ASSOCIATION FOR CREATINE DEFICIENCIES

BUILDING STRENGTH
changing lives

# 2021 CCDS Virtual Conference

## Program Agenda & Abstracts

2021 | CCDS
VIRTUAL CONFERENCE

**SESSION 1**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

---

**SANGEETHA IYER**
Association For Creatine Deficiencies

**Bio**

Dr. Sangeetha Iyer received her PhD in Molecular Pharmacology from the University of Pittsburgh and went on to complete her postdoctoral research at the University of Texas at Austin. She has over ten years of experience in model/assay development and drug screening for human disorders. Dr. Iyer is currently employed at Denali Therapeutics Inc, where she and her team develop assays for early as well as late stage therapeutic programs headed to the clinic. She works across multiple therapeutic modalities such as gene therapy, large molecules as well as small molecule drug candidates. Prior to Denali Therapeutics, Dr. Iyer was employed at Perlara PBC, a drug discovery company in San Francisco committed to finding therapeutics for rare genetic diseases. During her time there, she specialized in all aspects of the therapeutic discovery process- creating tools to study a specific disease, conducting drug discovery screens and identifying biomarkers for a successful transition to clinic. In her role, she also interacted with parents and foundations and laid the foundation for PerlQuests- a patient-driven personalized drug discovery program. With the assistance of clinical KOL's and parent advocates, she was involved in generating a roadmap for an n=1 trial for phosphomannomutase 2 deficiency that is currently underway. Since 2020, Dr. Iyer has been working with the Association of Creatine Deficiencies as their scientific consultant to refine their scientific research roadmap. She brings her expertise in working with rare disease patient groups, clinical KOL's and scientific discovery processes to her role with the ACD.

**2021 CCDS Virtual Conference Opening Comments**

**SESSION 1**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

---

**OLIVIER BRAISSANT**
Service of Clinical Chemistry, Lausanne University Hospital, Switzerland

**Bio**
Working on cerebral creatine for many years, I contributed to the understanding of how creatine can be transported from the periphery to the central nervous system, as well as how creatine can be synthesized and transported within the brain. Our work also contributed to better understanding creatine metabolism and transport in the brain under AGAT, GAMT and SLC6A8 deficiencies (CCDS). We are currently developing and working on several in vitro and in vivo models of CCDS, including a new knock-in rat model of creatine transporter deficiency on which we are developing new strategies of treatment through AAV gene therapy.

**Abstract**
"AAV Strategy To Treat Creatine Transporter Deficiency In The Slc6a8y389c KI Rat"
*Gabriella Fernandes-Pires, Lara Duran-Trio, Marc Lanzillo, Liliane Tenenbaum, Dunja Simicic, Cristina Cudalbu, Olivier Braissant*

Among the three primary creatine deficiencies, creatine transporter deficiency (CTD) is the sole devoid of treatment so far, despite 20 years of research. We have designed an in vivo model of CTD, the Slc6a8Y389C knock-in (KI) rat, in which we are developing adeno-associated virus (AAV) gene therapy as proof of concept for SLC6A8 deficiency treatment. We will present here AAV9 transduction of both WT and Slc6a8Y389C/y rat males with either the GFP or mCherry fluorescent proteins as well as with the functional Slc6a8 transporter.
WT and KI males were injected at postnatal day 11 intravenously (100µl ; 1013 AAV9 vg/ml) or intracisternally (10µl ; 1012 AAV9 vg/ml) with scAAV9-2YF-CMV-GFP, ssAAV9-2YF-CMV-mCherry or ssAAV9-2YF-CMV-SLC6A8-Flag suspensions. Animals were sacrificed at 5 and 16 weeks post-injection to assess AAV9 transduction efficacy and cellular tropism. Starting from 5 weeks post-injection, a subgroup of ssAAV9-2YF-CMV-SLC6A8-Flag-injected KI males were exposed to daily Cr treatment (2g/kg*day).
We observed a widespread GFP and mCherry transduction in most brain regions (cortex, hippocampus, medulla oblongata, cerebellum, spinal cord), as well as in peripheral organs (kidney, liver, heart, muscle). The SLC6A8-Flag transporter was also observed both in peripheral tissues and (at low levels) in CNS of KI-injected males. As compared to non-injected KI males, ssAAV9-2YF-CMV-SLC6A8-Flag-injected KI males showed partial reestablishment of body weight gain and BMI. While still ongoing and effort-demanding in terms of improvement, our strategy appears as promising to treat SLC6A8 deficiency.

**SESSION 1**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

---

**RIKKE BIRKEDAL**
Tallinn University of Technology, Department of Cybernetics

**Bio**
Since I received my Ph.D. in 2004 in comparative physiology from the University of Aarhus in Denmark, I have worked as a post doctoral student at INSERM-769 in France and The University of Manchester in the UK. From 2008, I have worked as a senior researcher in the Laboratory of Systems Biology in Tallinn, Estonia. Much of our work is focused on the cardiac phenotype of creatine deficient AGAT and GAMT mice. We are interested in the interplay between structure, metabolism, excitation-contraction coupling and mechanical performance of the heart, and how this is affected by the lack of creatine.

**Abstract**
"The Effect of Creatine Deficiency On Muscles and Heart In AGAT And GAMT Mice"

The work in our laboratory focuses on how creatine deficiency in AGAT and GAMT mice affects the skeletal muscles and, in particular, heart. In skeletal muscles, the extent of atrophy varies with the muscle type. In GAMT mice, hindleg muscles weigh 55-75 % of those in wildtype mice, and only small changes in metabolic marker enzymes are observed. In AGAT mice, the hindleg muscles weigh 25-65 % of those in wildtype mice and our measurements indicate a large shift in metabolism. The total heart weight is smaller, whereas the ratio of heart weight to body weight is larger in GAMT and AGAT mice. Also, there were no changes in metabolic marker enzymes or the respiration capacity in the heart. In cardiomyocytes from AGAT mice, the expression and location of hexokinase was altered suggesting changes in regulation and/or substrate utilization of mitochondria. Furthermore, the fluxes of calcium, which regulate excitation-contraction coupling of the heart, seemed to be slowed down. This adaptation of calcium fluxes to the absence of active creatine kinase is a subject of further studies. However, we demonstrated that the changes in calcium fluxes were fully reversed by creatine supplementation when given from the time of weaning.

**SESSION 1**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

---

**LARA DURAN**
CHUV-UNIL

**Bio**
Dr. Lara Duran-Trio is a neuroscientist with experience in the fields of development of central nervous system, neuronal plasticity and neurometabolism. She graduated with degrees in Biology and in Biochemistry and did a MScRes in Molecular Biomedicine. She did her PhD studying the pathological mechanisms of a rare and fatal neurometabolic disorder (the neurodegenerative epilepsy called Lafora Disease) and, as a postdoc, she joined the laboratory of Prof. Olivier Braissant at the Lausanne University Hospital (CHUV) to study the effects of another metabolic disorder, creatine transporter deficiency (CTD), in a new rat model. She is member of the Society for Neuroscience and member of the Managing Board of crowdfight.org

**Abstract**
"Slc6a8xy389c/Y KI Rat Males Exhibit Motor Dysfunction Linked With Muscle Cr Deficiency Without Muscle Atrophy And Morphological Alterations In Cerebellum"

Creatine is a nitrogenous organic acid and plays roles as fast phosphate energy buffer to replenish ATP, osmolyte, antioxidant, neuromodulator, and as a compound with anabolic and ergogenic properties in muscle. Creatine is taken from the diet or endogenously synthetized by the enzymes AGAT and GAMT, and specifically taken up by the transporter SLC6A8. Loss-of-function mutations in the genes encoding for the enzymes or the transporter cause Cerebral Creatine Deficiency Syndromes (CCDS). CCDS are characterized by brain creatine deficiency, intellectual disability with severe speech delay, behavioral troubles, epilepsy and motor dysfunction. Among CCDS, the X-linked creatine transporter deficiency (CTD) is the most prevalent with no efficient treatment so far. Different animal models of CTD show reduced brain creatine levels, cognitive deficiencies and together they cover other traits similar to those of patients. However, motor function was poorly explored in CTD models and some controversies in the phenotype exist in comparison with CTD patients. Our recently described Slc6a8y/Y389C knock-in (KI) rat model of CTD showed mild impaired motor function linked with reduced muscular mass without signs of muscle atrophy, creatine deficiency and increase GAA content in muscle, and morphological alterations in cerebellum. Our results point that such motor dysfunction is due to both nervous and muscle alterations.

**SESSION 2**
**9:30 – 10:30 am PDT, 18:30 – 19:30 CET**

---

**ELSA GHIRADINI**
CNR Institute of Neuroscience

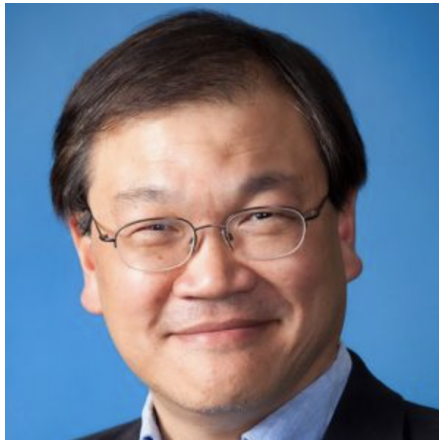
**Bio**

My long-term research interests involve the study of how neurons function and how alterations in this process contribute to human disease. I began my scientific journey at the University of Milan, Italy, where I dedicated my Master and PhD studies to understand how neurons fail to communicate and eventually die in a type of neurodegenerative conditions called prion diseases. During this period, I also spent some time in Germany, at the university of Kaiserslautern, where I studied how a type of non-neuronal brain cells called astrocytes participate in the process of neurotransmission. After my PhD I moved to the University of Cambridge, UK, to study human neurons derived from Induced Pluripotent Stem Cells (IPSCs). There, I improved my knowledge on neurodevelopment and stem cell biology and developed novel methods for generating IPSC-derived neurons with mature functional properties. As a natural result of the combination of my interests in brain pathology and neurodevelopment, I became interested in neurodevelopmental disorders as conditions with increasing impact on individuals and society and, often, with unmet medical needs. I am currently a Postdoc at the CNR Institute of Neuroscience, Pisa, Italy, where I work on Creatine Transporter Deficiency. The aim of my research is to understand how the lack of brain creatine leads to neurological dysfunction and to establish a gene therapy protocol to rescue the pathological phenotype.

**Abstract**

"Tackling Creatine Transporter Deficiency: New Insight Into Cell-specific Vulnerability And Development Of A Gene Therapy Approach"

Creatine Transporter Deficiency (CTD) is an X-linked neurodevelopmental disorder caused by mutations in the Creatine Transporter (CrT) gene presenting with cerebral creatine depletion, intellectual disability, behavioural problems, and epilepsy. To these days there is no cure for CTD, and the pathogenic mechanisms of the disease remain elusive, hampering the identification of good therapeutic targets. Achieving a better understanding of the bases of CTD and searching for therapies are therefore challenges that need to be addressed in parallel. We generated a mouse model which faithfully recapitulates the symptoms observed in patients. Based on this tool, we studied how creatine depletion affects the different cell populations of the brain. By combining single-cell RNA sequencing, electrophysiological techniques, and behavioural studies we found that creatine depletion alters gene expression in specific cell types, with a major impact on parvalbumin inhibitory neurons, causing structural and functional alteration in these cells. Creatine depletion in parvalbumin neurons is sufficient to cause cognitive impairment and increased susceptibility to epilepsy, indicating a fundamental role for these cells in the pathogenesis of CTD. We are also evaluating gene therapy as a possible treatment. We used Adeno-Associated Viral vectors to deliver the functional CrT gene (AAV/CrT) to newborn CTD mice. AAV/CrT administration resulted in the expression of transgenic CrT, increasing brain creatine levels and improving cognitive performance. However, toxicity was observed with high titres of the vector. We are currently optimising the vector dosage and design to obtain a widespread, physiological expression of CrT reducing the toxicity caused by creatine overload.



**SESSION 2**
**9:30 – 10:30 am PDT, 18:30 – 19:30 CET**

---

**ALEX KUAN**
University of Virginia School of Medicine, Department Of Neuroscience

**Bio**
Chia-Yi (Alex) Kuan, MD, PhD is a Professor of Neuroscience at the University of Virginia (UVA) School of Medicine. Dr. Kuan graduated from the National Taiwan University College of Medicine, and trained with Dr. Pasko Rakic, a premium developmental neurobiologist and Member of the National Academy of Science, for his graduate and post-graduate research at Yale University. Dr. Kuan served as faculty at the Cincinnati Children's Hospital Medical Center (2001-2012) and the Emory University Department of Pediatrics (2012-2017) before his recent relocation to UVA.

Dr. Kuan's research focuses on developmental brain disorders, especially on acute perinatal injury that could result in cerebral palsy. Dr. Kuan has published >70 research articles and serves on the editorial board of several leading neuroscience research journals, including Cerebral Cortex. Moreover, Dr. Kuan served on two National Institute of Health (NIH) study sections, Neurogenesis and Cell fate (NCF) and Developmental Brain Disorders (DBD), and travelled frequently to give research seminars.

**Abstract**
"The Importance Creatine Transporter For Maintaining the Brain Energetics and Stress-adaptation Homeostasis"

Creatine transporter (CrT) upholds the brain creatine (Cr) levels, but the impacts of its deficiency on brain energetics and stress adaptation remain uncertain. Neither are there effective treatments of CrT-deficiency, which is the second most common cause of X-linked intellectual disabilities. To address these issues, we produced CrT-deficient (CrT-/y) mice to assess their neurocytological defects and responses to a multitude of brain stress and insults. We found that CrT-deficient mice harbored dendritic spine and synaptic dysgenesis. Nurtured CrT-null mouse neonates maintained the baseline brain ATP level by pivoting towards the pAMPK/autophagy signaling from mTOR activity, which was amplified by starvation and correlated with reduction of the brain ATP levels in CrT-null mice. Likewise, CrT-null neonates showed an imbalance between autophagy/mTOR signaling pathways and greater susceptibility to cerebral hypoxia-ischemia and ischemic insults. Notably, intranasal administration of Cr after cerebral ischemia increased the brain Cr/NAA (N-acetylaspartate) ratio, partially averted the signaling imbalance, and reduced the infarct size more potently than intraperitoneal Cr injection. These findings suggest important functions of Cr and CrT in preserving the homeostasis of brain energetics in stress conditions. Further, intranasal Cr supplement may be an effective therapy of congenital CrT-deficiency and acute brain injury.

**SESSION 2**
**9:30 – 10:30 am PDT, 18:30 – 19:30 CET**

---

**MADELEINE HALL**
The Hospital for Sick Children

**Bio**
Madeleine Hall is an inspired biochemistry student at McGill University and research student in Genetics & Genome Biology at The Hospital for Sick Children in Toronto, Canada. Her interest in Creatine Deficiency Syndromes and involvement in research is driven by the curiosity in seeing how her biochemistry classes translate into helping improve lives. Being awarded a National Sciences and Engineering Research Council of Canada (NSERC) Undergraduate Student Research Award assisted her engagement in research.

**Abstract**
"Effect of Creatine Supplementation on AGAT Expression and Metabolic Intermediates in GAMT-Deficient Mice"

We investigated the effect of creatine supplementation on the expression of AGAT as well as creatine metabolites in a creatine-deficient mouse model. Wildtype and GAMT-deficient, 12-weeks old mice were fed with creatine-free or creatine-enriched (2% or 4%) mouse chow for ten weeks. Urine was collected weekly. Brain, kidney, liver, heart, and skeletal muscle were harvested after ten weeks.
In urine, HPLC analysis demonstrated increase of creatine from 1.600 to 4.370 and 14.500 µmol/mol creatinine in wildtype and from zero to 8,000 and 6,500 in mutant mice treated with 2% and 4% creatine, respectively. GAA decreased from 200 to 40 (2%) and 30 (4%) µmol/mol creatinine in wildtype and from 6,900 to 800 (2%) and 400 (4%) in mutant mice.
In kidney, LC-MSMS analysis demonstrated increase of creatine from 600 to 2,000 (2%) and 3,000 (4%) in wildtype and from 20 to 2,000 (2% and 4%) in mutants. GAA decreased from 200 to 100 (2%) and 80 (4%) in wildtype and from 1,000 to 400 (2% and 4%) in mutants. Quantitative PCR (qPCR) and Western blot analysis revealed marked decrease in AGAT gene and protein expression by ~50% (2% and 4%) in wildtype and mutant mice.
In summary, creatine metabolite analysis in urine and organ homogenates validates the GAMT-deficient model and confirms the efficacy of creatine supplementation through marked increases of creatine and persistent reductions in GAA and both in wildtype and mutant mice. The effect on GAA is likely caused by the creatine mediated suppression of AGAT.

**SESSION 3**
**10:45 – 11:45 am PDT, 19:45 – 20:45 CET**

---

**AURORE CURIE**
French National Reference Center For Rare Diseases With Intellectual Disability, Department Of Child Neurology, Woman Mother And Child Hospital, Lyon University Hospital

**Bio**
Aurore Curie is a child neurologist (MD, PhD) at the Child Neurology Department of Lyon Hospital (Assistant Professor) and the Reference Center for Intellectual Disability (ID) from rare causes (Co-Head). She is affiliated to the Lyon Neuroscience Research Center (CNRS UMR5292, Inserm U1028, Lyon, France) and also part of the DéfiScience national network for rare diseases of brain development and ID. She coordinates a French Inter University Diploma (DIU) on Neurodevelopmental Disorders. She has a strong expertise in genetics (especially in X-linked ID) and in neuroscience. She developed new outcome measure adapted to ID patients (HCL/CNRS patent). She contributed to the development of the research plateform "Cognitoscope". Her clinical and research expertise is dedicated to X-Linked ID and other ID from rare causes. She described cognitive profiles of neurodevelopmental disorders (including ARX, PQBP1, Rab-GDI, SLC6A8 mutated patients) using eye-tracking and neuroimaging analysis, and contributed to several multisite clinical trials for Fragile X syndrome. She also furthered our knowledge on placebo effect in ID patients, and the different trial plans that can be used in ID patients to test for an effect (Randomized controlled double blind Clinical Trials (RCT) but also n-of-1 trials, also called Single-Case Experimental Designs or SCEDs).

**Abstract**
"Efficacy Judgment Criteria for Clinical Trial in Creatine Transporter Deficiency"

Creatine Transporter Deficiency (CTD) is a rare genetic disorder related to SLC6A8 gene mutations, leading to moderate to severe Intellectual Disability (ID). Most of the cognitive tests were developed to distinguish typically developing persons and ID patients, leading to a floor effect in the latter who systematically fail these tests. Therefore, these tests are not adapted to capture the potential effect of a drug within ID patient group. As new avenues are emerging for treatment in CTD, it is necessary to identify objective, reliable and sensitive outcome measures for use in future clinical trials.
To address the lack of outcome measures adapted to CTD patients, we developed new quantitative and objective measures appropriate for mild to severe ID patients. We present here these new tests that could constitute useful biomarkers in designing future studies.
We developed new outcome measures on tablet, testing elementary visuo-spatial perception, and non-verbal reasoning. We also developed eye-tracking tasks: (i) to analyze the exploration of social visual scenes, (ii) to compute a relative preference for social clip index while seeing a series of clips side by side, one social, and one non-social. This task will assess patients' social interest. All these new tasks were performed on adults (n=20), and typically developed children older than 3 (n=47).
These outcome measure would be interesting to be used as evaluation criteria in drug trials and cognitive rehabilitation programs.

**SESSION 3**
**10:45 – 11:45 am PDT, 19:45 – 20:45 CET**

---

**BETH POTTER**
University of Ottawa

**Bio**
Beth Potter has a PhD in Epidemiology from the University of Western Ontario. She is currently an Associate Professor of Epidemiology and Public Health and the University Research Chair in Health Services for Children with Rare Diseases at the University of Ottawa. She is also an Affiliate Investigator at the Children's Hospital of Eastern Ontario. Her research focuses on developing evidence to improve health care for children with rare genetic diseases, particularly inherited metabolic diseases, and to inform newborn screening programs. Dr. Potter's current studies focus on experiences with family-centred care and the use of disease registries to facilitate observational and experimental studies. She is Principal Investigator for the Canadian Inherited Metabolic Diseases Research Network (www.cimdrn.ca), and INFORM RARE (www.informrare.ca), two Canadian research networks that bring together stakeholders (patients and their family members, clinical providers, methodologists, and policy makers) across pediatric centres to focus on the research questions and outcomes that matter most to rare disease decision-makers.

**Abstract**
"Core Outcome Sets: Engaging Patients and Families in Consensus Methods to Identify the Outcomes That Matter Most"

Objectives: Core outcome sets (COSs) aim to harmonize the collection of outcomes across studies in a disease area to promote meaningful evidence generation and improved patient-centred care. We developed COSs for two rare inherited metabolic diseases in children under 12 years of age. Our approach engaged patients and family members as both research partners and study participants.
Study methods: For each of the two COSs, we first identified candidate outcomes by conducting a systematic literature review of published studies. Subsequently, parents of children with the metabolic diseases, clinicians, and policy advisors participated in a multi-round internet-based Delphi consensus survey. In round 1, participants rated the importance of each outcome on a 9-point scale. In rounds 2 and 3, participants revisited their ratings based on aggregate results. Final COSs were selected by discussion and through voting at an in-person multi-stakeholder workshop.
Patient engagement methods: Two patient partner co-investigators worked with the principal investigator throughout the study to co-develop a patient engagement strategy that enabled the meaningful contributions of seven family advisors. The patient engagement strategy included in-person training and inclusive support for advisors. The family advisors were involved at key points in COS development, for example, in co-developing the surveys and participating in the workshop.
Conclusion: Our final COSs will be incorporated into evaluative studies to inform care for children with these rare conditions. Our approach to patient and family engagement was considered valuable to all team members and could be applied in other rare disease settings.

**SESSION 3**
**10:45 – 11:45 am PDT, 19:45 – 20:45 CET**

---

**MARK LEVIN**
NHLBI, NIH

**Bio**
Dr. Levin is a board certified pediatric cardiologist who completed medical school at Boston University, pediatrics residency at Columbia Presbyterian in NYC, and pediatric cardiology fellowship at Children's Hospital of Philadelphia. He subsequently completed advanced training in non-invasive clinical cardiac electrophysiology also at Children's Hospital of Philadelphia as well as post-doctoral research fellowships in molecular cardiology at the University of Pennsylvania and cellular ion channel physiology at Washington University.

**Abstract**
"Cardiac Abnormalities in Patients and Animal Models of Creatine Transporter Deficiency"

Purpose: Creatine transporter deficiency (CTD) is a rare X-linked disorder of creatine transport caused by pathogenic variants in SLC6A8 (Xq28). CTD features include developmental delay, seizures and autism spectrum disorder. This study was designed to investigate CTD cardiac phenotype and sudden death risk.
Methods: We performed a cross-sectional analysis of CTD males between 2017-2020. Subjects underwent evaluation with ECG, echocardiography and ambulatory ECG with comparable analysis in creatine transporter deficient mice (Slc6a8-/y) using ECG, echocardiography, exercise testing and indirect calorimetry.
Results: Eighteen subjects with CTD [18 males, age 7.4 (3.8) years] were evaluated: seven subjects (39%) had QTc≥470msec: 510.3±29.0 vs. 448.3±15.9, P<0.0001. The QTc≥470msec cohort had increased left ventricular internal dimension(diastole) ((LVIDd) Z-score: 0.22±0.74, n=7 vs. -0.93±1.0, n=11, P=0.0059), and diminished left ventricular posterior wall dimension(diastole)((LVPWDd, in mm): 5.0±0.6, n=7 vs. 5.7±0.8, n=11, P=0.0183), when compared to subjects with normal or borderline QTc prolongation. Similar ECG and echocardiographic abnormalities were seen in Slc6a8-/y mice. Additionally, Slc6a8-/y mice had diminished survival (65%).
Conclusions: Prolonged QTc and abnormal echocardiographic parameters consistent with developing cardiomyopathy are seen in some male subjects with CTD. Slc6a8-/y mice recapitulated these cardiac abnormalities. Male CTD subjects may be at increased risk for cardiac dysfunction and sudden death.

**SESSION 4**
**12:00 – 1:00 pm PDT, 21:00 – 22:00 CET**

---

**STEVE BAKER**
University of Utah

**Bio**
Steven Baker is an assistant professor in the department of pathology at the University of Utah. After completing a bachelor of science in biological sciences at Cornell University, he joined the MD/PhD training program at Baylor College of Medicine. He performed his graduate studies in the laboratory of Dr. Huda Zoghbi studying the molecular basis of Rett syndrome. He continued his medical training as a clinical pathology resident and then transfusion medicine fellow at Stanford University. While at Stanford he carried out research on Cerebral Creatine Deficiency Syndrome (CCDS) as a post-doctoral fellow in the laboratory of Dr. Thomas Montine. His work on CCDS focused on utilizing single cell sequencing resources and immunofluorescence to characterize the creatine synthetic pathway in mammals. Following the completion of his medical training, Dr. Baker joined the faculty of the University of Utah as an associate medical director of transfusion medicine and medical director of the immunohematology reference laboratory at ARUP.

**Abstract**
"GATM and GAMT Synthesize Creatine Locally Throughout the Mammalian Body and within Oligodendrocytes of the Brain"

The enzymes glycine amidinotransferase, mitochondrial (GATM also known as AGAT) and guanidinoacetate N-methyltransferase (GAMT) function together to synthesize creatine from arginine, glycine, and S-Adenosyl methionine. Deficiency in either enzyme or the creatine transporter, CT1, results in a devastating neurological disorder, Cerebral Creatine Deficiency Syndrome (CCDS). To better understand the pathophysiology of CCDS, we mapped the distribution of GATM and GAMT at single cell resolution, leveraging RNA sequencing analysis combined with in vivo immunofluorescence (IF). Using the mouse as a model system, we find that GATM and GAMT are coexpressed in several tissues with distinct and overlapping cellular sources, implicating local synthesis as an important mechanism of creatine metabolism in numerous organs. Extending previous findings at the RNA level, our analysis demonstrates that oligodendrocytes express the highest level of Gatm and Gamt of any cell type in the body. We confirm this finding in the mouse brain by IF, where GATM localizes to the mitochondria of oligodendrocytes, whereas both oligodendrocytes and cerebral cortical neurons express GAMT. Interestingly, the latter is devoid of GATM. Single nucleus assay for transposase-accessible chromatin sequencing (snATAC-seq) analysis of 4 brain regions highlights a similar primacy of oligodendrocytes in the expression of GATM and GAMT in the human central nervous system. Importantly, an active putative regulatory element within intron 2 of human GATM is detected in oligodendrocytes but not neurons.

**SESSION 4**
**12:00 – 1:00 pm PDT, 21:00 – 22:00 CET**

---

**MATTHEW SKELTON**
Cincinnati Children's Research Foundation

**Bio**
Dr. Skelton has been involved in research on CTD for over 10 years. He developed the first creatine transporter knockout mouse in 2011 and has published 12 papers on the CTD mouse model. His lab focuses on the mechanisms that underlie the behavioral effects of CTD. Dr. Skelton was the proud recipient of the first ACD Care Grant and has also received funding from the NIH and several pharmaceutical companies.

**Abstract**
"Examining Executive Function in Creatine Transporter Knockout Mice"

The first paper describing the cognitive deficit in Slc6a8 knockout mice was published 10 years ago this year. In that paper, we showed that Slc6a8 knockout (KO) mice have spatial learning deficits, reductions in novel object memory, and reduced freezing in conditioned and contextual fear paradigms. A criticism of these tasks is that they generally do not have correlative task in humans, reducing translational value. In addition, they omit behaviors related to executive function. In this study, we examined working memory by measuring spontaneous and forced alternation in the Y-maze. There were no differences in Slc6a8 KO mice in this task. We also evaluated attention-related behaviors using 5-choice serial reaction time test (5CSRTT). The 5CSRTT is an operant task that requires the mouse to make a correct decision to obtain a reward (strawberry milk). It is similar to the human 5-choice continuous performance task, providing great translational value. Brain-specific Slc6a8 KO mice had more omissions to respond when shorter stimulus durations were presented, a sign of inattentiveness. Interestingly, when the intertrial interval was extended, the KO mice showed fewer premature responses, which suggests that the mice have reduced impulsivity. The KO mice did not show deficits during training trials, suggesting there were able to learn the task and were motivated for reward. Future studies will examine working memory using delayed match to place testing in trained mice. These data show that Slc6a8 deficient mice have reductions in executive function and that the 5CSRTT may be a reliable and translationally relevant task to test potential therapies for CTD.

**SESSION 4**
**12:00 – 1:00 pm PDT, 21:00 – 22:00 CET**

---

**GERALD LIPSHUTZ**
David Geffen School of Medicine at UCLA

**Bio**

Gerald Lipshutz, MD, MS, received his medical degree from the UCLA School of Medicine and completed his postgraduate training at the University of California San Francisco School of Medicine. Dr. Lipshutz is a Professor-in-Residence within the Departments of Surgery and the Department of Molecular and Medical Pharmacology. He is also a member of the Intellectual and Developmental Disabilities Institute at UCLA along with the Broad Center; he presently holds the Goldwyn Chair. Within the David Geffen School of Medicine, he is the Chairman of the Academic Medicine College. His clinical specialty and interests include liver and pancreas transplantation and gene and cell therapies for single gene metabolic disorders of the liver. Dr. Lipshutz has been an invited participant in several NIH conferences and has served as a grant reviewer for both Wellcome Trust and the National Institutes of Health where he is presently a standing member of the GDD Study Section.
In addition to authoring over 70 peer-reviewed manuscripts, Dr. Lipshutz also serves on the editorial board for Molecular Therapy, Methods and Clinical Development, and Gene Therapy. He is a member of several surgical, transplant, and gene and cell therapy societies including ASGCT and SIMD. As a principal investigator, Dr. Lipshutz's research focuses on regenerative medicine technologies for the investigation and treatment of urea cycle disorders and creatine deficiency disorders; he aims to develop new therapies that would replace liver transplantation for single-enzyme metabolic deficiencies. He is also a PI for multiple NIH grants and industry-sponsored studies for gene therapies.

**Abstract**

"Control of GAA and Restoration of Creatine Levels with Gene Therapy for Guanidinoacetate Methyltransferase Deficiency"

Typical characteristics of guanidinoacetate methyltransferase (GAMT) deficiency include features of autism, self-mutilation, intellectual disability and seizures; approximately 40% will also have a disorder of movement. Guanidinoacetic acid (GAA) neurotoxicity in part has been implicated in the pathophysiology. We sought to develop a gene therapy approach for deficiency of GAMT. Our group developed a viral vector strategy utilizing a serotype-specific adeno-associated viral vector expressing human codon-optimized GAMT under a liver-specific promoter. By intravenous administration, we delivered vectors to 8-12 week old adult GAMT-deficient mice after performing dose-finding studies. Following for 12 months, mice early on gained weight to nearly match that of their wild type littermate controls. Monthly serial collection of blood demonstrated a marked early and sustained reduction of GAA and essentially a normalization of plasma creatine levels. Urine GAA was also markedly reduced. Behavioral studies demonstrated resolution of abnormalities in grip strength and learning detected in the Barnes maze studies. Furthermore, PET-CT imaging of the brain of treated mice demonstrated resolution in impaired glucose utilization. Examination of the liver by in situ hybridization demonstrated marked expression of GAMT mRNA in the murine liver and also demonstrated panhepatic expression of GAMT protein by immunostaining. In conclusion, we have developed a gene therapy approach that normalizes the marked elevation of GAA (with reduced creatine) in guanidinoacetate methyltransferase deficiency and at the same time resolved abnormalities in the behavioral phenotype. These findings have important implications for the development of a new therapy for this disorder of creatine metabolism.

**KEYNOTE SPEAKER**
**1:00 – 2:00 pm PDT, 22:00 – 23:00 CET**

---

**STEVEN GRAY**
University of Texas Southwestern Medical Center

**Bio**
Dr. Steven Gray earned his Ph.D. in molecular biology from Vanderbilt University in 2006, after receiving a B.S. degree with honors from Auburn University. He performed a postdoctoral fellowship focusing on gene therapy in the laboratory of Jude Samulski at UNC Chapel Hill. He is currently an Associate Professor in the Department of Pediatrics at the University of Texas Southwestern Medical Center. Dr. Gray is the director of the UTSW Viral Vector Facility and maintains affiliations with the Department of Molecular Biology, the Department of Neurology and Neurotherapeutics, the Eugene McDermott Center for Human Growth and Development, and the Hamon Center for Regenerative Science and Medicine at UT Southwestern. Dr. Gray's core expertise is in AAV gene therapy vector engineering, followed by optimizing approaches to deliver a gene to the nervous system. His research focus has also included preclinical studies to development AAV-based treatments for neurological diseases, some of which have translated into clinical trials.

**Abstract**
"AAV-mediated Gene Therapy for Neurological Disorders"

Gene therapy for central nervous system (CNS) disorders has seen a recent resurgence with the discovery of adeno-associated virus (AAV) vectors that are capable of crossing the blood-brain barrier (BBB), such as AAV9. The Gray lab has been focused on examining the translational potential of AAV9 to treat inherited CNS disorders. Initial studies demonstrated that AAV9 can achieve dose-dependent, widespread gene transfer to neurons and astrocytes in mice as well as in non-human primates, when injected intravenously or intrathecally. Using AAV9-mediated gene transfer as a platform approach to treat an inherited CNS disease, in 2015 Dr. Gray and colleagues at the NIH initiated a Phase I clinical to test intrathecal administration of scAAV9/JeT-GAN in patients with Giant Axonal Neuropathy. Using the same technology and approach, clinical trials for CLN7 Batten disease and GM2 gangliosidosis have recently begun. Additional studies from Dr. Gray's lab support the initiation clinical trials for Batten disease (CLN1, CLN5), Aspartylglucosaminuria, Krabbe disease, Charcot-Marie-Tooth disease type 4J, and Multiple Sulfatase Deficiency. AAV9, delivered intrathecally or intravenously, is emerging as a platform approach capable of treating an increasing number of nervous system diseases.

**SESSION 5**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

---

**ALANA O'BRIEN**
The Eric Wang Lab at the University of Florida

**Bio**

Alana O'Brien is a third year Ph.D. student in the Biomedical Sciences program at the University of Florida. She completed her bachelor's degree in biology at the University of Florida before joining the Eric Wang Lab in the Center for Neurogenetics to begin her graduate career. O'Brien's research focuses on RNA processing and short tandem repeat expansion disorders, and she is interested in the mechanisms behind repeat expansion. She connected with the ACD through the CZI Network to investigate the RNA processing and protein expression of SLC6A8 in a patient with Creatine Transporter Deficiency.

**Abstract**

"Characterization of SLC6A8 Mis-splicing and Protein Expression in a Patient with Creatine Transporter Deficiency"

Creatine Transporter Deficiency (CTD) is a disorder caused by the dysfunction of the SLC6A8 creatine membrane transporter. If creatine transporter function is deficient, there is inadequate creatine uptake into organs that require it, such as the brain and the muscles, and creatine builds up in the bloodstream. Many mutations in the SLC6A8 gene have been linked to CTD, but it is unclear mechanistically how these individual mutations cause deficiencies at the mRNA or protein level. We characterized the splicing patterns and protein expression of SLC6A8 in cells derived from a patient with CTD. This patient has the chrX:152959835-152960127 deletion, which removes part of exon 10, intron 10, exon 11, and part of intron 11 of the SLC6A8 gene. This deletion spans two out of the 12 transmembrane domains of the SLC6A8 protein. RT-PCR was performed on patient fibroblasts, with primers targeting the region from exon 9 to exon 12. Results showed an SLC6A8 mRNA product in which exon 9 and exon 12 are spliced together. Skipping of exons 10 and 11 does not result in a frameshift, so the presence of an altered SLC6A8 protein remained a possibility. A western blot was performed on the patient fibroblasts using an SLC6A8 antibody targeting the N-terminus in order to determine whether protein was still expressed. SLC6A8 was detected at the expected size in the patient fibroblasts, but these results are preliminary and additional work needs to be done to validate the results of the western blot. The potential expression of SLC6A8 protein with altered function in patients with CTD could give rise to therapeutic approaches that utilize its presence.

**SESSION 5**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

**ALOISE MABONDZO**
CEA

**Bio**

Dr Aloïse Mabondzo joined the French alternative energies and Atomic Energy Commission (CEA), the Life Science Division, in May of 1998 as the leader of a neurovascular pharmacology Lab with a strong focus on in vitro blood-brain barrier (BBB) modeling and pathophysiology of the brain. His Lab developed in vitro screening tools to fully characterize and optimise molecules for brain penetration. His innovative research made possible the development of research programs in the neuroscience field : Alzheimer's disease, nanotoxicology, ischemic hypoxia encephalopathy, creatine transporter deficiency. Dr Aloïse Mabondzo is author or co-author of 62 articles in peer reviewed journals, 7 patents. He directed 14 PhD students, and 7 postdoctoral positions were part of his team. As a Neuroscientist, Dr Mabondzo aims to bridge the gap between experimental research and clinical therapy for cerebral diseases. He is also the cofounder of CERES BRAIN THERAPEUTICS, a spin-off from the CEA, committed to focus its resources to provide CTD patients with a therapeutic solution to deliver creatine into the brain. Ceres' first medicine candidate obtained the Orphan Drug Designation by the EMA and FDA.

**Abstract**

"Non-Invasive Nose-to-Bain Delivery of CBT101 Highlights a Widespread Brain Creatine Content in Non Human-Primates"

Introduction: Creatine (Cr) transporter deficiency (CTD) is an inherited metabolic disease caused by mutation of the SLC6A8 gene encoding the Cr transporter (CrT) in charge of Cr transport across the blood brain barrier and into neurons. Cr is essential for proper brain function, has a crucial role in energy storage and transmission, and has anti-apoptotic, antioxidant, neuroprotector and neuromodulator effects. Several therapeutic approaches have been attempted to address the critical issue of Cr absence in brain cells of CTD patients without any success. Whereas no treatment is available for CTD to date, dodecyl creatine ester or CBT101 might be a therapeutic option for CTD patients based on previously published preclinical in vitro and in vivo data.
To demonstrate the efficacy of the CBT101 nasal dosing to deliver creatine into neurons, we conducted an experiment in non-human primates.
Material & methods: CBT101 was labelled with D2 and C13 and formulated in a dedicated emulsion. CBT101 was then administered via a nasal spray into the nose of 2 cynomolgus daily for one month. Labelled CBT101, creatine and creatinine were quantified by mass spectrometry in plasma, Cerebral Spinal Fluid (CSF), and in different brain areas and cellular subtypes.
Results: Labelled CBT101 and creatine were found in all brain areas and more precisely into neurons.
Conclusion: These promising findings of the presence of CBT101 and of the active compound, creatine, in brain and in neurons are a new step toward a therapeutic solution for patients suffering from CTD.

**SESSION 5**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

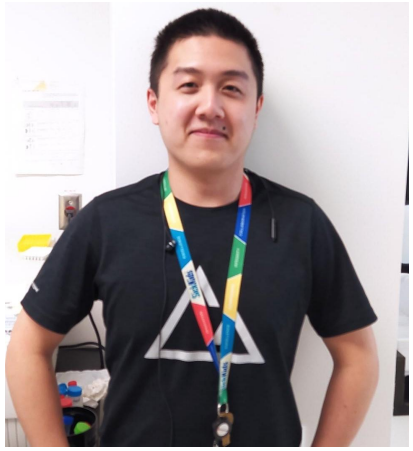
---

**NICOLA LONGO**
University of Utah

**Bio**
Dr. Nicola Longo is a Professor of Pediatrics and Adjunct Professor of Pathology, Nutrition and Integrative Physiology at the University of Utah in Salt Lake City, UT. He is also the Chief of the Division of Medical Genetics, Director of the Metabolic Service, Director of the Training Program in Medical Biochemical Genetics and Medical co-Director of the Biochemical Genetics Lab at ARUP Laboratories in Salt Lake City. His research concerns the molecular bases of metabolic disorders, their identification through newborn screening, their natural history, and the development of novel therapies.

**Abstract**
"Small Molecules for the Treatment of GAMT Deficiency"

Guanidinoacetate methyltransferase (GAMT) deficiency is one of the three inborn errors of metabolism resulting in cerebral creatine deficiency. GAMT deficiency can cause intellectual disability, autistic behavior, seizures, moderate to severe speech delay, hypotonia, and movement disorders. Treatment of GAMT deficiency includes repletion of cerebral creatine (with supplements) and reduction of guanidinoacetate concentrations. Guanidinoacetate, synthesized from arginine and glycine, is reduced using an arginine/protein-restricted diet, high-dose ornithine supplementation (ornithine inhibits guanidinoacetate synthesis) and sodium benzoate (that binds to and reduces the concentration of glycine). Even with therapy, guanidinoacetate concentration remains elevated several folds above normal, explaining the incomplete response of GAMT deficiency to existing therapies. To develop inhibitors, we purified the AGAT (arginine:glycine amidino transferase) enzyme that synthesizes guanidinoacetate after a genetic modification to remove the mitochondrial targeting sequence and to make it more soluble. We developed and validated an assay capable of measuring production of ornithine and guanidinoacetate from AGAT and we are testing a series of 100 putative inhibitors designed and synthesized based on the crystal structure of the AGAT enzymes. Our results show that the assay is robust and reproducible with production of equimolar amounts of guanidinoacetate and ornithine, a pre-requisite before testing putative inhibitors capable of reducing guanidinoacetate synthesis.



**SESSION 5**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

---

**ALEX LEE**
University of Toronto

**Bio**
My name is Alex and I am a MSc candidate in the Department of Biochemistry at the University of Toronto. Currently, I work in the lab of Dr. Andreas Schulze at The Hospital for Sick Children.
My research involves determining the mechanism by which creatine acts to regulate the expression of Arginine: Glycine Amidinotransferase (AGAT). While it is known that creatine can repress the expression of AGAT, the mechanism by which this occurs and how this process is regulated is not well understood. Currently, I am investigating whether creatine acts on AGAT in a transcriptional and/or post-transcriptional manner by studying its rate of transcription and stability of mRNA. In addition, I am also evaluating which amino acids within the AGAT protein are key in facilitating its repression by creatine.

**Abstract**
"Identifying the Mechanism by which Creatine Represses Expression of AGAT"

Creatine is a molecule that facilitates the recycling of adenosine triphosphate (ATP), the primary source of cellular energy, and acts to buffer imbalances in its level to maintain cellular homeostasis. The synthesis of creatine occurs in a 2-step pathway that involves two enzymes: Arginine: Glycine Amidinotransferase (AGAT) and Guanidinoacetate N-Methyltransferase (GAMT).
While it is currently known that creatine can inhibit the expression of AGAT, the mechanism by which this occurs as well as how this process is regulated is not well understood.
In order to investigate this question, we used a technique called nascent RNA labeling to measure the rate of mRNA transcription as well as the rate of its decay. This technique involves incubating cells with 5-ethynyl uridine (EU) which is a modified uridine molecule that is incorporated into RNA as transcription occurs. By doing so, this allows us to selectively identify newly transcribed AGAT mRNA and the effects that creatine has on its transcription and stability. Preliminary data has indicated that the presence of creatine acts to repress the expression of AGAT by reducing its rate of transcription, thereby resulting in lower levels of AGAT mRNA. In addition, we have also observed that creatine can also inhibit AGAT in a post-transcription manner by reducing the stability of its mRNA.
The significance of this research is that by understanding how AGAT expression is regulated, this can provide us with additional information on how to modulate its activity and develop better therapeutic techniques for treating patients with creatine deficiency syndromes.

**SESSION 5**
**8:00 – 9:30 am PDT, 17:00 – 18:30 CET**

---

**CLAIRE STEPPAN**
PFIZER

**Bio**
Claire Steppan is a Research Fellow in Discovery Sciences at Pfizer and the EFPIA lead for two IMI consortia focused on Solute Carriers, RESOLUTE and REsolution. Claire has 17 years of experience in pharmacology and drug discovery driving over 12 small molecules to clinical trials for diseases ranging from diabetes, obesity, Alzheimer's disease, pain, gout, peripheral artery disease and depression. Her passion for Solute Carriers started with her work on SGLT2 and STEGALATRO (ertugliflozin), approved for the treatment of Type 2 Diabetes. Both within Pfizer and the scientific community, she is committed to unlocking the potential of Solute Carriers as drug targets for the treatment of human disease. She is also a member of the New York Academy of Sciences Biochemical Pharmacology Steering Committee.

**Abstract**
"Unlocking Solute Carriers Transporters: An Impact on the Scientific Community"

Solute carriers (SLCs) are the largest family of transport proteins located on cellular membranes. They act as cellular "gates" that control nutrient uptake and waste removal. Beside their critical role in maintaining cellular homeostasis and constituting paths for drug absorption, SLCs are associated with several diseases and hence they constitute emerging therapeutic targets. Despite their medical relevance, many SLCs are still considered 'orphans' in terms of substrate specificity or function.
The mission of the RESOLUTE-IMI consortium is to intensify worldwide research on SLCs and to establish them as a novel target class for medical research. RESOLUTE is achieving this by i) empowering the scientific community with biological tools, ii) carrying out a systematic deorphanisation campaign using multi-omics approaches and, iii) establishing robust transport assays.
While RESOLUTE is working efficiently on increasing the knowledge on SLCs, the new REsolution-IMI consortium aims at understanding how genetic variants in humans affect the function of SLCs. To that end, REsolution is gathering publicly available datasets and will combine them with novel experimental studies to assess the impact of genetic variants on SLC function.

By giving open-access to results, techniques and reagents, RESOLUTE and REsolution expect to accelerate research on SLCs and to create an unprecedented SLC database. This unique resource could act as a "compass" to allow the medical community and drug discoverers to prioritize those targets most clearly involved in specific human diseases.

**SESSION 6**
**9:30 – 10:30 am PDT, 18:30 – 19:30 CET**

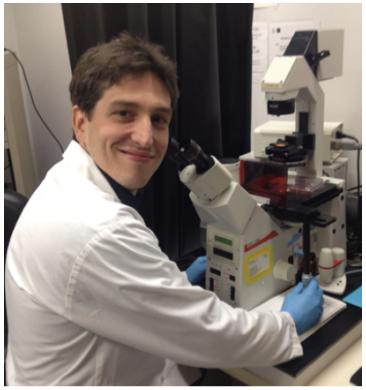
---

**LARA GECHIJAN**
Jnana Therapeutics

**Bio**
Lara Gechijian received her Ph.D. in Biomedical and Biological Sciences with a concentration in Therapeutics from Harvard Medical School in 2018. She conducted her graduate research in the labs of Dr. Jay Bradner and Dr. Nathanael Gray, where she focused on targeted degradation as an approach to developing novel cancer therapeutics. Lara is currently a biochemist at Jnana Therapeutics, a biotechnology company dedicated to developing new medicines for metabolic diseases.

**Abstract**
"A Novel Corrector for Variants of SLC6A8: a Therapeutic Opportunity for Creatine Transporter Deficiency"

Understanding the specific molecular defects linked to SLC6A8 variants and their impact on creatine transporter location and function is important for the development of therapeutics and predicting patient response. Many of the SLC6A8 variants characterized have a substantial defect in surface localization, but retain residual transport function, suggesting a viable hypothetical approach to identify molecular chaperones which could correct the functional deficit. Here, we describe the screening funnel that led to the identification of lead corrector molecules which augmented surface localization of SLC6A8 variants and increased creatine transport. We developed a translational pipeline that bridges an engineered cellular system to the endogenous SLC6A8 regulation and creatine transport, and finally to a knock-in P544L animal model of disease, to probe correction of SLC6A8 variants in each translational system. The P544L knockin mouse model recapitulates deficits in brain creatine to a degree that is commensurate with patients harboring the same SLC6A8 variant. Taken together our results describe the functional deficits of SLC6A8 variants, introduce a translational path for the discovery of therapeutics for CTD, and establish proof of mechanism for a corrector approach to treat CTD.



**SESSION 6**
**9:30 – 10:30 am PDT, 18:30 – 19:30 CET**

---

**PETER AXERIO-CILIES**
BC Children's Hospital

**Bio**

Peter Axerio-Cilies received a BS in Chemistry from the University of British Columbia after which he began his graduate studies in Pharmaceutical Sciences and his PhD in Neurology/Experimental Medicine. He is now a postdoctoral fellow at the University of British Columbia from the Department of Medicine and Psychiatry at the Djavad Mowafaghian Centre for Brain Health (UBC). His research in the last 12 years has been focused on various brain transporters and how they can be modulated by drugs and consequently used as treatments for epilepsy, Schizophrenia, Alzheimer's disease and other rare genetic diseases. He has recently engaged in the development of new treatments for creatine transporter (SLC6A8) deficiency. He has 16 years experience in generating lead drug prototypes for various protein targets associated with neurological disorders and rare genetic diseases (including solute carrier transporters), which have led to numerous successful patents and publications.

**Abstract**

"Towards the Discovery of Small Molecules that Restore Function to Defective SLC6A8 Creatine Transporter Variants"

Over 100 mutations in the SLC6A8 creatine transporter have been discovered in patients suffering from cerebral creatine deficiency syndrome (CCDS). Though the molecular effects of most mutations remain uncharacterized, current evidence suggests most enhance misfolding of the creatine transporter protein (CT1), which ultimately leads to a decrease in its expression and a loss of creatine transport. To identify small molecules that restore function to these variants, we have utilized structural modeling and virtual screening to search for small molecules that interact with CT1. Experimental characterization of ~75 of the top hits from the initial screen have identified 4 clinically approved drugs that generate a modest activation of CT1 and 3 that act as CT1 inhibitors. Compounds found to activate CT1 appear to do so without appreciably enhancing its plasma membrane expression. While these initial results are promising, the discovery of more potent effectors will require improved screening approaches. Towards this goal, we are currently developing counter-screening approach that accounts for how small molecules interact with CT1 in both its outward-facing and inward-facing conformations. In conjunction with current experimental data, these approaches will ultimately help to differentiate activators from inhibitors early in the screening process in a manner that may improve the chances of identifying potent activators of CCDS variants. Ongoing discovery efforts involve experimental characterization of additional hits from the preliminary screen, the development of enhanced screening methods, and the characterization of various other existing therapeutics that may potentially benefit certain CCDS patients.

**SESSION 6**
**9:30 – 10:30 am PDT, 18:30 – 19:30 CET**

---

**CHARLES KUNTZ**
The Schlebach Lab at Indiana University

**Bio**
Charles received a BS in Biochemistry from Indiana University in Bloomington, Indiana and a PhD in medicinal chemistry and molecular pharmacology at Purdue University in the laboratory of Prof. Eric Barker. At Purdue he studied the pharmacology and structural biology of the serotonin transporter using methods from computational chemistry and computational biology, such as protein structure prediction, molecular docking, and binding free energy calculations using molecular dynamics simulation. In 2018 he joined Prof. Jonathan Schlebach's laboratory at Indiana University where he uses computational biology approaches to understand the mutational sequence constraints of membrane proteins and how this contributes to disease. As the recipient of an ACD fellowship, Charles is engaged in studies searching for possible small molecule therapies for creatine deficiencies using virtual screening, molecular docking, and protein structure prediction and modeling. He is also a Visiting Scholar in the laboratory of Prof. Jens Meiler at Vanderbilt University, contributing to an ongoing collaboration between the Schlebach Lab and Meiler Lab to use machine learning methods to predict the effects of mutation on membrane protein trafficking.

**Abstract**
"Towards the Discovery of Small Molecules that Restore Function to Defective SLC6A8 Creatine Transporter Variants"

Over 100 mutations in the SLC6A8 creatine transporter have been discovered in patients suffering from cerebral creatine deficiency syndrome (CCDS). Though the molecular effects of most mutations remain uncharacterized, current evidence suggests most enhance misfolding of the creatine transporter protein (CT1), which ultimately leads to a decrease in its expression and a loss of creatine transport. To identify small molecules that restore function to these variants, we have utilized structural modeling and virtual screening to search for small molecules that interact with CT1. Experimental characterization of ~75 of the top hits from the initial screen have identified 4 clinically approved drugs that generate a modest activation of CT1 and 3 that act as CT1 inhibitors. Compounds found to activate CT1 appear to do so without appreciably enhancing its plasma membrane expression. While these initial results are promising, the discovery of more potent effectors will require improved screening approaches. Towards this goal, we are currently developing counter-screening approach that accounts for how small molecules interact with CT1 in both its outward-facing and inward-facing conformations. In conjunction with current experimental data, these approaches will ultimately help

to differentiate activators from inhibitors early in the screening process in a manner that may improve the chances of identifying potent activators of CCDS variants. Ongoing discovery efforts involve experimental characterization of additional hits from the preliminary screen, the development of enhanced screening methods, and the characterization of various other existing therapeutics that may potentially benefit certain CCDS patients.

**SESSION 7**
**10:45 – 11:15 am PDT, 19:45 – 20:15 CET**

---

**SOFIA BALOG**
Association for Creatine Deficiencies
"ACD Registry Update"

Background: The CreatineInfo Registry for Cerebral Creatine Deficiency Syndromes, launched in March 2021, is a Patient-reported Registry and Natural History Study created by ACD and hosted by the National Organization for Rare Disorders (NORD) for furthering research and empowering the Cerebral Creatine Deficiency Syndromes (CCDS) community.
This registry collects meaningful patient-reported longitudinal data including socio-demographic, diagnostics, management of care, and treatment and disease progression.Objective: Conduct a prospectively planned and efficient natural history study that will result in the most comprehensive understanding of CCDS and its course and pace over time.Methods: Patients or legally authorized representatives (LAR) of individuals with a CCDS diagnosis are invited to participate. A confirmed diagnosis and provision of Informed consent are required. Participants provide personal and medical information by answering online surveys and uploading medical information into a secure platform developed by NORD.
Discussion and Conclusion: The CreatineInfo registry has the potential to inform and accelerate research focusing on what is the most meaningful to patients and caregivers. The CreatineInfo registry will continue to engage the CCDS community to recognize and empower CCDS patients as active partners in research with the power to shape the CCDS research agenda.

**SESSION 7**
**10:45 – 11:15 am PDT, 19:45 – 20:15 CET**

---

**HEIDI WALLIS**
Association for Creatine Deficiencies

"Shortening the Road to CCDS Diagnosis"

**PLENARY SPEAKER**
**11:15 am – 12:00 pm PDT, 20:15 – 21:00 CET**

---

**MARZIA PASQUALI**
University of Utah/ARUP Laboratories

**Bio**
Dr. Pasquali is a professor of Pathology, the Program Director of the ACGME accredited Fellowship program in Clinical Biochemical Genetics at the University of Utah School of Medicine, and the Section Chief and Medical Director of Biochemical Genetics at ARUP Laboratories. Dr. Pasquali earned her degrees of doctor in pharmaceutical chemistry and technology and pharmacy doctor at the University of Parma School of Pharmacy in Italy. She trained in clinical biochemical genetics at Emory University, in Atlanta, Georgia where later served as the co-director of the Biochemical Genetics Laboratory. Dr. Pasquali is board certified in Clinical Biochemical Genetics. She is a member of the Society for Inherited Metabolic Disorders, the American College of Medical Genetics and Genomics, and several other professional societies. Her research interests are newborn screening, disorders of carnitine and creatine metabolism and transport, and lysosomal storage disorders.

**Abstract**
“Cerebral Creatine Deficiency Syndromes: Screening, Diagnosis, Monitoring. What have we learned?”

Cerebral creatine deficiency syndromes (CCDS) are characterized by lack of creatine in the brain. They are caused by defects in creatine synthesis or transport, and can result in intellectual disability, autistic-like behavior, seizures, speech and cognitive delays.
Therapy is effective if initiated early for L-arginine:glycine amidino transferase (AGAT) deficiency and guanidinoacetate methyltransferase (GAMT) deficiency. There is still no universal therapy for creatine transporter deficiency (CTD. Early identification is the key to prevent irreversible brain damage.
Newborn screening is effective for GAMT deficiency. Creatine and guanidinoacetate can be detected in newborn screening blood spots and guanidinoacetate is elevated (>3 micromolar, normal 1.20±0.43 micromolar) even at birth. Measurement of guanidinoacetate and creatine in plasma (but inconsistently in urine) can biochemically confirm or exclude the diagnosis that is then definitively established by genetic testing. There are no data for newborn screening in AGAT deficiency or for CTD. In patients clinically identified, guanidinoacetate levels are very low in patients with AGAT deficiency and in theory should be low (<0.25 micromolar) in newborn screening blood spots. The diagnosis of CTD is usually established in urine where the creatine/creatinine ratio is elevated. There is the need for

informative biomarkers that would enable detection of CTD in blood spots. The integration of biochemical testing with molecular techniques offers the opportunity to test directly positive blood spots for specific genetic disorders and could allow targeted identification of CTD patients in which a suitable biomarker is abnormal.

**SESSION 8**
**12:00 – 1:00 pm PDT, 21:00 – 22:00 CET**

---

**JACK SCHJELDERUP**
Haukeland University Hospital, Norway

**Bio**
I took my medical degree at the University of Bergen, Norway in1980. I am a specialist in neurology and family medicine. Previously, I have worked within general practice, psychiatry, a public institution for the mentally retarded, an emergency medicine/trauma policlinic, surgery, internal medicine, occupational medicine, neurology and neurosurgery. Since 2008, I have worked as a consultant in the department of neurohabilitation in Haukeland University hospital, Bergen, Norway. Our patients have congenital or early-acquired developmental disabilities, and the majority have intellectual disability. I am involved in diagnosing patients and in some cases treatment – for instance epilepsy, Tourette syndrome, mental disorders, behavioral problems and in this case creatine transporter defect (in collaboration with good colleagues). Our article about treating two patients with creatine transporter defect was published earlier this year. I am also a board member of the Norwegian Medical Association for Neurohabilitation.

**Abstract**
“Treatment experience in two adults with creatine transporter deficiency”

Background: Limited information exists on the adult course of Creatine transporter deficiency (CTD), and there were no previous treatment studies in adults. Methods: We report two half-brothers with CTD, 36 and 31 years at intervention start. Their clinical phenotypes were consistent with CTD, and intervention was indicated because of progressive disease course, with increased difficulties speaking, walking and eating, resulting in fatigue. We therefore performed a treatment trial in the older patient with arginine, glycine and a proprietary product containing creatine and betaine, and later a trial supplementing with betaine alone. Results: In the older patient, glycine and arginine were accompanied by adverse effects, while betaine containing proprietary product gave improved balance, speech and feeding. When supplementation stopped, his condition deteriorated, and improved again after starting betaine supplement. Betaine supplementation was also beneficial in the younger patient, reducing his exhaustion, feeding difficulties and weight loss, making him able to resume his protected work.

Discussion & conclusion: We report for the first time that betaine supplement was well tolerated and efficient in adults with CTD, while arginine and/or glycine possibly were accompanied by side effects. Thus, betaine is potentially a new useful treatment for CTD patients. We discuss possible underlying treatment mechanisms. Further studies of betaine's effects in well-designed studies are warranted.

**SESSION 8**
**12:00 – 1:00 pm PDT, 21:00 – 22:00 CET**

---

**JUDITH MILLER**
Children's Hospital of Philadelphia & University of Pennsylvania

**Bio**
Judith Miller, PhD, is a clinical psychologist with more than 25 years' experience in developmental disorders. She has a joint appointment as Assistant Professor in both the Psychiatry and Pediatrics departments at the Children's Hospital of Philadelphia (CHOP), which is affiliated with the Perelman School of Medicine at the University of Pennsylvania. She is also the Clinical Training Director at the Center for Autism Research, and the Associate Director for the Leadership in Education in Neurodevelopmental Disorders (LEND) program at CHOP. She has been studying Creatine Transporter Deficiency since 2015, and is the coordinating Principal Investigator for the Vigilan Observational Study.

**Abstract**
"Vigilan Observational Study of CTD – Study Progress and Updates"

This presentation will provide updates from the Vigilan Observational Study of Creatine Transporter Deficiency, the largest and longest-running natural history study of a creatine deficiency syndrome. Study goals remain to determine how children with CTD develop over time, and to identify the best measures for a future interventional study. We now have longitudinal data from 50 participants across our US and Canada sites. We will update results on first age of CTD symptoms, medical histories, language development, behavioral challenges, and cognitive and adaptive levels over time. We will also present our updated protocol battery.

**SESSION 8**
**12:00 – 1:00 pm PDT, 21:00 – 22:00 CET**

---

**MELANIE BRANDABUR**
Ultragenyx Pharmaceutical Inc.

**Bio**
Melanie Brandabur, MD received her BA degree from the University of Illinois in Urbana and her MD degree from Rush Medical College in Chicago. She completed her neurology residency and Movement Disorders and Neuropharmacology fellowship at Rush-Presbyterian-St. Luke's Medical Center in Chicago. This was followed by a post-doctoral basic sciences fellowship in Neurodegenerative Diseases.

Dr. Brandabur is currently a Senior Medical Director in Global Clinical Development at Ultragenyx Pharmaceutical Inc., where she works on the development of therapeutic agents for neurodevelopmental rare diseases. During her clinical career as a specialist in Parkinson's disease and Movement Disorders, Dr. Brandabur served as the Medical Director for three National Parkinson Foundation Centers of Excellence; at the University of Illinois, at Alexian Neurosciences Institute and at the Parkinson's Institute in Sunnyvale, California.

**Abstract**
"Creatine Transporter Deficiency Caregiver Perspectives and the Path to Diagnosis"

Creatine Transporter Deficiency (CTD) is a rare, X-linked, recessive disorder caused by mutations in the SLC6A8 gene resulting in blockage of creatine transport, primarily in the brain. Clinical CTD symptoms include intellectual disability, delays in speech/motor development, language impairment, behavioral issues, and seizures. Caregivers of patients with CTD participated in a virtual advisory board and/or provided information through an online discussion series and online survey that focused on the path to diagnosis, symptoms, and patient impacts. Nine caregivers participated in the advisory board and reported experience with 10 family members diagnosed with CTD (7 males, 3 females). These nine caregivers described a prolonged path to diagnosis due to nonspecific disease signs and symptoms. In the online survey, 37 caregivers provided data for 40 unique children (age range: 1-24 years) with CTD (37 males, 3 females). These 37 caregivers reported that their children were, on average, 1.4 years old (median 0.8 years) when they began displaying symptoms (first symptoms age range: birth-10.3 years) and 5.7 years old (median 4.0 years) when they were diagnosed with CTD (diagnosis age range: 0.2-18 years). Most frequent initial symptoms from the online

survey included gross motor delay (80.0%), speech delay/impairments (62.5%), and global developmental delay (60.0%). Together, results from both the advisory board and online survey highlight the broad spectrum of CTD symptom presentation, challenges to receiving a CTD diagnosis, and CTD symptom impact. These findings also provide preliminary insight into the burden of CTD and the need for better diagnostic tools and improved treatment.

**SESSION 9**
**1:00 – 1:15 pm PDT, 22:00 – 22:15 CET**

---

**LAURA TRUTOIU**
ASSOCIATION FOR CREATINE DEFICIENCIES

**Bio**

Laura Trutoiu is a computer scientist and researcher. She holds a PhD from the Robotics Institute at Carnegie Mellon University and a BA in Computer Science from Mount Holyoke College. She has conducted research in several industry labs including Disney Research, Industrial Light and Magic, Oculus Research & Facebook, and currently Magic Leap. Her research spans computer graphics, human perception, and sensing and interaction for virtual and mixed reality systems. Laura lives in Seattle with her husband Amar (CMU PhD), and their son Rohan. Rohan was diagnosed with Creatine Transporter Defect in June 2017 when he was 2 and a half years old. Laura is passionate about supporting the potential for scientific discovery to help and serve the rare diseases community in general and creatine deficiencies in particular.

**“Closing Remarks: The CCDS Road From Diagnosis to Therapies”**