Recent Advances in Rett Syndrome

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Bibliography:

Clinical Care:

1. The Rett Syndrome Handbook, available in the website of the International Rett Syndrome Foundation:
   http://www.rettsyndrome.org/family-support/newly-diagnosed/the-rett-syndrome-handbook


Clinical Outcome and medical Issues

Baikie, G., M. Ravikumara, J. Downs, N. Naseem, K. Wong, A. Percy, J. Lane, B. Weiss, C. Ellaway, K.


Genetic


Therapy research


BDNF- IGF-1


Other MeCP2


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RETT SYNDROME UPDATE

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Learning Objectives

- Brief Review Of Diagnosis and last diagnostic criteria 2010
- Key Research Studies on Rett
- Brief description MeCP2 function
- Promising Research Advances on Treatment

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History of Rett Syndrome

Dr. Andreas Rett identified females who had a unique pattern of Neurodevelopment in 1966.
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History of Rett Syndrome

- Dr. Andreas Rett, first description 1966
- Diagnosis Criteria
  - 1994 Rett Syndrome is included in DSM IV as a PDD
- Rett Syndrome was removed from DSM V

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Diagnostic Criteria 2010-Main criteria

1. Partial or complete loss of acquired purposeful hand skills
2. Partial or complete loss of acquired spoken language
3. Gait abnormalities: Impaired (dyspraxic) or absence of ability.
4. Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms
5. Consider diagnosis when postnatal deceleration of head growth observed


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Diagnostic Criteria 2010

Required for typical or classic RTT
1. A period of regression followed by recovery or stabilization
2. All main criteria and all exclusion criteria
3. Supportive criteria are not required, although often present in typical RTT

Required for atypical or variant RTT
1. A period of regression followed by recovery or stabilization
2. At least 2 of the 4 main criteria
3. 5 out of 11 supportive criteria

ANN NEUROL 2010;68:944–950, Jeffrey L. Neul, MD, PhD et al.
Diagnostic Criteria 2010

Supportive criteria for atypical RTT
1. Breathing disturbances when awake
2. Bruxism when awake
3. Impaired deep pattern
4. Abnormal muscle tone
5. Peripheral vasomotor disturbances
6. Seizures/convulsions
7. Growth retardation
8. Small cold hands and feet
9. Inappropriate laughing/screaming spells
10. Diminished response to pain
11. Intense eye communication - “eye pointing”

Exclusion criteria for typical RTT

- 1. Brain injury secondary to trauma (peri- or postnatally), neurometabolic disease, or severe infection that causes neurological problems
- 2. Grossly abnormal psychomotor development in first 6 months of life
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Staging system for classical Rett syndrome.


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History of Rett Syndrome

- MeCP2 identified in 1999 in Huda Zoghbi’s lab
  Amir et al, Nat Genet 1999;23:185–188

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MeCP2 mutations

- MeCP2 contains three functional domains:
  1. A methyl-binding domain (MBD) on the N-terminus allowing binding to DNA
  2. A nuclear localization sequence allowing trafficking of MeCP2 to the nucleus
  3. A transcriptional repression domain (TRD), which modulates gene transcription.

- 80% have MeCP2 mutation:
  - Missense mutations: 39%
  - Nonsense mutations: 28%
  - Frame shift mutations: 17%
  - Large deletions: 14.5%
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2001 - KO mice (MeCP2⁻⁻) at Huda Zoghbi lab

- Develops progressive symptoms
  - Paws wringing movements
  - Poor motor coordination
  - Smaller brain
  - Abnormal breathing
- Early death

Shahbazian MD, et al.

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Brain changes in Rett Syndrome

- Decreased size of the neurones
- Immature Neurones
- Smaller Nucleus
- Reduced Dendrites
- Reduced synapsis
- Reduced spines.

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2007. Dr. Adrian Bird’s Mice model

- Mecp2 gene is silenced by insertion of a lox-Stop cassette
  - Mice develops Rett
  - Abnormal gait, hind-limb clasping, tremor, irregular breathing, and poor general condition
  - Early death
- MECP2 is activated with Tamoxifen
  - When MECP2 is activated with Tamoxifen
    - Increase MeCP2 in the brain
    - Decreased symptoms, long survival

Guy, J et al. Science Vol313, 1143-1147
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MeCP2 Reversal of deficit
Guy, J et al. Science Vol313, 1143-1147

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Male
Female

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MeCP2 Reversal of deficit
Guy, J et al. Science Vol313, 1143-1147

Developmental absence of MeCP2 does not irreversibly damage neurons.
MeCP2 function

**MeCP2**
- Binds to DNA
- Activates or inhibits transcription
- Increased MeCP2 → Increased DNA Methylation
- Methylation is associated with gene inactivation
- Binds to BDNF exon IV
- Binding is methylation-dependent

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Fig. 1. Significant gene expression changes in hypothalamis of MeCP2 mouse models.
Chahrour M et al. Science 2008;320:1224-1229
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Chahrour M et al. Science 2008;320:1224-1229

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MeCP2

- MeCP2 is a vertebrate invention
- MeCP2 is involved in brain plasticity
- In MeCP2- development is OK when experience is not important
- MeCP2 is more critical in older mice
- Tam-Cre model at different ages
- Other proteins are MeCP2 partners (N-Cor, Sind3A, GPS2 and others)

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Developmental Stages

- Early Stages in Rett results from Glutamate-mediated excitotoxicity during synaptogenesis
- Late phase hypo-connectivity of excitatory circuit
- Therapies may need to be different at different stages of the disease

Colleen Niswender, PhD Vanderbilt Univ. Medical Center, 2014 IRSA Research Symposium
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Balancing the Excitatory/Inhibitory Balance

- In MeCP2 mice model
  - BDNF: Inhibits excitatory in Brain Stem
  - Enhances Excitatory activity in Cortex
  - Prefrontal Cortex = NMDA activity, dendrites
  - Brainstem = Activity

David Katz, Case Western Reserve University, Colleen Niswender, PHD Vanderbilt Uni. Medical Center, 2014 ISEA Research Symposium

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MeCP2

- Type of Mutation is associated with severity
  - More Severe:
  - Less Severe and Atypical Rett:
    - p.Arg133Cys, p.Arg294X, p.Arg306Cys, 3° truncations and other point mutations, were relatively less severe in both typical and atypical RTT.


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Other MeCP2 Clinical presentations

- X-linked MR
- Fatal encephalopathy
- Autistic spectrum disorders
- Mild learning disability
- Normal carrier
- Angelman phenotype
- Somatic mosaicism
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Males with Rett/MECP2

- Few cases described
- Early presentation
- Severe mental retardation
- Some Rett like symptoms:
  - Hand stereotypies
  - Breathing abnormalities

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MeCP2 duplication

- Duplications in Xq28: severe mental disability, delayed milestones, absence of language, hypotonia replaced by spasticity and retractions, and recurrent and often severe infections.
  - 2% of Males with MR

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Cyclin-Dependent Kinase-Like 5 (CDKL5) in Xp22.13

- Early-onset, often intractable epileptic seizures (17% of infantile spasm)
- Severe mental retardation
- Most patients also show impaired social interaction with avoidance of eye-to-eye contact
- Some clinical features of Rett syndrome (RTT):
  - Stereotypic hand movements
  - Lack of purposeful hand use
  - Acquired microcephaly
  - Hypotonias
  - One case with classic Rett
Atypical Rett
(Forkhead box G1 - FOXG1 Deletion)

The Forkhead box G1 (FOXG1) is a transcription factor that promotes progenitor proliferation and suppresses premature neurogenesis.

Typical stereotypic hand movements with hand-washing and hand-mouthing activities

Early Symptoms
- Microcephaly at birth
- Hypotonia
- 2-4% of Atypical Rett Sx

Current Research on Treatment of Rett Syndrome

Treatments Proposals: genetics
- Gene Therapy: replacement
  - Challenges in Rett
    - Excess of MeCP2 can have severe adverse effects
    - Patients do have 50% of the chromosomes with normal MeCP2 gene
    - How to deliver only to the cells that need it?
- Aminoglycoside
  - Gentamicin, Amikacin
    - Shown to read through Missense Mutations (30 - 40% of girls with Rett Syndrome have missense mutation)
    - So far not helpful
Gene Therapy

Adeno-associated virus serotype 9 (AAV9) vector

- Used AAV9 with MeCP2 cDNA under control of a fragment of its own promoter (scAAV9/MeCP2)
- 3% of cells expressed hMeCP2, these cells 65% more GABA
- Increased survival (50% more)
- Increase MeCP2 in brain (Cortex, hippocampus)
- Increased in heart, liver, kidney

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Gene Therapy - AAV9 with MeCP2 cDNA


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IGF-1 and BDNF
Insuline like growth factor-1 (IGF-1)

- MeCP2 controls BDNF (Brain derived Neuro-trophic Factor)
- RTT mice have decreased BDNF
- BDNF deficient mice have RTT like symptoms (hand clasp, loss weight)
- Over-expression of BDNF in KO mice (Rett) improves motor and respiratory abnormalities.
- BDNF over-expression in hippocampal neurons prevents dendritic atrophy caused by Rett-associated MECP2 mutations.
- Synapsis remains immature but can be "activated"
- Molecules that increased BDNF can improve synapsis and function.
- IGF increases BDNF
Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice with active peptide fragment of Insulin-like Growth Factor 1 (IGF-1).

Tropea D et al. PNAS 2009;106:2029-2034

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Changes in brain structure in MeCP2 mutant mice and the effects of IGF-1 treatment.

Tropea D et al. PNAS 2009;106:2029-2034

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Mecasermin (recombinant human IGF-1)

Walter Kaufmann, MD

- Safety Study with 12 girls (3 to 10 years) Post regression
- Daily injections, twice a day
- 4 weeks ascending dose
- 20 weeks Open Label

Findings:
- No side effect
- Increase IGF in serum and CSF
- Some improvement in cardio-respiratory measurements.
- Some improvement in resp-cardio in sleep

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Mecasermin (recombinant human IGF-1)
Walter Kaufmann, MD
- Improved anxiety
- Improved social avoidance behaviors
- Less breath-holding

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Mecasermin (recombinant human IGF-1)
Walter Kaufmann, MD
- No improvement in irritability, aggressiveness, disruptive/hyperactive behavior, communication, and motor domains
- Started Phase 2 (N=30)
  - Double blind, cross over
  - 20 weeks

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NNZ-2566
Jeffrey Neul, MD, PhD
- Tropea study used a tripeptide [1-3] IGF-1
- NNZ-2566 is a peptidase-resistant (no digested in stomach) analogue to [1-3] IGF-1
- Helped in mice
- On Phase 1 Clinical Study
  - Adults 16-45 y
  - 5 days in hospital, multiple measurements (behavior, EEG, physiologic, ECG, breathing, etc)
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Lab research

<table>
<thead>
<tr>
<th>Treatment</th>
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<tbody>
<tr>
<td>MeCP2</td>
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<td>IGF-BP</td>
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<td>BDNF antisense RNA (BDNF-AS)</td>
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<td>BD2-4 activates TrkB (receptor for BDNF)</td>
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<tr>
<td>Abnormal Cholesterol</td>
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<td>Statins</td>
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</tbody>
</table>

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Glutamate- NMDA receptors

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Dextromethorphan

- Glutamate increased in patients with RTT at younger ages
- Glutamate increased in Mice model (Bird)
- NMDA receptors are increased in young patients
- Glutamate activates NMDA receptors
- Overflow of Glutamate have toxic effect
- Dextromethorphan is a non-competitive NMDA receptor antagonist
- Test for rapid metabolizers (P450 system)
Dextromethorphan (ongoing study)
Naidu, S @ John Hopkins

- Selected girls with high spike activity in EEG.
- 35 patients randomized, very low dose (control), low dose (2.5 mg), high dose (5 mg)
- No adverse effects
- Increased Receptive Language at high dose.
- Some increase in Expressive language (trend) both doses
- On the Mullen scale there was improvement in Receptive language
- Improved visual perception on the high dose.
- Seizure frequency reduced in 6/10 children with szs below the age of 10 yrs
- Better gait (low dose)
- Parents report that made girls more alert
Breathing Abnormalities
- Hyperventilation
- Breath holding
- Apneas: Decreased O2 can result in cyanosis and fainting
- Dysregulation occurs during breathholding as well as during "normal" breathing
- In general better during sleep
- Likely explanation for sudden death

Autonomic System:
Breathing and heart
RS girls have autonomic dysfunction
- Most patients with RS have apnea, shallow breathing, or hyperventilation, when awake and during sleep.
- Cold hands and feet
- They can have:
  - Bradycardia or tachycardia
  - Abnormal cardiac conduction
Air Swallowing

- 60% have air bloat,
- Apneas and air bloat are often worse when individuals are distressed
- May be reduced with anxiolytic medications.

Breathing:

- Naltrexone (opioid receptor antagonist) if central apneas with cyanosis. Can improve disorganized breathing
  - One study showed a decline in motor function and more rapid progression of the disorder suggesting a deleterious effect.
- Buspirone (one case described) Serotoninergic agonist. Improved breathing and oxygen. Also increase alertness noticed.
- Few case reports of improvement with Fluoxetine

Breathing: No treatment yet, but

- Norepinephrine:
  - RTT have low synthesis of nor-epinephrine in Brain Stem
  - Desipramine (antidepressant) inhibit the re-uptake of Nor-epi, so more stays in the synapsis.
  - In mice increases life span and improves apnea

  Study being conducted in Europe
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**Sarizotan (Bissonette, J et al, OHSU)**

- Is a Serotonin 1A Receptor Agonist, D2 partial agonist.
- Is in Phase III for treatment of L-dopa induced dyskinesia in Parkinson
- Respiratory abnormalities in RTT are due to lack of inhibition
- Findings in Rett Mice
- One injection: Corrected breathing (87 % less apneas, more regular RR). But, decreased motor activity in WT and Null/y.
- Treatment for 7 days in +/- corrected breathing without decreased motor activity

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**Use of Hands**

- Lack of use of hands—hand stereotypies
  - Use of elbow bracing to limit handwringing
  - Increases use of hands
- Apraxia
  - VERY DELAYED reaction time

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**Growth, Short stature**

- Some girls with RS can present with severe failure to thrive during the period of regression.
- A decrease in growth is apparent in 85-90% of the patients, inspite of normal or even increased appetite.
- No evidence of thyroid hormones, estrogens or growth hormone deficiency.
Feeding and Nutrition

Patients with feeding problems with less preference to hard-to-chew foods such as meats.

- Have prolonged feeding times (92%),
- They lack of self-feeding skills (92%),
- Have poor oral motor control (69%).


25% have GT

Rett Syndrome and Gastrointestinal Function

- GI Motility: Esophageal dysphagia
- Gastro-Esophageal Reflux
- Delayed gastric emptying
- Constipation.
- Gallbladder stones
- Air swallow

Heart

- Prolonged QT.
- Obtain Electrocardiogram - ECG
- Consult with cardiology if abnormal

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**Sleep**

- Abnormal sleep is very common (80%)
- Sleep pattern
  - Awakening – laughing
  - Abnormal sleep cycles
- Apneas
  - Snoring
- More problems in cases with a large deletion of the MECP2 gene

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**Sleep**

- No specific treatment:
- Routine: Decrease stimulation and maintain schedule
- Medications
  - Melatonin
  - Other: Trazodone, Klonopin, Ambien

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**Behavior: Agitation-aggressive**

- Look for a cause (medical, environment)
- Responds to functional communication / Applied behavioral analysis
- Use calming activities
- No specific medication
- Depending on main behavior problem
  - Antipsychotic medication
    - Risperidone
    - Zyprexa (Olanzapine)
  - Naltrexone (Tranxene)
Motor impairment

- Decrease balance and motor control
  - Physical therapy, keep them walking
  - Walking aids, other therapies (hydro, hippo)
- Contractures
  - Maintain range of motion, orthosis (AFO)
  - Botulinum toxin
- Hypotonia
  - Carnitine (can also improve sleep and alertness)
- Dystonia
  - Baclofen, Valium, Klonopin

Communication

- Eye movements can be used for communication
  - Augmentative Communication, i.e.
    - Tobii
    - EagleEye
    - [www.opportunityfoundationamerica.org/eagleeyes/]
  - Make choices

Others

- Few case reports of increased sensitivity to anesthesia with long recovery time
**Orthopedics:**

<table>
<thead>
<tr>
<th>Scoliosis</th>
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<tr>
<td>□ Very Frequent (45 to 75%)</td>
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<td>□ Frequency increases with age (stage IV)</td>
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<td>□ Can progress after 18 years of age</td>
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<td>□ Poor response to conservative treatment</td>
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<tr>
<td>□ TLSO – Thoraco-Lumbar Sacral Orthosis</td>
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<tr>
<td>□ Often needs surgery</td>
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<tr>
<td>□ Surgery if more than 40 degrees.</td>
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Contractures