



# RETT SYNDROME RESEARCH, TOWARDS THE FUTURE

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**ROME**

**27-29 September 2018**

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The aim of this scientific meeting is to provide a platform to discuss **recent advances** in basic, translational and clinical research on **Rett syndrome**.

*The meeting is also intended to promote and foster collaborations.*

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# RETT SYNDROME RESEARCH, TOWARDS THE FUTURE

Rome  
27-29 September 2018

*rettrome2018.org*

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## Introduction

### Because a cure is possible

*by Salvatore Franzè, President of Pro RETT Research*

When the Board Members of Pro RETT Ricerca met to start organizing this meeting, the choice of a title that could express our intentions was of primary importance.

Rett Syndrome: Towards the Future.

Towards the future, therefore, starting from the certainty that a cure is possible.

The testimonies collected in the next pages are the demonstration of how many steps forward have been made since 2007 when Adrian Bird proved that Rett syndrome is not irreversible.

During the meeting, researchers from laboratories all over the world will get involved by bringing to the attention of the scientific community the findings of their work and the challenges that await ahead. We hope, therefore, that the efforts put in the organization of this meeting will lead to novel collaborations that will foster research on Rett syndrome towards new horizons, with great motivation, information and combinations that only these moments of collective comparison can help arise.

We had a similar approach thinking to families. We would like to offer them an insight on the actual state of research on Rett syndrome. We strongly believe that having a detailed overview of the projects carried out in major laboratories in various research institutions can contribute, first of all, in maintaining the courage which is needed to continue fighting, but also to guide choices and decisions related to family contingencies. Expertise, therefore, is a beacon which illuminates what may sometimes seem like a dead-end tunnel.

Like many of you, I am the father of a child with Rett syndrome. Her name is Giorgia and she is seven years old. During my time with Pro RETT Ricerca I realized that one of my biggest mistakes would be to think of myself as 'alone', because I'm not. By my side there are hundreds of fathers and mothers who, like me and with me, face this battle with determination, looking towards the future, fighting for the future of our daughters along with the many researchers who have joined our initiative.

**Because a cure is possible and we must find it: together.**

# Introduzione

## Perché la cura è possibile

*di Salvatore Franzè, Presidente Pro RETT Ricerca*

Quando il Direttivo di Pro RETT Ricerca si è riunito per dare il via all'organizzazione di questo meeting la scelta di un titolo capace di esplicitare i nostri intenti era di primaria importanza.

Rett Syndrome: Towards the Future.

Verso il futuro, quindi, partendo dalla certezza che una cura è possibile.

Le testimonianze raccolte nelle prossime pagine sono la dimostrazione di quanti siano stati i passi in avanti fatti dal 2007 a oggi, ossia da quando Adrian Bird ha dimostrato che la sindrome di Rett non è irreversibile.

Nei giorni del meeting interverranno ricercatori provenienti da laboratori di tutto il mondo, portando all'attenzione della comunità scientifica presente i risultati del proprio lavoro e le sfide che li attenderanno nel prossimo futuro. Il nostro augurio, quindi, è che dall'impegno messo nell'organizzazione di questo incontro possano nascere collaborazioni utili a rilanciare la ricerca verso nuovi orizzonti, forte di stimoli, informazioni e commistioni che solo nei momenti di confronto collettivo possono nascere e proliferare.

Abbiamo fatto un ragionamento analogo pensando alle famiglie, cercando di offrire loro uno spaccato sullo stato di fatto della ricerca sulla sindrome di Rett. Siamo fortemente convinti che avere una panoramica dettagliata dei progetti portati avanti nei laboratori delle più importanti strutture di ricerca mondiali possa contribuire, in primis, a mantenere alto il coraggio indispensabile per continuare a combattere, ma anche a orientare scelte e decisioni legate alle contingenze familiari. La conoscenza, dunque, come faro per illuminare quello che, a volte, può sembrare un tunnel senza via d'uscita.

Io, come molti di voi, sono padre di una bambina con la sindrome di Rett. Si chiama Giorgia e ha sette anni. Nel tempo trascorso in Pro RETT Ricerca ho capito che se c'è una cosa che posso sbagliare è quella di credermi "solo": non lo sono. Al mio fianco ci sono centinaia di padri e madri che, come me e con me, affrontano questa battaglia con determinazione, guardando al futuro, lottando per il futuro delle nostre figlie insieme a tanti dei ricercatori che hanno aderito alla nostra iniziativa.

**Perché la cura è possibile e dobbiamo trovarla: insieme.**

# Programme

## September 27, 2018

- 08.45-10.45 **Registration**  
10.45-11.00 **Welcome and opening remarks**

### Session 1

#### Basic research on MeCP2: from its structural domains to its essential molecular functions

Chair: James Eubanks

- 11.00-11.40 **The molecular basis of Rett syndrome**  
Adrian Bird, *University of Edinburgh (UK)*
- 11.40-12.10 **The two isoforms of MeCP2 in search of a function**  
Juan Ausio, *University of Victoria (Canada)*
- 12.10-12.30 **Lack of Methyl-CpG binding protein 2 (MeCP2) affects cell fate refinement during embryonic cortical development**  
Francesco Bedogni, *San Raffaele Scientific Institute (Italy)*
- 12.30-12.45 **Importin alpha5 is a new in vivo target for modulation of MeCP2 pathways**  
Nicolas Panayotis, *Weizmann Institute of Science (Israel)*
- 12.45-13.00 **Brain protein changes in *Mecp2* mouse mutant models: effects on disease progression of *Mecp2* brain specific gene reactivation**  
Alessio Cortelazzo, *Azienda Ospedaliera Universitaria Senese (Italy)*
- 13.00-14.30 **Lunch break**

September 27, 2018

Session 2

Latest news from the development of novel cellular and animal models in  
Rett research

Chair: Juan Ausio

- 14.30-15.10 **“Seq-ing” pathogenic insights into Rett syndrome**  
Zhaolan (Joe) Zhou, *University of Pennsylvania (USA)*
- 15.10-15.40 **Is precision medicine relevant for the treatment of Rett syndrome?  
Suggestions from a novel mouse model of *Mecp2***  
Nicoletta Landsberger, *University of Milan and San Raffaele Scientific Institute  
(Italy)*
- 15.40-16.00 **Dysregulation of autophagy and proteasome leads to altered proteostasis in  
Rett syndrome**  
Diego Sbardella, *University of Rome (Italy)*
- 16.00-16.20 **Alteration of SMAD3 signaling decreases hippocampal neuronal survival in  
*Cdkl5* KO mice**  
Claudia Fuchs, *University of Bologna (Italy)*
- 16.20-16.40 **Coffee break**

September 27, 2018

Session 3

**New insights in neuronal and non-neuronal dysfunctions generated by MeCP2 deficiency**

Chair: Enrico Tongiorgi

- 16.40-17.20 **Autonomic dysfunctions and approaches to therapy in Rett syndrome**  
Jeffrey Lorenz Neul, *Vanderbilt University Medical Center (USA)*
- 17.20-17.50 **Dysfunction of the hippocampus-prefrontal cortex projection and its role in social deficits in Rett syndrome**  
Lucas Pozzo-Miller, *The University of Alabama at Birmingham (USA)*
- 17.50-18.10 **Loss of Mecp2 causes atypical synaptic and molecular plasticity of parvalbumin-expressing interneurons reflecting Rett syndrome-like sensorimotor defects**  
Maurizio Giustetto, *University of Turin (Italy)*
- 18.10-18.30 **The surveillance receptor TRPM2: a novel therapeutic target for Rett syndrome?**  
James Eubanks, *Krembil Research Institute Toronto (Canada)*
- 18.30-18.50 **Tuning of ATM activity to control the delayed GABA development and neuronal hyper-excitability in neurodevelopmental disorders**  
Flavia Antonucci, *University of Milan (Italy)*
- 19.00-20.30 **Finger food and poster session**

September 28, 2018

Session 4

Treating Rett syndrome:  
future approaches and novel challenges

Chair: Nino Ramirez

- 8.45-9.15     **An update on gene therapies for Rett syndrome**  
Stuart Cobb, *University of Edinburgh (UK)*
- 9.15-9.45     ***In vivo* and *in vitro* models for X chromosome reactivation**  
Joost Gribnau, *Erasmus MC (NL)*
- 9.45-10.15   **From user- to genome-friendly CRISPR/Cas9**  
Antonio Casini, *University of Trento (Italy)*
- 10.15-10.35   **Toward gene editing in Rett syndrome**  
Alessandra Renieri, *University of Siena (Italy)*
- 10.35-11.00   **Coffee break**

September 28, 2018

Session 5

What is going on: preclinical studies in *MECP2* related disorders

Chair: Maurizio Giustetto

- 11.00-11.30 **IRSF's Scout Program: an *in vivo* screen for potential drugs to treat Rett syndrome**  
Steve Kaminsky, *Rettsyndrome.org (USA)*
- 11.30-12.00 **Repurposing Mirtazapine to target Rett syndrome**  
Enrico Tongiorgi, *University of Trieste (Italy)*
- 12.00-12.20 **The phytocannabinoid cannabidivarin (CBDV) rescues behavioural alterations and brain atrophy in *Mecp2*-308 hemizygous male mice**  
Daniele Vigli, *Istituto Superiore di Sanità (Italy)*
- 12.20-12.40 **Calcineurin-huntingtin pathway restores BDNF trafficking and improves *Mecp2* knock-out mice symptoms**  
Jean Christophe Roux, *Aix Marseille University (France)*
- 12.40-14.15 **Lunch break**

What is going on: news from ongoing clinical studies and trials

Chair: Nicoletta Landsberger

- 14.15-14.55 **Rett syndrome: closing the gap to clinical trials**  
Alan K. Percy, *The University of Alabama at Birmingham (USA)*
- 14.55-15.35 **Clinical trials in Rett syndrome: a critical appraisal**  
Walter E. Kaufmann, *Emory University & Mind Institute/University of California Davis (USA)*
- 15.35-16.05 **Sarizotan in the treatment of respiratory abnormalities in patients with Rett syndrome: new findings from an international 6-month, randomized, double-blinded, placebo-controlled phase III trial (stars)**  
Ravi Anand, *Newron Pharmaceutical SpA (Italy)*
- 16.05-16.30 **Coffee break**
- 16.30-17.00 **Dysautonomia in Rett syndrome: from bench to bedside**  
Jan-Marino Ramirez, *University of Washington (USA)*
- 17.00-17.20 **Behavioral and cardiac reactivity to sensory stimulation in Rett syndrome**  
Breanne Byiers, *University of Minnesota (USA)*
- 17.20-17.40 **Oculomotor function in individuals with Rett syndrome**  
Gillian Townend, *Rett Expertise Centre Netherlands (NL)*
- 17.40-18.00 **GABBR2 mutations determine phenotype in Rett syndrome and epileptic encephalopathy**  
Murim Choi, *Seoul National University College of Medicine (PRK)*
- 18.30 **Short tour of Rome by bus followed by a social dinner in front of the Colosseum** (dress code: casual)

September 29, 2018

In traduzione simultanea

**La ricerca sulla sindrome di Rett: lo stato attuale e le prospettive future**

Moderatori: Salvatore Franze, Nicoletta Landsberger

- 9.30-10.00 **Introduzione e riassunto delle giornate precedenti**  
Salvatore Franzè, *Presidente Pro RETT Ricerca*  
Nicoletta Landsberger, *Università Statale di Milano e Istituto Scientifico San Raffaele (Italia)*
- 10.00-10.25 **Conoscenze e lacune da colmare per i prossimi trial clinici**  
Alan K. Percy, *The University of Alabama at Birmingham (USA)*
- 10.25-10.50 **Valutazione critica dei trial clinici per la sindrome di Rett**  
Walter E. Kaufmann, *Emory University & Mind Institute/University of California Davis (USA)*
- 10.50-11.15 **Disfunzioni autonome e approcci terapeutici per la sindrome di Rett**  
Jeffrey Lorenz Neul, *Vanderbilt University Medical Center (USA)*
- 11.15-11.30 **Coffee break**
- 11.30-11.55 **Il Sarizotan per il trattamento delle anomalie respiratorie nei pazienti affetti da sindrome di Rett: i risultati ottenuti da un trial clinico internazionale di fase III**  
Ravi Anand, *Newron Pharmaceutical SpA (Italia)*
- 11.55-12.20 **Lo stato attuale delle terapie geniche per la sindrome di Rett**  
Stuart Cobb, *University of Edinburgh (UK)*
- 12.20-12.40 **Oltre i limiti del CRISPR/Cas9: miglioramento della fedeltà e del “delivery”**  
Antonio Casini, *Università di Trento (Italia)*
- 12.40-13.45 **Lunch break**

September 29, 2018

In traduzione simultanea

**La ricerca sulla sindrome di Rett: lo stato attuale e le prospettive future**

Moderatori: Salvatore Franze, Nicoletta Landsberger

- 13.45-14.10 **Il Programma Scout di IRSF - uno screening *in vivo* alla ricerca di farmaci utili al trattamento della sindrome di Rett**  
Steve Kaminsky, *Rettsyndrome.org (USA)*
- 14.10-14.30 **I benefici di un nuovo approccio terapeutico in un modello sperimentale di sindrome di Rett**  
Angelisa Frasca, *Università Statale di Milano (Italia)*
- 14.30-14.50 **Linee guida internazionali per la comunicazione con i pazienti affetti da sindrome di Rett**  
Gillian Townend, *Rett Expertise Centre Netherlands (NL)*
- 14.50-15.10 **Il riposizionamento della Mirtazapina per il trattamento della sindrome di Rett**  
Enrico Tongiorgi, *Università di Trieste (Italia)*
- 15.10-15.30 **Una vita intrappolata: un faro sul bisogno inascoltato di accrescere la consapevolezza sulla sindrome di Rett e di fornire informazioni alle politiche pubbliche; uno studio internazionale sulla sindrome di Rett e sull'onere della malattia**  
  
Dennis Dionne, *Newron Pharmaceuticals SpA (Italia)*
- 15.30 **Saluti e chiusura dei lavori**

## **Oral presentations**

(chronological order, only oral presentations approved by the speakers for publication)

Basic research on MeCP2: from its structural domains  
to its essential molecular functions

The molecular basis of Rett syndrome

Adrian Bird

*The Wellcome Trust Centre for Cell Biology, University of Edinburgh (UK)*



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**Basic research on MeCP2: from its structural domains  
to its essential molecular functions****The two isoforms of MeCP2 in search of a function****Juan Ausio***University of Victoria, Department of Biochemistry, Victoria, Canada*

MeCP2 is a chromatin-binding protein and its mutations are associated with Rett syndrome, a severe neurological disorder. The *Mecp2* gene encodes two isoforms, MeCP2-E1 and MeCP2-E2 with a 96% amino acid identity. Previous studies have shown a brain region-specific expression of these isoforms which, in addition to their different nuclear localization and differential expression during brain development, suggest they might have non-overlapping molecular mechanisms. However, the functions of MeCP2 E1 and E2 remain largely unexplored. Here, we show that the biophysically distinct structural characteristics of the N-terminal domain (NTD) of MeCP2 E1 and E2 can modulate the ability of the methyl binding domain (MBD) to interact with DNA. Our proteomics data indicates that both isoforms exhibit unique interacting protein partners. Cyclohexamide-chase assays combined with functional (circadian/nuclear depolarization) assays demonstrate the occurrence of different turnover rates and binding dynamics between the two isoforms. Moreover, genome-wide analysis using ChIP-seq provide evidence for a shared as well as a specific involvement in the regulation of different sets of genes. Our findings help gaining insight into the especial functional complexity of MeCP2 by dissecting differential aspects of its two isoforms.

- - -

Basic research on MeCP2: from its structural domains  
to its essential molecular functions

**Lack of Methyl-CpG binding protein 2 (MeCP2) affects cell fate refinement during embryonic cortical development**

*Clementina Cobolli Gigli (1), Linda Scaramuzza (1), Riccardo Rossi (2), Nicoletta Landsberger (1,3), Francesco Bedogni (1)*

The X-linked Methyl-CpG-Binding Protein 2 (MeCP2) gene encodes for a multifunctional protein ubiquitously expressed from developmental stages to adulthood. Mutations in MECP2 are linked to Rett syndrome (RTT), the most common genetic cause of severe intellectual disability in females. Although MECP2 plays a crucial role in the maintenance of proper neuronal functionalities, several evidences now suggest that early signs of the pathology can be observed (in both humans and animal models) long before the typical RTT symptoms become overt. We focused on the development of the embryonic cerebral cortex of *Mecp2* null animals, as our published transcriptional analyses suggest that the mechanisms of embryonic corticogenesis are delayed. Based on this observation, we assessed the dynamics of neuronal differentiation in embryonic and early postnatal cortical tissues. Our data show that the expression of transcripts that are typical of neuronal progenitors is retained by null newborn neurons, while the level of transcripts expressed by maturing or fully functional neurons are reduced in null cortexes. Altogether, our data suggest that already during embryonic and early post-natal life lack of *Mecp2* affects the ability of newborn neurons to drop the transcription of genes that are outdated (like those defining cortical progenitors) while acquiring new, more refined, postmitotic identities. We believe this evidence demonstrates that the transcriptional noise typically affecting adult *Mecp2* null tissues is a feature already displayed at an early stage of differentiation. The impairments displayed by adult RTT animal models can thus be considered the worsening of a condition that is already generated during embryogenesis.

1: San Raffaele Rett Research Unit, San Raffaele Scientific Institute, Milan, Italy

2: Istituto Nazionale Genetica Molecolare, Milan, Italy

3: Università degli Studi di Milano, Biometra, Milan, Italy

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**Basic research on MeCP2: from its structural domains  
to its essential molecular functions****Importin alpha5 is a new in vivo target for modulation of MeCP2 pathways**

**Panayotis N.** (1), **Sheinin A.** (2), **Dagan S.Y.** (1), **Koley S.** (1), **Marvaldi L.** (1), **Tsoory M.M.** (3), **Rother F.** (4), **Hartmann E.** (4,5), **Bader M.** (4,5), **Michaevlevski I.** (2,7), **Fainzilber M.** (1)

MeCP2 (Methyl-CpG binding Protein 2) is a multifunctional and activity-dependent modulator of the nervous system epigenome. Alterations in MeCP2 levels are implicated in Rett syndrome (RTT) and MeCP2 duplication syndrome (MDS). As a transcriptional regulator, MeCP2's subcellular locus of action is the nucleus, however nuclear import pathways for MeCP2 have not been targeted in vivo as yet. The importins family of nuclear import factors (also known as karyopherins) have pivotal roles in subcellular transport in neurons, including in axon-to-soma, synapse-to-nucleus and nucleocytoplasmic transport. During the course of comprehensive behavioral analyses of a battery of importin alpha mouse mutants, we identified an importin alpha5 knockout line with significantly reduced anxiety levels and a presynaptic deficit at the Shaffer collaterals in the hippocampus. Strikingly, hippocampal gene expression analyses in this line highlighted changed expression in MeCP2-responsive genes, and immunohistochemistry revealed changes in the nucleocytoplasmic distribution of MeCP2 in importin alpha5 knockout hippocampal neurons. Our investigation highlights a critical role of importin alpha5 for the transport of MeCP2 in neurons, and suggests that targeting importin alpha5/MeCP2 dependent pathways might provide new avenues for therapeutic development for RTT and MDS.

1: Dept. of Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel

2: Sagol School of Neuroscience, Tel Aviv University, Israel

3: Dept. of Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel

4: Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

5: Institute of Biology, University of Lübeck, Germany

6: Ariel University, Israel

- - -

Basic research on MeCP2: from its structural domains  
to its essential molecular functions

Brain protein changes in *Mecp2* mouse mutant models: effects on disease progression of  
*Mecp2* brain specific gene reactivation

Alessio Cortelazzo (1, 2, 3), Claudio De Felice (4), Jacky Guy (5), Anna Maria Timperio (6), Lello Zolla (6), Roberto Guerranti (2, 3), Silvia Leoncini (1, 7), Cinzia Signorini (7), Thierry Durand (8), Joussef Hayek (1)

**Background:** Rett syndrome (RTT) is a progressive neurodevelopmental disorder and a leading cause of severe intellectual disability in the female. De novo mutations in the X-linked methyl-CpG binding protein 2 (MECP2) gene are the main cause of RTT. Several animal models have been developed to understand disease progression and pathogenesis in the search of possible therapeutic targets. Limited efforts have been reported on the effects of brain gene reactivation on global protein expression.

**Methods:** Here, a whole proteome analysis using liquid chromatography coupled with tandem mass spectrometry was performed to identify potential molecular targets in a deficient *Mecp2* mouse model (*Mecp2*<sup>stop/y</sup>). Pre-symptomatic and symptomatic *Mecp2* deficient mice were examined in order to better understand the relationship between protein changes and disease progression. Furthermore, a model in which *Mecp2* deficiency was rescued by brain specific reactivation of the gene was evaluated to evidence possible therapeutic targets.

**Results:** A total of 18 to 20 proteins were identified as differentially expressed in the pre-symptomatic and symptomatic mice, respectively. In both groups, the majority of proteins are related to energy metabolic pathways and proteostasis processes. A total of 12 novel protein markers in the *Mecp2* rescued mouse model were identified as potential therapeutic targets. The majority of protein changes of likely pathogenic significance belong to proteostasis or energy metabolic pathways. The remaining changes refer to proteins involved in redox regulation, NO regulation and neurodevelopment.

**Conclusions:** These findings indicate that *Mecp2* mutations mainly affect energy metabolism, proteostasis, redox regulation and neuronal development.

- 1: Child Neuropsychiatry Unit, University Hospital, Azienda Ospedaliera Universitaria Senese (AOUS), Siena, Italy
- 2: Department of Medical Biotechnologies, University of Siena, Italy
- 3: Clinical Pathology Laboratory Unit, University Hospital, AOUS, Siena, Italy
- 4: Neonatal Intensive Care Unit, University Hospital, AOUS, Siena, Italy
- 5: Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, UK
- 6: Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy
- 7: Department of Molecular and Developmental Medicine, University of Siena, Italy
- 8: Institut des Biomolécules Max Mousseron, Université de Montpellier, ENSCM, France

- - -

**“Seq-ing” pathogenic insights into Rett syndrome****Zhaolan (Joe) Zhou***University of Pennsylvania, USA*

Rett syndrome (RTT) is a neurological disorder caused by mutations in the X-linked MECP2 gene. It represents one of the most common causes of intellectual disability among young girls. MeCP2 is a ubiquitously expressed nuclear protein that binds to chromatin and modulates gene transcription. To gain insights into the molecular etiology of Rett syndrome, we recently engineered genetically modified mice whereby MeCP2 is labeled with biotin using Cre-Lox recombination. To understand the molecular impact of RTT-associated mutations on cell type-specific gene expression *in vivo*, we also developed tagged knockin mice bearing one of two frequent and molecularly distinct RTT missense mutations, T158M and R106W. When combined with Fluorescence-Activated Cell Sorting (FACS), this strategy effectively circumvents the cellular heterogeneity of the mouse brain and allows for the isolation of neuronal nuclei from cell types of interest. Thus, we systematically profiled the nuclear transcriptome from multiple distinct neuronal cell types in MeCP2 wild-type, T158M, and R106W mutant of both male and female mice, respectively. By examining differentially expressed genes in MeCP2 mutant mice within the context of neuronal gene expression, we identified underlying transcriptional features that correlate with the severity of the mutation in different cell types. We also found low-expressing cell type-enriched genes are preferentially disrupted by MeCP2 mutations, and upregulated and downregulated genes demarcate distinct functional categories relevant to RTT phenotypes, despite that individual gene expression changes are largely specific to each MeCP2 mutation and each cell type. Furthermore, we uncovered that genome-wide transcriptional changes in the nucleus are opposed by post-transcriptional compensation of RNAs in a gene length-dependent manner. Finally, our approach allows us to circumvent genetic heterogeneity associated with random X-chromosome inactivation in heterozygous females and profile the transcriptome of neighboring wild-type and mutant neurons, thereby discerning cell autonomous from non-cell autonomous transcriptional effects. Our study across different neuronal settings leads to a contextualized model by which cell and non-cell autonomous transcriptional changes in different cell types contribute to the molecular severity of neuronal deficits observed in Rett syndrome and supports personalized therapeutic interventions.

- - -

**Is precision medicine relevant for the treatment of Rett syndrome? Suggestions from a novel mouse model of *Mecp2*****Nicoletta Landsberger***University of Milan and San Raffaele Scientific Institute (Italy)*

The generation of several mouse models carrying different *Mecp2* alterations proved instrumental to investigate MeCP2 functions and its involvement in Rett syndrome and *MECP2* related disorders. *Mecp2*-null animals were the first model generated and still represent the mostly used one. However, it has recently become evident that when *Mecp2* is absent, its functions are replaced by compensatory mechanisms that might mask and confound the consequences of its loss. These results highlight the importance of producing other models with less severe genetic lesions, possibly mimicking human mutations. We will present the generation, behavioral and molecular characterization of a novel mouse model of *Mecp2* bearing the human mutation Y120D, which is localized in the methyl-binding domain.

In line with the clinical features described for the Y120D RTT patient and with the important role of Y120 in MeCP2 activities, the Y120D mutation in mice leads to a harsh condition that is only slightly less severe compared to the total absence of the protein. As expected, this mutation alters the interaction of the protein with chromatin but surprisingly it also impairs its association with corepressors independently on the involved interacting domains.

These features, which become overt only in the mature brain, cause a more accessible and transcriptionally active chromatin structure; conversely, in the *Mecp2*-null brain we find a less accessible and transcriptionally inactive chromatin.

Our results demonstrate that different *MECP2* mutations can produce concordant neurological phenotypes but discordant molecular features, therefore suggesting the importance of clarifying whether treatment of *MECP2*-related diseases should consider a personalized approach in which patients are stratified based on the molecular consequences of their genetic lesion.

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**Dysregulation of autophagy and proteasome leads to altered proteostasis in Rett syndrome**

**Diego Sbardella (1), Grazia Raffaella Tundo (1), Chiara Ciaccio (1), Giuseppe Grasso (2), Alessio Cortellazzo (3), Marta Elena Santarone (4), Luisa Campagnolo (4), Augusto Orlandi (4), Cinzia Galasso (4), Paolo Curatolo (4), Claudio De Felice (5), Joussef Hayek (6), Maurizio D'Esposito (7, 8), Giuseppe Valacchi (9), Massimo Coletta (1), Stefano Marini (1)**

Autophagy and the Ubiquitin Proteasome System (UPS) are the major intracellular proteolytic pathways which handle proteostasis under resting and stressful metabolic conditions. Thus, dys-regulation of these systems compromises cell homeostasis leading to several pathological conditions. Remarkably, several studies point to a critical role of the basal constitutive (e.g. that under resting metabolic conditions) functionality of these pathways in the development of the nervous tissues by stimulating neurons viability, axon sprouting and dendritic spine arborisation. Herewith we report that both autophagy and the central proteolytic machinery of the UPS, the proteasome, are dysregulated in primary cells isolated from Rett subjects harbouring different MeCP2 mutations.

With respect to autophagy, skin primary fibroblasts of Rett subjects were found to be incompetent in generating autophagosomes under nutrient starvation. Moreover, mitochondria were found to be retained in mature erythrocytes of some Rett subjects (organelles clearance during the maturation of circulating reticulocytes to mature erythrocytes is an autophagy-mediated process). Finally, the immuno-staining of the p62/SQSTM1 and ubiquitin autophagy reporter molecules were increased in the cerebellum of MeCP2 KO murine models of the disease during the transition from the asymptomatic to the symptomatic stage of the disease. Regarding the proteasome, two main alterations were documented in erythrocytes and skin primary fibroblasts. The proteasome particles of Rett erythrocytes and fibroblasts were found to bear some putative structural defect which is matter of investigation by mass spectrometry. Most notably, the biogenesis of the proteasome particles was found to be strongly impaired in Rett fibroblasts: a selective post-transcriptional down-regulation of the expression of some proteasome core subunits, in the absence of adequate intracellular level of *wild-type* MeCP2, has been identified at the root of the low abundance of mature proteasome particles in the cytosol of Rett cells. Thus, we report molecular evidence that, in primary cells isolated from patients harbouring MeCP2 mutations, both autophagy and the proteasome are defective, suggesting that the Rett Syndrome onset and progression could be characterized by a severe impairment of intracellular proteostasis.

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**Alteration of SMAD3 signaling decreases hippocampal neuronal survival in Cdkl5 KO mice**

**Claudia Fuchs, Giorgio Medici, Laura Gennaccaro, Giuseppe Galvani, Stefania Trazzi and Elisabetta Ciani**

*Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy*

Cyclin-dependent kinase-like 5 (CDKL5) mutations are found in severe neurodevelopmental disorders, including the early-seizure variant of Rett syndrome (RTT). CDKL5 loss-of-function is accompanied by intractable epilepsy and causes several clinical features that overlap with RTT, including intellectual disability, motor dysfunctions, and autism. The roles exerted by CDKL5 in the central nervous system (CNS), and the molecular mechanisms underlying its effects, are still largely unknown. By exploiting an animal model of CDKL5 deficiency disorder (CDD), the Cdkl5 knockout (KO) mouse, we recently found that, in the absence of CDKL5, Smad3 levels and activity are reduced in hippocampal neurons. Transforming growth factor (TGF- $\beta$ ) signaling, through Smad3, contributes significantly to neuron survival and differentiation as well as to neuroprotection after traumatic and excitotoxic brain injuries. The aim of our study was to evaluate whether reduced Smad3 activity contributes to the neuropathology due to CDKL5 deficiency. We found that treatment with TGF- $\beta$  restored the Smad3-dependent transduction pathway, and thus normalized defective survival and maturation of Cdkl5 KO hippocampal neurons. Comparison of the response of wild-type and Cdkl5 null mice to NMDA-induced excitotoxicity showed a more pronounced loss of neuronal viability in Cdkl5 mutant mice. We found that, while Cdkl5 null mice displayed similar seizure susceptibility to NMDA compared to wild-type mice, neuronal density in the hippocampal CA1 region was more greatly reduced in Cdkl5 null mice than in wild-type mice post NMDA-injection. Cleaved caspase-3 staining also demonstrated increased neuronal degeneration at early time points after excitotoxic injury in the hippocampus of Cdkl5 null mice. Taken together, our results suggest that Smad3 deregulation may play a role in the impaired survival and maturation that characterizes Cdkl5 KO hippocampal neurons and in the greater vulnerability of Cdkl5 null neurons to excitotoxic stimuli. Since TGF- $\beta$  signaling through Smad3 is a critical regulator of key events in brain development, it is therefore valid to hypothesize that aberrant TGF- $\beta$ /Smad3 signaling might contribute to the etiology of CDD.

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### Autonomic dysfunctions and approaches to therapy in Rett syndrome

**Jeffrey Lorenz Neul**

*Vanderbilt University Medical Center (USA)*

Rett syndrome (RTT) is a neurodevelopmental disorder primarily caused by mutations in Methyl-CpG-binding protein 2 (MECP2). A variety of mouse models of RTT have been generated, and they show remarkable face validity. The Neul lab has characterized a variety of autonomic and physiological features in mouse models of RTT including breathing problems, cardiac rhythm abnormalities, and bladder dysfunction. In this lecture, Dr. Neul will review autonomic abnormalities and describe work on understanding the pathophysiological basis of these problems in RTT, as well as outlining approaches to therapy for this disorder.

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**Dysfunction of the hippocampus-prefrontal cortex projection and its role in social deficits in Rett syndrome****Lucas Pozzo-Miller***Department of Neurobiology, School of Medicine at The University of Alabama, Birmingham (USA)*

The ventral hippocampus (vHIP) of *Mecp2* knockout (KO) mice is hyperactive due to an excitation/inhibition (E/I) imbalance of synaptic activity. In contrast, several cortical regions are hypoactive in *Mecp2* KO mice. CA1 pyramidal neurons of the vHIP project their axons to the medial prefrontal cortex (mPFC), forming glutamatergic synapses onto excitatory pyramidal neurons and inhibitory GABAergic interneurons. Because altering the E/I balance in the mPFC causes impairments in social behaviors that are reminiscent of autism spectrum disorders, I will present experiments that test whether vHIP hyperactivity propagates to and alters mPFC function in *Mecp2* KO mice. Electrical stimulation of identified vHIP fibers evokes voltage-sensitive dye signals with larger amplitude and spatial spread in *Mecp2* KO slices, while selective optogenetic stimulation of vHIP afferents evokes larger excitatory postsynaptic currents in layer 5 mPFC pyramidal neurons. Furthermore, selective chemogenetic excitation of vHIP neurons projecting to the mPFC causes deficits of social memory in WT mice that are similar to those observed in *Mecp2* KO mice, while chemogenetic silencing of those same neurons improves social memory in *Mecp2* KO mice. We conclude that hyperactive hippocampal afferents alter network activity in the mPFC of *Mecp2* KO mice by affecting the E/I balance within the mPFC circuit, which leads to impaired social memory in Rett mice.

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**Loss of Mecp2 causes atypical synaptic and molecular plasticity of parvalbumin-expressing interneurons reflecting Rett syndrome-like sensorimotor defects**

Morello N. (1), Schina R. (1), Pilotto F. (1), Phillips M. (2), Melani R. (3), Plicato O. (1), Pizzorusso T. (3,4), Pozzo-Miller L. (2), Giustetto M. (1,5)

Rett syndrome (RTT) is caused in most cases by loss-of-function mutations in the X-linked gene encoding methyl CpG-binding protein 2 (MECP2). Understanding the pathological processes impacting sensory-motor control represents a major challenge for clinical management of individuals affected by RTT, but the underlying molecular and neuronal modifications remain unclear. We find that symptomatic male *Mecp2* knockout (KO) mice show atypically elevated parvalbumin (PV) expression in both somatosensory (S1) and motor (M1) cortices together with excessive excitatory inputs converging onto PV-expressing interneurons (INs). In accordance, high-speed voltage-sensitive dye imaging shows reduced amplitude and spatial spread of synaptically induced neuronal depolarizations in S1 of *Mecp2* KO mice. Moreover, motor learning-dependent changes of PV expression and structural synaptic plasticity typically occurring on PV INs in M1 are impaired in symptomatic *Mecp2* KO mice. Finally, we find similar abnormalities of PV networks plasticity in symptomatic female *Mecp2* heterozygous mice. These results indicate that in *Mecp2* mutant mice the configuration of PV INs network is shifted toward an atypical plasticity state in relevant cortical areas compatible with the sensory-motor dysfunctions characteristics of RTT.

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**The surveillance receptor TRPM2: a novel therapeutic target for Rett syndrome?****James Eubanks***Division of Genetics and Development Krembil Research Institute Toronto, Canada*

Previous work has revealed cells lacking MeCP2 display indications of elevated metabolic and oxidative stress, as well as signs of altered mitochondrial function. Such alterations can cause the activation of normally quiescent surveillance receptor systems, such as the TRPM2 Transient receptor potential channel, whose activities tend to promote energy conservation by negatively regulating mTORC1. Given hypoactive mTORC1 activity has been observed in several Rett syndrome model systems, we investigated whether TRPM2 expression and/or signalling would be elevated in the MeCP2-null brain. Indeed, Western blot analysis revealed a robust induction of TRPM2 in cortical and hippocampal regions of MeCP2-null mice, which correlated with a hypo-phosphorylation of the known TRPM2 target Protein kinase B (Akt). To test therapeutic potential, we generated MeCP2-deficient mice that were also heterozygous for TRPM2. The lifespan and RTT-like phenotypic behaviours of these mice were dramatically improved, and the hypo-phosphorylation of Protein Kinase B, p70-S6 Kinase, and ribosomal protein S6 were so rescued. Collectively these results implicate the transient receptor potential channel TRPM2 in Rett syndrome pathogenesis, and provide evidence that it could be targeted for therapeutic benefit.

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**Tuning of ATM activity to control the delayed GABA development and neuronal hyper-excitability in neurodevelopmental disorders**

*Lara Pizzamiglio (1,4), Elisa Focchi (1), Silvia Ferrara (1), Nicoletta Landsberger (1), Michela Matteoli (2, 3), Elisabetta Menna (2, 3), Flavia Antonucci (1)*

Several evidences indicate that the pharmacological promotion of inhibition may be a promising target in cognitive and neurodevelopmental disorders. Recently, we identified Ataxia Telangiectasia Mutated (ATM) as a novel kinase that orchestrates GABA development. In particular, ATM regulates the excitatory-to-inhibitory switch of GABA by controlling KCC2 expression. Interestingly, in *Atm* het/KO tissues we found an enhanced KCC2 and MeCP2 levels whereas and, vice versa, in Rett *Mecp2* KO hippocampi we measured a low KCC2 expression and increased ATM signal. Thus, starting by these remises we tested the effect of a selective ATM inhibitor called KU, in wt and *Mecp2* KO neurons. In wt cells and mice, application of KU promotes KCC2 expression and accelerates the excitatory-to-inhibitory switch of GABA. Accordingly, in *Mecp2* KO neurons a single treatment with KU normalizes development of GABAergic system by increasing expression of KCC2. The resulted neuronal network is more inhibited upon KU administration and less susceptible to generate hyper-excitability. In fact, electrophysiological experiments indicate that the hyper-excitability promoted by the pharmacological removal of  $Mg^{++}$  from external solution occurs in *Mecp2* KO neurons but not in *Mecp2* KO cells treated with KU. These results allow us to define i) ATM as a new target protein in Rett pathology and ii) KU as a new molecule potentially effective in the treatment of neurological disorders linked to a delayed GABA development as the case of Rett pathology.

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## An update on gene therapies for Rett syndrome

### Stuart Cobb

*Patrick Wild Center & Simons Initiative for the Developing Brain Centre for Discovery Brain Sciences at Edinburgh Medical School: Biomedical Sciences of University of Edinburgh, UK*

Rett syndrome is a severe neurological disorder resulting from loss-of-functions mutations in the MECP2 gene. The expression of MeCP2 protein, especially within the nervous system, is essential throughout life for normal function. Gene therapy targets the root-cause of the Rett syndrome by restoring functional MeCP2 to cells. Several independent reports have now supported the concept of viral vector-mediated gene transfer in rodent studies and work is advanced in translating these findings into a safe and effective gene therapy for Rett syndrome. The challenges for a successful therapy include the delivery and targeting of sufficient cells within the brain and constraining the expression levels of the vector-derived transgene within tolerable limits. The presentation will provide an update and overview of the current state of the Rett syndrome gene therapy efforts. It will also describe the various strategies to generate a next generation of regulated gene therapy cassettes with enhanced efficacy and safety properties. These include enhanced endogenous regulation, exploiting feedback mechanisms and developing minigene and RNA strategies.

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*In vivo* and *in vitro* models for X chromosome reactivation

**Joost Gribnau**

*Developmental Biology Department Erasmus MC, Rotterdam, The Netherlands*



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**From user- to genome-friendly CRISPR/Cas9****Antonio Casini, Anna Cereseto***Centre for Integrative Biology, University of Trento, Italy*

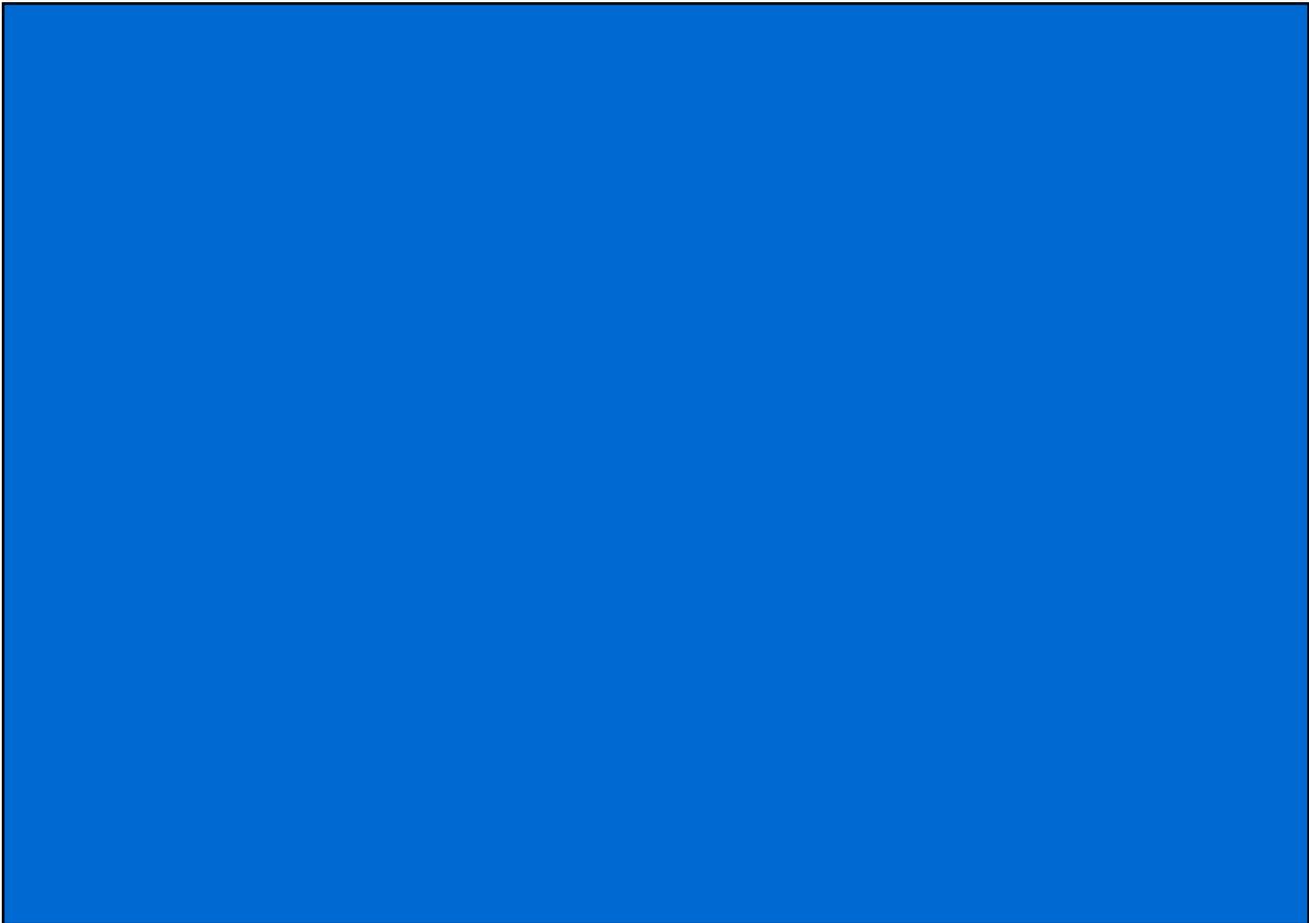
Therapeutic applications of Cas9 are still limited by inefficient in vivo delivery and unspecific cleavages. In the attempt to tackle these issues, we have generated more specific SpCas9 variants and a traceless-viral based delivery system. The identification of high-specificity SpCas9 variants from a random library was obtained through a yeast-based assay allowing simultaneous evaluation of on- and off-target activity. The combination of the best isolated substitutions together with structure-assisted prediction generated an evolved Cas9 (evoCas9), characterized by unprecedented fidelity. Long term expression of Cas9 correlates with increased off-target activity. Nevertheless, for in-vivo CRISPR/Cas9 applications such as in gene therapy clinical the existing delivery systems, mainly viral vectors, produce permanent expression of the nuclease. We have recently developed systems for CRISPR/Cas9 delivery that ensure transient nuclease expression thus limiting the non-specific cleavages. At least two delivery systems have been validated to reduce off-target: a Self-Limiting Cas9 circuitry introduced into a lentiviral vector for Enhanced Safety and specificity (lentiSLiCES) and VSV-G Enveloped vesicles carrying CRISPR/Cas9 ribo-nuclear protein. These recent strategies towards safer and efficient genome editing obtained by combining precise Cas9 variants and their traceless delivery will be presented.

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Treating Rett syndrome:  
future approaches and novel challenges

Toward gene editing in Rett syndrome

**A. Renieri, S. Croci, S. Daga, F. Lorenzetti, F.T. Papa, F. Donati, C. Lo Rizzo, D. Lopergolo, M. Doria, A. Auricchio, S. Conticello, I. Meloni**  
*Medical Genetics, University of Siena, Policlinico Le Scotte, Italy*



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What is going on: preclinical studies in *MECP2* related disorders

IRSF's Scout Program: an *in vivo* screen for potential drugs to treat Rett syndrome

**Steve Kaminsky**

*International Rett Syndrome Foundation, Rettsyndrome.org*



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What is going on: preclinical studies in MeCP2 related disorders

Repurposing Mirtazapine to target Rett syndrome

**Enrico Tongiorgi**

*Cellular and Developmental Neurobiology Lab at Department of Life Sciences, University of Trieste, Italy*



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What is going on: preclinical studies in MeCP2 related disorders

The phytocannabinoid cannabidiol (CBDV) rescues behavioural alterations and brain atrophy in *Mecp2*-308 hemizygous male mice

Vigli D. (1), Cosentino L. (2), Raggi C. (2), Laviola G. (1), Woolley-Roberts M. (3), De Filippis B. (1)



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- 3: GW Research Ltd, Cambridge, UK

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**Calcineurin-huntingtin pathway restores BDNF trafficking and improves *Mecp2* knock-out mice symptoms**

*Yann Ehinger (1), Julie Bruyère (2), Nicolas Panayotis (1), Yah-Se Abada (3), Lydia Saidi (1), Valerie Matagne (1), Benoit Delatour (3), Laurent Villard (1), Frédéric Saudou (2, 4), Jean-Christophe Roux (1)*



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## What is going on: news from ongoing clinical studies and trials

### Rett syndrome: closing the gap to clinical trials

**Alan K. Percy**

*Civitan International Research Center and Department of Pediatrics, University of Alabama at Birmingham, USA*

Since identification, nearly two decades ago, of mutations in the gene for most individuals with Rett syndrome (RTT), advances in basic and clinical research have been astonishing. Expansion of the number of clinical trials involving potential disease-modifying pharmaceutical agents has been quite dramatic. Further, replacing the aberrant gene or reactivating the normal gene offers the greatest potential for 'cure'. Recent progress in gene replacement suggests that such clinical trials are feasible.

As a precursor to such trials, a thorough understanding of the complex clinical picture represented by mutations in MECP2 is essential. We know that the MECP2 gene exerts important neurobiologic influences, as already described, from a very early age. As such, we must be mindful that efficacy of clinical trials is likely to depend on the impact of such mutations on both developmental progress and secondary changes such as seizures, scoliosis, and other medical issues. Therein lies the rub. The effect of any treatment strategy in a one year-old could be very different from that in a five, ten, or twenty year-old. This is because the natural history of RTT provides a remarkable pattern of changes in physical and cognitive abilities and raises important medical issues. In this regard, natural history studies (NHS) are essential precursors to effective clinical trials. Results from the US NHS which has been gathering information for more than twelve years, as well as similar studies from Europe and elsewhere, offer some insights. Results from the US will be presented in some detail as well as important gaps in knowledge such as meaningful biomarkers and outcome measures.

The utilization of varied approaches including gene-based, pharmaceutical, and neurohabilitation-based strategies may be essential. This will require expertise, as it has to this point, from multiple disciplines to achieve the desired goal of reversing the impact of this devastating disorder. Lest we forget, the support and involvement of families affected by RTT is integral.

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## Clinical trials in Rett syndrome: a critical appraisal

**Walter E. Kaufmann**

*Emory University & Mind Institute/University of California Davis (USA)*

Rett syndrome (RTT) is experiencing a revolution in treatment research. Multiple therapeutic strategies, based mainly on targeting neurobiological mechanisms underlying MeCP2 deficiency, are being tested. Although most preclinical and clinical studies focus on abnormalities downstream of MeCP2, new approaches targeting the gene are beginning to emerge. In fact, the first trial attempting to deliver a functional MECP2 using a non-replicating adeno-associated virus (AAV) capsid system is scheduled for the near future.

At present, most completed and ongoing drug trials have studied general modulators of synaptic function and neural metabolism, as well as drugs that influence specific neurotransmitter systems (most of which are abnormal in the evolution of RTT). Only drugs related to IGF-1, either the full-length molecule or the active peptide, have reported positive results. Effectiveness has been in general modest and variable in terms of symptom targets. Nonetheless, a phase 3 trial of trofinetide, a modified form of the IGF-1's active peptide, will be the first pivotal study in RTT and is planned for early 2019.

These milestone trials bring hope and opportunities for affected girls and women affected by the disorder, and have provided valuable information about study design and implementation. Nevertheless, some of the challenges previously experienced in clinical trials for other neurodevelopmental disorders such as fragile X syndrome have also emerged in RTT intervention studies. The most important is the limited number of appropriately validated outcome measures and biomarkers. These are critical measures for adequate selection of cohorts and, more important, for detecting positive response to treatment in early stages of drug development. As the RTT field moves into phase 3 trials, the shortcomings of outcome measures continue to be an issue. Another major concern in RTT is the evolution of the disorder, which suggests that targeting the developmental regression period will be the most effective way to modify the course of the disease. Identification of patients at early stages and interpretation of treatment effects during regression represent major challenges.

The potential availability in the near future of therapies that can substantially improve multiple symptoms, or affect the progression of RTT, represents an important achievement in the field. In order to sustain the progress in RTT translational research, an appropriate balance between development of outcome measures and biomarkers and implementation of clinical trials needs to be found. It is also critical to balance expectations and realities, so the RTT community can cooperate more effectively in the development of new treatments.

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What is going on: news from ongoing clinical studies and trials

Sarizotan in the treatment of respiratory abnormalities in patients with Rett syndrome: new findings from an international 6-month, randomized, double-blinded, placebo-controlled phase III trial (stars)

Anand R. (1), Giuliani R. (2), Lucini V. (2), Forrest E.C. (2), Graham S.M. (3), Hartman R.D. (4)





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- 3: Newron Pharmaceuticals US, Inc., Morristown, NJ, USA
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## Dysautonomia in Rett syndrome: from bench to bedside

**Jan-Marino Ramirez**

*Departments of Neurological Surgery and Pediatrics, University of Washington School of Medicine, Seattle, USA*

Dysautonomia is one of the most devastating characteristics of Rett Syndrome (RTT). The clinical phenotype includes disordered breathing, which is characterized by two key features: repeated breath-holds and breathing irregularities. Dysautonomia also includes disturbances in cardiorespiratory coupling. During breathholds, RTT patients show sudden heart rate increases that are uncoupled from breathing. The heart rate of RTT patients is generally increased and heart-rate irregularity decreased. A major issue is the individual variability: some children show more, others less signs of dysautonomia. Children can have good and bad days. Individual variability is also seen in the response to medications such as Busperone. While the degree of x-inactivation and the specific types of *Mecp2* mutation are critical factors driving the clinical phenotype, genetics is only one aspect contributing to dysautonomia. Dysautonomia is associated with intermittent hypoxia and oxidative stress which itself is a driver of dysautonomia. Untangling what aspects of the clinical phenotypes are caused by the *Mecp2* mutations, versus a vicious cycle that is associated with intermittent hypoxia will be critical to develop effective therapeutic strategies. Here we describe the dysautonomia phenotype of Rett Syndrome patients, and relate the clinical insights to the cellular mechanisms underlying cardiorespiratory control. Using mouse models we characterize the cellular mechanisms underlying cardiorespiratory coupling in WT and mutant mice, and unravel the effects of intermittent hypoxia and the reversal by antioxidant treatment. This comparative approach provides critical insights into the determinants of dysautonomia and ideas for future therapeutic strategies.

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## What is going on: news from ongoing clinical studies and trials

### Behavioral and cardiac reactivity to sensory stimulation in Rett syndrome

**Byiers B, Merbler A., Hoch J., Barney C.**

*Department of Educational Psychology, University of Minnesota, Minneapolis, USA*

Delayed or absent response to painful experiences is a commonly reported phenomenon in Rett syndrome (RTT), but much of the existing evidence is based on parent report of behavioral pain responses. The degree to which reduced behavioral responding to putatively painful experiences is attributable to decreased experience of pain versus decreased capacity to express the pain experience is currently unclear. Therefore, the purpose of the current study was to examine behavioral and cardiac changes among a sample of individuals with RTT in response to a set of sensory stimuli. A modified quantitative sensory test (mQST) consisting of six standardized, calibrated sensory stimuli (light touch, pin prick, cool, pressure, repeated von Frey, and heat) was conducted with a sample of 11 individuals with RTT and confirmed MECP2 mutations (aged 4 to 35 years) and a comparison sample of 15 typically-developing (TD) preschoolers. Data on behavioral reactivity and heart rate (HR) was collected during each stimulus application. Changes in HR and heart rate variability (HRV) during each stimulus application were compared to a resting baseline. The RTT group showed significantly elevated HR during the repeated von Frey and heat stimuli, whereas the TD group showed only significant elevation only during heat (and significant decreases during several stimuli). For HRV, the RTT group showed significant decreases during the pressure stimulus, whereas the TD group showed significant increases during light touch, cool, and pressure, and a significant decrease during heat. Significant differences in HR and HRV patterns among individuals with low and high behavioral reactivity were observed in both groups. These results suggest that behavioral and HR reactivity can be used to evaluate sensory reactivity among individuals with RTT. The elevated HR change in response to the repeated von Frey stimulus is consistent with a preclinical study showing hyperreactivity to mechanical sensory stimulation in a rat model of RTT (Bhattacharjee et al., 2017). The differences in patterns of HRV between groups warrant further investigation. Further investigation is needed to determine relations between behavioral and physiological reactivity and symptom severity and/or mutation type.

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Oculomotor function in individuals with Rett syndrome

Gillian Townend (1), Raymond van de Berg (1, 2, 3), Laurèl de Breet (4), Monique Hiemstra (4), Laura Wagter (4), Eric Smeets (1), Josine Widdershoven (2), Herman Kingma (2, 3), Leopold Curfs (1)



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- 2: Division of Balance Disorders, Department of Otorhinolaryngology and Head and Neck Surgery, Maastricht University Medical Center, The Netherlands
- 3: Faculty of Physics, Tomsk State University, Tomsk, Russia
- 4: Faculty of Medicine, Maastricht University, The Netherlands

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**GABBR2 mutations determine phenotype in Rett syndrome and epileptic encephalopathy****Murim Choi***Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea*

Rett syndrome (RTT) and epileptic encephalopathy (EE) are devastating neurodevelopmental disorders with distinct diagnostic criteria. However, highly heterogeneous and overlapping clinical features often allocate patients into the boundary of the two conditions, complicating accurate diagnosis and appropriate medical interventions. Therefore, we investigated the specific molecular mechanism that allows an understanding of the pathogenesis and relationship of these two conditions. We screened novel genetic factors from 34 RTT-like patients without MECP2 mutations, which account for ~90% of RTT cases, by whole-exome sequencing. The biological function of the patient-specific novel variants was assessed in cell culture and *Xenopus tropicalis* models. We identified a recurring de novo variant in GABAB receptor R2 (GABBR2) that reduces the receptor function, whereas different GABBR2 variants in EE patients possess a more profound effect in reducing receptor activity and are more responsive to agonist rescue in an animal model. Furthermore, we recruited additional GABBR2 mutation carriers with similar Rett-like symptoms and constructing mouse models of GABBR2 mutations. GABBR2 is a genetic factor that determines RTT- or EE-like phenotype expression depending on the variant positions. GABBR2-mediated c-aminobutyric acid signaling is a crucial factor in determining the severity and nature of neurodevelopmental phenotypes.

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**Associazione per la ricerca sulla  
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