CREATIVE

KICKING OFF:

VIRTUAL SOCIAL HOUR
SUNDAY, AUGUST 2 | 1:00-2:00 PM PT

JOINED BY KEYNOTE
DR. JUDITH MILLER
SUNDAY, AUGUST 2 | 2:00-3:00 PM PT

FOLLOWED BY:

CCDS | 2020
VIRTUAL CONFERENCE

2- DAY EVENT | AUGUST 7-8, 2020

Community Social Hour

The Association for Creatine Deficiencies is kicking off our virtual events with some community building following our signature Walk for Stength. **Join us on August 2nd from 1:00-2:00 PM (PT)** for a Virtual Social Hour. This event will allow you to connect virtually with other CCDS community members and share commonalities and experiences related to your CCDS journey.

Keynote Speaker: Dr. Judith Miller

Lessons Learned From 2 Years of the Vigilan Natural History Study

2:00-3:00PM PT

About Dr. Miller

Judith Miller, Ph.D., MS
Clinical Training Director, Center for Autism Research
Assistant Professor of Psychology in Psychiatry
Perelman School of Medicine, University of Pennsylvania

Judith Miller, PhD is a clinical psychologist who studies diagnostic and clinical features of ASD across the lifespan. Her work includes studies of early identification, differential diagnosis between autism and other disorders such as anxiety and intellectual disorders, and identification of autism within the context of known genetic disorders. She hopes to identify those behaviors that are specifically unique to autism, in order to better understand the strengths and challenges faced by individuals and families. In addition to her research, Dr. Miller is the Clinical Training Director for CAR and the Autism Director for CHOP's Leadership Education in Neurodevelopmental Disorders (LEND) program, training the next generation of healthcare providers and advocates to provide the support and resources needed for individuals with ASD to reach their potential.

Virtual Conference Overview

Join us virtually for two half-days of research talks on cerebral creatine deficiency syndromes. We believe that in order for creatine deficiencies to one day be easily diagnosed and fully treated, we must openly collabrate and sare data information.

We have a select set of talks that will showcase the latest published (or soon to be published) results relevant to all three creatine deficiencies. Featured speakers include world-renowned scientists already part of our research network (such as Dr. Nicola Longo and Dr. Sylvia Stockler) as well as outstanding researchers that are new to our community (such as Dr. Jonathan Schelbach and Dr. Claire Colas). Speakers represent institutions across the US, Canada, Italy Switzerland, and Austria. Topics include:

- Gene Therapy
- Drug Repurposing
- Computational Models
- Clinical Findings from Vigilan Observational Study
- Updates from Industry Experts

ACD ASSOCIATION FOR CREATINE DEFICIENCIES

CCDS | 2020 VIRTUAL CONFERENCE

Virtual Conference Schedule *(Times in Pacific Time PT)*

Sunday, August 2 | 2:00-3:00 PM PT

Dr. Judith Miller, Children's Hospital of Pennsylvania | **Keynote: Lessons Learned From 2 Years of the Vigilan Natural History Study**

Friday, August 7 | 12:00-5:00 PM PT

12:00-12:20 PM PT	ACD	Welcome & Intro
12:00-12:40 PM PT	Ethan Perlstein, Perlara	Family-Driven n-of-1 Drug Repurposing for Inborn Errors of Metabolism
12:40-12:50 PM PT	Q&A	
1:00-1:20 PM PT	Peter Axerio-Cilies Sylvia Stockler, UBC	Drug Screening for Creatine Transporter Deiciency
1:20-1:40 PM PT	Aloïse Mabondzo, Ceres Brain Theraputics	Confirmation of CBT101 Efficacy in a 2nd Motel of Ko Mice to Treat Creatine Transporter Deficiency
1:40-1:50 PM PT	Q&A	
2:00-2:20 PM PT	Lee Graves, University of North Carolina	Lyn Kinase Regulates Creatine Uptake in an Imatinib-Resistant CML Cell Line
2:20-2:40 PM PT	Fady Hannah-Shmouni, NIH	Cardiac Manifestations of Creatine Transporter Deficiency
2:40-2:50 PM PT	Q&A	
3:00-3:20 PM PT	Matt Skelton, Cincinnati Children's	Ketogenic Diet in Mice
3:20-3:40 PM PT	Gai Ayalon, Ultragenyx	Creatine Transporter Deficiency Syndrome: An Advanced Preclinical Program
3:40-3:50 PM PT	Q&A	
4:00-4:20 PM PT	Jonathan Schelbach, Indiana University	Classification of the Molecular Defects Associated with Pathogenic Variants of the SLC6A8 Creatine Transporter
4:20-4:40 PM PT	Lara Gechijian, Jnana Therapeutics	Characterization of the Molecular Features of Patient-Defined Variants of SCL6A8 in CTD
4:40-4:50 PM PT	Q&A	

Saturday, August 8 | 7:00-11:30 AM PT

7:00-7:20 AM PT	Laura Baroncelli, CNR Pisa	Creatine Transporter Deficiency: New Insights on Cell-Specific Vulnerability to Metabolic Failure
7:20-7:40 AM PT	Olivier Braissant, University Hospital of Lausanne	The SLC6A8Y389C/y Creatine Transporter-Deficient Rat: From Characterization to Strategies of Treatment
7:40-7:50 AM PT	Q&A	
8:00-8:20 AM PT	Jagdeep Walia, Queen's University	Gene Therapy for Creatine Deficiency Disorders
8:20-8:40 AM PT	Ameya Sanjay Kasture, University of Vienna	4-Phenylbutyrate Rescues Several CTD-Linked Misfolded Creatine Transporter-1 Variants
8:40-8:50 AM PT	Q&A	
9:00-9:20 AM PT	Claire Colas, University of Vienna	Novel Structural Models of the Creatine Transporter Rationalize its Structural Determinants of Binding
9:20-9:40 AM PT	Emil Alexov, Clemson University	Computational Analysis of Missense Mutations in Creatine Transporter Protein Associated with Creatine Deficiency Syndrome
9:40-9:50 AM PT	Q&A	
10:00-10:20 AM PT	Axel Neu, UKE	Muscle Phenotype of AGAT- and GAMT-Deficient Mice After Simvastatin Exposure
10:20-10:40 AM PT	Nicola Longo, University of Utah	Small Molecule Approach for the Treatment of GAMT Deficiency
10:40-10:50 AM PT	Q&A	
11:00-11:30 AM PT	Sangeetha Iyer, ACD	ACD Research Strategy & Closing Remarks

Friday, August 7 | 12:00-12:20 PM PT
Welcome & Intro

Laura Trutoiu, PhD Director of Research
Association for Creatine Deficiencies

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), their cat Spocky, 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.

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Abstract:
It is our great pleasure to welcome you to the CCDS 2020 Virtual Conference. As a parent-led organization, our responsibility is to support the widest range possible of relevant research efforts in the scientific community, including biotech and industry, in order to maximize our chances of getting treatments. This virtual meeting is featuring 20 speakers covering the latest research on cerebral creatine deficiencies. As part of this welcome talk we will share some of the ACD initiatives and success stories from the past year. Thank you for being part of our community!

** Talk will be recorded and made available post conference.*

Friday, August 7 | 12:20-12:40 PM PT
Family-Driven n-of-1 Drug Repurposing for Inborn Errors of Metabolism*

Ethan Perlstein, Ph.D.
Perlara

Ethan Perlstein is received a Ph.D. in 2006 from Harvard University (Department of Molecular and Cell Biology) while working in the laboratory of Professor Stuart Schreiber. He completed an independent postdoctoral fellowship at the Lewis-Sigler Institute at Princeton University from 2007 to 2012. Since its founding in 2014, he is CEO of Perlara PBC, the first biotech PBC that partners with highly motivated families to cure rare genetic diseases. In 2019, he joined the Christopher & Dana Reeve Foundation as their first Chief Scientific Officer, but due to unexpected COVID-related financial constraints, his team was let go in Spring 2020. Recently, he became CEO of a new nonprofit, co-founded by Dr. Tim Yu and Julia Vitarello, whose mission is to make personal programmable medicines accessible to everyone.

Abstract:
Perlara is the first-ever biotech public benefit corporation/PBC with a "n-of-1, one for all" mission to cure genetic diseases of metabolism and in so doing discover drugs that safely slow aging and extend healthspan. Perlara works in partnership with entrepreneurial families to codevelop repurposed orphan drugs for rare metabolic diseases thereby uncovering drug targets and disease modifiers with blockbuster expansion opportunities. I will present two case studies showing how yeast, worm, fly and fibroblast models of two Congenital Disorders of Glycosylation, PMM2-CDG and NGLY1-CDDG, made possible a repeatable and cost-effective path from drug repurposing screens in the lab to a single patient IND in under a year.

** Talk will be recorded and made available post conference.*

Friday, August 7 | 1:00-1:20 PM PT
Drug Screening for Creatine Transporter Deficiency

Sylvia Stockler, MD, and Peter Axerio-Cilies, PhD
BC Children's Hospital & Department of Pediatrics UBC; Djavad Mowafaghian Centre for Brain Health, UBC

Dr. Stockler-Ipsiroglu is a Professor of Pediatrics at the Department of Pediatrics (UBC) and head of the Division for Biochemical Genetics at BC Children's Hospital. She authored the first description of GAMT and AGAT deficiency and has published several articles on cerebral creatine deficiency syndromes. Her research focuses on diagnosis and treatment of genetic conditions causing intellectual disability as well as on the evaluation of outcomes of treatment outcomes using innovative trial methodologies and outcome measures.

Dr. Axerio-Cilies from the Department of Medicine and Psychiatry at the Djavad Mowafaghian Centre for Brain Health (UBC) has recently engaged in the development of new treatments for creatine transporter (SLC6A8) deficiency. He has extensive 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. Work done in collaboration with Matt Cheng who is an undergraduate student at the University of British Columbia and University of Pennsylvania, who performed the SLC6A8 database analysis. We are indebted Dr. Lee-Jun C. Wong for allowing analysis of the SLC6A8 variants identified at the Baylor Medical Genetics Laboratory.

Abstract
Creatine transporter (SLC6A8) deficiency causes intellectual disability, autism and seizures. Treatment with creatine and its metabolic precursors arginine and glycine are ineffective in improving clinical outcomes. To find new treatments, we have developed a drug discovery pipeline that incorporates a series of biological and structural techniques, for example 14C-creatine uptake, immunofluorescence, measurement of electrogenic transporter properties, surface biotinylation, computer-assisted drug design techniques and X-ray crystallography, that allows us to assess SLC6A8 function and expression in various mammalian cell lines. We will identify candidate molecules for potential rescue of SLC6A8 function and test them in our experimental setting. We used the LOV and the Baylor Database (Lee-Jun C. Wong) to select SLC6A8 variants. Grouping these variants according to pathogenicity/functional validation and number of affected patients, we selected two deletion variants (c.1006_1008del and c.1222_1224del, observed in 26 and 19 individuals respectively), and two missense variants (c.1661C>T, c.1169C>T, each observed in 13 individuals) for further research. As a proof-of-principle study, we showed an absence of activity during SLC6A8 mediated creatine transport in one of these variants. Drug candidates which recover mutant SLC6A8 function and/or expression will be tested on individuals carrying the respective variant, with a single experimental study design (n=1 trials) using core, personalized, and personally meaningful outcomes. In this joint presentation, Dr. Axerio-Cilies will present principles of drug screening and the general experimental design. Dr. Stockler will show how to select core, personalized and personally meaningful outcomes for single experimental study designs used in the clinical validation of our drug candidates.

Friday, August 7 | 1:20-1:40 PM PT
Confirmation of CBT101 Efficacy in a 2nd Model of Ko Mice to Treat Creatine Transporter Deficiency

Aloïse Mabondzo, PhD, HDR
Ceres Brain Therapeutics

Dr. Aloïse Mabondzo joined the 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 has developed fully characterized in vitro screening tools allowing the optimization of the molecules under development for brain penetration. His innovative research has made possible the development of research programs in the neuroscience field: Alzheimer's disease, nanotoxicology, ischemic hypoxia encephalopathy, X-linked creatine transporter deficiency disease. Dr. Aloïse Mabondzo is author or co-author of 59 articles in peer-reviewed journals, six patents, gave lectures as a lecturer and as well as guest speaker, poster presentation in the scientific congress, and he often reviews articles for scientific journals. He has directed twelve Ph.D. students, and six postdoctoral positions have been 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 a co-founder of CERES BRAIN THERAPEUTICS, a spin-off from the French alternative energies and Atomic Energy Commission (CEA), committed to focusing its resources to the preclinical development of advance drug over coming years in order to provide CTD patients with a therapeutic solution to deliver creatine in the brain.

Abstract
The creatine (Cr) transporter (CrTD) deficiency is an X-linked inherited metabolic disorder characterized by cerebral Cr deficiency, which results in intellectual disability associated with epilepsy and autistic behaviour. No satisfactory treatments are available for CrTD. We propose an innovative therapeutic approach to increase Cr content within neurons and improve cognitive functions. We developed and improved a new pharmaceutical formulation comprising creatine fatty ester and safe excipients. Keeping in mind that we expect in the future to treat CrTD patients with moderate to severe intellectual disability, behavioral disorder and occasionally epilepsy, we focused on the assessment of cognitive function of a second new CrT Ko mouse model after administration of CBT101 by the nasal route. We demonstrated that CrT Ko mice showed a marked memory deficit ($P<0.01$) using a Novel Object Recognition test. Remarkably, CBT101 treatment significantly improves this deficit ($P<0.05$). No difference was evidenced between WT and CBT101 treated mice (88% of WT memory recovery). The spontaneous alternation of treated CrT Ko mice using the Y maze was also investigated. Young WT mice alternation rate was about 64.2±4.2% of total arm choices. In CrT Ko mice, it dropped to the level of 44±6.2%; $P<0.001$, demonstrating that CrT disruption in the mouse model could reproduce the early pathological phenotype of CrTD patients. CBT101 treatment improves the deficit memory of Ko mice (55.2±7.8%). To summarize, we demonstrated improvement of declarative memory abilities, spatial memory, and motor abilities in a mouse model for CTD and confirmed our previous reported findings.

Friday, August 7 | 2:00-2:20 PM PT

Lyn Kinase Regulates Creatine Uptake in an Imatinib-Resistant CML Cell Line*

Lee M. Graves, PhD
University of North Carolina

Dr. Lee Graves is a Professor in the Department of Pharmacology at the University of North Carolina at Chapel Hill. He is currently the Faculty Director of the UNC Michael Hooker Proteomics Facility at UNC. His laboratory applies proteomics to study the biological mechanisms of disease as well as the pharmacological responses to targeted therapeutics. Over the last 25 years, his lab has focused on cellular adaptations to protein kinase inhibitors in cancer biology and other diseases. Recently his research has combined these studies with metabolomics analyses to gain a deeper understanding of mechanisms of acquired drug resistance. He has served on the Editorial Board of multiple journals (JBC, Mol Pharm, BBA-GEN), been a co-founder of two companies (KinoDyn, ViroKyn), and has trained multiple graduate students and post-doctoral fellows.

Abstract:
We previously demonstrated that an imatinib-resistant leukemia cell line (Myl-R), contained elevated intracellular creatine pools compared to imatinib-sensitive Myl cells. We performed stable isotope metabolic labeling, media creatine depletion, and phosphoproteomics experiments to investigate the origin of increased creatine pools in Myl-R cells. Inhibition of the Na+/K+-ATPase pump (ouabain, digitoxin), depletion of extracellular creatine or inhibition of Lyn kinase (ponatinib, dasatinib), demonstrated that enhanced creatine accumulation in Myl-R cells was dependent on uptake from the growth media. Western blot analyses showed increased Lyn activity and phosphorylation of the Na+/K+-ATPase in Myl-R cells, whereas Lyn inhibition or knockdown decreased Na+/K+-ATPase phosphorylation and creatine accumulation. Thus our data suggests that Lyn can affect creatine uptake through phosphorylation of the Na+/K+-ATPase pump and identify kinase and phosphorylation-dependent regulation of the Na+/K+-ATPase as pivotal in regulating creatine uptake and energy metabolism in these cells.

** Talk will be recorded and made available post conference*

Friday, August 7 | 2:20-2:40 PM PT

Cardiac Manifestations of Creatine Transporter Deficiency*

Fady Hannah-Shmouni, MD FRCPC
NIH

Fady Hannah-Shmouni, M.D., DABIM, AHSCP, FRCPC, is a clinician investigator in endocrinology, hypertension, and biochemical genetics with clinical and research interests in the diagnosis and management of familial endocrinopathies, endocrine hypertension disorders, and inborn metabolism errors. For NICHD, he serves as director of the Office of Education's Graduate Medical Education (GME); chief of the internal medicine, endocrinology, and genetics inpatient ward and outpatient endocrinology; associate program director of NIH's Inter-Institutes Endocrinology & Metabolism Fellowship Program; and principal investigator of endocrine genetic and hypertension disorders for the Stratakis Laboratory's section on endocrinology and genetics. He is the principal investigator on two NICHD protocols: "Clinical and Molecular Characteristics of Primary Aldosteronism in Blacks" and "Observational Study of Males with Creatine Transporter Deficiency."

Abstract
The clinical spectrum of cardiac manifestation in CTD is largely unknown. The SLC6A8 transport system is highly expressed in skeletal and cardiac muscles, however, observable muscle weakness or cardiomyopathy is not a known feature of CTD. Several cardiac manifestations in males with CTD were reported in the literature, including premature ventricular contractions, mild cardiomyopathy, and long QT syndrome (QT time 495 ms, reference 350–440). Unpublished reports of pacemaker placement in a case of long QTc, left ventricular non compaction, and cardiomyopathy have been observed amongst clinicians caring for patients with CTD. However, to date, causality between cardiac manifestations and CTD have not been ascertained. Modalities for early cardiac disease detection, monitoring and potential therapeutic interventions are needed. Using clinical and mouse data, we hypothesized that in CTD, cardiac creatine deficiency is associated with cardiac electrocardiographic and mechanical changes. In this talk, I will update the CTD community about our ongoing clinical and mouse studies pertaining to cardiac manifestations in males patients with CTD.

** Talk will be recorded and made available post conference*

Friday, August 7 | 3:00-3:20 PM PT
The Effect of a Ketogenic Diet on Slc6a8 Knockout Mice*

Matthew R. Skelton, PhD
Cincinnati Children's Research Foundation

Dr. Skelton received his Ph.D. in Molecular and Developmental Biology from the University of Cincinnati in 2006. His graduate work focused on the effects of prenatal MDMA exposure on learning and memory in rats. As a post-doctoral fellow at CCRF, Dr. Skelton characterized the first Slc6a8 knockout model. Dr. Skelton joined the CCRF faculty in 2011 where his lab focuses on the biological underpinnings of the cognitive deficits caused by CTD. He has over 50 publications (12 related to creatine) and has received funding from the NIH, pharmaceutical companies, and private foundations. He has routinely served as a reviewer for academic journals and grant review committees. Dr. Skelton has proudly served on the ACD SMAB since its inception and is even prouder to be the first recipient of an ACD grant in 2018.

** Talk will be recorded and made available post conference*

Abstract:
Of the three known causes of creatine deficiency syndromes, creatine transporter deficiency (CTD) is the only one where individuals do not benefit from creatine supplementation. Restoring Cr in the brain requires a treatment that gets Cr past the blood brain barrier and then into the correct cells. Together, these challenges-while not impossible-present a difficult treatment paradigm. Therefore, it may be beneficial to target secondary effects of Cr deficiency. Creatine transporter knockout (Crt-KO) mice have lower brain ATP levels than control mice, which makes it a potential therapeutic target. The ketogenic diet (KD) has been used to improve metabolic (ATP-producing) function in the brain and has been used as a therapeutic for certain types of drug-resistant epilepsy. Based on our data and the posited use of the KD, we hypothesized that placing Crt-KO mice on a KD could increase brain ATP and improve cognitive function. To mimic an early-life start, we started Crt-KO and control mice on the KD immediately after weaning. Early-life exposure to the KD lead to significant mortality in the Crt-KO mice-though this may have been due to secondary (housing) effects. In cognitive testing, the KD did not improve the performance of the Crt-KO mice. Together, starting Crt-KO mice on a KD immediately following weaning had negative effects on the well-being of the mice with no improvement in cognitive function. Further work is needed to test the effect of the KD in older mice and other testing parameters related to the KD in Crt-KO mice.

Friday, August 7 | 3:20-3:40 PM PT
Creatine Transporter Deficiency Syndrome: An Advanced Preclinical Program

Gai Ayalon, PhD
Ultragenyx Pharmaceutical Inc.

Gai Ayalon, Ph.D., is a neuroscientist and a Senior Director at Ultragenyx Pharmaceutical Inc., a Bay Area, California company dedicated to developing therapeutics for rare and ultra-rare diseases. He is currently leading teams dedicated to developing treatments for neurodevelopmental disorders. Previously, Dr. Ayalon was a scientist at Genentech, where he led drug discovery programs focused on immunotherapeutic approaches to neurodegenerative diseases. Dr. Ayalon received his Ph.D. from the Hebrew University Medical School in Jerusalem, Israel, and conducted his postdoctoral research at Duke University Medical Center.

Abstract
Creatine Transporter Deficiency (CTD) is a rare X-linked inherited disorder, caused by loss of function mutations in the SLC6A8 gene, which encodes the creatine transporter (CrT). CrT protein expression at the blood-brain barrier and in neurons helps supplement intraneuronal creatine pools in the brain, which are required for maintaining ATP and cellular energy homeostasis. Without functional CrT, creatine pools become depleted, thus impairing the normal metabolic function in these cells. Patients with CTD experience global developmental delay, mild to severe intellectual disability, autistic features, behavioral disabilities, and delayed/absent language development. UX068 is a small molecule prodrug that traverses the blood-brain barrier and cell membranes and releases free creatine to neurons and glial cells. This mechanism of action bypasses the CrT altogether and is designed to provide a source of brain creatine independent of the specific mutation in the SLC6A8 gene. UX068 has been studied in a novel SLC6A8 knock-out CTD rat model which was developed utilizing CRISPR/Cas9 technology, and in non-human primates. Data from these UX068 preclinical studies demonstrate creatine delivery throughout the brain. In this presentation, we will share highlights of preclinical results, as well as provide an overview of the ongoing activities that Ultragenyx is engaged in for CTD.

Friday, August 7 | 4:00-4:20 PM PT

Classification of the Molecular Defects Associated with Pathogenic Variants of the SLC6A8 Creatine Transporter*

Jonathan Schlebach, PhD
Indiana University, Bloomington

Jonathan received a BS in Biochemistry from the University of Illinois at Urbana-Champaign in 2007 after which he began his graduate studies at Purdue University in the laboratory of Chiwook Park. In 2012 he received a PhD for his studies on the kinetics and thermodynamics of integral membrane protein folding. Jonathan went on to pursue postdoctoral training in the laboratory of Charles R. Sanders at the Vanderbilt University School of Medicine, where he was awarded a Ruth L. Kirschstein National Research Service Award from the NIH for his studies of integral membrane protein misfolding and disease.
His current studies involve the physiochemical coupling between the folding and trafficking of integral membrane proteins in the cell. His laboratory utilizes an interdisciplinary array of biophysical, analytical, and cellular techniques to gain mechanistic insights into the key reactions that modulate the cellular proteostasis of integral membrane proteins and ultimately give rise to the molecular basis of disease. His group is also interested in adapting these tools and perspectives to address emerging issues in precision medicine.

Abstract:
To date, more than 80 mutations in the SLC6A8 creatine transporter (hCRT1) have been identified in creatine transporter deficiency (CTD) patients. Though these mutations have the same effect on creatine uptake, they disrupt the hCRT1 protein in different ways. An understanding of the differences between these mutations may offer key advantages in efforts to develop and target of corrective therapeutics. To survey the effects of these mutations, we quantitatively profiled the cellular processing, trafficking, expression, and function of eight pathogenic CTD variants in relation to the wild type (WT) and one neutral isoform. All eight CTD variants exhibit measurable expression defects that likely contribute to the resulting loss of creatine uptake. However, the magnitudes of their specific effects on the expression and trafficking of hCRT1 vary considerably, and we find that the loss of uptake associated with two of these variants primarily arises from the disruption of the creatine pocket. Our observations suggest these mutational effects parallel those of mutations responsible for Cystic Fibrosis (CF). Recent breakthroughs in the treatment of CF suggest a clear path for the development of targeted therapeutics. Based on these considerations and our own results, we suggest potential strategies for the identification and development of novel CTD therapeutics.

** Talk will be recorded and made available post conference*

Friday, August 7 | 4:20-4:40 PM PT

Characterization of the Molecular Features of Patient-Defined Variants of SLC6A8 in CTD

Lara Gechijian, PhD, Senior Scientist
Jnana Therapeutics

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
Additional SLC6A8 variants associated with creatine transporter deficiency (CTD) continue to be identified and now number more than 80 variants in total. Understanding the specific molecular defects linked to these SLC6A8 variants and their impact on creatine transporter location and function is important for the development of therapeutics and predicting patient response. We developed two cellular systems to further characterize SLC6A8 patient-defined variants. In one system, we overexpressed a cross-section of SLC6A8 variants and compared them based on surface localization and creatine transport capacity. For translational purposes, a more physiological cellular model was developed using the CRISPR/cas9 technology to make precise edits in the genome to deliver individual SLC6A8 variants to the endogenous genetic locus. Herein we report a comparison of the surface localization and creatine transport capacity for a cross-section of patient variants. Additionally, for several variants, we compare the creatine transport function from the overexpression and knock-in systems. These two models can be used to characterize the molecular features of patient-defined variants as well as aid in the development of novel therapeutics for CTD.

Saturday, August 8 | 7:00-7:20 AM PT

Creatine Transporter Deficiency: New Insights on Cell-Specific Vulnerability to Metabolic Failure*

Laura Baroncelli, PhD
Neuroscience Institute CNR Pisa

Dr. Baroncelli graduated in Biology from the University of Pisa in 2005 and trained in the PhD program in Neurobiology at the Scuola Normale Superiore from 2006 to 2009. Following a fellowship at Scuola Normale Superiore, she was awarded in 2010 a two-year post-doctoral fellowship at the Accademia Nazionale dei Lincei, in Italy. Since 2011, she is Tenured Researcher at the Neuroscience Institute (IN) of CNR in Pisa. Recently, she was awarded a six-month travel grant within the program "Post-Doctoral Fellowship- 2017" of Fondazione Umberto Veronesi for a training period with two-photon microscopy at the University Medical Center of Göttingen. Her scientific production was highly fruitful leading to the publication of 36 original research papers in international peer-reviewed journals (H index: 18). She was awarded personal fundings by Fondazione Roma, LUMOS Pharma, Italian Ministry of Health, Lejeune Foundation and Telethon for the study of creatine-related disorders. She is also Academic Editor of Neural Plasticity and Scientific Report, and reviewer for various international journals and national agencies.

Abstract:
Creatine Transporter Deficiency (CTD) is an X-linked inherited metabolic disorder presenting with cerebral creatine (Cr) deficiency, early intellectual disability, epilepsy and autistic-like behaviour. Although rare, CTD represents a major issue in health care, leading to a significant decrease of life expectancy and causing chronic illnesses with a large impact on patient quality of life and health-care system. There is no cure for this devastating disorder. Despite much knowledge about the natural history of CTD and the role of Cr in energy metabolism, little is known about the brain alterations underlying the impairment of multiple functional domains in CTD. To provide a characterization of brain morphological and neurofunctional alterations associated to CTD, we used an integrated approach combining neuroanatomical, electrophysiological and behavioural techniques. These analyses were performed in ubiquitary CrT ko mice and conditional transgenic mice carrying CrT deletion only in parvalbuminergic interneurons.We report that anomalies in GABAergic neurotransmission, particularly depending on the failure of parvalbuminergic interneurons, constitute a pathological hallmark of CTD. Consistently, CTD mice show a very specific EEG signature and a severe epileptic phenotype, as assessed through behavioural observation and video-EEG recordings in awake animals. We demonstrated that aberrant development and function of a selective neuronal population contribute to the etiology of CTD disorder. These findings will allow us to identify new potential targets for pharmacological treatment. Moreover, EEG spectral signature could be used as classifying non-invasive biomarker for evaluation of brain function in CTD and treatment assessment. Importantly, EEG is routinely included in the follow-up of patients, increasing the translational value of this biomarker.

** Talk will be recorded and made available post conference*

Saturday, August 8 | 7:20-7:40 AM PT

The Slc6a8Y389C/y Creatine Transporter-Deficient Rat: From Characterization to Strategies of Treatment*

Prof Olivier Braissant, PhD
Service of Clinical Chemistry, University Hospital of Lausanne, Switzerland

Working on cerebral creatine for many years, I contributed to the understanding of how creatine can be transported from 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 understand 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 which I will present at the CCDS 2020 Virtual Conference.

** Talk will be recorded and made available post conference*

Abstract
The Slc6a8Y389C/y creatine transporter-deficient knock-in (KI) rat was established based on one same missense point mutation described in human and abolishing completely the Cr transporter activity. KI male rats show a strong decrease in brain creatine (-85%), decreased body weight gain from weaning, and autism-characteristic features under behavioral experiments. They also show structural changes in the brain tissue, such as disorganized astrocytic fibers and thinner granular layer of cerebellar cortex. We are currently developing strategies of treatment of this creatine transporter-deficient rat through AAV transduction of the functional SLC6A8 transporter in its brain.

Saturday, August 8 | 8:00-8:20 AM PT
Gene Therapy for Creatine Deficiency Disorders*

Jagdeep S Walia, MD, FRCPC, FCCMG
Queen's University

Dr. Jagdeep Walia is a full-time faculty, Head of the Division of Medical Genetics, and Director of research in the Department of Pediatrics at Queen's University. He is an Associate Professor and a Medical Geneticist. Dr. Walia is a graduate of the Guru Nanak Dev University School of Medicine. He did his post-doctoral fellowship at the University of Toronto and Medical Genetics training at the University of Manitoba. He joined the Department in 2012 and also started a research program in 2013. His work in Medical Genetics includes consultation on a broad range of genetic problems in children and adults including Cancer, Prenatal, Metabolics, and General Genetics. He teaches undergraduate students and residents in genetics.

He has an active clinical and basic genetics research program. His lab is focused on developing novel gene therapy approaches for inherited and acquired neurodegenerative disorders especially focusing on GM2-gangliosidoses and Creatine Deficiency disorders.

Abstract:
Our lab focuses on developing gene therapy for neurodegenerative conditions. In past few years one of our main foci was to develop gene therapeutics for a group of disorders called GM2 Gangliosidosis and we achieved excellent preclinical results. Our product is now in clinical translation stage with the clinical trial planned to start by the end of 2020. Now our focus is to develop gene therapeutics for all 3 primary creatine deficiency disorders, in collaboration with Dr. Steve Gray and Dr. Andreas Schulze. We have already developed multiple vectors for all three conditions. In vitro and in vivo testing of these vectors will be done next to assess the efficiency of these vectors in disease specific mouse models. The results from these experiments may open pathways for a clinical trial for these disorders.

** Talk will be recorded and made available post conference*

Saturday, August 8 | 8:20-8:40 AM PT
4-Phenylbutyrate Rescues Several CTD-Linked Misfolded Creatine Transporter-1 Variants *

Ameya Kasture, PhD
University of Vienna, Vienna, Austria

Ameya Kasture is a postdoctoral researcher at the University of Vienna, Vienna, Austria. His research examines the use of pharmacological compounds to remedy protein misfolding. He primarily focuses on a group of proteins called solute carrier 6 (SLC6) transporters, which include transporters for creatine, amino acids, biogenic amines, and osmolytes. Mutations in the genes encoding the SLC6 transporters are linked to various diseases. In the case of creatine transporter, the disease-causing mutations retain the transporter in the endoplasmic reticulum, thereby affecting its surface availability. Pharmacological approaches can be used to restore the surface availability of at least some of the disease-causing mutations. Additionally, he uses Drosophila melanogaster as a model organism to understand dopamine and serotonin neurotransmission. He also teaches undergraduate and graduate students at the Department of Neuroscience and Developmental Biology, University of Vienna. He completed an MSc in Molecular Neuroscience at the University of Bristol, UK, and a Ph.D. in Cell Communication in Health and Disease at the Medical University of Vienna, Austria.

** Talk will be recorded and made available post conference*

Abstract
Creatine and creatine phosphate play crucial role in managing energy metabolism in cells. Creatine requirement of body is party fulfilled by creatine synthesis in liver and kidney, and partly by dietary sources. Uptake of creatine into tissues such as skeletal muscles, cardiac muscles and brain relies on creatine transporter. A non-functional transporter in hemizygous males result in creatine transporter deficiency syndrome (CTD) manifested by a varying cluster of neurological symptoms that include epilepsy, mental retardation, autism, development delay and motor dysfunction. Mutations in the coding sequence of the human creatine transporter-1 (hCRT-1/SLC6A8) gene result in CTD. We assessed 16 clinically relevant hCRT-1 mutants in cell culture setup and found that the mutants stay retained in the endoplasmic reticulum. 4-phenylbutyrate (4-PBA), a clinically approved drug, was tested for its chaperoning potential on cells expressing the hCRT-1 variants. We found that 4-PBA restored the activity of several hCRT-1 variants. One representative mutant (hCRT-1-P544L) was studied in hippocampal culture neurons and we found that 4-PBA restored the transporter trafficking to neurite extensions. Our work highlights that the clinically available 4-PBA could also prove beneficial in the management of some of the disease causing hCRT-1 mutants. Additionally, our studies justify the search for additional pharmacochaperones to restore folding of 4-PBA-unresponsive hCRT-1 mutants.

Saturday, August 8 | 9:00-9:20 AM PT

Novel Structural Models of the Creatine Transporter Rationalize its Structural Determinants of Binding*

Dr. Claire Colas, Ph.D.
University of Vienna

Claire Colas is a postdoctoral scientist at in the Pharmacoinformatics research group (University of Vienna). She completed her PhD in 2011 at the Pasteur Institute in Paris, France and worked for two years as a postdoctoral scientist at the Institut de Chimie des Substances Naturelles at Gif-sur-Yvette, France. Since 2013, Dr. Colas' research has been focused on the structural characterization of Solute Carrier (SLC) transporters. First at the mount Sinai School of Medicine in New-York City (2013-2018) and then in Vienna (2018-present), Dr Colas has been working on distinct SLC families, involved in various diseases and disorders.

Dr Colas uses various computational methods such as homology modeling and molecular docking to explore the structural determinants defining the substrate specificities of SLCs.

Abstract:
Creatine is a crucial metabolite that plays a fundamental role in ATP homeostasis in tissues with high- energy demands. The creatine transporter (CreaT, SLC6A8) belongs to the solute carrier 6 (SLC6) transporters family, and more particularly to the GABA transporters (GATs) subfamily. Understanding the molecular determinants of specificity within the SLC6 transporters in general, and the GATs in particular is very challenging due to the high similarity of these proteins. in the study presented here, our efforts focused on finding key structural features involved in binding selectivity for CreaT using structure-based computational methods. Due to the lack of three-dimensional structures of SLC6A8, our approach was based on the realization of two reliable homology models of CreaT using the structures of two templates, i.e. the human serotonin transporter (hSERT) and the prokaryotic leucine transporter (LeuT). Our models reveal that an optimal complementarity between the shape of the binding site and the size of the ligands is necessary for transport. These findings provide a framework for a deeper understanding of substrate selectivity of the SLC6 family and other LeuT fold transporters.

** Talk will be recorded and made available post conference*

Saturday, August 8 | 9:20-9:40 AM PT

Computational Analysis of Missesnse Mutations in Creatine Transporter Protein Associated with Creatine Deficiency Syndrome*

Professor Emil Alexov, PhD
Clemson University

Emil Alexov is a professor at Clemson University, SC, USA. He is affiliated with the Departments of Physics and Astronomy, Material Sciences, and the School of Nursing HealthCare Genetics. His research is focused on understanding the molecular effects caused by human DNA variants associated with diseases and developing treatments to eliminate these effects. Of particular interest are monogenic diseases and X-linked disorders. He also develops methods to predict the pathogenicity of mutations and their effect on the thermodynamic properties of macromolecules and their assemblages.

Abstract
Cerebral creatine deficiency syndromes (CCDS) are frequently caused by mutations in the creatine transporter gene, SLC6A8. The mutations result in a blockage of the transportation of creatine to the brain and muscle. Creatine transporter protein is a membrane protein that transports creatine across the membrane, but little is known about the details of the reaction and the pathway of creatine. Here we address these questions by modeling the creatine pathway by the means of steered MD simulations in parallel with modeling the effect on protein stability. The outcome is used to assess the molecular effects of known pathogenic and benign mutations and to group pathogenic mutations according to their molecular effect into several categories. It is demonstrated that numerous genotypes result in the same molecular effect and thus can be targeted with the same therapeutic solution.

** Talk will be recorded and made available post conference*

Saturday, August 8 | 10:00-10:20 AM PT

Muscle Phenotype of AGAT- and GAMT-Deficient Mice After Simvastatin Exposure

Axel Neu, MD
Department of Pediatrics, UKE, Hamburg, Germany

Axel Neu is a child neurologist at the university hospital Hamburg Eppendorf. His main interests are ion channel disorders and disorders of creatine metabolism. His research group Experimental Neuropediatrics works on the functional consequences of creatine deficiency in transgenic mouse models.

Abstract:
Statin-induced myopathy affects more than 10 million people worldwide. L-arginine:glycine amidinotransferase (AGAT) gene was associated with statin-induced myopathy in two populations, but the causal link is still unclear. In cerebrovascular patients treated with statin, lower hArg and GAA plasma concentrations were found than in non-statin patients, indicating suppressed AGAT expression and/or activity. This observation suggests that statin-induced myopathy may be associated with AGAT expression and/or activity in muscle cells.

We studied simvastatin-induced myopathy in AGAT- and GAMT-deficient mice. We found that simvastatin induced muscle damage and reduced AGAT expression in wildtype mice. Simvastatin-induced motor impairment was exacerbated in AGAT-deficient mice compared with AGAT-overexpressing GAMT-/- mice. Cr supplementation itself improved muscle strength independent of AGAT expression. Our results from clinical and animal studies suggest that AGAT expression/activity and its product Cr influence statin-induced myopathy independent of each other. Further clinical pharmacological studies are needed to elucidate the underlying mechanism(s) and to evaluate whether supplementation with Cr, or possibly GAA, in patients under statin medication may reduce the risk of muscular side effects.

Saturday, August 8 | 10:20-10:40 AM PT

Small Molecule Approach for the Treatment of GAMT Deficiency*

Nicola Longo, MD, PhD
University of Utah

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 natural history, and their identification through newborn screening.
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.

** Talk will be recorded and made available post conference*

Abstract
Guanidinoacetate methyltransferase (GAMT) deficiency impairs the synthesis of creatine and results in the accumulation of guanidinoacetate that 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 guanidino acetate in the brain, improving the outcome of patients with GAMT deficiency.
Our goal is to identify novel, safe and effective AGAT inhibitors to prevent or reduce synthesis of guanidinoacetate. To accomplish this objective, we utilized the proprietary artificial intelligence (AI)- assisted computational screening platform at Atomwise Inc to predict binding of small molecules to the enzyme based on the published AGAT crystal structures at < 2.5 Å resolutions. The top 100 compounds identified from this screening will be tested in vitro using the purified recombinant human AGAT in which the 37 amino acid leader was replaced with a His6 tag at the N-terminus. Addition of glycine and arginine to the enzyme results in the production of ornithine. This and the production of guanidinoacetate will be monitored using UPLC-MS/MS. These studies will identify compounds capable of reducing guanidinoacetate production that could be used (after further optimization) as a novel treatment for GAMT deficiency.

Saturday, August 8 | 11:00-11:30 AM PT
ACD Research Strategy & Closing Remarks

Sangeetha Iyer, PhD
ACD Scientific Advisor

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.

Abstract:
Dr Iyer will summarize the conference main themes and take home messages. Furthermore, Dr Iyer will discuss the ACD research strategy and initiatives to fund, build, and share tools and resources that can accelerate the discovery of treatments for patients living with creatine deficiencies. Overall, the ACD approach is collaborative and relies on open science to shorten the timelines required for development.

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