“Interrogating Rett Syndrome: developing ideas for research that matters”

Angus Clarke,

Clinical Genetics, Prifysgol Caerdydd, Cymru

Research into Rett Syndrome

Four Factors:

- Unique clinical entity
- Biology is fascinating, intriguing
- The patients (and their families) are waiting
- An effective, rational treatment may just be a possibility

Research THAT MATTERS

The research you would do if your daughter had Rett syndrome:

Research THAT MATTERS:

- Natural History
- Genotype-Phenotype research (and other loci)
- Outcome Measures for Possible Trials
- Molecular Mechanisms
- Potential therapies
- Experiences and Ethics

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- Progress in recognising (? understanding) the underlying pathology
- Recognition of wider range of phenotypes associated with the same pathology
- Appropriate clinical indications for further investigation

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- Diagnostic landscape

Initial Diagnostic Criteria

- Normal development to 6 months
- Developmental stagnation
- Regression - social contact, hand use
- Hand stereotypies
- Recovery of social contact
- Persisting profound cognitive impairment
- Gait and truncal ataxia
- (Only girls)

Other associated features

- Muscle tone, including spasticity in legs
- Ventilatory rhythm
- Vasomotor disturbances including cool, atrophic feet
- Seizures
- Scoliosis
- Impaired growth
  - including 4th metatarsal

Rett syndrome is primarily a ***CLINICAL*** diagnosis

with a highly characteristic timecourse and evolution, although:

(i) recognition of clinical features before 6 months in (otherwise) classic cases,

(ii) molecular studies have shown us that there are variant forms of Rett syndrome

Variant / Atypical forms

- Forme fruste (late stagnation, no regression)
- Preserved speech (Zapella)
- Congenital onset (no regression)
- Early onset of seizures (Hagberg)
- Angelman-like
- Male cases (some with 47,XXY)

How was RTT not “spotted” until 1966 ?

- 6-12 months: “She’ll catch up”
- Acute regression
  - degenerative cerebral disease
  - undiagnosed encephalopathy
  - childhood psychosis or autism
- Dystonic, ataxic or diplegic cerebral palsy; microcephaly and epilepsy

Recognition of the **temporal pattern**

”Early concerns”

Systemic disease

Non-progressive intellectual impairment

? Dysmorphism – dx by genetic test or opinion

Regression – severe autism

- diagnosis by biochemical methods

  Leigh’s disease

- diagnosis by MRI

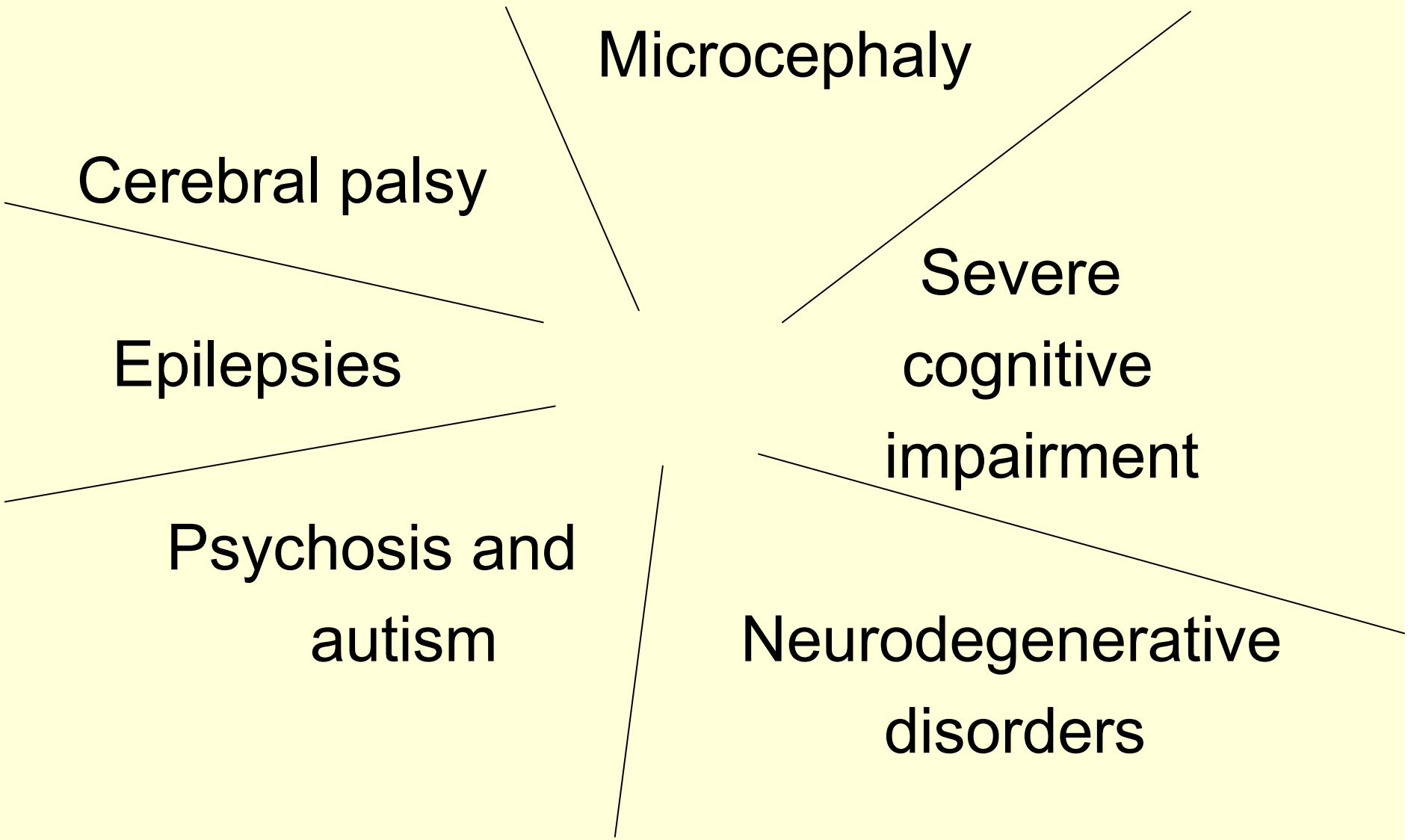
  Brain tumour

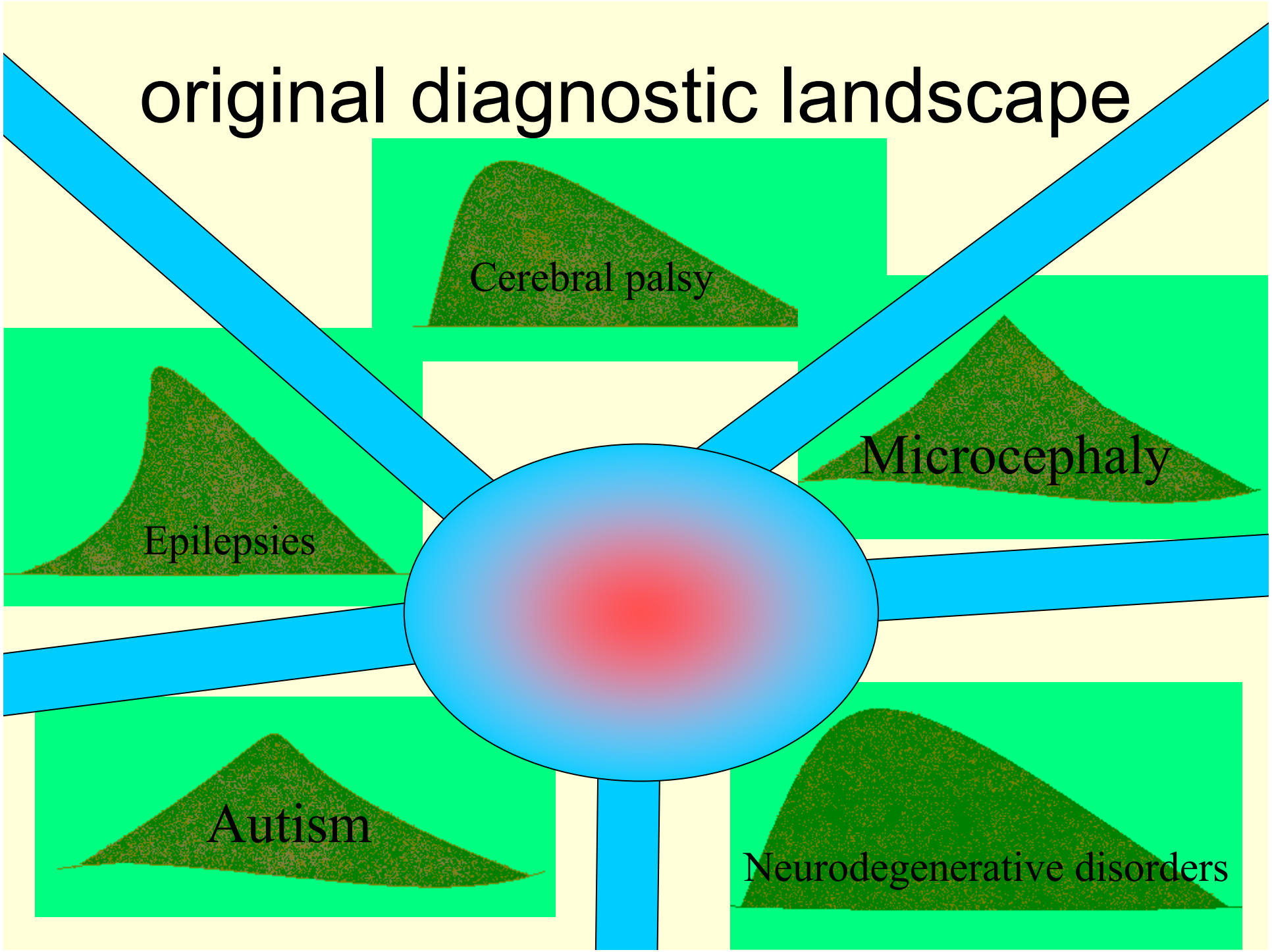
Neurological problem – seizures

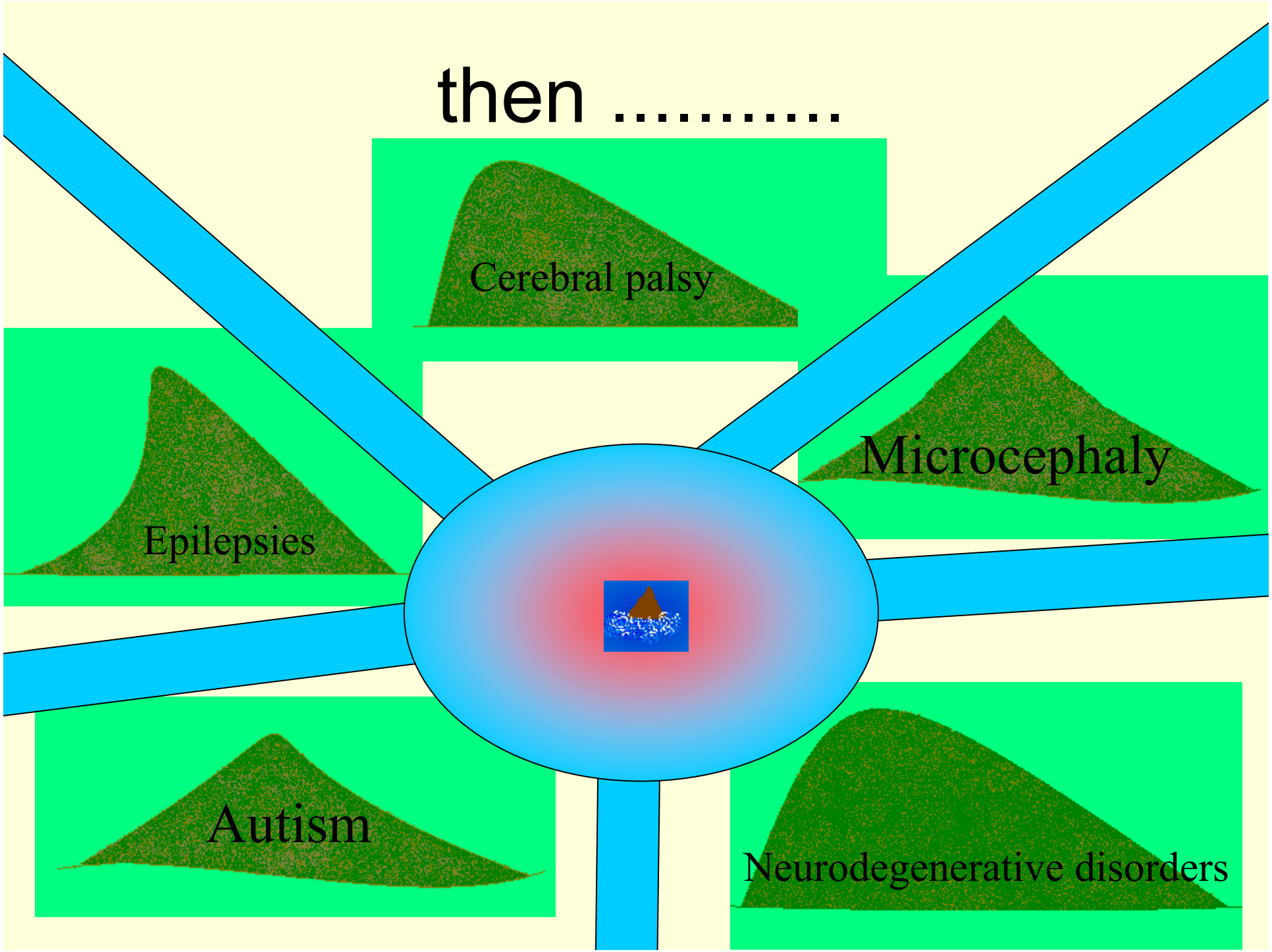
- microcephaly
- cerebral palsy

Socio-emotional problem

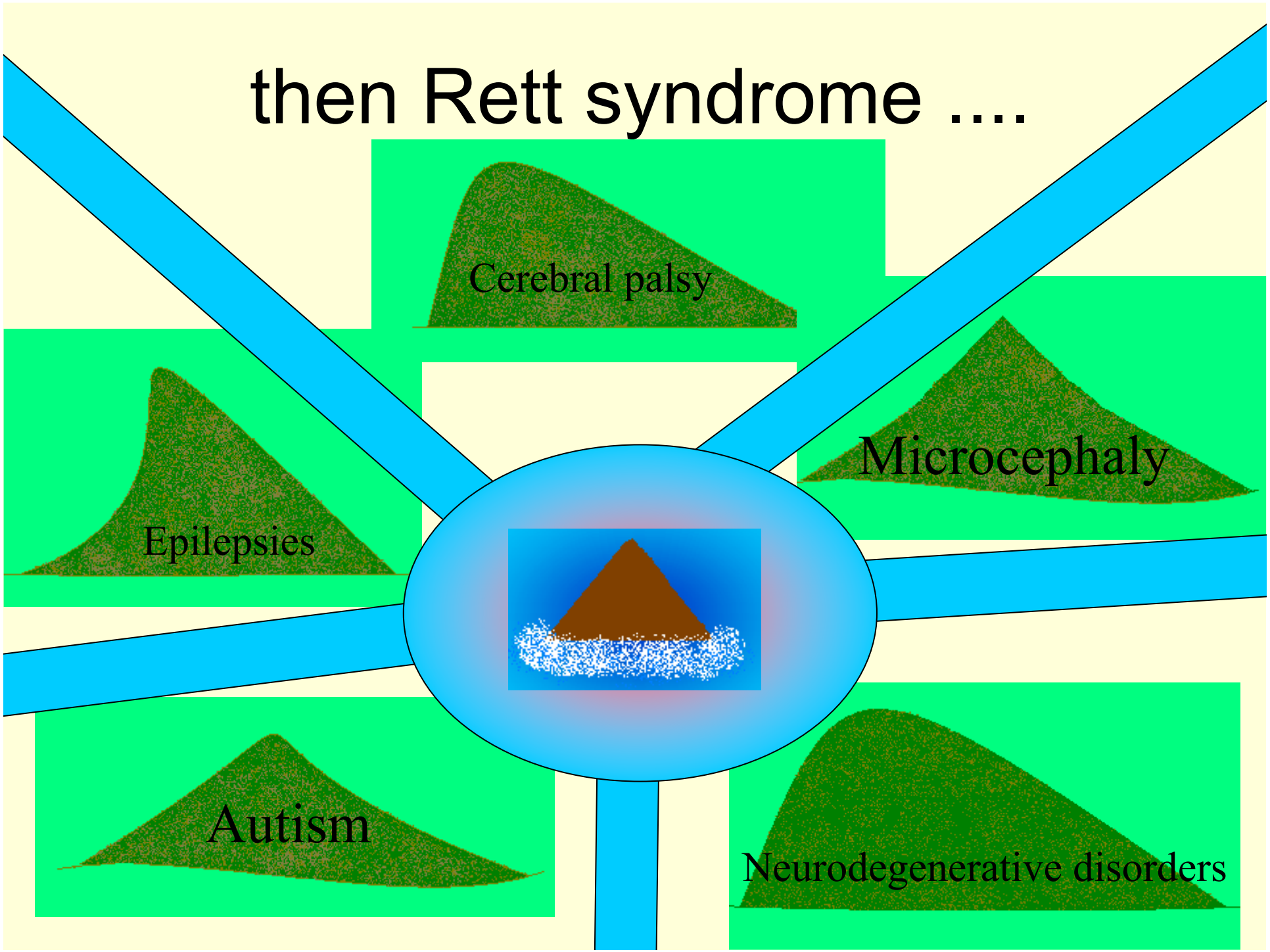
original diagnostic landscape

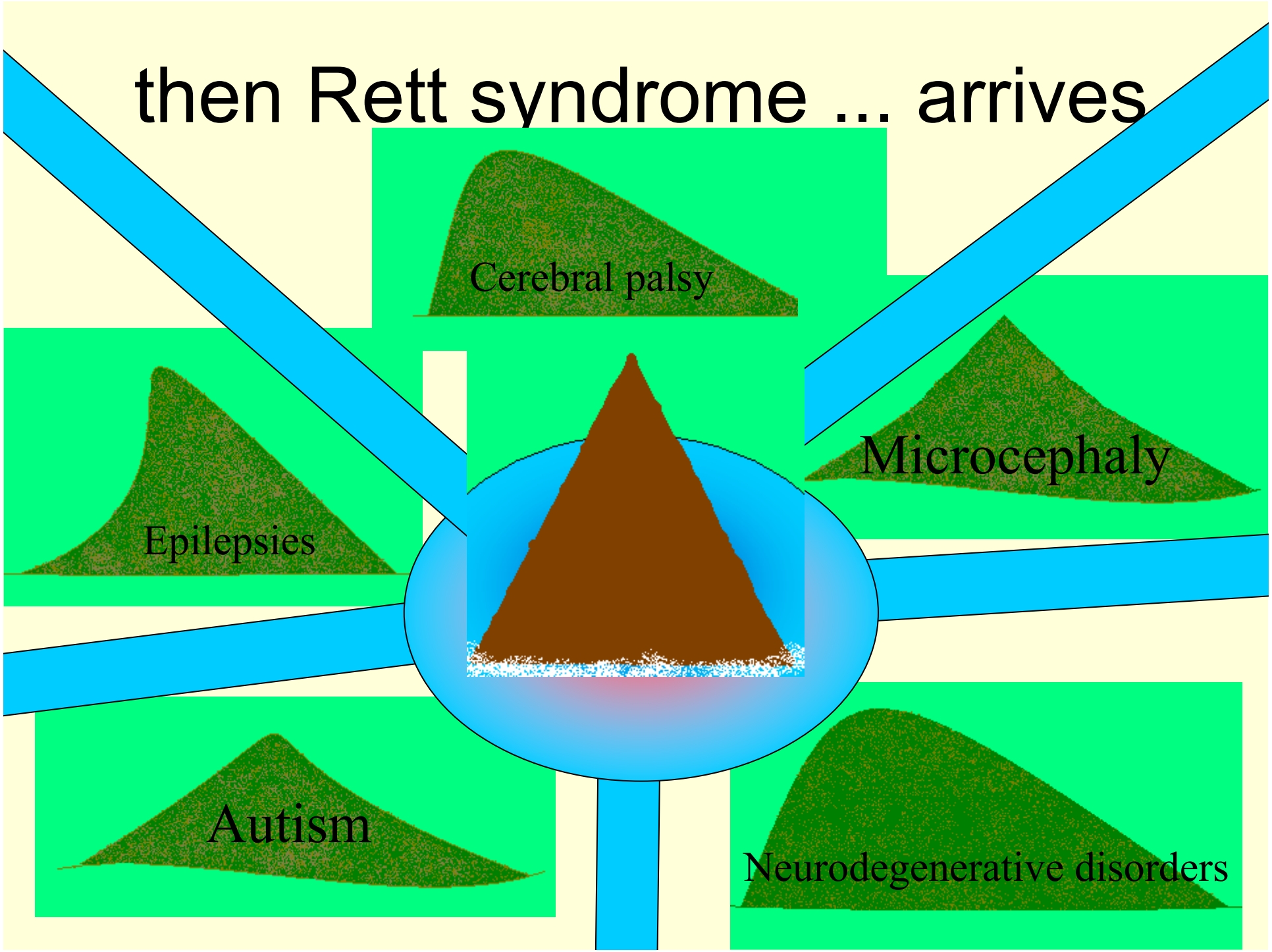



original diagnostic landscape
Cerebral palsy
Epilepsies
Microcephaly
Autism
Neurodegenerative disorders

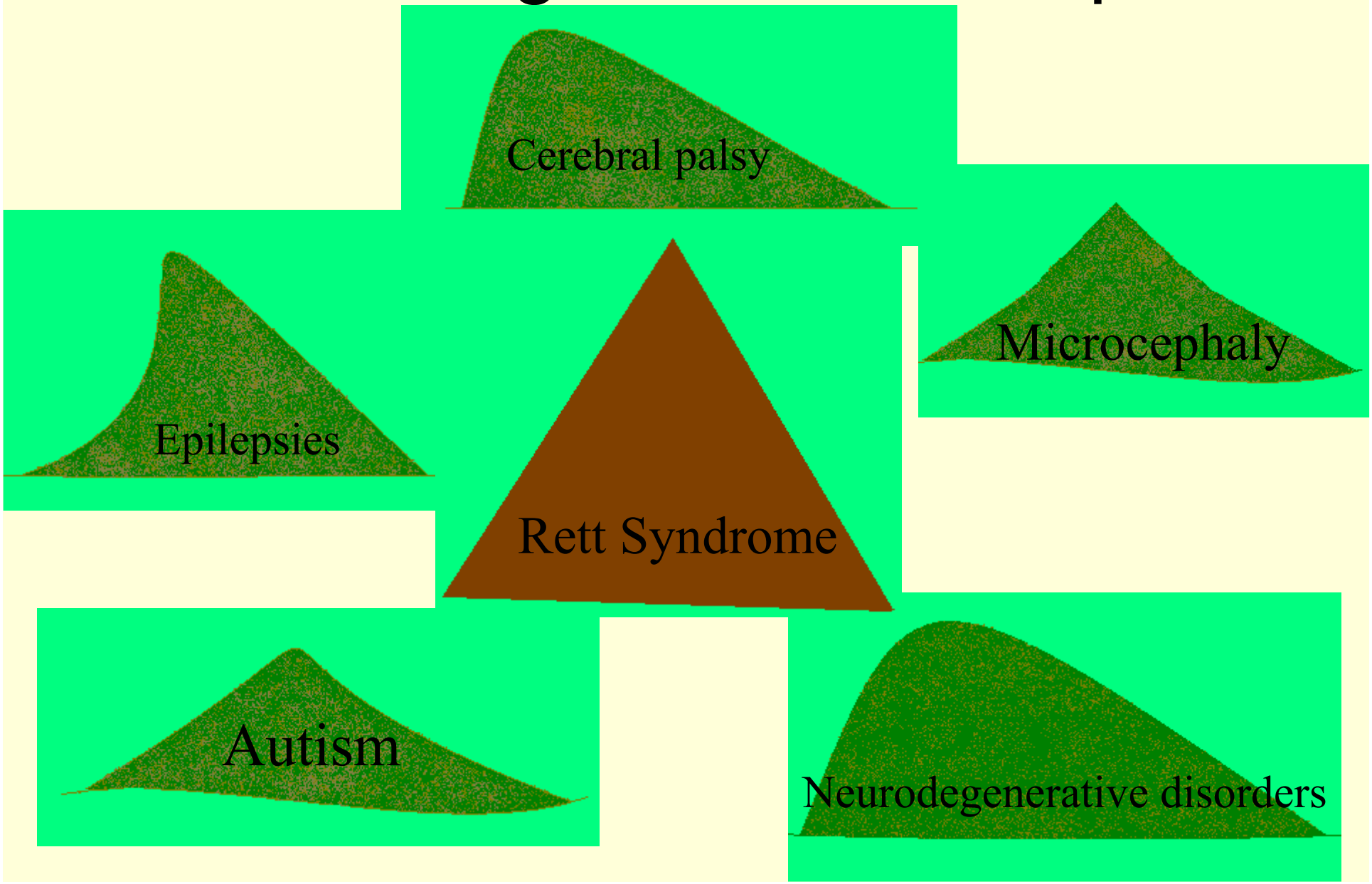

then .....
Cerebral palsy
Epilepsies
Microcephaly
Autism
Neurodegenerative disorders

then Rett syndrome ....

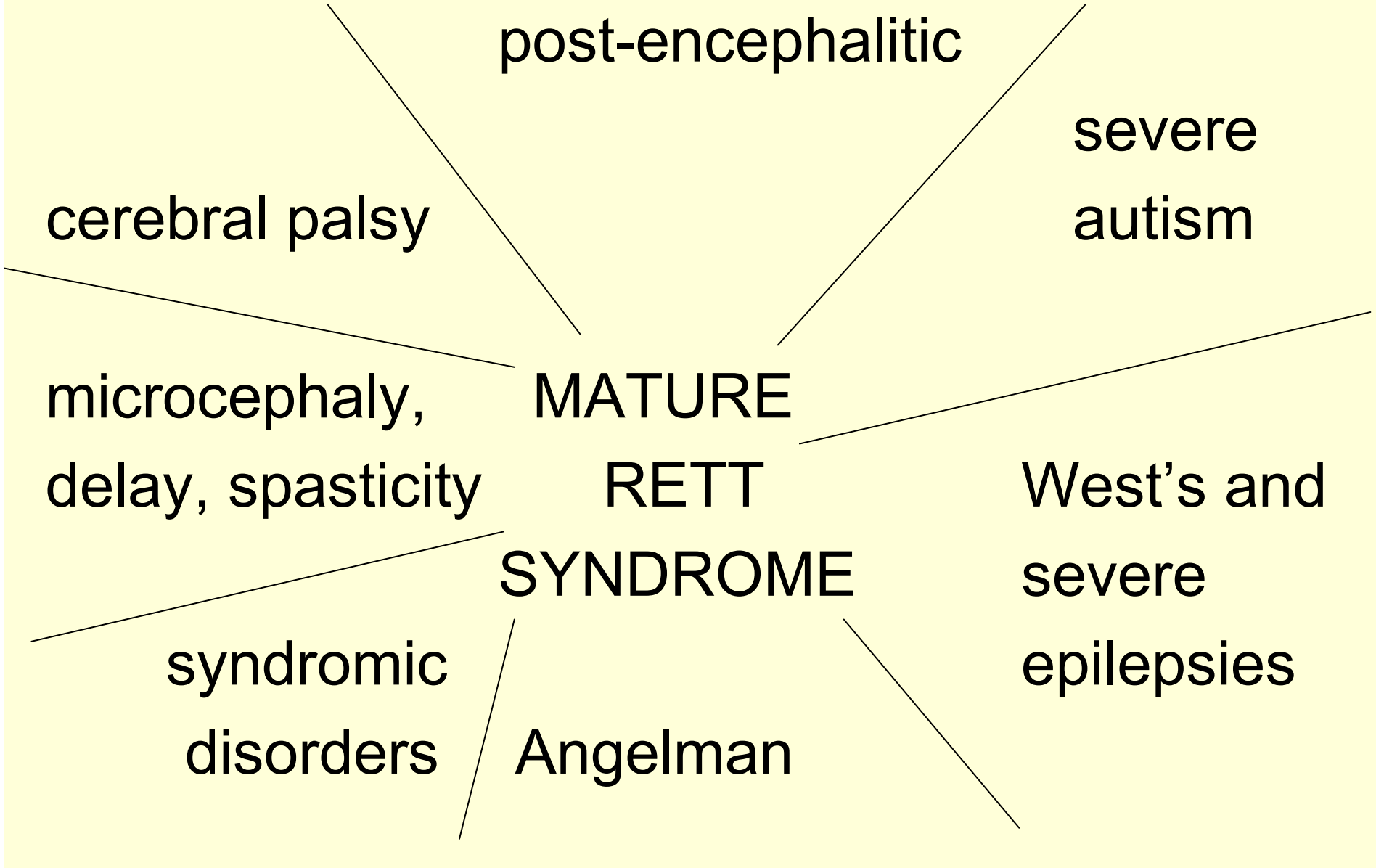



then Rett syndrome ... arrives
Cerebral palsy
Epilepsies
Microcephaly
Autism
Neurodegenerative disorders

revised diagnostic landscape ...



the later diagnostic landscape ...



the diagnostic landscape - 4

”***MECP2* disease**”

(UBE3A)
Angelman

forme – mild – CLASSIC – severe – early - congenital

fruste RTT RTT RTT fits onset

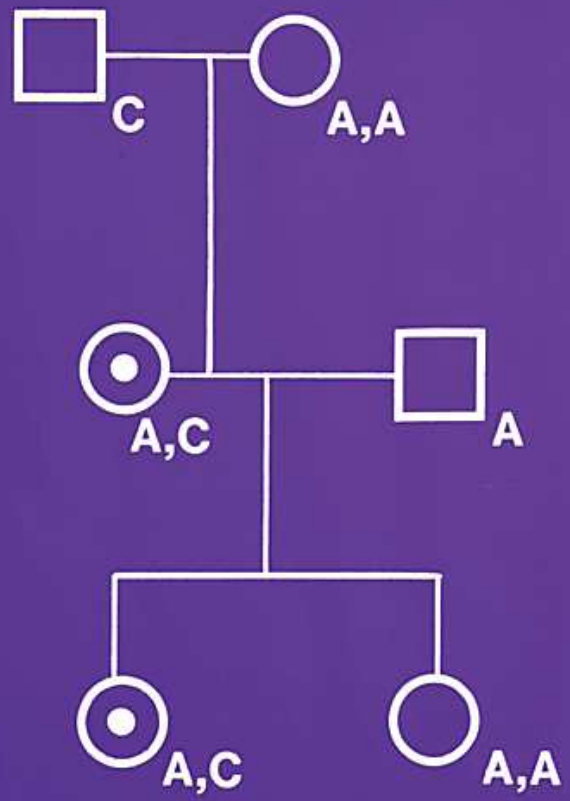
(no regression) (with regression) (no regression)

infantile spasms
(CDKL5)
(TSC1/2)

Cerebral atrophy "*with hyperammonaemia*" ?

- Initial observations not supported
- BUT many cases found to have some metabolic anomalies, although
  - Although often variable and inconsistent
  - Typically an increase in blood lactic acid or ammonia or in 24 hour orotic acid excretion
- One very unusual family:

Rett Syndrome Sisters : Oct study



Search for genetic basis

- X-linked dominant, male-lethal disorder would account for unusual pattern of occurrence
  - Sporadic
  - Occurring only in females

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- Wide clinical variability from X inactivation
  - Apparent in familial cases and MZ twins

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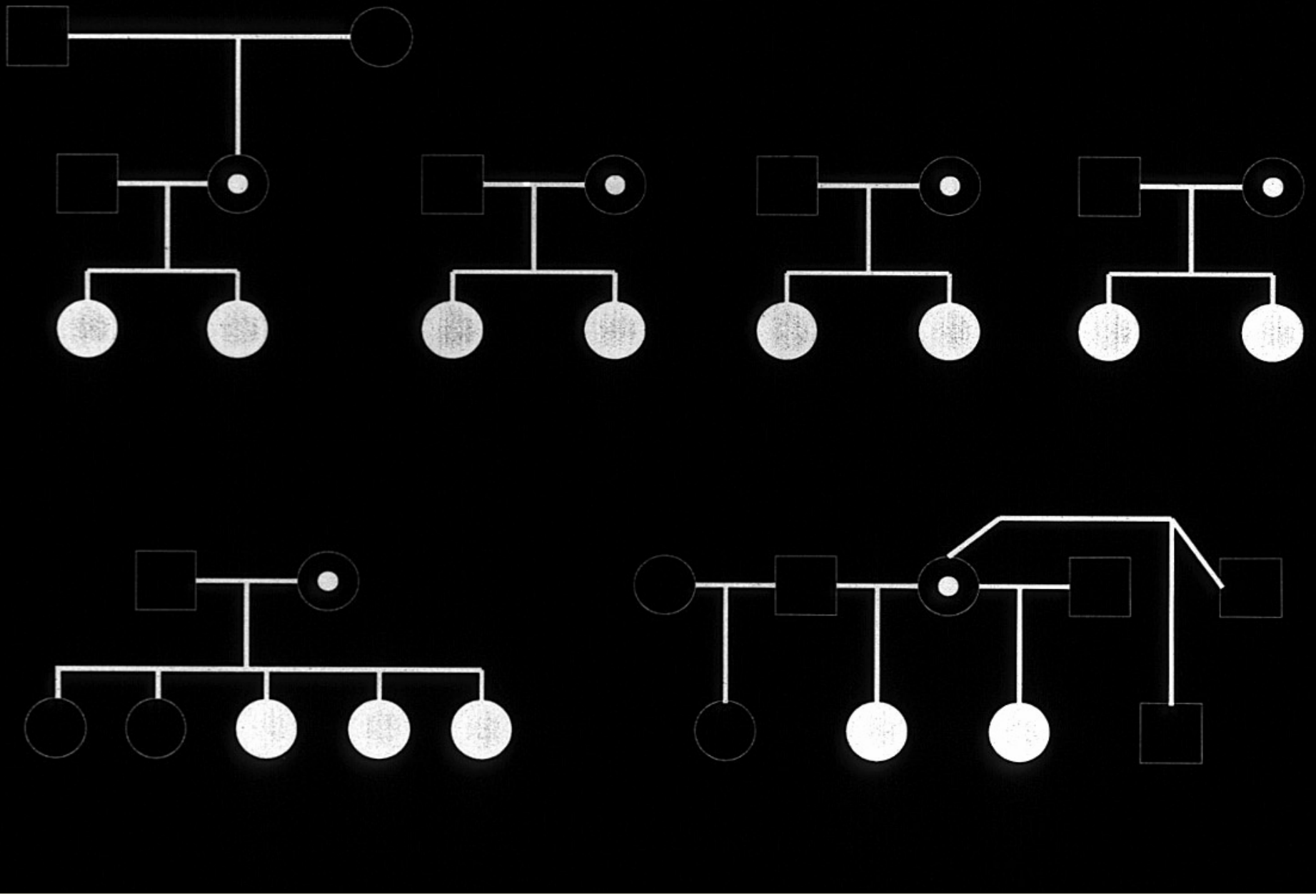
  - Few families
  - Familial cases perhaps atypical

    (criteria *too* relaxed ??)

FAMILIAL RETT SYNDROME: SISTER - SISTER PAIRS



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- Xq28 a likely region
  - Amir et al 1999 => *MECP2* gene

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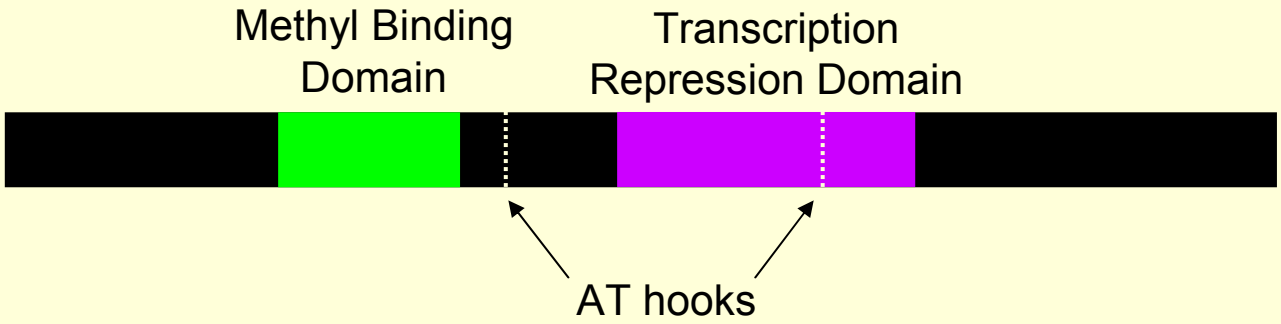
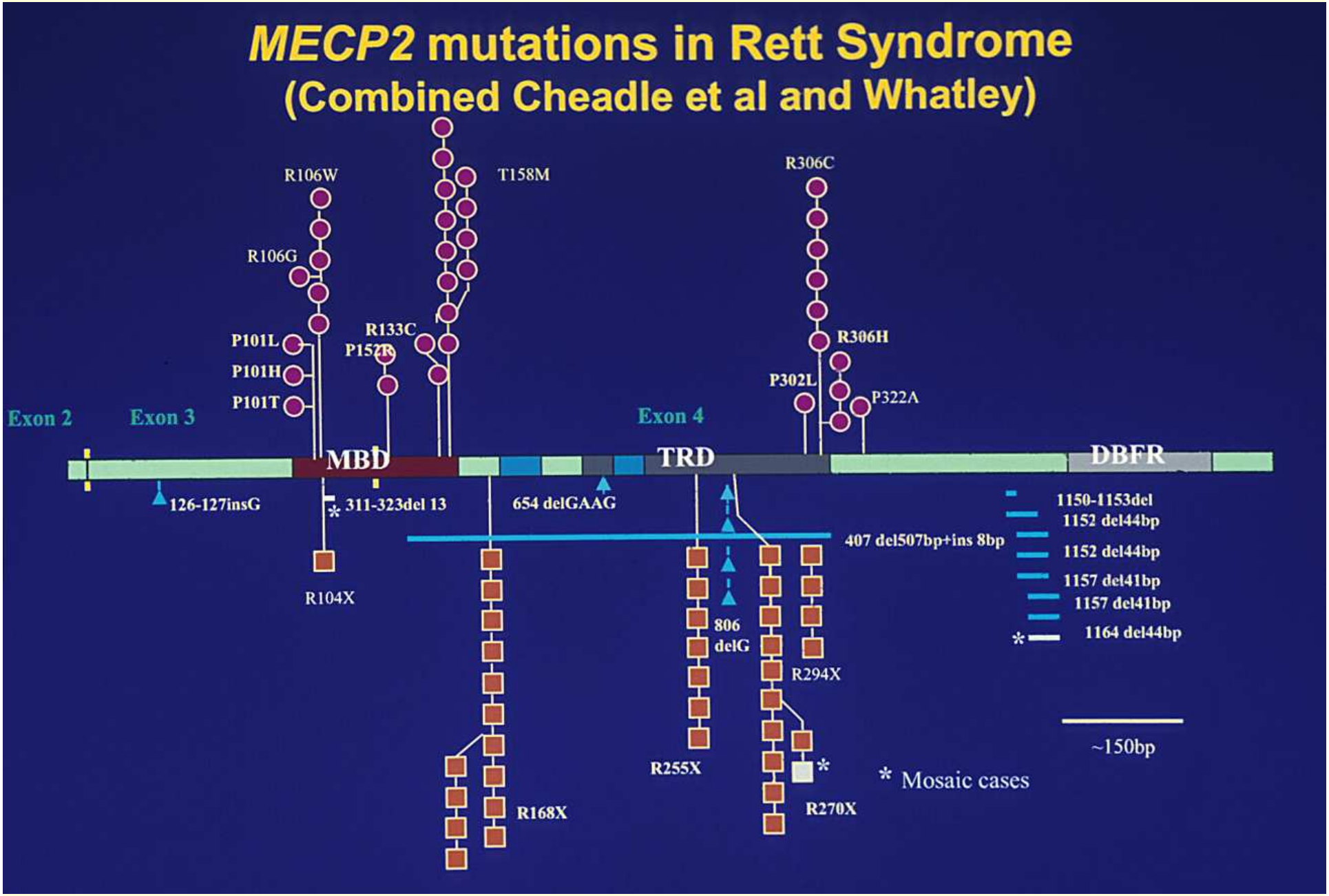
- ***Rapid*** progress in the molecular biology
- ***Steady*** progress in diagnostic utility
- ***Slower*** progress in understanding the pathogenesis

Rett syndrome is caused by mutations in *MECP2*



- Methyl-CpG-binding protein 2
- Global transcription repressor
- Locus at Xq28

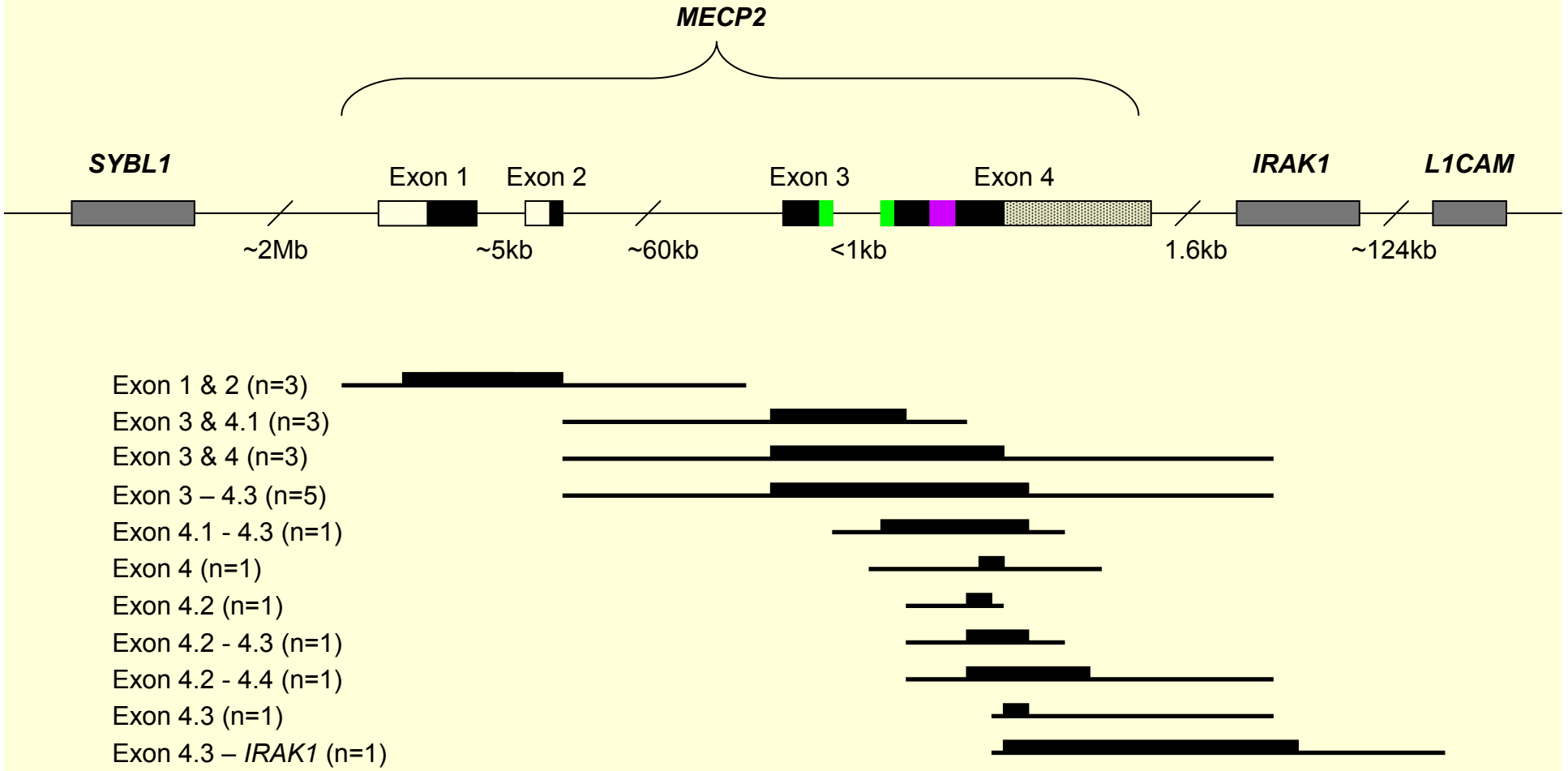
Amir *et al* 1999


MECP2 mutations in Rett Syndrome
(Combined Cheadle et al and Whatley)
R106W
T158M
R306C
R106G
R133C
P101L
P157R
R306H
P101H
P302L
P322A
P101T
Exon 2
Exon 3
Exon 4
MBD
TRD
DBFR
126-127insG
311-323del 13
654 delGAAG
1150-1153del
1152 del44bp
407 del507bp+ins 8bp
1152 del44bp
1157 del41bp
1157 del41bp
1164 del44bp
R104X
806
delG
R294X
R255X
R168X
R270X
~150bp
* Mosaic cases

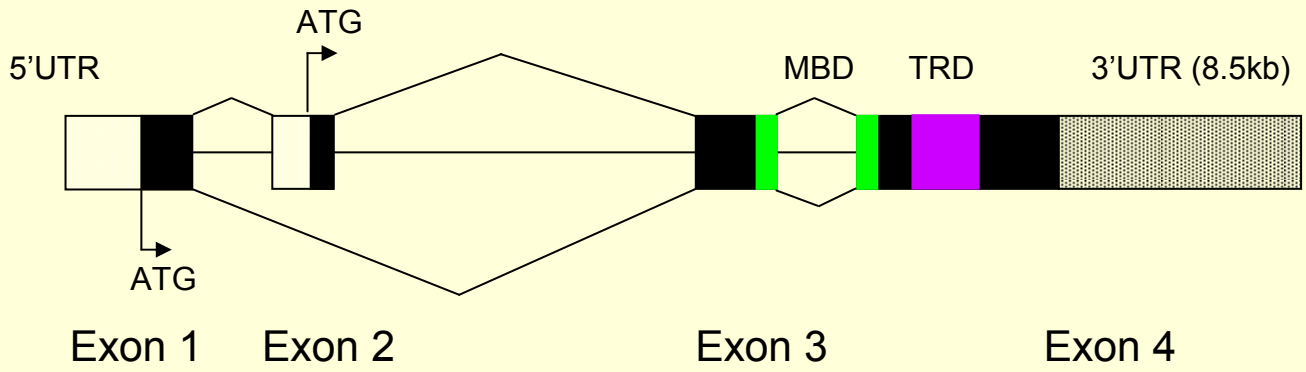
Correlation between the mutation and the disorder

- truncating vs missense mutations
- early truncating vs late truncating mutations
- some common mutations associated statistically with greater or lesser severity
  - R133C and C-terminal deletions milder
  - R270X more severe

Large deletions in *MECP2*



MeCP2 has 2 isoforms



- Previously exon 1 was thought to be non coding
- Now known that exon 1 – containing isoform is 10x more abundant in the brain

Mnatzakanian *et al* 2004, Kriaucionis and Bird 2004

Mild Rett syndrome

- Walk
- Swim
- Ride a bike
- Talk
- Use hands – self-feed, write
- Better growth
- Greater survival
- But significant learning disability

How do the mutations cause the disease ?

- MeCP2 deficiency => ~2 fold up-regulation of many genes (Ballestar *et al* 2005)
- Rett syndrome is a disease of “chromatin configuration” that “should” have global consequences
  - hard to understand how mutation leads to the very ***specific*** disease phenotype

Some of the MeCP2 target genes

- *UBE3A/GABRB3* (Samaco *et al* 2005, Makedonski *et al* 2005)
  related to Angelman phenotype
- *BDNF* (Chen *et al* 2003, Martinowich *et al* 2003)
  neuronal plasticity, eating behaviour
- *DLX5* (Horike *et al* 2005)
  silent chromatin loop, GABA synthesis
- *FMR1* (Harikrishnan *et al* 2005)
- *Hairy2a* (Stancheva *et al* 2003)
- Glucocorticoid response elements

Diagnostic Applications of *MECP2* testing

- Classical Rett Syndrome

  >85% mutations

- 'Atypical' Rett syndrome

  50% mutations

- Early seizure variant

  <10% mutations, nil (so far) with infantile spasms

- Is the mutation pathogenic ?

  – *de novo* ? synonymous ? conserved ?

  – present in healthy male ?

Family Consequences of Mutation Testing for RTT

- Confirmation of diagnosis
  - Reproductive confidence in face of mosaicism
  - But still an emotional kick
- "Disconfirmation" of diagnosis
  - An anomalous category
  - A different emotional kick
- "Disconfirmation of normality" when *MECP2* mutation found in absence of RTT

Diagnostic Test => 2 x 2 Table

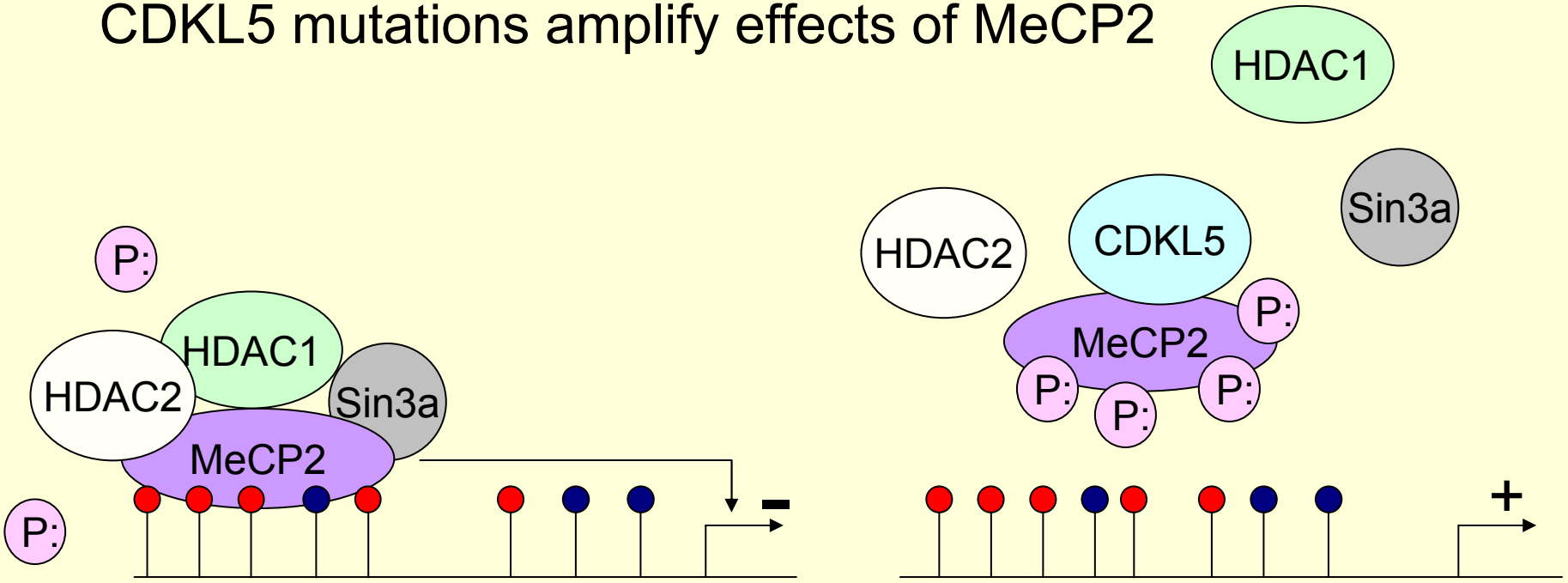
Mutation Test / Clinical assessment	Test Positive: mutation found	Test Negative: mutation ***NOT*** found
Clinical diagnosis: typical or 'atypical' case of RTT	expected	***! New and anomalous***
Clinical diagnosis: **NOT** typical of RTT	***! New and anomalous***	expected

No (*MECP2*) mutation ? …

No *MECP2* mutation ? …

- Look harder in *MECP2* (promoter, 3'UTR, miRNA binding sites, …)
- In the mouse, duplications of *MECP2* in males associated with some features of RTT
- In boys, duplications of Xq28 associated with delay (and ? Some features of RTT)
  - Duplications including *MECP2, filamin A, …*
- In girls, *CDKL5* gene disrupted in girls with infantile spasms and ? RTT or autism
  - Mutations in 3/20 girls with early seizure variant of RTT, including infantile spasms (15%)

Presence of CDKL5 results in phosphorylation of MeCP2, releasing it from the methylated CpGs – CDKL5 mutations amplify effects of MeCP2



Inactive state

Active state

Pathogenesis

What could plausibly count as an “explanation” of RTT pathogenesis ?

RTT Pathogenesis

- Descriptive explanations

  Pattern recognition

  = natural history

- Mechanistic / Linear explanations

  A => B => C => D; upstream and target loci

  = science

- Systemic explanations

  Complex web of interactions; neuronal plasticity

  ……. = ? despair or reality ?

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- Mutations in *MECP2* can be associated with classic or atypical RTT, non-RTT, or normal female phenotypes
- Mutations at other (interacting?) loci result in related disorders

(BUT <u>do</u> remember that families can suffer distress from - or during - our attempts to understand the disease)

Back to research areas:

Natural History

- Ageing – pattern of problems
  - Value of surveillance
  - Need to monitor osteoporosis ?
  - Neurophysiological changes (spasticity and late motor deterioration)
  - (Very) late deterioration ?
- Response to conventional treatments:
  - Seizures, ‘Rett episodes’
  - Scoliosis
  - Agitation and mood
  - Environmental triggers for episodes, behaviours

Genotype-Phenotype research and other loci

- We do not need a single, ‘global’ Rett syndrome database
- Joint (even global) collaborative analysis of anonymised data is possible without a global database – ***but needs coordination***
- The fact of X chromosome inactivation makes a focus on genotype-phenotype analysis futile for ***predictive*** prognosis of the individual case

InterRett (and EuroRett?)

- Can coordinate analyses of pooled, anonymised data from national registers
- Need to support, strengthen and coordinate the national databases and registers:
  - Data quality: checking, updating, …
  - Compatibility of IT systems and data fields
  - Links with Family Support Groups
  - Identify potential participants in therapeutic trials **within a country**

Genotype-Phenotype research: modifiers and other loci

- Phenotypic subgroups are emerging

  CDKL5, FOXG1, …
- Remember modifying polymorphisms as with BDNF: ? effect of other loci, eg HIPK2, PARP1.
- SNPs and CNVs – as modifiers within mutation categories –

  BUT may need parent-of-origin information
- CNVs may identify new loci

Progression and Outcomes for Clinical Trials

- Seizure severity and activity scale
  - Include “Rett episodes” and “challenging behaviours”
- Scoliosis
- Agitation, mood
- Nutrition
- Cognition

Neurophysiological outcome measures

- Transcranial magnetic stimulation (with surface EMG on biceps, triceps, ….)
- Sensory evoked potentials
  - SSER, ABR, VER
- Rett episodes and autonomic events
  - System of carer scoring severity
  - Monitoring respiratory patterns

Molecular Mechanisms

- Neuronal iPS cells … as best material for ChIP on chip: where does MeCP2 bind ?
- Detailed studies of MeCP2 interaction with other DNA sequences and proteins
- miRNA and the 3'UTR
- Synapse biology + proteome …

Potential therapies

- Read-through of stop codons, PTC124 …
- TAT-MeCP2 fusion protein …
- High-throughput screening of small molecules
- ‘Transmitters’ etc given orally or IM

- WHAT IF a treatment was really very nearly ‘complete’

Experiences, Ethics and Engagement

- Family experiences => a needs assessment
- Ways of helping RTT support groups from becoming too optimistic about progress
- Preparation of family support groups to give consent for clinical trials
  - equipoise
  - concept of the RCT
- Imagine the experiences of patients undergoing treatment. How do we prepare for this ?

Ideas to Forget ??

- More and bigger genotype-phenotype studies of each mutation
- Mapping disease features to specific areas of the brain ???

  (is there such a 1:1 correlation ?)
- Osteoporosis mechanisms - unless this really is a major clinical problem …

Acknowledgements

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- Drs Hayley Archer and Julie Evans
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