2022 CCDS Scientific & Patient Symposium

Program Agenda & Abstracts

ACD
ASSOCIATION FOR
CREATINE DEFICIENCIES

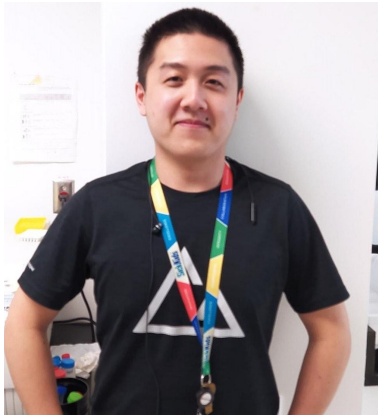
8:30 – 8:45 am MDT (June 26, 2022)

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.

2022 Opening Remarks & Session Overview



8:45 – 9:00 am MDT (June 26, 2022)

ALEX LEE
Department of Biochemistry, 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). Currently, I am investigating whether the mechanism by which creatine acts on AGAT 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 and localization to the mitochondria.

Identifying the mechanism by which creatine represses expression of AGAT

Alex Lee

Creatine is a molecule that facilitates the recycling of adenosine triphosphate (ATP), the primary source of cellular energy, and acts to buffer imbalances in ATP levels to maintain its 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 known that creatine can inhibit the expression of AGAT, the mechanisms and regulation by which this process occurs is not well understood.

In order to investigate this question, we used nascent RNA labeling to measure the rate of synthesis and degradation of mRNA transcripts. Our results have indicated that the presence of creatine acts to repress the expression of AGAT by reducing its rate of transcription. This observation has been further validated through the use of nuclei isolation experiments which show that creatine results in reduced levels of AGAT mRNA within the nuclei. In addition, we have also observed that creatine may inhibit AGAT at the post-transcriptional level by reducing the stability of its mRNA. Furthermore, we have identified a region within the N-terminal AGAT protein that is involved in creatine mediated repression and mitochondrial targeting. Through mutagenesis and immunofluorescence techniques, we aim to identify residues within the region that are essential for the aforementioned processes.

Through this research, we hope to better understand how AGAT expression is regulated as this will provide us insight regarding how to modulate its activity and develop better ways for treating patients with creatine deficiencies.

9:00 – 9:15 am MDT (June 26, 2022)

CHARLES KUNTZ
Indiana University, Bloomington

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. The Class A GPCR rhodopsin has primarily been utilized in these studies as a model system and as an important object in itself as rhodopsin mutations can lead to retinitis pigmentosa, a form of congenital night blindness. 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. Charles is hoping to broaden the scope of his expertise into GPCR pharmacology in collaboration with Prof. Joshua Ziarek in the search for allosteric sites in neurotensin receptor 1 and with Prof. Michael VanNieuwenhzhe in the search for allosteric modulators of the mu-opioid receptor.

Towards the discovery of small molecules that restore function to defective SLC6A8 creatine transporter variants

Charles P. Kuntz & Jonathan P. Schlebach

Over 100 mutations in the SLC6A8 creatine transporter have been discovered in patients suffering from cerebral creatine deficiency syndrome (CCDS), which is associated with a spectrum of neurological phenotypes ranging from intellectual disability to epilepsy. Though the molecular effects of most of these mutations remain uncharacterized, current evidence suggests most compromise creatine transport by enhancing the misfolding of the creatine transporter protein (CT1). Previous biochemical investigations have revealed that drugs that specifically bind to the inward-facing (IF) conformation of related transporter proteins can rescue misfolding phenotypes. We therefore believe this class of molecules could potentially help restore the activity of misfolded CT1 variants. In our first year of ACD support, we developed computational methods to search for molecules that selectively bind to the IF conformation of CT1. In our second year of support, we have begun to apply these approaches to identify promising therapeutic lead compounds. We have first used the Arthor database mining tool (arthor.docking.org) to identify a library of 2,310 commercially available analogs of β-guanidinopropionate, which is a creatine-like CT1 inhibitor that naturally binds to the outward-facing (OF) conformation of the transporter. We are currently applying our structural docking approaches to search this library of molecules for modifications that confer specific binding to the IF conformation over the OF conformation and shift the activity of the molecule from inhibition to activation. As a

secondary approach, we are searching for other metabolites and/ or approved drugs that may also stabilize the IF conformation of CT1. In this presentation, we will discuss our most promising lead compounds and our ongoing plans to experimentally validate their biochemical effects. Our results will provide fundamental insights into the chemical properties that differentiate CT1 substrates from inhibitors and pharmacological chaperones while identifying new avenues for therapeutic development.

9:15 – 9:30 am MDT (June 26, 2022)

MANUEL CERNIGOJ
Axxam S.p.A.

Bio

2017 - Graduated in Molecular Biotechnology and Bioinformatics at the University of Milan, Master thesis on Direct reprogramming of dermal fibroblast to Medium Spiny Neurons at Elena cattaneo's Laboratory (University of Milan)
2017/2020 - PhD in Molecular and Cell Biology at the University of Milan, Prof. Elena Cattaneo's Laboratory
Main project: Genetic engineering of hESC with dTAG (protein degradation cassette) as tool to study Huntington's Disease
Side project: Genetic engineering of hESC and HEK cells with different fluorescent protein tags genetating a multicolor reporter line for genes involved in Huntington's Disease
2020/2021 - Post doc. at Elena Cattaneo's Laboratory working on design of a pooled screening based on different expansions of the Huntington's gene
2021/present - Cell Biology Scientist at AXXAM S.p.a. working on cell line generation and biological assay development

A high throughput screening assay for CTD drug discovery

Manuel Cernigoj & Viviana Agus

9:30 – 9:45 am MDT (June 26, 2022)

LÉA BROCA-BRISSON
University Paris-Saclay, CEA, Department of Medicines and Technologies for Health (MTS), Neurovascular unit and Therapeutic Innovation Laboratory (LENIT), Gif-sur-Yvette cedex 91191, France

Bio

Léa Broca-Brisson joined the CEA in February 2020 as a PhD student, under the supervision of Dr Aloïse Mabondzo, at the Neurovascular unit and Therapeutic Innovation Laboratory. Her research concerns the development of human brain organoids from induced pluripotent stem cells from CTD patients in order to determine the efficacy of therapeutic agents as treatment for CTD.As a cell biologist, Léa Broca-Brisson is interested in the understanding of the functions of cells, their interactions with the environment and their potential dysfunctions. She aims to develop more predictive models to better study pathophysiology and determine the efficacy of drug candidates.

Modelling creatine transporter deficiency using brain organoids reveals molecular targets associated with inherited intellectual deficiency and acquired neurodegenerative diseases

Léa Broca-Brisson, Rania Harati, Anne-Cécile Guyot, Narciso Costa, Balazs Sarkadi, Agota Apati, Orsolya Mozner, Jean Armengaud, Rifat A. Hamoudi, & Aloïse Mabondzo

CTD is an X-linked intellectual disability caused by mutations in the SLC6A8 gene encoding the creatine transporter, resulting in a lack of creatine within the brain and cellular energy perturbation of neuronal cells. There are significant roadblocks in understanding and developing treatments for the known causes of intellectual disabilities (ID). Rodent models may have inconsistent phenotypes and low translational validity. Human post-mortem samples may be difficult to obtain and frequently lack proper control groups, limiting the investigation of potential mechanisms. Therefore, it is of utmost interest to develop high-fidelity models that allow in-depth molecular characterization and validation of translational relevant data.

This work focuses on the development of a pharmacological model of brain organoids differentiated from human induced pluripotent stem cells from CTD patients in order to identify readably measurable biomarkers for measuring the success of drugs under development. CTD patient-derived organoids have been characterized in terms of cell population and did not show increase in creatine concentration when incubated in creatine-supplemented media. Shotgun proteomics performed with high-resolution tandem mass spectrometry and state-of-the-art bioinformatics analysis and mathematical modelling identified differentially expressed proteins between healthy and CTD brain organoids. Among the 4,219 proteins identified, we highlighted those associated with ID, autism and acquired neurodegenerative diseases. Additional analysis of the molecular mechanisms and corresponding pathways provided key insights into some of the mechanisms involved with this deficiency. This study showed that CTD-derived organoids are high-fidelity model that can be used to test potential treatments and better understand the mechanisms that underlie CTD.

10:15 – 10:30 am MDT (June 26, 2022)

EVANDRO FERRADA
CeMM, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

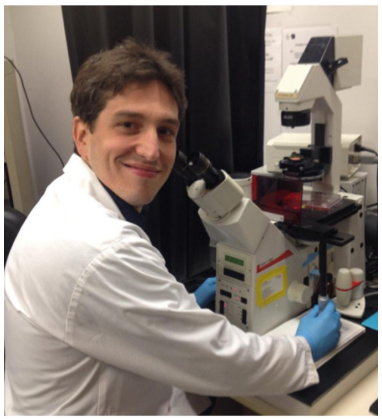
Bio

Dr. Evandro Ferrada obtained his PhD at the University of Zurich, working on molecular evolution. He carried out postdoctoral work at the Santa Fe Institute in New Mexico, and at the Department of Genome Sciences of the University of Washington, Seattle, USA. As a researcher at CeMM, he brings expertise on molecular evolution, evolutionary systems biology, and the integration of large-scale data analysis. In June 2021 he joined the REsolution Consortium where he is contributing to the analysis of human genetic variation and to the functional characterization of solute carriers using large-scale mutagenesis data.

Integrating computational and experimental evidence for the characterization of single amino acid variants of the creatine transporter (SLC6A8)

Ferrada, E., Osthushenrich, T., Macnamara, A., Malarstig, A., Garofoli, A., Steuer, B., Klimek, C., Azzollini, L., Scarabottolo, L., Wiedmer, T., & Superti-Furga, G.

As part of the REsolution consortium, a public-private partnership funded by the Innovative Health Initiative (IHI) of the European Union and the European Federation of Pharmaceutical Industries and Associations (EFPIA), we are studying the functional significance of single amino acid variants of the creatine transporter (ie., SLC6A8). To describe broadly the mutational landscape of members of the SLC6 family of transporters, we performed structural modeling of single amino acid variants and studied their effect on thermodynamic stability and structural dynamics. Similarly, we identified signatures of natural selection which provide independent evidence of sites involved in the molecular function of the transporter. We focus on a representative set of 30 variants of biomedical relevance identified by compiling human genetics data from publicly available biomedical databases, including genome-wide studies, and the literature. These variants of biomedical relevance were studied experimentally to characterize both their subcellular localization and functional activity. We foresee the integration of these approaches into a predictive model for the functional effect of SLC6A8 variants of interest to translational medicine.



10:30 – 10:45 am MDT (June 26, 2022)

PETER AXERIO-CILIES
BC Children's Hospital Institute, University of British Columbia

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, publications and clinical trials.

Discovery of small molecules that restore function to defective SLC6A8 creatine transporter variants

Peter Axerio-Cilies & Sylvia Stockler

SLC6A8-related intellectual disability causes developmental and neurologic deficits in 3% of males with X-linked ID. SLC6A8 encodes the cerebral creatine transporter and over 100 genetic variants are currently known that induce misfolding and degradation, resulting in loss of cellular surface activity and compromising creatine uptake and utilization in the brain. Currently, there is no causal treatment. As a recipient of an ACD Fellowship, Dr. Axerio-Cilies, in collaboration with Dr. Stöckler, has initiated a drug-repurposing pipeline to identify approved drugs and natural products that can restore functional activity of dysfunctional SLC6A8 transporters caused by mutations. We used computer-aided drug design, in-vitro functional assays and expression assays to screen drugs that functionally correct transporter activity. In addition, in collaboration with Drs. Schlebach and Kuntz (Indiana University) we are working towards identifying drugs that enhance surface expression of variants impaired in this process. Our research focuses on variants which have been selected according to type of mutation/likelihood to respond to small molecules, frequency, and occurrence in patients available for clinical trials. We have made great strides towards understanding the necessary structural features required for restoration of dysfunctional variants using in-silico modelling in combination with drug screening. In-vitro, we have evaluated the functional landscape of 16 variants of clinical significance, and we have identified 6 'hit' functional correctors that partially restore function and will be used to inform future rounds of screening to increase drug potency. We will test these drugs against these variants using uptake and subcellular localization studies in different cell lines.

10:45 – 11:00 am MDT (June 26, 2022)

MAURIZIO BALESTRINO
Università di Genova, Italy

Bio

1980: Degree in Medicine and Surgery, with honors1984: Specialist in Neurology Diploma 1980-1983: Resident in Neurology, University of Genova.1983-1986: Research Associate, Dept of Physiology, Duke Univ. Medical Center 1987 -1993: National Research Council (Genova, Italy), external collaborator. Consultant Neurologist, Italian Army, Genova. Italian Public Health System, several outpatients facilities -Neurologist Specialist Visiting scientist at Duke University, Univ. of Washington, Univ. of Ottawa.1993-2019: Senior Researcher, Institute of Neurology (currently Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Sciences), University of Genova. Chief of the Laboratory of Experimental Neurophysiology.2019-present: Associate Professor in Neurology at the University of Genoa, Italy in the Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Sciences. Chief of the Laboratory of Experimental Neurophysiology.99 printed papers (source: PUBMED); H-index: 25 (source: Scopus)

In vitro effectiveness of a prodrug of the creatine precursor guanidinoacetic acid against block of the creatine transporter

Enrico Adriano, Annalisa Salis, Gianluca Damonte, Enrico Millo, & Maurizio Balestrino

Diacetyl guanidinoacetic acid ethyl ester (diacetyl-GAAE) is a lipophilic derivative of the creatine precursor guanidinoacetate (GAA). The latter has been suggested to be epileptogenic or toxic to the nervous system, nevertheless it was administered to humans with no adverse effects. Furthermore, increased GAA content in guanidinoacetate methyltransferase (GAMT) deficiency is probably even beneficial, improving muscle function. In our experiments, 11.5 μM GAA (the highest concentration in the cerebrospinal fluid of GAMT-deficient patients) did not change the postsynaptic compound action potential. Even 1 or 2mM had no effects, while 4mM caused a reversible decrease of the potential. Guanidinoacetate increased creatine and phosphocreatine, but not after block of the creatine transporter. To allow brain delivery of GAA in creatine transporter deficiency, we synthesized diacetyl-GAAE. In brain slices, 0.1 mM did not cause electrophysiological changes and improved tissue viability after block of the creatine transporter. However, diacetyl-GAAE did not increase creatine nor phosphocreatine in brain slices after block of creatine transporter. We conclude that (1) upon acute administration GAA is neither epileptogenic nor neurotoxic; (2) Diacetyl-GAAE improves tissue viability after block of the creatine transporter, but not through increase of creatine or phosphocreatine. Diacetyl-GAAE might give rise to a GAA-phosphoGAA system that vicariates the missing creatine-phosphocreatine one. A lipophilic GAA prodrug might be useful in creatine transporter deficiency.

11:15 am – 12:00 pm MDT (June 26, 2022)

TERRY JO BICHELL
COMBINEDBrain

Bio

Terry Jo Vetters Bichell is the Founder/Director of COMBINEDBrain, a patient-advocacy led organization formed in 2019 with a mission to identify outcome measures and biomarkers for neurodevelopmental disorders. Dr. Bichell has a B.A. from Johns Hopkins University and a bachelors in nursing from St. Louis University. Dr. Bichell earned a masters degree in public health and a certificate of nurse-midwifery from Boston University, where she was a Maternal and Child Health Leadership Scholar. She worked as a nurse-midwife in San Diego, and was a scientific advisor to the CASA Midwifery School in San Miguel de Allende, Mexico, until her fifth child, Lou, was diagnosed with Angelman syndrome in April, 2000. Soon after her son's diagnosis, she was inspired by Dr. Art Beaudet at the first international Angelman conference in Tampere, Finland in July, 2000, to help move bench research into clinical trials. Dr. Bichell was on the team of investigators who launched the first Angelman clinical trial (Folic acid in 2001), the NIH-funded Natural History trials, and the Levodopa trial. Dr. Bichell earned a PhD in neuroscience from Vanderbilt University in 2016, studying gene-environment interactions in Huntington's disease rodent models, She was the founding director of the Angelman Biomarkers and Outcome Measures Alliance (A-BOM) from 2016-2018. Dr. Bichell has published studies on clinical aspects of Angelman syndrome as well as Angelman rodent models concerning Gabrb3 mutation, Ube3a gene expression and circadian rhythms. In addition, Dr. Bichell originated Alphabet Therapy, a method to teach literacy skills to children with Angelman syndrome. Dr. Bichell, her husband, Dr. David Bichell, and their son, Lou, reside on a small horse farm in Nashville, TN along with several musician/caregivers who help with Lou. Their four adult daughters (and grandson) visit often, in person or on face-time.

Keynote Address: "Knowledge is Power: Connecting and Collaborating to Cure Rare Diseases"

Terry Jo Bichell

Diagnosis day is often both a horrible and wonderful milestone. People who have a rare disease, or parent someone with a rare disease, have all received very bad news at some point in their lives - it is a pivotal moment, a difficult call to action. For me it was a relief to have a name for what was wrong with my son, and it showed me a path towards a goal. Since that day I have been working towards a cure for Angelman syndrome, first by running a clinical trial. Then, by running a natural history trial. Then by getting a PhD in molecular neuroscience, dissecting mice, doing western blots, and programming induced pluripotent stem cells. Along the way, I realized that there were potholes in the path that could stall the road to a cure, and they weren't scientific potholes. Our disorder had no newborn testing, no ICD-10 code, no biorepository and no actual understanding of what it meant to have Angelman syndrome over a lifetime and for a whole family. What we did have were two foundations who hated each other, and a few amazing clinicians and brilliant scientists who could be brought together to work on pre-competitive projects that would push us all faster to a cure. In 2019 I formed COMBINEDBrain, a non-profit with a big and hopeful mission - to push dozens of

rare genetic neurodevelopmental disorders together towards cures. The Association for Creatine Deficiencies is already working collaboratively, has already made so many pro-active choices, and already has filled in a lot of the potholes. I am here to tell you about my experiences and learn from yours.

1:00 – 1:15 pm MDT (June 26, 2022)

NICOLA LONGO
University of Utah

Bio

Dr. Nicola Longo received his M.D. and Ph.D. in molecular biology and pathology from the University of Parma, School of Medicine in Italy. He then trained in Pediatrics, Medical and Biochemical Genetics at Emory University in Atlanta, Georgia, USA. Currently, Dr. Longo is 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 Chief of the Division of Medical Genetics, Director of the Metabolic Service in the Department of Pediatrics, 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. He has a long-standing interest in membrane transporters for which he has worked on amino acid, glucose, carnitine and creatine transporters. He follows several patients with brain creatine deficiency and has an active interest in developing new methods to facilitate their detection by newborn screening, improving existing therapies and developing new ones for these conditions.

Small molecule therapy for GAMT deficiency

Bijina Balakrishnan, Filippo Ingoglia, Kent Lai, Marzia Pasquali, Niel Henriksen, & Nicola Longo

Guanidinoacetate methyltransferase (GAMT) deficiency is a recessive disorder of the synthesis of creatine that causes the accumulation of guanidinoacetate (GAA) and other guanidino compounds, which can be toxic for the brain. Affected patients present with intellectual disability, seizures, and autistic-like features. Current therapies can restore creatine levels and can reduce, but not normalize levels of guanidinoacetate. As a result, patients can still suffer from brain damage despite maximal therapy and early detection. Inhibition of arginine: glycine amidinotransferase (AGAT) should suppress the synthesis of guanidinoacetate and could normalize levels of guanidinoacetate in the brain, improving the outcome of patients with GAMT deficiency.

To identify novel, safe and effective AGAT inhibitors to prevent or reduce synthesis of guanidinoacetate, we performed a virtual screen using a diverse compound library composed of ~3M compounds against the crystal structure of AGAT (PDB 4JDW). Our virtual screening method employs a neural network for predicting ligand binding affinities, trained using a large curated dataset of experimental binding affinities and 3D receptor/ligand structures. Based on the predicted binding affinity and computed drug-like properties, we selected 96 compounds for further evaluation of their abilities to inhibit recombinant human AGAT enzyme with its leader sequence removed. These compounds were added to the AGAT enzyme reaction mix at a final concentration of 1mM, and the production of guanidinoacetate and ornithine were measured by an UPLC-MS/MS assay. To date, we have tested 92 compounds and found that 10 compounds inhibited more than 75% of control AGAT activity in vitro. Among these, three inhibited more than 92% of control AGAT activity, which is close to the efficacy of 1mM ornithine that is used clinically to reduce GAA production. Initial examination of the chemical structures of the strongest inhibitors revealed a backbone based on arginine,

the natural substrate of the enzyme. Future characterization and optimization of selected small molecules in vitro followed by testing in cellular and animal models of GAMT deficiency can identify novel, potent and safe small molecule AGAT inhibitors that could be used to treat patients with GAMT deficiency.

1:15 – 1:30 pm MDT (June 26, 2022)

MATTHEW SKELTON
Department of Pediatrics, University of Cincinnati College of Medicine and Division of Neurology, Cincinnati Children's Research Foundation, Cincinnati, OH

Bio

I have been involved in creatine research for over a decade when Dr. Ton Degrauw, who discovered CTD, asked my post-doctoral advisors (Drs. Charles Vorhees and Michael Williams) to develop a mouse model of CTD. We published the first results from this mouse model in 2011. Our mouse model is a high-fidelity model of CTD, showing significant cognitive and metabolic deficits. This important mouse model has been used to test several potential therapies for CTD and to better understand the role of Cr in neurology, cancer, inflammation, and metabolism. The goal of our work is to better understand the role of Cr in the brain and develop the most relevant mouse models for CTD, providing an important resource for the scientific community to design treatments for this important disorder. I have published 12 papers related to Cr function in the brain. I have been invited to give lectures on our work in Cr research at both national and international venues, hosted by academic, pharmaceutical partners, and patient advocacy groups. I am one of the inaugural members of the Scientific and Medical Advisory Board for the Association of Creatine Deficiencies and am a member of the Gene Therapy Consortium. I am also proud to be the first recipient of an ACD research grant, which we have used to better understand the role of Cr in the brain and to attempt to develop AAV vectors to restore SLC6A8 function to the brain of our mice.

Mice with a P544L mutation in the Slc6a8 gene have reduced brain Cr, spatial learning deficits, and impaired nest building

Matthew R. Skelton, Marla K. Perna, Aaron Williams, Lara Gechijian, & Heather S. Blanchette

In humans, mutations of the creatine transporter (CrT; SLC6A8) gene lead to moderate to severe intellectual disability, epilepsy, and lack of language development. There is no treatment for CrT deficiency (CTD) and there is still much to be learned about how the lack of Cr causes such profound neurological deficits. To better understand the underlying causes of CTD, there have been two mouse models developed where large portions of the Slc6a8 gene have been removed. These models show significant learning and memory deficits, suggesting they are high fidelity models of the CTD phenotype. While these models have a similar phenotype to CTD patients, the deletion of large segments of the gene do not model the genotype of CTD patients, who typically have point mutations. To better model the genotype of CTD patients, knock-in mice with a proline to leucine substitution at amino acid 544 (P544L) underwent a cognitive test battery similar to those used in the larger knockout models. The P544L knock-in (Slc6a8PL) mice had a reduction in brain Cr levels and body mass compared with WT mice. In the MWM, Slc6a8PL mice had similar latencies and path length to the platform but showed an impaired navigation strategy compared with controls. The Slc6a8PL mice also had spatial memory deficits during one phase of testing. Nest building was reduced in Slc6a8PL mice compared with controls, an indication of hippocampal dysfunction. No differences were observed in spontaneous alternation or object recognition. Together, these data suggest that Slc6a8PL mice have mild cognitive deficits and could be a useful model to better understand and treat CTD.

1:30 – 1:45 pm MDT (June 26, 2022)

HONG-RU CHEN
Department of Neurosciences, University of Virginia School of Medicine

Bio

Hong-Ru Chen is the assistant professor of Department of Neuroscience, University of Virginia School of Medicine, USA. She obtained her PhD degree from the Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taiwan and did her postdoctoral training in Department of Pediatrics, Emory University School of Medicine, USA. At present, her research primarily focuses on investigating how neurons and monocyte-derived pathological microglia interact to mediate neural physiology and behavior in various neurological conditions and how creatine transporter deficiency affects the brain energetics in murine models.

Creatine transporter deficiency impairs stress adaptation and brain energetics homeostasis

Hong-Ru Chen, Xiaohui Zhang-Brotzge, Yury M. Morozov, Yuancheng Li, Siming Wang, Helen Heju Zhang, Irena S. Kuan, Elizabeth M. Fugate, Hui Mao, Yu-Yo Sun, Pasko Rakic, Diana M. Lindquist, Ton DeGrauw, & Chia-Yi Kuan

The creatine transporter (CrT) maintains brain creatine (Cr) levels, but the effects of its deficiency on energetics adaptation under stress remain unclear. There are also no effective treatments for CrT deficiency, the second most common cause of X-linked intellectual disabilities. Herein, we examined the consequences of CrT deficiency in brain energetics and stress-adaptation responses plus the effects of intranasal Cr supplementation. We found that CrT-deficient (CrT–/y) mice harbored dendritic spine and synaptic dysgenesis. Nurtured newborn CrT–/y mice maintained baseline brain ATP levels, with a trend toward signaling imbalance between the p-AMPK/autophagy and mTOR pathways. Starvation elevated the signaling imbalance and reduced brain ATP levels in P3 CrT–/y mice. Similarly, CrT–/y neurons and P10 CrT–/y mice showed an imbalance between autophagy and 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/N- acetylaspartate ratio, partially averted the signaling imbalance, and reduced infarct size more potently than intraperitoneal Cr injection. These findings suggest important functions for CrT and Cr in preserving the homeostasis of brain energetics in stress conditions. Moreover, intranasal Cr supplementation may be an effective treatment for congenital CrT deficiency and acute brain injury.

2:00 – 2:15 pm MDT (June 26, 2022)

JAGDEEP WALIA
Queen's University

Bio

Dr. Jagdeep S Walia is a Clinical Geneticist and a full Professor at Queen's University in the Department of Pediatrics. He received his MBBS in medicine at Guru Nanak Dev University School of Medicine, Amritsar, India. Following postdoctoral research in genetics at the University of Toronto, he completed residency in Medical Genetics at the University of Manitoba, Canada. He joined Queen's University as an Assistant Professor, Department of Pediatrics in 2012, and was promoted to Head of Medical Genetics at the Kingston Health Science Centre in 2015. In 2016, he was appointed as the Director of Research for Pediatrics. In addition to working in the clinic and running the research lab he is active in knowledge translation and education. He has published multiple peer reviewed manuscripts. He has organized multiple advocacy events, like Genetics Patient and Family Day, to facilitate learning and support opportunities for individuals and families with genetic conditions. He is a member of the Ontario Genetics Secretariat Committee, focused on developing a provincial strategy for genetics services and practice in Ontario. He is also treasurer and secretary of Garrod association, a national body of biochemical geneticists in Canada.

Future clinical gene therapy trials for creatine deficiency disorders: What parents shall be aware of?

Jagdeep S. Walia

We are all hoping that one day clinical gene therapy trial for creatine deficiency disorders will begin and may prove to be one time treatment for life. We are looking forward for the best outcome of our research, but parents specifically may have very high hopes from such trials whenever trial will happen. Through this talk directed to the parents and caregivers, Dr. Walia will discuss different scenarios in the outcome of clinical trials including possible successes, complications, and unpredicted events in the beginning of the journey.

Panel Discussion: "Clinical Trials and Outcomes"
2:15 – 3:00 pm MDT (June 26, 2022)

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.

ANDREAS SCHULZE
University of Toronto

Bio

Dr. Schulze received his MD and PhD in Biochemistry at the University of Leipzig, Germany. He has worked at the Hospital for Sick Children in Toronto, Canada since 2007 and has established his own research group with a focus on Cerebral Creatine Deficiency Syndromes, Regulation of Creatine Synthesis, Pathophysiology of Guanidino Compounds, and Small Molecule Treatments. He is Director of the Newborn Screening Program at the Hospital for Sick Children, as well as the Section Head of Metabolic Genetics. Dr. Schulze was the first to report and describe the full biochemical spectrum in GAMT Deficiency and has an ongoing interest in advancing research of creatine deficiencies.

SAADET ANDREWS
University of Alberta

Bio

Dr. Saadet Andrews' research focuses on creatine deficiency disorders, pyridoxine-dependent epilepsy and epilepsy genetics. Andrews and her team characterized the first knock-out aldh7a1 zebrafish to study neuropathogenesis of pyridoxine dependent epilepsy caused by mutations in ALDH7A1. This model will serve for drug screening to identify novel treatment for pyridoxine dependent epilepsy; gene discovery for epilepsy and developmental delay in childhood; gene discovery for neonatal encephalopathy.

AURORE CURIE
French National Reference Center for Rare Diseases with Intellectual Disability, Department of Child Neurology, Woman Mother and Child Hospital, Lyon University Hospital, Bron, France; Lyon Neuroscience Research Center, CNRS UMR5292, Inserm U1028, Lyon, France; Claude Bernard Lyon 1 University, Lyon, France

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 platform "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).

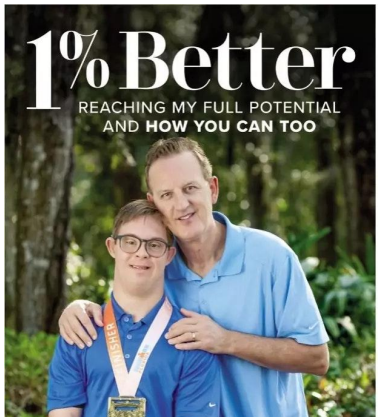
3:00 – 3:15 pm MDT (June 26, 2022)

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.

ACD's Vision for the Future



4:00 – 5:00 pm MDT (June 26, 2022)

CHRIS NIKIC & NIK NIKIC

Bio

Chris Nikic set a new world record by being the first person with Down Syndrome to complete a 140.6-mile Ironman. His mission is to inspire others like him to pursue their dreams and goals. His greatest achievement is showing others how shifting your focus from your disabilities to your abilities and pursuing your dreams with an unwavering tenacity, a positive attitude and a no-quit grit can help anyone achieve their goals and dreams.

Achievements: • Ironman 140.6 Finisher • Guinness Book Record Holder • Boston & NYC Marathon Finisher • Author Book: 1% Better – Reaching My Full Potential and How You Can Too

Special ACD Warrior of Hope Session: "1% Better - The Road to Full Potential"

Chris Nikic & Nik Nikic

Father and son deliver a substantive and inspiring speech that shares Chris's story and Nik's underlying principles of success, coaching and execution to achieve your full potential.

An accomplishment of this magnitude requires solid foundational principles, a well-designed and executed plan. Nik provides the underlying principles and framework while Chris brings it to life with his story.

Hear the compelling story of how they worked together, so Chris could pursue his dreams and make his goal of becoming the 1st person with Down Syndrome to do a 140.6 IRONMAN® Triathlon.

8:30 – 8:45 am MDT (June 27, 2022)

MARZIA PASQUALI
University of Utah and 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.

Evolving landscape of newborn screening for creatine deficiency syndromes

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 and intervention are the key to prevent irreversible brain damage. Newborn screening is available for GAMT deficiency in which elevated levels of guanidinoacetate are identifiable at birth. The first baby with GAMT deficiency was identified in Utah by NBS in 2020, shortly after a second baby was identified in NY. In May 2022, GAMT deficiency has been recommended for inclusion in the Recommended Uniform Screening Panel. AGAT deficiency is characterized by low guanidinoacetate, which might be challenging to detect by NBS. The knowledge that will be gained with universal screening for GAMT deficiency should allow to define appropriate decision limits for the detection of this very rare conditions. There is not currently a good biochemical marker for CTD in dried blood spots. Alternative strategies could be used to detect CTD at birth.

8:45 – 9:00 am MDT (June 27, 2022)

LARA DURAN-TRIO
Lausanne University Hospital (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

Females' characterization in the Slc6a8Y389C rat model of CTD

Lara Duran-Trio, Gabriella Fernandes-Pires, Dunja Simicic, Marc Lanzillo, Clothilde Roux-Petronelli, Pierre-Alain Binz, Carmen Sandi, Cristina Cudalbu & Olivier Braissant

Creatine (Cr) is an organic acid essential for recycling ATP. Cr is synthesized by a 2-step pathway (AGAT and GAMT) and transported by SLC6A8 (also called CrT). Cerebral Cr deficiency syndromes (CCDS), due to AGAT, GAMT or CrT deficiencies, are metabolic diseases causing severe neurodevelopmental delays and intellectual disability, characterized by brain Cr deficiency. Among CCDS, the X-linked CrT deficiency (CTD) is the most prevalent with no efficient treatment so far. Increasing number of human and animal studies contributes to the understanding of CTD pathology and the roles of Cr and CrT. However, most of them are focused in males and little is known about female carriers and how CrT deficiency affect them. We present the preliminary results of the females' first characterization of the Slc6a8Y389C rat model of CTD using heterozygous and homozygous females.

Brain Cr deficiency was found in all homozygous females, while heterozygous ones showed a broad spectrum of Cr levels. Similar as Slc6a8Y389C males, only homozygous females presented increased urinary Cr/Crn ratio, reduced body weight and motor dysfunction. However, spontaneous alternation and grooming behavior were not affected in any type of mutant females. Interestingly, different patterns of anxiety-like behavior were shown depending on the zygosity and the test used. Our first results validate the Slc6a8Y389C rat females as a promising tool to better understand CTD, provide new insights about CTD pathology and reveal sex and/or zygosity effects. They highlight the importance of including females in the study of CTD and possibly in other diseases.

9:00 – 9:15 am MDT (June 27, 2022)

GABRIELLA FERNANDES-PIRES
Lausanne University Hospital (CHUV)

Bio

I graduated with degree in Biomedical Sciences and I did my MSc in Biomedical Sciences at University of Bern, Switzerland. I joined the laboratory of Prof. Olivier Braissant at the Lausanne University Hospital (CHUV) to do my Ph.D. The aim of my thesis is to develop a new strategy for treatment of creatine deficiency by gene therapy in a new rat model.

AAV strategy to treat creatine transporter deficiency in the Slc6a8Y389C/y KI rat

Gabriella Fernandes-Pires, Lara Duran-Trio, Marc Lanzillo, Liliane Tenenbaum, Cristina Cudalbu, Carmen Sandi, & Olivier Braissant

For creatine transporter deficiency (CTD), current treatment strategies benefit one-third of patients. However, as their phenotype is not completely reversed, and for the other two-thirds of CTD patients, the development of novel more effective therapies is needed. We chose gene therapy by adeno-associated virus (AAV) as a new strategy to treat SLC6A8 deficiency in our Slc6a8Y389C/y knock-in rat model. We will present here preliminary results of ssAAV9-2YF-CMV-SLC6A8 transduction in injected Slc6a8Y389C/y rat males.

WT and KI males were injected at P11 intracisternally with ssAAV9-2YF-CMV-SLC6A8-Flag (10µl; 1013 vg/ml). Animals were sacrificed at 5 and 14 weeks post-injection to assess Slc6a8 transporter transduction. Starting from 5 weeks post-injection, a subgroup of injected KI animals were treated with Cr (2g/kg*day; drinking water). In vivo brain 1H-MRS scans were performed at week 5 and 14 weeks post-injection. Behavioral tests were performed starting from week 12 post-injection before MRS scans.

AAV-injected and Cr-treated KI animals presented a significant increased in body weight gain as compared to their non-injected KI littermates. Furthermore, the BMI in KI animals injected and Cr-treated was increased compared to non-injected KI animals. In open-field, injected and Cr-treated KI animals move significantly more and with more velocity compared to not injected KI animals.

Our results suggest that our AAV-driven strategy, by restoring Cr in CNS, is encouraging and may become clinically available with the ultimate goal to treat the so far untreatable SLC6A8 deficiency.

9:15 – 9:30 am MDT (June 27, 2022)

LAURA BARONCELLI
Neuroscience Institute CNR

Bio

Laura Baroncelli graduated in Biology from the University of Pisa in 2005 and received her doctorate degree in Neurobiology from the Scuola Normale Superiore in 2009. Following a fellowship at Scuola Normale Superiore, in 2010, she was awarded a two-year post-doctoral fellowship at the Accademia Nazionale dei Lincei in Italy. Since 2011, she has been a tenured researcher at the Neuroscience Institute (IN) of CNR in Pisa. She has published over 50 original research papers in international peer-reviewed journals. She was awarded funding by Fondazione Roma, LUMOS Pharma, Italian Ministry of Health, Lejeune Foundation, and Telethon for the study of creatine-related disorders. She is also an Academic Editor of Neural Plasticity and Scientific Report, and reviewer for various international journals and national agencies.

Creatine transporter deficiency: The long journey to successful therapy

Ghirardini Elsa, Calugi Francesco, Sagona Giulia, Di Vetta Federica, Roberta Battini, Giovanni Cioni, Tommaso Pizzorusso, & Laura Baroncelli

Although rare, Creatine Transporter Deficiency (CTD) has a major impact on patients and caregivers. There is no cure for this disorder, and the pathogenic mechanisms of the disease remain still elusive, hampering the identification of suitable therapeutic targets. Thus, achieving a better understanding of the neurobiological bases of CTD and searching for therapies are challenges that need to be addressed in parallel by the scientific community. Using a knockout murine model, we studied how Cr depletion affects the development and function of different cell population in the brain. We found that parvalbumin (PV) inhibitory interneurons are particularly vulnerable to the metabolic disruption induced by Cr deficit, leading to structural and functional alterations in these cells. Also, the selective loss-of-function of CrT in PV neurons is sufficient to cause cognitive impairment and increased susceptibility to epilepsy, demonstrating the fundamental role of these cells in the pathogenesis of CTD. Thus, PV neurons might represent an ideal target for a pharmacological approach in CTD. A second line of research in the lab is investigating gene therapy as a possible disease-modifying treatment. Using AAV-mediated delivery, we found that the administration of a functional copy of the CrT gene to KO mice results in the expression of transgenic CrT in the brain, increasing Cr levels and improving the cognitive performance of mice. However, CrT overexpression brings toxic effects, indicating that the therapeutic window for CTD is narrower than expected.

9:30 – 9:45 am MDT (June 27, 2022)

GERALD LIPSHUTZ
David Geffen School of Medicine at UCLA, Los Angeles, California, USA

Bio

Gerald S. Lipshutz, MD, MS, received his medical degree from the University of California Los Angeles (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. Clinically, within the David Geffen School of Medicine his clinical 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 National Institutes of Health (NIH) conferences and has served as a grant reviewer for both Wellcome Trust and the US National Institutes of Health where he is presently a standing member of the GDD Study Section.

AAV-based gene therapy restores cerebral creatine, reduces guanidinoacetate, and resolves of behavioral abnormalities in a mouse model of GAMT deficiency

Suhail Khoja, Jenna Lambert, Matthew Nitzahn, Adam Eliav, YuChen Zhang, Mikayla Tamboline, Colleen T. Le, Eram Nasser, Puja Patel, Irina Zhuravka, Lindsay M. Lueptow, Ilona Tkachyova, Shili Xu, Itzhak Nissim, Andreas Schulze, & Gerald S. Lipshutz

We previously presented early results of our studies of gene therapy for GAMT deficiency. Now we have completed studies where data was collected over 12 months including male and female mice. Utilizing an AAV-based gene therapy approach to express GAMT in hepatocytes, in situ hybridization and immunostaining demonstrated pan-hepatic GAMT expression. Serial collection of blood demonstrated a marked early and sustained reduction of guanidinoacetate (GAA) with normalization of plasma creatine and markedly reduced urinary GAA levels; this persisted over the year of study. The terminal time point demonstrated marked improvement in cerebral and myocardial creatine levels. In conjunction with the biochemical findings, treated mice gained weight to nearly match their wild-type littermates, and behavioral studies demonstrated resolution of abnormalities. While our studies were likely underpowered to detect statistically significant abnormalities in cerebellar function by rotarod testing, Gamt-deficient mice did demonstrate a reduction in grip strength of the fore- and hind-paws; these motor performance issues were also resolved with gene therapy. Finally, Gamt-deficient mice demonstrated reduced brain glucose consumption; this was nearly resolved with liver-based GAMT gene therapy and was superior to mouse chow with creatine supplementation. In conclusion, a gene therapy approach can result in long-term reduction of GAA levels with increased creatine in guanidinoacetate N-methyltransferase deficiency and at the same time can resolve the behavioral and motor abnormalities in a murine model of the disorder. These findings have important implications for the development of a new therapy for this abnormality of creatine metabolism.

10:15 – 11:00 am MDT (June 27, 2022)

KIM CECIL
Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine

Bio

Dr. Kim M. Cecil completed her bachelor's degree in Chemistry and Mathematics from Kentucky Wesleyan College, Master's and Doctorate in Chemistry from Vanderbilt University in Nashville, TN. After completing a post-doctoral fellowship in Magnetic Resonance Spectroscopy at the University of Pennsylvania in 1998, she was recruited to Cincinnati Children's Hospital Medical Center, where she serves as the Director of Basic and Translational Radiology Research. Her academic affiliations are with the Departments of Radiology, Pediatrics, Environmental and Public Health Sciences at the University of Cincinnati College of Medicine. Dr. Cecil's research efforts focus on the application of magnetic resonance spectroscopy and imaging in several populations by characterizing the features of inborn errors in metabolism and evaluating the effects of environmental neurotoxicants (such as lead, air pollution, and flame retardants), on brain anatomy and function. With Ton deGrauw, MD, PhD, and Gajja Salomons, PhD, Dr. Cecil published the first two studies identifying a 6 year old boy with creatine transporter deficiency. The first report featured the almost complete absence of creatine in the brain, as revealed by magnetic resonance spectroscopy. The second provided the genetic description of the condition. Dr. Cecil has subsequently published several articles describing the diagnostic features of creatine deficiency syndromes in children and adults. She is a member of the Joint Steering Committee for the Vigilan Study.

Recognizing females with creatine transporter deficiency syndrome

Kim M. Cecil, Katherine Morey, & Barbara Hallinan

Creatine transporter deficiency (CTD) is recognized as an X-linked genetic disorder caused by a variant in the SLC6A8 gene located on the X chromosome (Xq28). This condition varies in severity with characteristics that may include intellectual disabilities, speech delay, autism, attention deficit hyperactivity and gastrointestinal problems. While CTD primarily affects males, females may also demonstrate severe phenotypes. Noninvasive screening of creatine deficiency syndromes (both CTD and synthesis disorders) in males and females has exploited atypical concentrations of creatine and related molecules, such as creatinine. However, such screening of females with CTD can be compromised as urine creatine/creatinine levels often have values falling within typical ranges. Also, females with CTD may not demonstrate the characteristic dramatic reduction of creatine concentrations in the brain visualized without using rigorous quantification of in vivo proton magnetic resonance spectroscopy (MRS). In this presentation, we present the features of a heterozygous female with a severe form of CTD ultimately identified at age 8 years with exome sequencing and pathogenicity further confirmed with quantitative MRS.

10:30 – 10:45 am MDT (June 27, 2022)

DENISE KAY
Newborn Screening Program, Division of Genetics, Wadsworth Center, New York State Department of Health

Bio

PhD in Biology: Rensselaer Polytechnic Institute, Troy, NY (2004), Postdoctoral training in genetic epidemiology / human genetics / genetics of complex disease: Wadsworth Center, New York State Department of Health Current: Research Scientist, New York State Newborn Screening Program, Wadsworth Center, New York State Department of Health

Universal GAMT newborn screening in New York state: The first three years

Denise M. Kay, PhD, Mark A. Morrissey, PhD, Matthew Wojcik, MS, & Michele Caggana ScD, FACMG

Background: Guanidinoacetate methyltransferase (GAMT) deficiency is a rare cerebral creatine disorder. Despite available treatment and several pilot newborn screening studies, GAMT deficiency is not included on the Recommended Uniform [Newborn] Screening Panel (RUSP).

Methods: Based on availability of an accurate screening method, minimal impact on the Newborn Screening (NBS) system, and reported benefit of early treatment, the New York State (NYS) NBS Program implemented universal GAMT screening on 10/1/2018. Guanidinoacetate (GUAC) and creatine levels are measured in dried blood spots using mass spectrometry. Infants with elevated GUAC and GUAC:creatine ratio are considered positive and referred for evaluation and diagnosis.

Results: Through 3/31/2022, 749,787 infants have been screened: 24 were referred and 435 had borderline results requiring repeat screening. Among 24 referrals, one was confirmed to have GAMT deficiency. This infant was referred by NBS on day of life (dol) 8 and was clinically asymptomatic. Treatment was initiated on dol 17. An older unscreened sibling noted to have hypotonia, developmental delay and lack of speech of unknown etiology was also tested and confirmed. At last report to our program, the infant remained asymptomatic, and the sibling demonstrated some improvement.

Conclusions: GAMT deficiency is detectable via NBS without a significant increase in program workload or cost. The screen is accurate, with a low false positive referral rate. GAMT deficiency was renominated to the RUSP and is currently undergoing evidence review. Identification of one infant with GAMT deficiency in NYS and another in Utah provides support for inclusion.

10:45 – 11:00 am MDT (June 27, 2022)

JENNY GOLDSTEIN
University of North Carolina at Chapel Hill

Bio

Jenny Goldstein, PhD, CGC, is a co-chair of the ClinGen Cerebral Creatine Deficiency Syndromes Variant Curation Expert Panel (CCDS VCEP). Jenny's interest in the CCDS began in 2004 when she joined the Biochemical Genetics Laboratory at Duke University Medical Center to work on research studies in metabolic disorders. As part of this work, Jenny was coordinator for a study to measure the level of guanidinoacetate in newborn blood spots from individuals with GAMT deficiency, with the goal of determining the utility of this method for newborn screening (El-Gharbawy et al, 2013, Mol Genet Metab. 109:215-7). During this time, Jenny also worked as a genetic counselor in the Metabolic Genetics Clinic. She provided care for several years to a family with a child with GAMT deficiency and to 2 families with X-linked creatine transporter deficiency, and contributed data to publications on GAMT deficiency and X-linked creatine transporter deficiency (Bruun et al, 2018, Metab Brain Dis. 33:875-884; Khaikin et al, Eur J Paediatr Neurol. 2018, 22:369-379). Jenny began working as a senior biocurator for the Clinical Genome Resource (ClinGen) at the University of North Carolina at Chapel Hill in 2016, and feels fortunate that she has been able to maintain her connection with CCDS by working with the ClinGen CCDS VCEP, and by volunteering as an ambassador for the Association for Creatine Deficiencies.

The ClinGen Variant Curation Expert Panel for Cerebral Creatine Deficiency Disorders: Classifying the clinical significance of variants in GATM, GAMT, and SLC6A8

Jenny Goldstein, Amanda Thomas-Wilson, Vimla Aggarwal, Taraka Donti, Elena Feofanova, Nicole Liang, Daniel Reich, Raquel Fernandez, Meredith Weaver, Laura Trutoiu, Heidi Wallis, Juliann Savatt, Simona Bianconi, Emily Groopman, Nicola Longo, Sarah Young, & Saadet Mercimek-Andrews

Background: The 2015 American College of Medical Genetics/Association of Molecular Pathology (ACMG/AMP) guidelines facilitate variant classification. However, discrepancies in variant classification may occur due to the availability and weighting of different types of evidence by different laboratories. To address these challenges, the Clinical Genome Resource (ClinGen), funded by the National Institutes of Health, assembles Variant Curation Expert Panels (VCEPs) which adapt the ACMG/AMP guidelines to classify variants in genes of interest. Classifications are submitted to ClinVar as part of a public, FDA-approved human genomic database.

Methods: The ClinGen Cerebral Creatine Deficiency Syndromes (CCDS) VCEP, composed of experts and curators with interest in these disorders, was started in September 2018 to develop guidelines for classification of variants in GATM, GAMT, and SLC6A8. Variant classifications and supporting evidence for 90 pilot variants were discussed on monthly group calls. Final guidelines were submitted to the ClinGen Sequence Variant Interpretation Working Group for approval.

Results: CCDS gene-specific variant classification guidelines were developed including allele frequency thresholds, phenotype specifications, and functional assay requirements. The CCDS VCEP's pilot variant classifications agreed with those of available ClinGen submitters, supporting the utility of the VCEP's guidelines.

Conclusion: The CCDS VCEP's variant classification guidelines will facilitate clinical variant interpretation, helping to provide accurate information to families. In addition, the VCEP has begun collaborations with the Association for Creatine Deficiencies (ACD) and GenomeConnect to use anonymized ACD registry data, such as genetic variants, and pre-treatment biochemical and magnetic resonance spectroscopy (MRS) data, to further inform the variant classification process.

11:15 – 11:30 am MDT (June 27, 2022)

ABBY D'CRUZ

ACD Special Interest Session: "Probably Genetic"

11:30 – 11:45 am MDT (June 27, 2022)

CAROLE CHEHOWAH
Xtraordinaire

Bio
Carole is a mum of a 32 years old DTC girl and has been involved in Xtraordinaire for 15 years. Carole's background involving working in the finance industry for almost 30 years and has been very involved in the association related to rare disease and ID.

Xtraordinaire - Focus on the CTD families in France
Carole Chehowah & Franck de Franco

Our presentation will give an update of the French families with DTC, a screenshot of the population.

Then, the results of a survey: The most difficult in your day-to-day life; What is the most important, if and when there is a treatment? What has been the evolution of your children over the last couple of years: the positive(s) and the negative(s). What is your main source of concern for the future? And your main hope?

1:00 – 1:15 pm MDT (June 27, 2022)

KIM SOESBERGEN
Vrije Universiteit Amsterdam

Bio

Kim Soesbergen is a sister of a Creatine Transporter Deficiency (CTD) patient. This personal experience together with her master's program Neurosciences inspired her to develop the CTD Guideline. This Guideline attempts to bridge the gap between science and society by explaining the disease in an understandable manner. Kim is currently finishing her master's program at the Vrije Universiteit Amsterdam with a final research internship. For this, she is researching an Alzheimer's disease related gene in induced pluripotent stem cell derived microglia at Icahn School of Medicine, Mount Sinai, New York.

Creatine transporter deficiency: Bridging the gap between science and society

Kim Soesbergen

In order to bridge the gap between science and society, a CTD Guideline was developed. Within this Guideline, scientific information about the mutation, symptoms, diagnostics, treatment, and research on CTD is presented in layman's language. Additionally, the Guideline holds new data obtained through an international questionnaire (n = 41) among caregivers of CTD patients. This includes information about CTD patients' use of therapy and medication. Moreover, it includes data on the subjective experiences of caregivers of CTD patients with regard to receiving the diagnosis, finding appropriate patient care, and understanding the disease. These results showed that 54% of caregivers experienced or still experience difficulties understanding the disease. The Guideline aims to improve this for current and future caregivers of CTD patients.

1:15 – 1:30 pm MDT (June 27, 2022)

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

Bio

Judith Miller, PhD, is a clinical psychologist with 25 years' experience in developmental disorders. She is the coordinating Principal Investigator for the Vigilan Observational Study of Creatine Transporter Deficiency. 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. Her research focuses on screening, diagnosis, and outcomes across the lifespan for individuals with neurodevelopmental disorders.

Measuring family adjustments and accommodations in intellectual and developmental disorders

Judith S. Miller, Manisha Udhnani, Angel Wang, Lindsey Becker, Audrey Thurm, Elizabeth Berry-Kravis, & the Vigilan Observational Study Team

Background and Objective: Parents of children with intellectual developmental disabilities (IDD) adjust their lives to maximize success and reduce stress.- We set out to measure family accommodations and determine whether parents would view this as valuable.

Methods: 133 Items were developed based on previous parent interview data in Creatine Transporter Deficiency (CTD). Items covered several themes, including behavior, daily impact, and shifts in long-term plans. Feedback was sought from clinicians, parent, and biostatistician, with consensus the measure could be applicable to other IDDs. We then distributed it through clinics and advocacy groups for specific IDD populations.

Results: 500 parents from IDD populations participated (child Mage = 12.0, SD = 8.1, range: 1-55); 416 also provided feedback. Most parents rated the questionnaire as "excellent" (n=131); or "good" (n=245); and the items as "very useful" (n=158) or "moderately useful" (n=178). Comments were overall very positive, including "I felt this questionnaire was written specifically for me. I was thinking 'oh my God, somebody gets it'." "Thank you for taking the time to understand the caregivers. This is far more important than the general public knows." Parents also gave several suggestions for question clarity and tailoring item sets to age and ability.

Conclusion: Results suggest this is an important topic for research and clinical care. Future directions include item-level analyses, and examining the potential to affect clinical decision-making or measure response to treatment.

1:30 – 1:45 pm MDT (June 27, 2022)

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.

Oral medications and creatine transporter deficiency (CTD): Impact of clinical symptoms and perspectives of caregivers and clinicians

Melanie Brandabur, Kristin Voorhees, Tricia Cimms, Susan Blair, Hilary Beggs, & Francesco Bibbiani

Delivering oral therapy for individuals with CTD may be challenging due to difficulties with feeding, swallowing, gastrointestinal (GI) function, and sensory sensitivities. We reviewed data from Ultragenyx-led projects, including a caregiver advisory board, a burden-of-disease survey, CTD Caregiver Leadership Council meetings, and clinician interviews, along with Vigilan Natural History Study data to understand these challenges. Caregivers and clinicians report that maintaining adequate nutrition and hydration is challenging in individuals with CTD. Some infants have frequent vomiting and may require G-tube placement. GI issues such as choking/gagging, vomiting, and diarrhea can persist, and behavioral issues often emerge in adolescence. These difficulties can affect medication adherence. Parents report that their children have taste and texture preferences, including strong aversions to extreme temperature or grittiness. Milk and juice are commonly accepted; high-carbohydrate foods are often preferred. Caregivers and clinicians report most individuals with CTD are on oral medications but have difficulty swallowing pills. Though liquid formulations are generally acceptable if part of daily routine and delivered in smaller amounts via syringe, unpleasant tastes or gritty textures may be rejected. Review of clinical literature, burden-of-disease survey, and Vigilan data confirm use of oral medications/supplements and GI issues, and indicate that vomiting, dehydration, and G-tube placement are relatively frequent causes of hospitalizations. Further understanding this landscape will help Ultragenyx and others develop potential therapies that are easier for caregivers to administer and for individuals with CTD to tolerate and adhere to over time. Collectively, these learnings suggest a manageable pathway for future CTD oral therapies.

1:45 – 2:00 pm MDT (June 27, 2022)

LAURA VOSS
University of Utah

Bio

Laura Voss graduated in May 2022 with her Master of Science degree in Genetic Counseling from the University of Utah. She is passionate about helping patients understand complex genetic concepts and making genetic testing more accessible. She first fell in love with genetics while working as a research technician in a developmental biology lab investigating the biogenesis of specialized cell-type specific organelles using C. elegans as a model organism. She was co-first author on the manuscript "Asymmetric organelle positioning during epithelial polarization of C. elegans intestinal cells" (2022) and first author on the manuscript, "An ABCG Transporter Functions in Functions in Rab Localization and Lysosome-Related Organelle Biogenesis in Caenorhabditis elegans" (2020). For her graduate research project, Laura partnered with the Association for Creatine Deficiency and her research focused on the development, launch, and initial findings of the CreatineInfo Registry.

The Creatine Info Registry: Demographics and initial findings

Laura Voss, Nicola Longo, Marzia Pasquali, Laura Trutoiu, & Emily Reinhardt

Cerebral creatine deficiency syndromes (CCDS) are a set of rare inherited metabolic disorders that are characterized by mild to severe intellectual disability, global developmental delay, and speech-language disorders. In this report, we outline the initial findings of the Association for Creatine Deficiency's (ACD) CreatineInfo Registry, an online registry for CCDS launched in March 2021. It was developed through the National Organization of Rare Disorders (NORD) IAMRARE Registry Program. Aggregate data are summarized for the first 103 patients; 69% were diagnosed with creatine transporter deficiency (CTD) and 31% with guanidinoacetate methyltransferase (GAMT) deficiency. Participants represent 15 countries, with ages ranging from 1 to 43.7 years. Both conditions have a similar average age of onset of around 9 months of age, however, individuals with CTD tend to receive a diagnosis earlier (4.9±4.6 years) than those with GAMT deficiency (8.5±10.7 years). The most common symptoms for both GAMT deficiency and CTD were global developmental delay, seizures, and speech delay. Additional findings included failure to thrive and decreased sensitivity to pain. Most participants were diagnosed via genetic testing, but sometimes MRI/MRS and biochemical testing. Participants and families felt like the diagnostic delay is too long (4.1±4.6 years in CTD; 7.8±10.6 years in GAMT deficiency) and a better strategy for diagnosis is necessary. With the advent of newborn screening for GAMT deficiency, patients can be diagnosed and treated earlier. The CreatineInfo Registry aims to provide a better understanding of the natural history of CCDS and provide a valuable resource for current and future research.

2:00 – 2:15 am MDT (June 27, 2022)

AURORE CURIE
French National Reference Center for Rare Diseases with Intellectual Disability, Department of Child Neurology, Woman Mother and Child Hospital, Lyon University Hospital, Bron, France; Lyon Neuroscience Research Center, CNRS UMR5292, Inserm U1028, Lyon, France; Claude Bernard Lyon 1 University, Lyon, France

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 platform "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).

Outcome measures for clinical trial in creatine transporter deficiency

Aurore Curie, Fahra Gheurbi, Michaël Pommier, Tiphaine Courtalon, Amandine Brun, Léa Saverat, Florian Ducret, Axelle Poulain, Marion Buchy, Solène Roudet, Nathalie Bedoin, Jean-Baptiste Van der Henst, Laure Pisella, Eric Chabanat, Anne Cheylus, & Vincent des Portes

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 non-verbal reasoning with 3 levels of increasing difficulty, implicit rule learning, and elementary visuo-spatial perception. 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=30), and on typically developed children aged 3 to 8 (n=120). These outcome measure would be interesting to be used as evaluation criteria in drug trials and cognitive rehabilitation programs.

2:30 – 2:45 pm MDT (June 27, 2022)

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.

Patient Meaningful Outcomes for CCDS

2:45 – 3:15 pm MDT (June 27, 2022)

HEIDI WALLIS
Association for Creatine Deficiencies

Bio

Heidi lives in Salt Lake City, Utah with her husband, Trey, and their four children. Samantha (18) was diagnosed with GAMT deficiency at 5-1/2 years of age and Louis (10) was diagnosed with GAMT deficiency and began treatment shortly after birth. Heidi served as a volunteer board member for the ACD from 2015-2022 prior to being hired as the Executive Director. Heidi's vision for the future is that all creatine deficiencies will be screened for at birth and that each disorder will have a safe and effective treatment. Heidi's background is in Business Management and prior to working for ACD was a grant analyst and project manager in the Utah Newborn Screening Informatics program. Heidi serves as a member of the Utah Newborn Screening Advisory Committee and is the Utah team co-lead for the Mountain States Regional Genetics Network.

Building upon PMOs for CCDS Core Outcome Sets & Closing Remarks

4:00 – 5:00 pm MDT (June 27, 2022)

SARAH BUCHANAN
Carmen B. Pingree Autism Center of Learning

Bio

Sarah is the Clinical Director at the Carmen B. Pingree Autism Center of Learning. The Pingree Center is an intensive day treatment program that uses strategies based in Applied Behavior Analysis (ABA) to improve the independence of individuals diagnosed with autism spectrum disorder and other co-occurring behavioral health conditions. Sarah received her Bachelor's degree in Psychology, and Master's Degree in Special Education from the University of Utah. She became certified as a Board-Certified Behavior Analyst in 2016. Sarah has worked at the Pingree Center since 2000 and has had the opportunity to work in various roles throughout that time, giving her insight and a broader perspective of effective treatment models. She has provided services both in-home and in-clinic including direct care, supervision, and parent and staff training. Sarah is passionate about helping families and the clients she serves meet their individual goals and improve their quality of life.

Parent and Caregiver Focus Session: "Practical behavior management strategies"

Sarah Buchanan

This presentation will cover practical strategies to address challenging behavior in home and community environments. We will discuss ways to set clear expectations, prevent and prepare for challenging behavior, and what to do when difficult behavior occurs.