A Research Update from the Rett Rat Model Working Group

Executive Summary:

The importance of the Rett rat model is highly significant for drug development for Rett syndrome, and Rettsyndrome.org took the initiative to make this model a priority in research by providing funds to five (5) investigators in the Rett Rat Model Working Group. Their overall goal was to characterize the rat model in symptoms that are known to be affected in Rett syndrome. Collectively, the group has presented this work through platform and poster presentations at research meetings and most recently, three (3) articles about the rat model have been published in peer-reviewed journals. Rettsyndrome.org is pleased that in just 2 years we have accelerated research and now know more about the rat model, which is likely to be a key model of Rett syndrome for drug development.

Background:

In 2014, Rettsyndrome.org sent out a special call for Letters of Interest in regards to using the MeCP2 Knockout Rat Model generated by Sage laboratories.

The importance of the Rett rat model is highly significant for drug development for Rett syndrome. For example, preclinical testing in two animal models (mice and rat) would increase the translatability of potential compounds to drug studies in human clinical trials. Rettsyndrome.org was aware that the Rett rat animal model had been available for some time, but was largely underutilized in the field due to the licensing fees and other associated high costs of studies in rats. With these challenges in mind, Rettsyndrome.org decided to increase the availability of this rat model to researchers by lowering their cost burden by paying for the licensing fees and other associated costs.

The Board of Directors approved to fund the Rettsyndrome.org Rat Model Working Group to a sum of $35,000.

Members of the Working Group include:

- Daniela Brunner, PhD at PyschoGenics, Inc.
- Chun Jiang, PhD at Georgia State University
- Lucas Pozzo-Miller, PhD, The University of Alabama at Birmingham
- Michelle Olsen, PhD, The University of Alabama at Birmingham
- Rodney Samaco, PhD at Baylor College of Medicine

The initial goal of the Working Group included characterization of the Rett rat model. Did this model have the same phenotypes, behaviors, or symptoms as seen in the Rett mouse model or in human diagnosed with Rett syndrome? The group looked at motor, social behavior, cognition, and respiratory issues in the rats, as well as cellular and molecular analysis of brain tissue and cell types.
**Results:**

Collectively, the group has presented this work through platform and poster presentations at research meetings, and most recently, three papers about the rat model have been published in peer-reviewed journals. Below we list a summary of the findings from the working group.

**Growth and development:**
Male rats displayed growth retardation. Body and brain weights of mutant male rats were lower compared to normal littermates (Jiang, Olsen). For heterozygous female rats, the animals tended to gain weight but still exhibited a lower brain weight compared to control animals (Olsen). To note, dental malocclusion developed frequently in male mutant animals (80%) and infrequently in heterozygous females (Jiang, Olsen).

*Evaluation of dental malocclusion in affected males must be considered in future studies when evaluating other motor and behavioral phenotypes due to weight loss and nutritional deficits.*

**Survival:**
A half of the mutant male rats died in 2 months. Most of the RTT-like symptoms were comparable to those seen in mutant male mice, while some appeared more or less severe (Jiang, Olsen). Spontaneous death of females was not observed over the course of study (18 months, Olsen).

**Motor:**
Male rats displayed severely reduced locomotion, hindlimb clasping, weaker forelimb grip strength and deficits in motor coordination assessed by rotor rod and gait analysis (Olsen). Female rats display reduced exploratory-based locomotor activity as early as 4 weeks after birth (Samaco), and deficits in motor coordination were also reported at older ages (Olsen).

*Severe motor deficits should be considered in future studies utilizing mutant male animals. The significant phenotypes displayed may confound interpretation of behavioral data.*

**Respiration:**
Respiratory abnormalities are partly recapitulated in juvenile and adult mutant male rats, which demonstrate decreased respiratory frequency and increased apneas compare to normal littermate controls (Jiang). Females mutant rats show increased frequency. However, there was no overtly apneic phenotype as seen in the mouse models and humans. Furthermore, respiratory problems are not progressive in the rat model, and in humans, breathing problems may actually be more severe at younger ages (Olsen).

**Seizures:**
Seizures were observed on occasion in mutant male rats as early as 4 weeks after birth, and in older mutant females already showing other symptoms exhibited seizures upon behavioral testing or handling (Olsen).

**Psychomotor Regression:**
Psychomotor regression in RTT, defined as the loss of acquired skills such as purposeful hand use, has yet to be well-modeled in MeCP2 mice. In the rat model, this regression can be measured by the seed opening task. Both RTT and normal rats roughly 100 seconds to complete the task. Over time, the normal rats learned to do this more and more quickly, while the time for RTT rats gradually increased, indicating a regression in the acquired psychomotor skill (Samaco).

**Social Interactions:**
Juvenile play is a test of social interaction among the rat model. Young female mutant rats were less engaged and less active in specific aspects of play when paired with a normal rat, yet showed normal movement and can smell normally (Samaco). Indirect social interaction tests showed that young female rats were also
impaired (Samaco); male rats were impaired in this type of test also but the severe motor defect may potentially confound performance (Jiang).

**Other behavioral evaluations:**
Young female rats showed abnormal anxiety-like behavior, altered learning and memory performance, normal sensorimotor gating. Some of these features are different from the mouse models. There was no evidence for perseverative behavior in one test (Samaco).

**Cellular/Molecular:**
Neurons from a specific brain region in the brain stem of mutant male rats showed excessive firing activity. These neurons are important for control of breathing and motor skills (Jiang).

The combination of analyzing both Mecp2 mutant rat and mouse models may provide a significant advantage for identifying downstream changes in other biological pathways relevant to Rett syndrome included biochemical and molecular changes. If similar changes occur in the 2 models, perhaps these changes will have higher predictive validity with respect to transcriptional and perhaps other molecular/biochemical changes in human RTT brain. In that regard, the only genes (from the hypothalamus) that were predictive of changes in RTT human brain were upregulated in either both Mecp2 rodent models or each rodent model alone (Samaco).

**Conclusions:**
The collective data demonstrate that *Mecp2* rat models are complementary tools with unique features for the studying Rett syndrome.

Utilizing a second rodent model for Rett syndrome has advantages. It can be used for juvenile toxicology studies of drugs that are being advanced to clinical trial. Without such studies, drugs cannot be tested in a pediatric population. Another advantage is that the rat model allows researchers to broaden research to other affected domains that are hard to observe in the mouse model due to their size or cognitive skills. All three articles clearly indicate that there is merit in using the rat model of Rett syndrome. It will expand our understanding of the Mecp2 mutation in the pathology of Rett syndrome which had begun with the mouse model. With two rodent models in our toolbox, there may be better predictive validity of biochemical targets for drug development that will lead to treatments for Rett syndrome.

**Journal Articles:**

1. **Loss of MeCP2 in the rat models regression, impaired sociability and transcriptional deficits of Rett syndrome.**
   
   Hum Mol Genet. 2016 Jun 30
   PMID: 27365498

2. **MeCP2 deficiency results in robust Rett-like behavioral and motor deficits in male and female rats.**
   
   Hum Mol Genet. 2016 Jun 21
   Patterson KC, Hawkins VE, Arps KM, Mulkey DK, Olsen ML.
   PMID: 27329765

   J Neurodev Disord. 2016 Jun 16
   PMID: 27313794

Platform Talk Presentation:
4. At the 14th Rett Syndrome Research Symposium in June 2016, Rodney Samaco, PhD of Baylor College of Medicine presented “Loss of MeCP2 in the rat models regression, impaired sociability and transcriptional deficits of Rett syndrome.”

Poster Presentation
5. Daniela Brunner, PhD and PsychoGenics team presented a poster “Assessing the Mecp2 (Bird) Model of Rett Syndrome Across Species, Sex, and Age” at the Society for Neuroscience meeting in 2015. Click here for the poster.


7. Michelle Olsen, PhD and her group presented a poster “Motor and behavioral phenotypes in a novel transgenic rat model of Rett Syndrome” at the Society for Neuroscience meeting in 2015.

Related Press Releases:
“Researchers advance rat model to enhance understanding of Rett syndrome”
https://scienceblog.com/486015/researchers-advance-rat-model-enhance-understanding-rett-syndrome/

“NRI researchers advance a novel rat model of Rett syndrome”
http://nri.texaschildrens.org/faculty_research/samaco_HMG.aspx