

World Congress Update on Therapies- those mice are working hard for you!



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Goal

To present a quick overview of emerging molecular and targeted pharmacologic treatment strategies for Rett syndrome based on our ever increasing understanding of the role of MECP2 in the nervous system and how loss of MECP2 affects neuronal function.

Disclaimer: I am bound by confidentiality agreement from disclosing specifics on PTC Therapeutics and other drug trials.

Mutations lead to loss of MECP2 function

Mutation in DNA



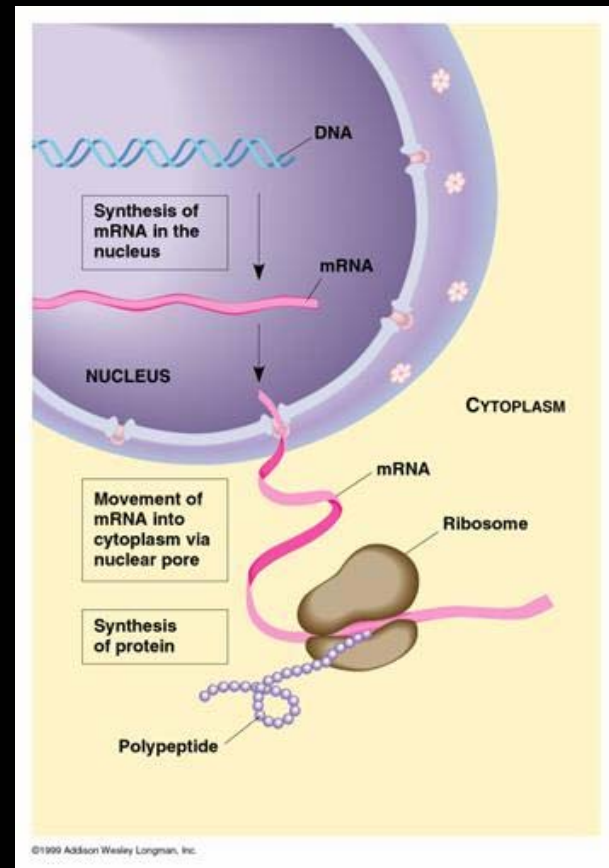
Incomplete or poorly functional protein



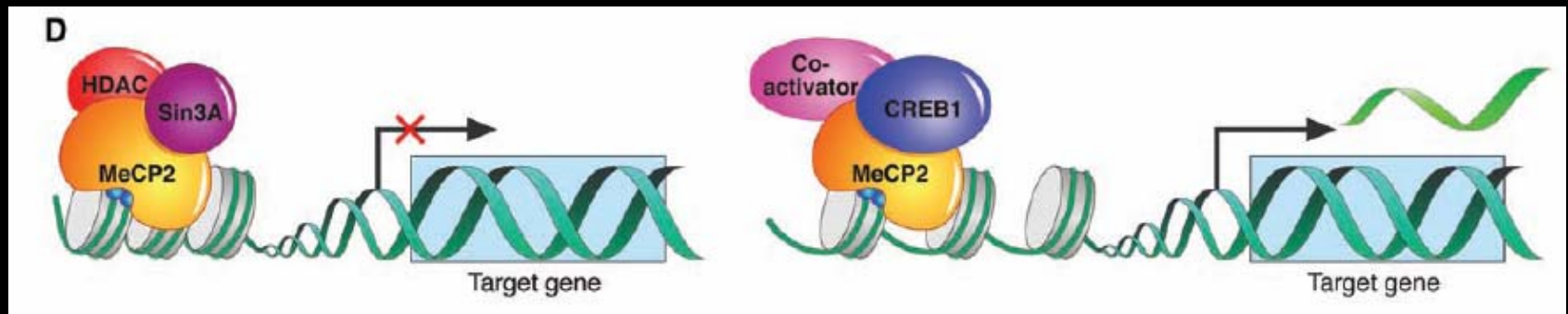
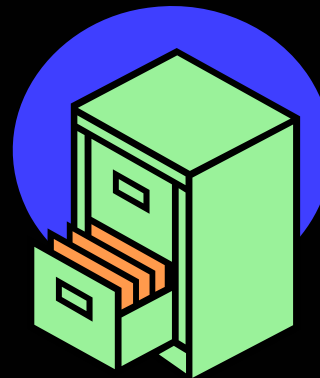
Impaired function of cells missing Mecp2 protein



Tissue effects



MECP2 protein turns other genes off and on and organizes DNA



Effects of Loss of MECP2 function



- Low level loss of regulation of gene expression.
- Just beginning to identify important targets that are directly regulated.
- Likely 1000's of genes are secondarily affected.

Challenges across the board for therapies in Rett syndrome

- No clear-cut reliable outcome measure for all the girls.
 - Natural history study may be very important.
- Girls will be heterogeneous in background (ethnicity, medications, treatments etc.)
- But the mice can help us here.....

Outcome measures in Rett syndrome



Treatment

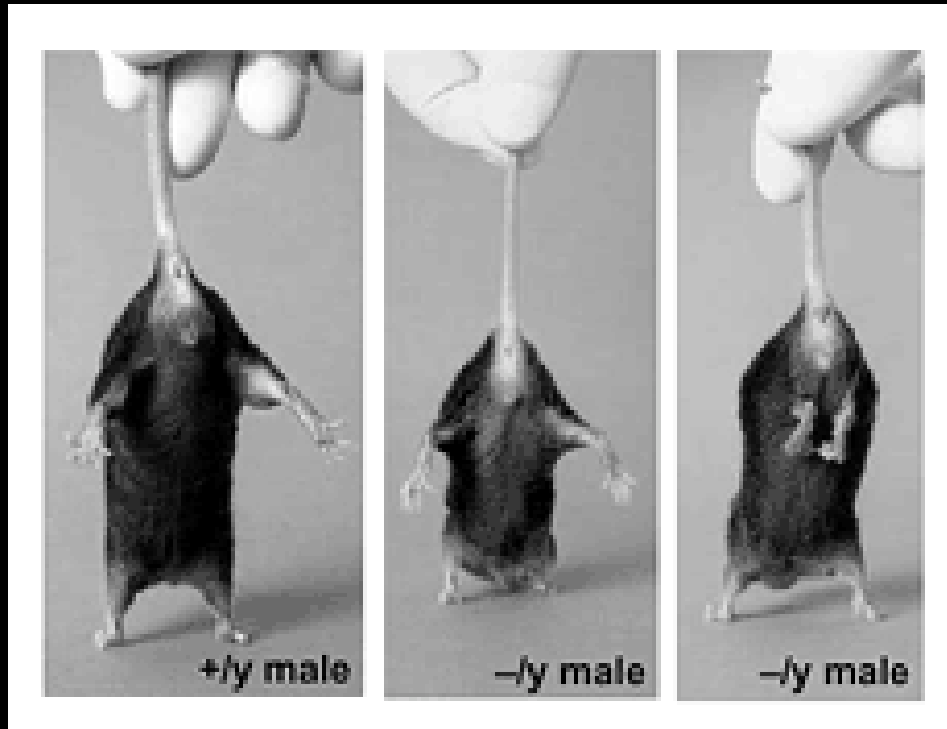


Measure of
“function”

Repeat Measure
of “function”

Is she better?

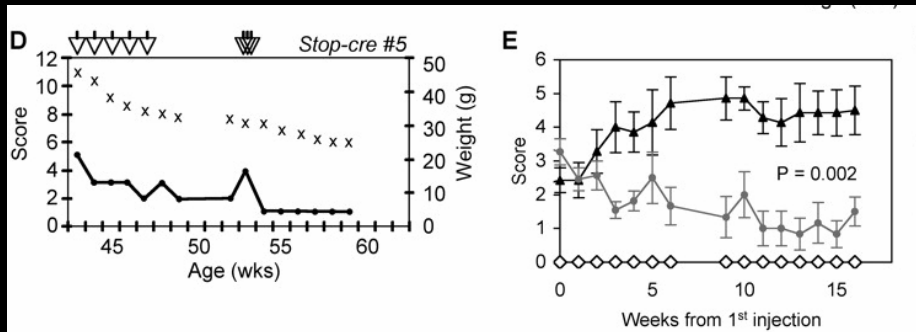
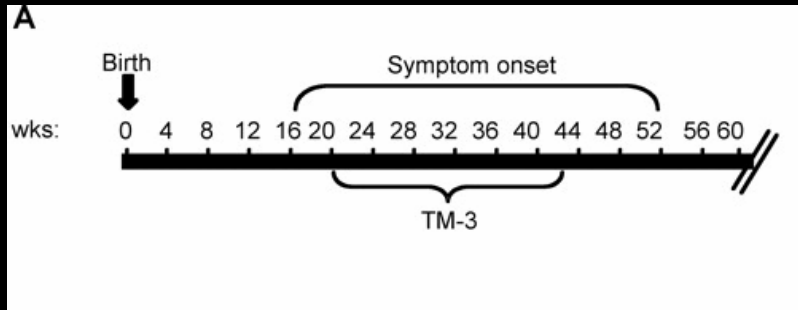
Mouse models are critical for finding new therapies



Guy et al, Nature Genetics (27) 322-326, 2001.

- Mecp2 “knockout” strains, Mecp2^{308/y}
- Dissection of “neuronal” phenotype
 - Anatomy
 - Behavioral
 - Electrophysiologic
- Testing new therapies
- Importantly- if a drug shows an effect in mice, we can’t directly assume it can be used in the girls.

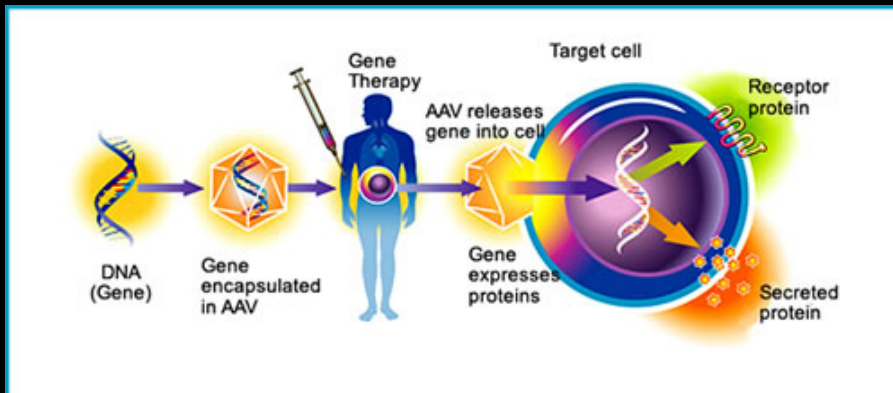
Reversal of symptoms in female mice



Guy et al., Science 2007

- In female mice, by breeding in a new *Mecp2* gene that could be turned on, Dr. Bird showed that it reversed symptoms.
- At World Congress, Dr. Bird reported that the females are dying of old age now and lived normal healthy mouse lives.
- Take home: the neurons in the mice were not irreversibly damaged by the process of RTT.

What about gene therapy for Rett syndrome?



Works best when the responsible gene is known

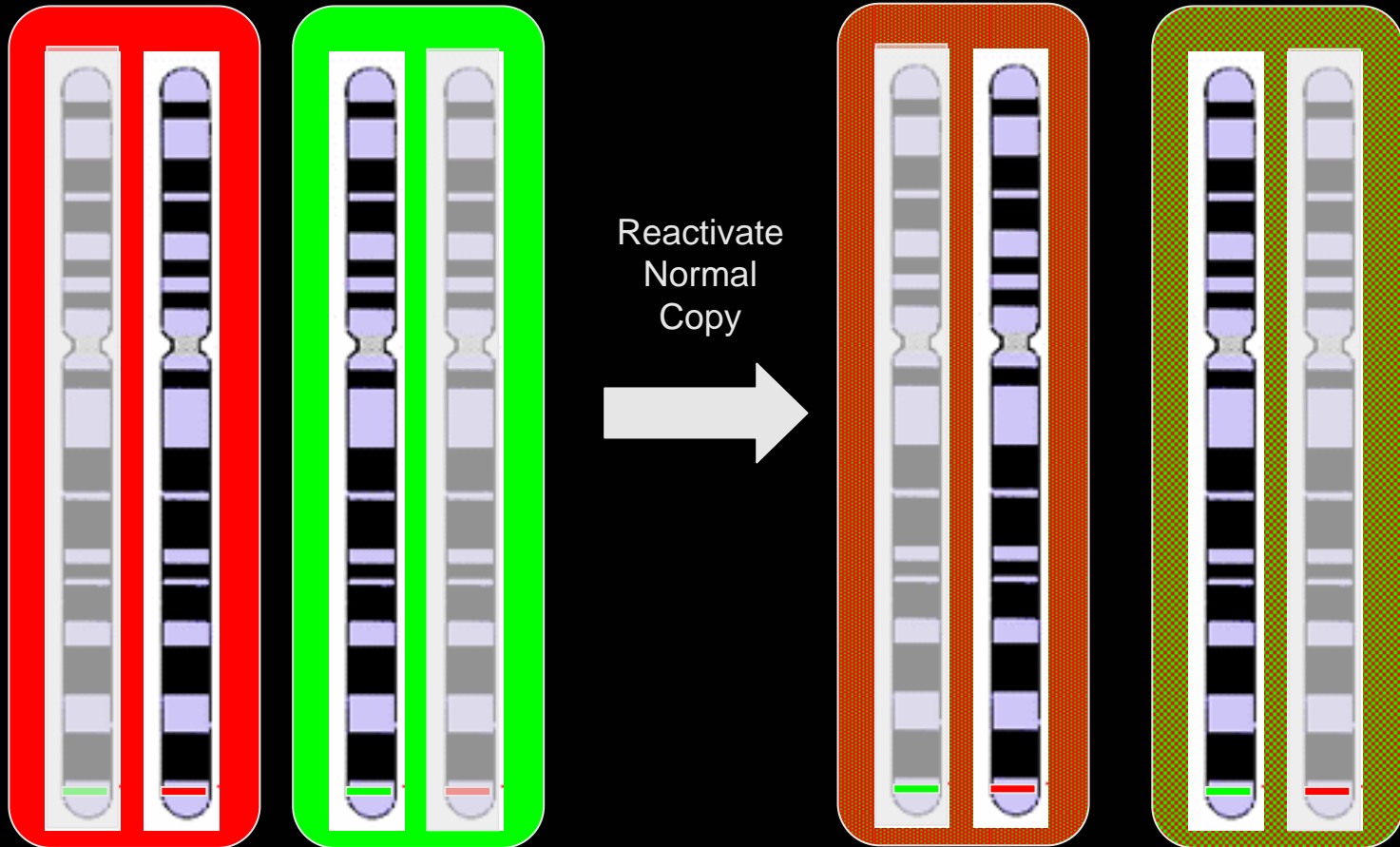
- +/- The role of the protein encoded by the gene is known
- + Adding a normal gene will fix the problem

The affected tissues are known and accessible

- girls already express normal gene in 1/2 cells in their brains
- Would only add a small population of new cells
- Hard to get repaired gene into nerve cells.

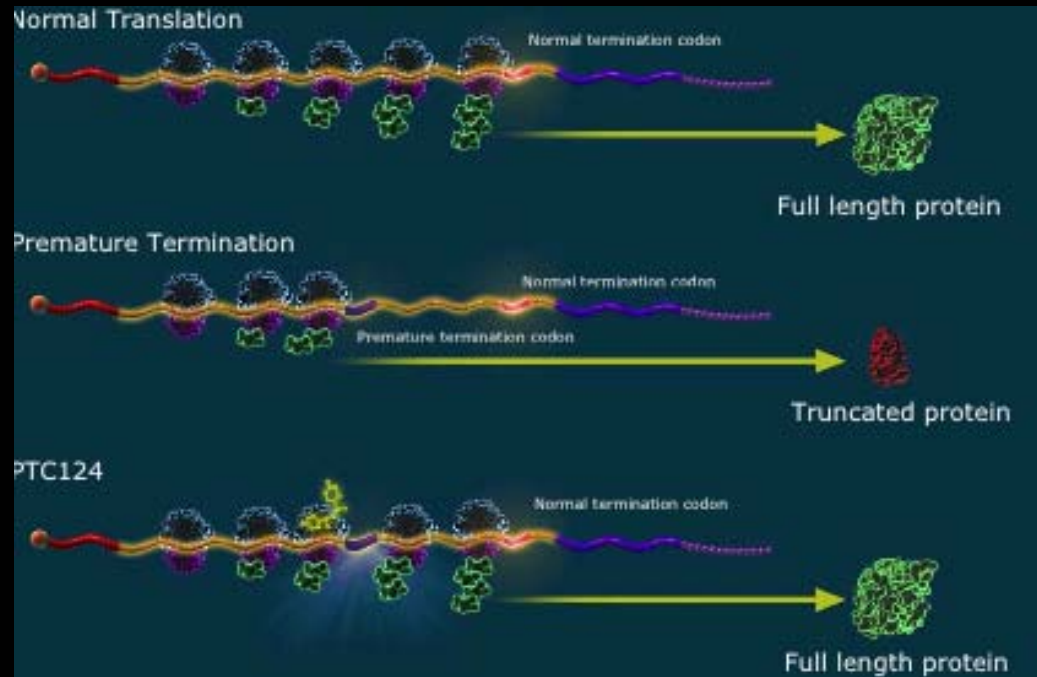


Modify X-inactivation



Post-transcriptional repair: aminoglycosides

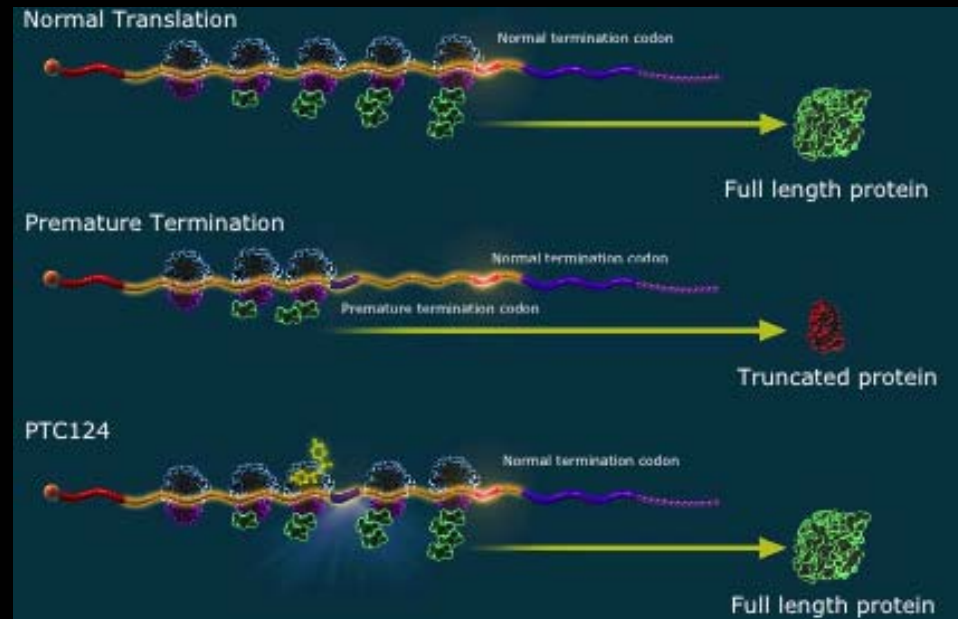
- Aminoglycoside antibiotics allow “read-through” of premature stop signals
 - Nonsense mutations
 - Problems with toxicity
 - Need to be given IV
- Puts a missense mutation at the “stop” site.



http://www.ptcbio.com/3.1.1_genetic_disorders.aspx

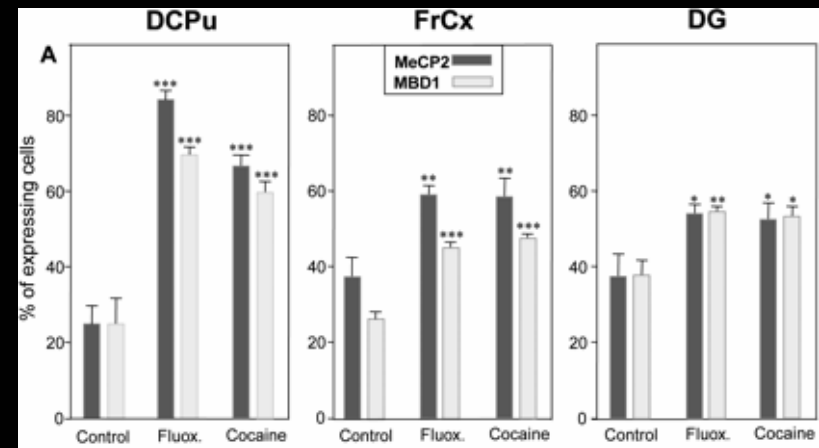
Post-transcriptional repair for nonsense mutations

- PTC124: Developed by PTC Therapeutics with similar action.
 - Oral, Less toxic
 - Being tested in cystic fibrosis and Duchenne Muscular Dystrophy with very promising results
 - Phase 2 trials- increased walking duration in DMD, improved chloride conductance in CF
- Another set of compounds with readthrough activity developed by NIH for treating spinal muscular atrophy.



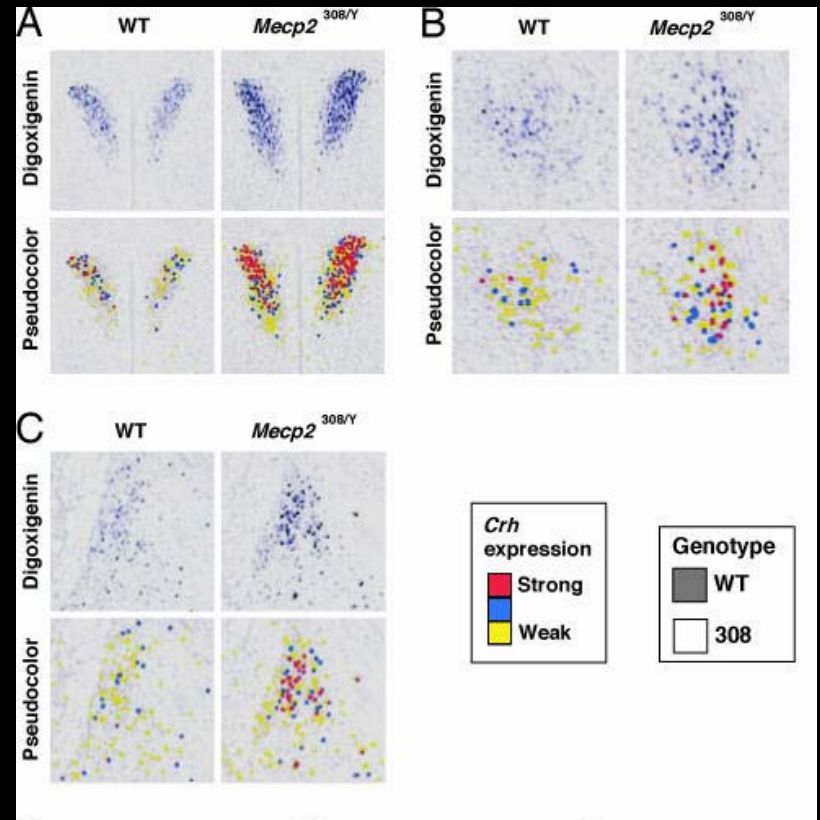
Fluoxetine (Prozac) and Cocaine upregulate MECP2 expression

- Adult rats
- Serotonin-elevating agents
- Chronic treatment (10days) with fluoxetine or cocaine increased expression of MECP2.
- In the figure, the number of cells making Mecp2 and a related protein, MBD1, were assessed in 3 regions of the brain after treatment with the drugs. Both drugs increased the numbers of cells making Mecp2. **Of course, we are not advocating cocaine!!**



Clues for treatment based on mouse models

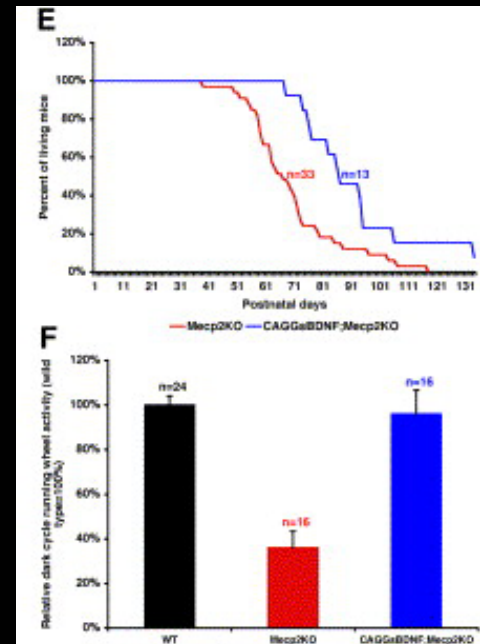
- Increased anxiety hormones and anxiety behaviors in mice with mutations in *Mecp2*
- Using more antianxiety medications in girls.
- Potential need for a real clinical trial for this.
- In the figure, increased levels of corticotropin releasing hormone (a stress hormone) were seen in multiple areas of the brain in a mouse model of Rett syndrome.



McGill et al, 2007PNAS

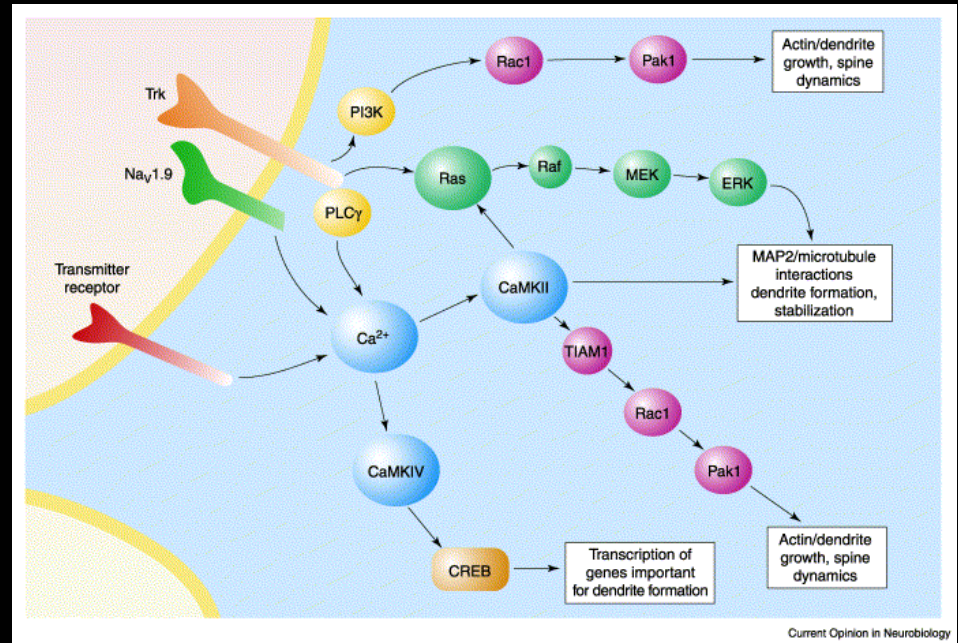
Neurobiological approaches

- MECP2 deficiency associated with low levels of growth factor for nerve cells, bdnf.
- Breed to mice with increased levels of bdnf, symptoms are better.
- In the figure, (E) the Mecp2 knockout mice show a shortened lifespan (red line) and this is increased when they are bred with mice that make excess Bdnf (blue line). In F, the knockout animals (red bar) are less active than normal mice (black bar) and their activity increases if the levels of Bdnf are increased by breeding them with the Bdnf overexpressing mice (blue bars).



Attacking BDNF

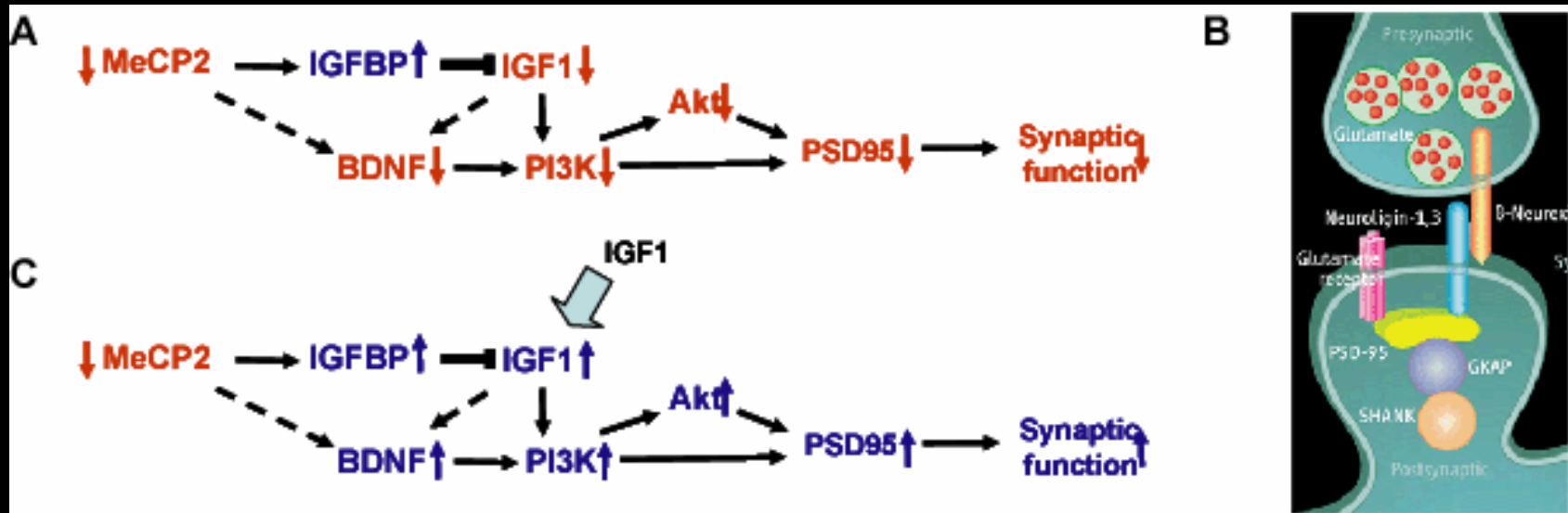
- Increase BDNF levels
- “BDNF-mimics”
- Activate receptor for BDNF
- Stimulate downstream pathways
- Importantly- lots of other disorders are interested in these too so it's like the Verizon network here in terms of who all is working on Rett syndrome!



Miller and Kaplan, Current Opinion in Neurobiology 13: 391, 2003

David Katz, PhD

New clinical trial



rh-IGF1 as a therapy for RTT

- Identified by M. Sur and colleagues (MIT) effective in partially restoring function in MecP2 mutant mice
- FDA approved for growth disorders in children
- Current protocol in IRB review
- Plan to enroll 30 pre-pubertal children in double-blind controlled cross-over study
- 20 weeks on treatment, 20 weeks on placebo
- Primary outcomes are autonomic function
- Safety and tolerability data paramount
- Secondary outcomes are neurological function, seizures
- Single site pilot study at Children's Hospital Boston

Omar Kwaja, MD

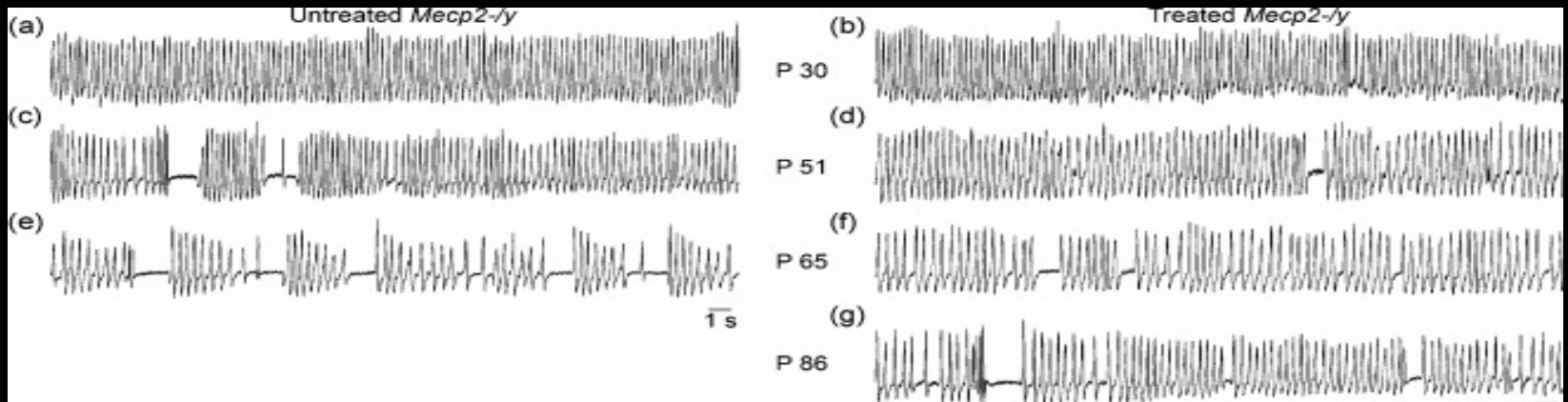
Other treatments applied to Mecp2 deficient mice with positive effects

- Choline: Increase cholinergic activity, improved motor coordination in male
- AMPAKINE: improved respiratory symptoms
- Desipramine: inhibitor of norepinephrine reuptake
- IGF-1
- Environmental enrichment



Nag and Berger-Sweeney, NBD 2007
Ward et al, NBD 2008
Nag et al., Brain Research, 2008
Ricceri et al. World Congress

Oral desipramine ameliorates respiratory phenotype and improves survival

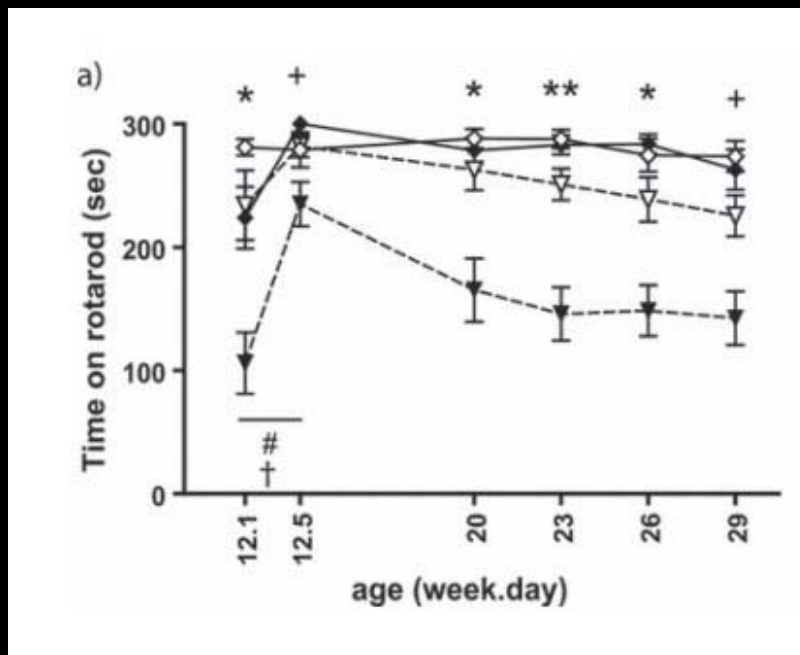


- Male mice
- Improved respiratory symptoms, but not failure to thrive, microcephaly, or reduced locomotion
- Increased lifespan (67 ± 4 to 90 ± 8 days)
- In the figure, the breathing patterns for the mice are shown. The untreated knockout mice are on the left and have pauses in breathing, the number of pauses decreases and the breathing pattern is more normal after treatment.

Desipramine clinical trial (France)

- Desipramine chlorhydrate.
 - age group is 6-18 years old.
 - 36 girls will be included.
- contact info is Pr Josette Mancini from Marseille Children's Hospital (Dept of Pediatric Neurology):
josette.mancini@ap-hm.fr
- Importantly... desipramine can have side effects that need to be specifically monitored in children with Rett syndrome (cardiac and constipation).

Environmental enrichment ameliorates motor coordination deficits in Mecp2 mutant mice



Kondo et al, EJN 2008

- Environmental enrichment beginning at 4 wks
- Assessments
 - Rotorod to measure coordination
 - Locomotor activity
 - BDNF levels
- Improvement in coordination for females
- In the figure, the females with the Mecp2 mutation are represented by the filled diamonds and are now in the same range as the normal females

Trials at KKI- Dr. Naidu

- *Dextromethorphan*
- *Aricept*
- **Contact Info**
- (443) 923-2778
- emailing Dr. Genila Bibat,
bibat@kennedykrieger.org

Conclusions

- Gene based therapies are potential avenues of treatment in the distant future.
 - Treatments may vary by mutation type: nonsense, missense, frameshift
- Current investigations into neurobiological underpinnings of CNS dysfunction opening doors to new therapies in the immediate future.
 - Repurposing existing drugs (using an existing drug for a new purpose)
 - Development of new agents
 - Role of environmental enrichment (supports early intervention programs)
- It will be critical to have mutation information to be included in trials.

Therapies: future

- Neurobiological approaches
 - **Moving fastest**
 - Will likely be applicable for girls/women (or boys) with Rett syndrome, regardless of mutation type.
 - May be age or clinical stage dependent.
 - Mutation status will need to be known.
- Genetic approaches
 - **Maybe** mutation class specific (nonsense, missense, frameshift, deletion).
 - Some approaches, such as those to increase amount of MECP2 made, are not “mutation-dependent”.

Thanks

- IRSF, Kathryn, Paige, MaryJoyce, Jennifer, and all those mice and all those graduate students, post-docs, lab-techs, and their PIs.