

INTERNATIONAL RETT SYNDROME FOUNDATION
11TH ANNUAL RETT SYNDROME SYMPOSIUM



IRSF

INTERNATIONAL
RETT SYNDROME
FOUNDATION

INTRODUCTION

The International Rett Syndrome Foundation (IRSF) hosted the 11th Annual Rett syndrome Symposium from June 27th through June 29th 2010 at the Lansdowne Resort and Spa, Leesburg, Virginia. This year the symposium was chaired by David M. Katz, PhD (Case Western Reserve University, School of Medicine) and Yi Eve Sun, PhD (University of California – Los Angeles).

The symposium brought together about 25 presenters who led a day and a half of in-depth sessions attended by nearly 150 researchers and scientists, IRSF staff, and families affected by the disease. The meeting featured sessions on the regulation and function of MeCP2, the pathophysiology of the disease and potential links with other Autism Spectrum Disorders (ASDs), as well as new advances in the development of treatments.

Most notably, new relationships and collaborations were forged as researchers had a chance to examine and contribute their knowledge to the work of the other members of the group. For the second year in a row, the closing session on treatment strategies attracted a full house demonstrating the field's increasing move towards the application of bench science to the plight of the patient.

NEW SESSION ON AUTISM SPECTRUM DISORDERS ATTRACTS KEEN INTEREST

The 2010 symposium introduced a new session on the potential links between several ASDs including Rett syndrome. This session provided researchers an opportunity to discuss similarities and differences in the pathogenesis and phenotypic characteristics of multiple ASDs.

Presentations covered a broad range of topical issues including regulation of MeCP2 function by activity dependent phosphorylation, genetic reversal of neuronal pathophysiology in mice, PTEN/mTOR signaling in neurodevelopmental disorders and iPS cells from autism spectrum disorder patients.

THE “MANHATTAN PROJECT” FOR THE 21ST CENTURY

The symposium also featured an expanded session on recent advances in therapeutics for RTT symptom treatment and reversal. The “Manhattan Project for the 21st Century,” was the analogy used to describe the development of therapeutics for Rett syndrome and other rare and neglected diseases by Dr. Alan Kozikowski, a medicinal chemist based at the University of Illinois in Chicago. Dr. Kozikowski co-chaired the fourth and final session of the symposium. While the goal of developing therapeutics to reverse Rett syndrome is ambitious, the intense effort and potentially life-changing outcomes are no less paradigm-changing than the atom-splitting feat of the 1940s.

SELECTED PRESENTATION HIGHLIGHTS

This section contains brief descriptions of a sampling of the workshops presented at the symposium. Information included here represents presentations that have been generously provided for public viewing by the presenters.

Please Note: IRSF has made every effort to maintain confidentiality of all presenters' work and the information provided below is provided with permission by the presenters.

SESSION I. REGULATION AND FUNCTION OF MeCP₂

CO-CHAIRS: HUDA Y. ZOGHBI, MD, BAYLOR COLLEGE OF MEDICINE & YI EVE SUN, PHD, UNIVERSITY OF CALIFORNIA – LOS ANGELES

TOWARDS UNDERSTANDING THE PATHOGENESIS OF RETT SYNDROME

KEYNOTE SPEAKER: HUDA Y. ZOGHBI, MD, BAYLOR COLLEGE OF MEDICINE

Pre-eminent RTT researcher, Huda Y. Zoghbi MD (Baylor College of Medicine) delivered the meeting's keynote address. In her opening remarks, Dr. Zoghbi noted that about half the participants in the symposium were first-time attendees, and emphasized the importance of each individual participant to the work that is being done. "Superheroes are people who have powers we don't have. That's how the families see you," she told the group of scientists, researchers and clinicians gathered during the opening session.

She pointed out that some drugs commonly prescribed today are helpful in addressing the symptoms of Rett syndrome (RTT), such as SSRIs and anti-anxiolytics. The future, though, is in discoveries that specifically address RTT. The discovery of Methyl-CpG-binding protein (MeCP₂) has been critical to moving the search for treatments beyond existing therapies into addressing the disease at its source.

In her report, Towards an Understanding of the Pathogenesis of Rett syndrome, Dr. Zoghbi wrote, "To determine how MeCP₂ affects the function of specific neurons and to identify the anatomical origin of specific features of Rett syndrome, her laboratory has taken a genetic approach that deletes MeCP₂ from specific neurons in different regions of the brain. Mice with neuron-specific deletions of MeCP₂ present characteristic Rett syndrome symptoms as well as certain neuropsychiatric features that are sometimes masked in the constitutive deletions of MeCP₂ and in Rett syndrome. Molecular studies of mice lacking MeCP₂ in selected neurons are beginning to identify pathways that might be of therapeutic relevance to MeCP₂ disorders."

MECP2 AND ATRX INTERACTION AND FUNCTION IN THE MOUSE BRAIN

Nathalie Bérubé, PhD, University of Western Ontario, London, Canada

Dr. Bérubé explored the genetic basis for ATRX alpha thalassemia mental retardation syndrome – X-linked (only manifests in males) and its relationship to MeCP2 and cohesin. These chromatin regulators co-localize at the (H19) imprinting control region, and Dr. Bérubé concludes that “these findings suggest that ATRX, cohesin and MeCP2 cooperate to silence a subset of imprinted genes in the postnatal mouse brain.”

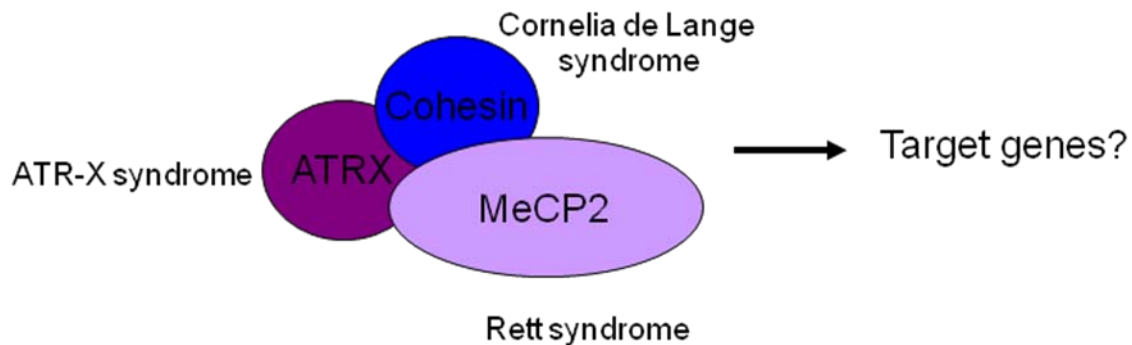


Figure 1: Relationships between ATRX Syndrome and Rett syndrome

LONG-RANGE CHROMATIN INTERACTIONS AND TRANSCRIPTION REGULATION

Yijun Ruan, PhD, Genome Institute of Singapore

Dr. Ruan's group has developed the Chromatin Interaction Analysis by Paired-End-Tag sequencing (ChIA-PET) to study transcription regulation mediated by potential long-range chromatin interactions in a genome-wide manner. The group has mapped the chromatin interaction network bound by the oestrogen receptor alpha (ERalpha) in the human genome.

“Our results suggest that long-range chromatin interaction is a primary mechanism for transcription regulation networks in mammalian genomes. We plan to use this approach to unravel the high order chromatin regulatory functions of MeCP2 neurons,” wrote Dr. Ruan.

MECP2 PROGRAMS PERSISTENT ADVERSE EFFECTS OF EARLY-LIFE STRESS

Dr. Dietmar Spengler, MD, Max-Planck Institute of Psychiatry

Dr. Spengler's work revolves around gene circuits underlying long-lasting stress responses in the central nervous system. Epigenetic marking of the arginine vasopressin (AVP) gene by early-life stress in mice underpins sustained expression and increased hypothalamic-pituitary-adrenal axis activity, triggering endocrine and behavioral alterations that are frequent features in depression.

Dr. Spengler wrote, "In a vicious circle, MeCP2 occupancy uncouples from the initial stimulus and leads to the hard-coding of early-life experience at the level of DNA methylation. The sequential order of these events demarcates the transition from a preliminary to a persistent, possibly irreversible, epigenetic memory and thus defines a critical time window for the timely therapy of severe trauma."

In this way, Dr. Spengler studies the relationship among early-life stress, its affect on methylation and MeCP2.

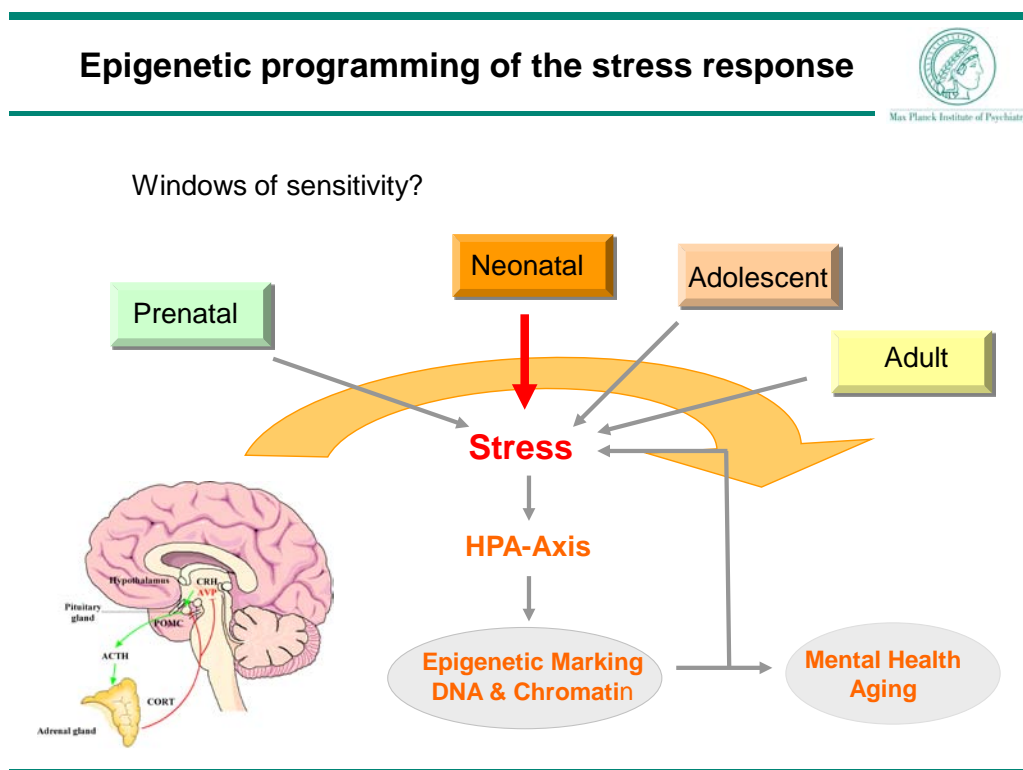


Figure 2: Effect of Early-Life Stressors Across Lifespan

SESSION II. PATHOPHYSIOLOGY OF RETT SYNDROME

CHAIR: JAMES EUBANKS, PhD, UNIVERSITY OF TORONTO

MODELING MeCP2 MUTATIONS AND POTENTIAL THERAPIES USING THE OLFACTORY SYSTEM

Alicia Degano, PhD, CIQUIBIC-CONICET, Universidad Nacional de Cordoba, Argentina and Johns Hopkins University, School of Medicine, Gabrielle Ronnett, MD, PhD, Johns Hopkins University, School of Medicine

Dr. Degano reported that the olfactory system is useful for analysis of neuro-developmental defects. She explained that researchers are limited in understanding the pathophysiology of many brain diseases because brain tissue is inaccessible during active phases of the disease process. However, the olfactory system has attributes that make it attractive for modeling neuronal development and CNS diseases, thus making the disease process more transparent to researchers.

ASPECTS OF SYNAPTIC AND STRUCTURAL PLASTICITY IN A MOUSE MODEL OF RETT SYNDROME

Stuart Cobb, DPhil., University of Glasgow

Dr. Cobb explained that while the exact function of MeCP2 is still unclear, it is believed that it is an important hub in regulating neuronal plasticity. Dr. Cobb explores the possibility that the neuronal plasticity deficit may respond to genetic or pharmacologic manipulation, meaning it may be a target for RTT therapies.

SESSION III. SHARED NEUROBIOLOGY OF RETT SYNDROME AND OTHER AUTISM SPECTRUM DISORDERS

CHAIR: EMANUEL DiCICCO-BLOOM, MD, UNIVERSITY OF MEDICINE & DENTISTRY OF NEW JERSEY, ROBERT WOOD JOHNSON MEDICAL SCHOOL

BEHAVIORAL PHENOTYPING ASSAYS TO TEST HYPOTHESES AND EVALUATE TREATMENT IN MOUSE MODELS OF AUTISM SPECTRUM DISORDERS

Jacqueline N. Crawley, PhD, Director, the Laboratory of Behavioral Neuroscience at the National Institute of Health, Bethesda, Maryland

Dr. Crawley's presentation started out with a cautionary note that mouse models cannot capture the uniquely human components of neurodevelopmental disorders, stating at the outset that there "will never be an autistic mouse," however she went on to explain the practical value of using mice in research.

Mouse models have three critical functions:

1. Establish face validity - generating mouse behavioral assays with analogies to the symptoms of autism
2. Establish construct validity - Mouse models are used to test hypotheses, e.g. genes linked to symptoms of autism, synapse development, neuroanatomical abnormalities, immune dysfunctions, or environmental toxins
3. Establish predictive validity - Robust mouse models offer translational tools to evaluate therapeutic efficacy

Researchers therefore need to consider which human characteristics of autism might have measurable features in mice and which human symptoms are most important to include in a mouse model.

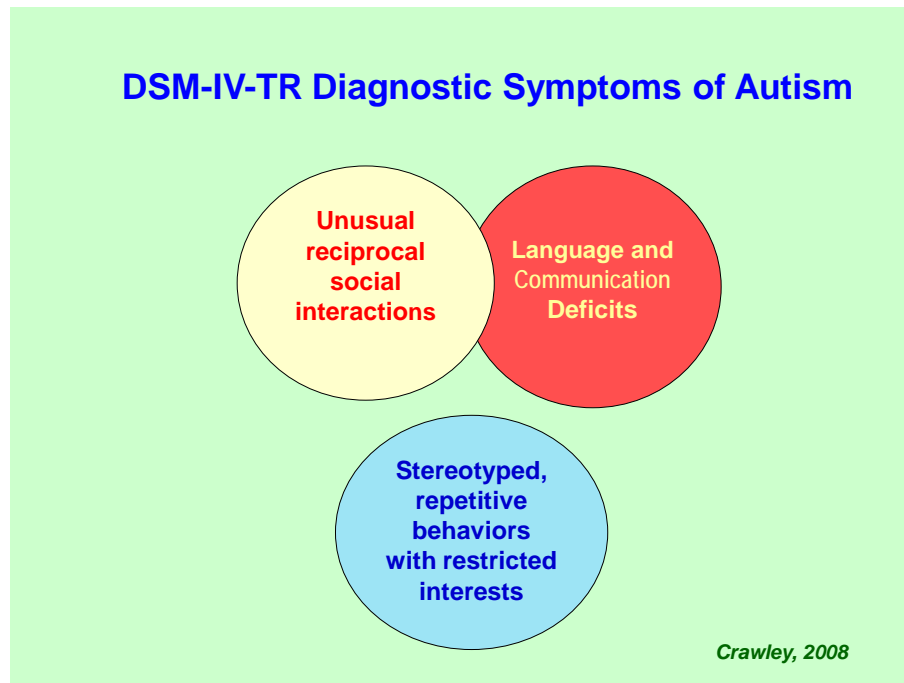


Figure 3. Diagnostic & Symptomatic Features of Autism

Associated symptoms include:

- Seizures
- Anxiety
- Low IQ
- Sleep disruption
- Hyperreactivity and hyporeactivity to sensory stimuli

Dr. Crawley's lab is developing mouse behavioral paradigms that have analogies to the three diagnostic symptoms of autism: social, language/communication and repetitive behaviors. Her laboratory is systematically applying these assays to phenotype mice with mutations in candidate genes for autism spectrum disorders. She concluded her presentation with encouraging news that: "Early preclinical results show that some autism-relevant behavioral phenotypes in the BTBR mouse model of autism are reversed by drug treatments and environmental interventions. These included a juvenile social peer intervention, treatment with an mGluR5 antagonist, and treatment with an AMPA receptor modulator."

SHARED NEUROBIOLOGY OF RETT SYNDROME AND AUTISM: INSIGHTS FROM IMAGING GENETICS

Ashley A. Van Zeeland, PhD, Scripps Translational Science Institute

Dr. Van Zeeland described imaging genetics as "a window into gene action within the living brain." She writes that the goals of imaging genetics are to:

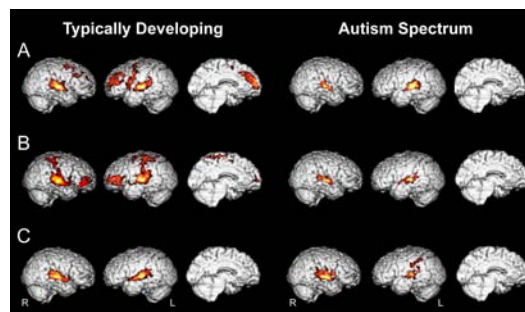
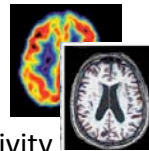
- Understand neuropathophysiology associated with genetic mutation
- Identify associations between genes and underlying neural circuits
- Identify potential drug targets based on neurobiology and genetics

While the gross morphological structural differences are recognized for both disorders i.e., microcephaly in RTT and macrocephaly in ASD, imaging genetics offers insights into the functional connectivity of neural circuits in both.

“The ability to combine brain imaging with genetics can yield greater insights into the ways the implicated risk genes impact the structure and function of neural circuits in these disorders,” she wrote.

Multiple Non-invasive Brain Imaging Modalities Provide Insight into Function and Structure

- Measure *in vivo* brain structure and function
- Modalities:
 - PET: metabolism, receptor density
 - MRI: structure
 - fMRI: function, functional connectivity



Scott-Van Zeeland et al., 2010 *Biol Psych*

Figure 4: Types of Imaging Modalities and Insights

SESSION IV. THERAPEUTIC STRATEGIES FOR THE TREATMENT OF RETT SYNDROME (MOLECULES & MODELS)

CO-CHAIRS: JOHN MCCALL, PhD, PHARMAC, LLC & ALAN P. KOZIKOWSKI, PhD, UNIVERSITY OF ILLINOIS – CHICAGO

IRSF TRANSLATIONAL RESEARCH WORKSHOP ON RETT SYNDROME: OVERVIEW

John McCall, PhD, President, PharMac LLC

To kick-off Session IV, Dr. McCall discussed the pathway of drug discovery, and the role of translational research and development in accelerating the movement of experimental therapies to trial and to market. Translational projects cross disciplines, since it involves a broad range of activities, including identification of valid biological targets, discovery of how to use the molecules identified, evaluating clinical efficacy, establishing proof of concept, and registering the endpoints to deliver a drug to market. He also explained that foundations, such as ISRF, and the National Institutes of Health (NIH) are increasingly focusing resources on translational research to optimize the value of their research portfolios.

Dr. McCall developed a flow chart (Figure 5) that describes the process of drug discovery from identifying a biological target to delivering an FDA-approved drug. The goal of translational research is to jump into the process at the “repurposing” stage. Drug “repurposing” allows a scientist to build on the work of others as identified in this chart, adapted from his presentation:

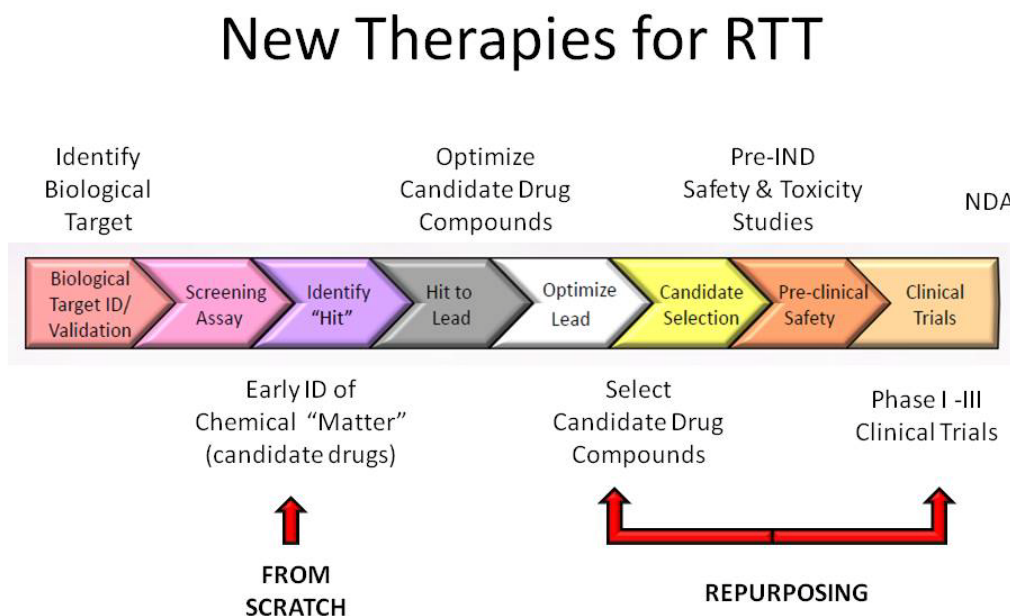


Figure 5: New Therapies for RTT – Pathway to drug discovery (Adapted)

Dr. McCall described the successful result of the translational research and development process as one that results in a marketable drug that is able to be prescribed by physicians and acquired by patients.

“A drug must be generally available at a reasonable price and paid by a third party payer,” he said.

Epigenetic Medicine – Developing Chromatin Remodelers for RTT

Alan P. Kozikowski, PhD, University of Illinois, Chicago, Drug Discovery Program, International Drug Discovery Institute

Dr. Kozikowski explored the potential of histone deacetylase inhibitors (HDACi) to modulate and possibly reverse MeCP2 deficiencies. HDAC₁, valproic acid, has already shown to be somewhat effective in this regard, although it is a non-selective HDAC. Research suggests that HDAC inhibitors may be used in combination with other therapies to achieve the best therapeutic result.

Further exploration of HDAC inhibitors isolated highly selective HDAC6 as a safe and effective choice.

BDNF-Targeted Therapies for Rett syndrome

David M. Katz, PhD, Department of Neurosciences, Case Western Reserve University, School of Medicine

Dr. Katz described the importance of BDNF in the treatment of Rett syndrome.

“Reduced expression of brain-derived neurotrophic factor has emerged as a potentially critical factor in the cascade of events leading from MeCP2 mutations to neuronal dysfunction. He notes three observations related to BDNF:

1. BDNF is required for maturation and function of neural symptoms affected in RTT
2. BDNF expression is reduced in RTT patients and mouse models of RTT
3. Increasing BDNF levels in RTT mice by genetic or pharmacologic means improves physiologic function and survival

Dr. Katz concludes that raising the level of BDNF signaling may have therapeutic value in Rett syndrome.

Development of BDNF Domain Mimetics Functioning as TrkB Ligands

Frank Longo, MD, PhD, George and Lucy Becker Professor, Chairman, Department of Neurology and Neurological Sciences, Stanford University

BDNF bind to the TrkB (Track B) tyrosine kinase receptor to promote neuronal survival, neurite outgrowth, and spine formation and synaptic plasticity. Researchers have been looking for a molecule that mimics selected domains within the BDNF ligand to bind and activate TrkB with specificity and high potency TrkB ligands have been applied to several in vitro and in vivo disease models including Rett syndrome. These have been found to inhibit neuronal degeneration and/or improve disease-related functional outcomes.

“These compounds are serving as leads to create novel derivatives for application in multiple disease settings,” Dr. Longo wrote. A first paper describing the initial set of compounds was published in 2010 in the Journal of Clinical Investigation.

Patient and Mouse iPS Cells to Study Rett syndrome

Aaron Cheung, HBSc., The Hospital for Sick Children, Toronto, Canada

Mr. Cheung described his work in the lab of Dr. James Ellis in which induced pluripotent stem cells (iPS) have been obtained from both patients and a mouse model, and then the cells were differentiated into affect neural lineages for *in vitro* phenotyping. He described his work, “Induced pluripotent stem cells (iPS) are reprogrammed from lineage committed somatic cells into a pluripotent state characteristic of embryonic stem cells.”

The promise of this work is that “patient-specific iPS cells have huge potential in regenerative medicine as an inexhaustible source of affected cells which can be used for in vitro applications such as disease modeling and drug screening, and potentially for cell replacement therapy,” he said.

About the Participants

Participants included: Huda Y. Zoghbi, MD (Baylor College of Medicine), Yi Eve Sun, PhD (University of California – Los Angeles), David M. Katz, PhD (Case Western Reserve University, School of Medicine), Nathalie Bérubé, PhD (University of Ontario), Yijun Ruan, PhD (Genome Institute of Singapore), Dietmar Spengler, MD (Max-Planck Institute of Psychiatry), Juliette Nectoux, PhD (Université Paris Descartes, Institut Cochin, INSERM), James Eubanks, PhD (University of Toronto), Alicia Degano, PhD (CIQUIBIC-CONICET, Universidad Nacional de Córdoba, Argentina & John’s Hopkins University, School of Medicine), Lisa Monteggia, PhD (University of Texas Southwestern Medical Center – Dallas), Stewart Cobb, DPhil. (University of Glasgow), Emanuel DiCicco-Bloom, MD (University of Medicine &

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