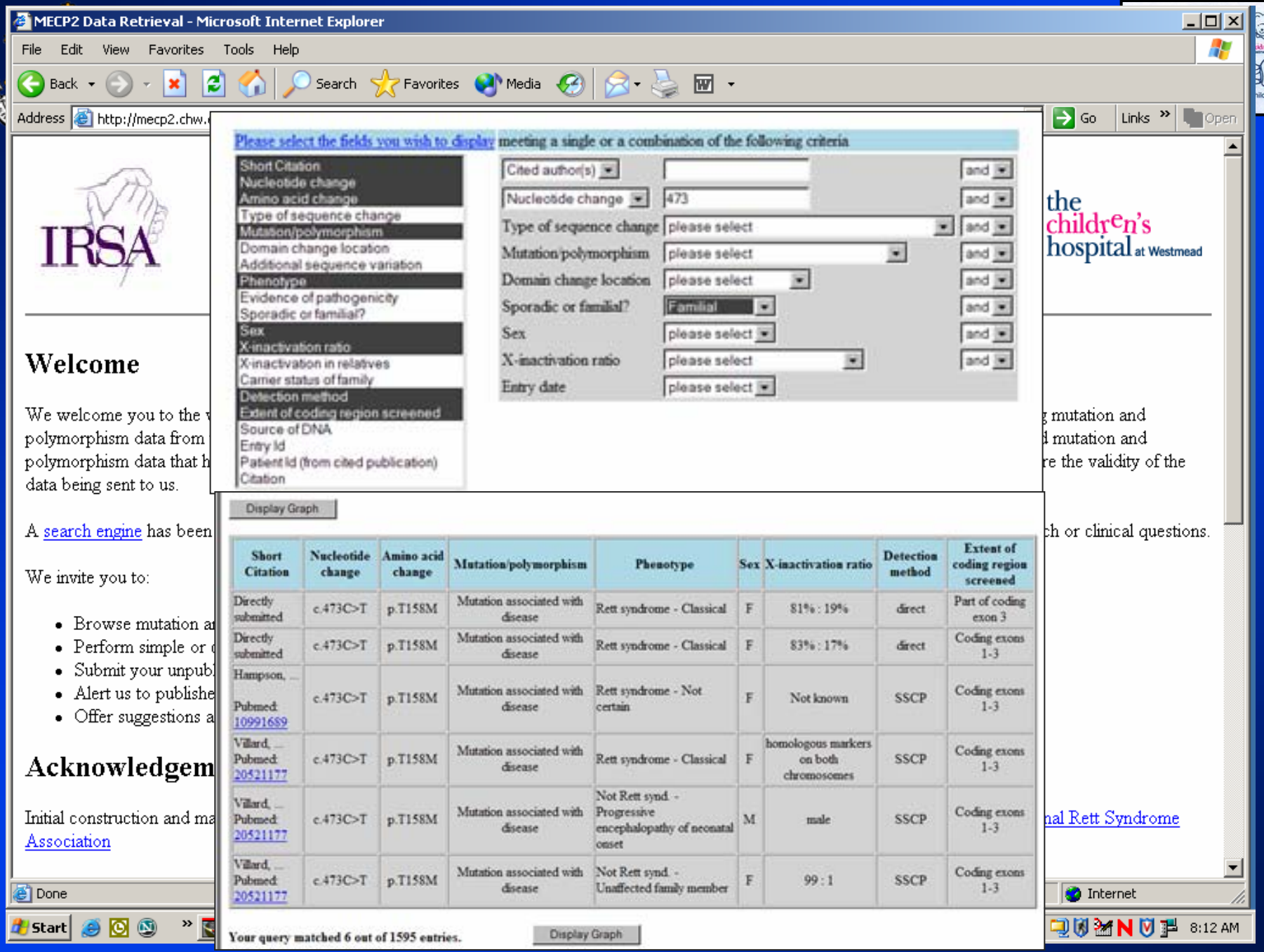
- **X-linked mental retardation:**
  - severe male congenital encephalopathy (Wan, AJHG 1999; Villard, Neurology 2000)
  - severe non-specific XLMR (Orrico, FEBS Lett 2000)
  - XLMR with progressive spasticity (Meloni, AJHG 2001)
  - MR in isolated male cases (2-3%?) (Couvert, Hum Mol Gen 2001)

- **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

- ***MECP2*** **mutation screening of a clinically well-characterised cohort of RTT patients** (Am J Med Genet, 2003)
  - 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
- **detailed evaluations of specific mutations** (J Med Genet, 2003; J Med Genet 2004)
- **development of clinical and mutation databases** (J Child Neurol, 2003; Hum Mut, 2003)


MECP2 Data Retrieval - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites Media
Address http://mecp2.chw.
Go Links Open
IRSA
the children's hospital at Westmead
Welcome
We welcome you to the
polymorphism data from
polymorphism data that h
data being sent to us.
A search engine has been
We invite you to:
Browse mutation a
Perform simple or
Submit your unpub
Alert us to publishe
Offer suggestions a
Acknowledgem
Initial construction and ma
Association
mutation and
mutation and
re the validity of the
ch or clinical questions.
nal Rett Syndrome
Please select the fields you wish to display meeting a single or a combination of the following criteria
Short Citation
Nucleotide change
Amino acid change
Type of sequence change
Mutation/polymorphism
Domain change location
Additional sequence variation
Phenotype
Evidence of pathogenicity
Sporadic or familial?
Sex
X-inactivation ratio
X-inactivation in relatives
Carrier status of family
Detection method
Extent of coding region screened
Source of DNA
Entry Id
Patient Id (from cited publication)
Citation
Cited author(s)
Nucleotide change 473
Type of sequence change please select
Mutation/polymorphism please select
Domain change location please select
Sporadic or familial? Familial
Sex please select
X-inactivation ratio please select
Entry date please select
and
Display Graph
Short Citation | Nucleotide change | Amino acid change | Mutation/polymorphism | Phenotype | Sex | X-inactivation ratio | Detection method | Extent of coding region screened
Directly submitted | c.473C>T | p.T158M | Mutation associated with disease | Rett syndrome - Classical | F | 81% : 19% | direct | Part of coding exon 3
Directly submitted | c.473C>T | p.T158M | Mutation associated with disease | Rett syndrome - Classical | F | 83% : 17% | direct | Coding exons 1-3
Hampson, ... Pubmed: 10991689 | c.473C>T | p.T158M | Mutation associated with disease | Rett syndrome - Not certain | F | Not known | SSCP | Coding exons 1-3
Villard, ... Pubmed: 20521177 | c.473C>T | p.T158M | Mutation associated with disease | Rett syndrome - Classical | F | homologous markers on both chromosomes | SSCP | Coding exons 1-3
Villard, ... Pubmed: 20521177 | c.473C>T | p.T158M | Mutation associated with disease | Not Rett synd. - Progressive encephalopathy of neonatal onset | M | male | SSCP | Coding exons 1-3
Villard, ... Pubmed: 20521177 | c.473C>T | p.T158M | Mutation associated with disease | Not Rett synd. - Unaffected family member | F | 99 : 1 | SSCP | Coding exons 1-3
Your query matched 6 out of 1595 entries.
Display Graph
Done
Internet
Start
8:12 AM

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