Issue 4 Summer 2019

UCDC Update

Newsletter of the Urea Cycle Disorders Consortium

Greetings from the UCDC Leadership

In this newsletter, we would like to update you about our current studies and the future direction of the consortium's research efforts. The UCDC continues to grow and two new clinical research sites, University of California San Francisco and Stanford University, were added to the consortium in 2017; both of these sites were funded by the National Urea Cycle Disorders Foundation. In the Fall of 2018, the UCDC submitted a competitive renewal grant application to the NIH for another 5 years of continued funding as part of the NIH Rare Diseases Clinical Research Network (RDCRN). In early 2019, we received notice that the grant application received an excellent score, and the UCDC will receive another 5-year round of funding, through 2024. Dr. Andrea Gropman, Dr. Sandesh Nagamani, and Cynthia Le Mons (NUCDF) will serve as overall principal investigators (PIs) of the consortium. Each of the 16 UCDC sites will continue to have a site PI and study coordinator. Many new projects have been proposed and there has been a slight change in the organizational structure. The organizational chart in figure 1 shows the leadership structure and projects proposed for the 2019-2024 funding cycle.

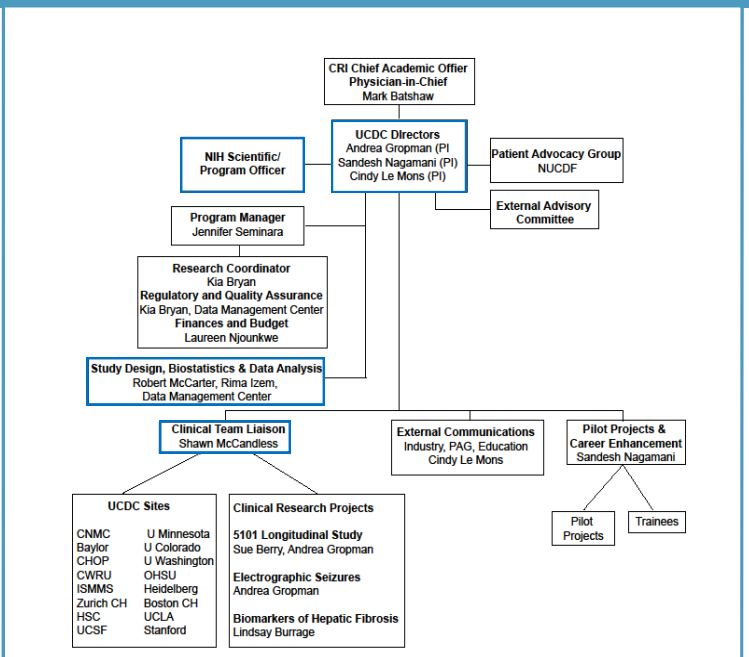


Figure 1: Organizational Structure of the Urea Cycle Disorders Consortium

The executive committee is bordered in blue.

We will continue to collect data for the Longitudinal Study in the next funding cycle, focusing on the impact of UCDs throughout the lifespan (e.g. pregnancy, transitioning to adulthood), the natural history of rare subtypes of UCDs, and comorbidities such as liver disease and seizures. Dr. Susan Berry from University of Minnesota will take the lead on the Longitudinal Study as the principal investigator (PI). The other two major projects will be 1) a study of electrographic (EEG) seizures in patients with UCDs led by Dr. Andrea Gropman from Children's National Medical Center and 2) noninvasive biomarkers (such as blood tests and MRI scans) of liver disease in UCDs led by Dr. Lindsay Burrage from Baylor College of Medicine. We are particularly proud that Dr. Burrage, who was one of our trainees supported by a training grant from the consortium, turned her work into a small pilot study, and is now leading a major component of the project. She is one of several young investigators supported by the UCDC in the past who are now active in UCD research as junior investigators. This demonstrates the importance of supporting the training of the next generation of UCD clinicians and researchers.

We are grateful for the opportunity to continue to build on the foundational research and move forward with new studies to improve the understanding of UCDs and develop new treatments. We are also grateful for commitments from the O'Malley Foundation, the Kettering Family Fund, and the National Urea Cycle Disorders Foundation for their continued financial support of the UCDC.

We hope that you will find this newsletter informative and encouraging. Thank you for your contribution to UCD research and for helping us meet the UCDC goal of improving quality of life for individuals with UCDs and their families by accelerating research to improve the understanding of urea cycle disorders.

Andrea Gropman, MD, Shawn McCandless, MD, Sandesh Nagamani, MD, and Cynthia Le Mons
Urea Cycle Disorders Consortium Leadership Team

What's Inside

The UCDC is funded by the National Institutes of Health (NIH), the O'Malley Foundation, the Dietmar-Hopp Foundation, the Rotenberg Family Fund, the Kettering Family Fund, and the National Urea Cycle Disorders Foundation.

Research Publication Summaries

Neuropsychological Functioning

Waisbren SE, Cuthbertson D, Burgard P, Holbert A, McCarter R, Cederbaum S, Members of the Urea Cycle Disorders Consortium. Biochemical markers and neuropsychological functioning in distal urea cycle disorders. J Inherit Metab Dis. 2018 Jul; 41(4): 657-667.

Key Point: Many clinicians focus on keeping ammonia levels low, but data shows that other biomarkers (glutamine, arginine and citrulline) may be equally important in influencing neuropsychological functioning. Cumulative exposure to the biomarkers included in the study proved to be highly sensitive indicators of neuropsychological outcomes, even when below the cut-off levels generally considered toxic. The importance of cumulative exposure supports early identification and confirms the need for well-controlled management of all biochemical abnormalities (and not just ammonia) that occur in urea cycle disorders.

The urea cycle breaks down ammonia into urea, which is then excreted (released) through urine; urea cycle disorders (UCDs) occur when a step in the urea cycle process is disrupted. Ammonia accumulation is shared amongst the different UCD types, but each individual UCD can have distinctive (unique) effects on the body. This report examines links between biochemical markers (ammonia, glutamine, arginine, citrulline) and neuropsychological test results in three UCD subtypes - argininosuccinic acid synthetase deficiency (ASD or citrullinemia type I), argininosuccinic acid lyase deficiency (ASA or ALD), and arginase deficiency (ARGD). Using data previously collected by the Urea Cycle Disorders Consortium's Longitudinal Study, the investigators evaluated the neuropsychological tests and lab results of 145 participants (Figure 2). The neurological tests measured for intelligence (IQ), verbal and visual abilities, motor function and memory. As a result, the mean full scale IQ was below the population mean of 100 ± 15 for all groups:

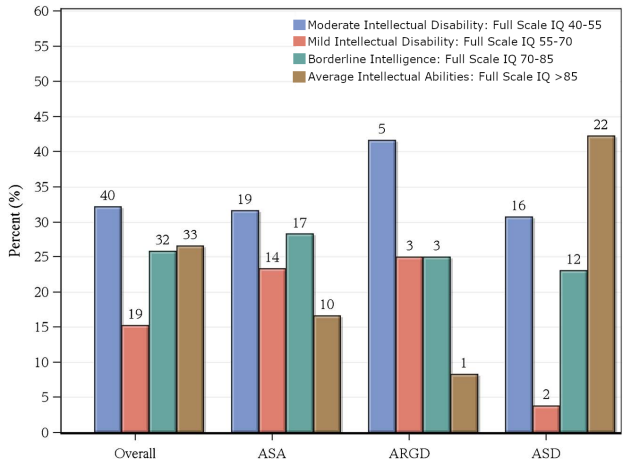


Figure 2: Distribution of full scale IQ for overall sample of patients.

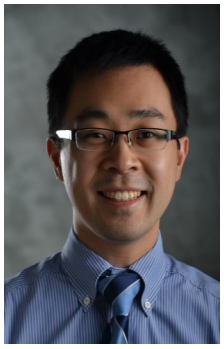
This bar chart showcases the percentage of overall (group 1), ASA (group 2), ARGD (group 3) and ASD (group 4) participants who have moderate intellectual disability (blue), mild intellectual disability (red), borderline intelligence (green) and average intellectual ability (brown). The numbers over the bars are the totals of participants within each IQ category.

(ASD = 79 ± 24; ASA = 71 ± 21; ARGD = 65 ± 19), and the greatest deficits were noted in visual performance and motor skills for all groups. The biomarkers that most reliably indicated poor overall neuropsychological performance were ammonia and citrulline for ASD participants; ammonia, glutamine, and citrulline for ASA participants; and arginine, ammonia and citrulline for ARGD participants. Recognizing biomarker profiles is useful in determining the most appropriate treatments.

Lessons Learned from UCD Study

Ah Mew N, Cnaan A, McCarter R, Choi H, Glass P, Rice K, Scavo L, Gillespie CW, Diaz GA, Berry GT, Wong D, Konczal L, McCandless SE, Coughlin CR, II, Weisfeld-Adams JD, Ficicioglu C, Yudkoff M, Enns GM, Lichter-Konecki U, Gallagher R, Tuchman M. Conducting an investigator-initiated randomized double-blinded intervention trial in acute decompensation of inborn errors of metabolism: Lessons from the N-Carbamylglutamate Consortium. Translational Science of Rare Diseases. 2018; 3: 157–170.

Key Point: Partnerships with patient advocacy groups such as the National Urea Cycle Disorders Foundation (NUCDF) are crucial for rare disease studies as they provide opportunities for researchers to educate and reach out to potential participants and their families and caregivers. Introspective publications such as this are valuable in the evaluation and improvement of study methods.



Children's National Health System Washington, D.C.
Nicholas Ah Mew, MD

Inborn errors of metabolism (IEMs), such as urea cycle disorders (UCDs), fall within the category of ultra-rare disorders in which 1 in 50,000 people have the disorder, many of whom die during childhood. This publication focuses on the challenges the N-carbamylglutamate Consortium (NCGC) faced while conducting two trials for N-carbamylglutamate (NCG), an approved treatment for N-acetylglutamate synthetase (NAGS) deficiency, one of the UCD subtypes that causes hyperammonemia. Among the topics discussed is the difficulty of recruiting and retaining eligible participants; enrollment numbers are largely affected by the small participant selection pools characteristic of rare diseases and missed opportunities to refer patients to open studies during routine clinical visits. It is preferable to enroll participants during non-crisis situations in order to avoid an exaggeration of the expected number of hyperammonemia events that could occur during a study. Partnerships with patient advocacy groups such as the National Urea Cycle Disorders Foundation (NUCDF) are crucial for rare disease studies as they provide opportunities for researchers to educate and reach out to potential participants and their families and caregivers. Introspective publications such as this are valuable in the evaluation and improvement of study methods.

UCDs in USA vs Europe

Posset R, Garbade SF, Boy N, Burlina AB, Dionisi-Vici C, Dobbelaere D, Garcia-Cazorla A, de Lonlay P, Teles EL, Vara R, Ah Mew N, Batshaw ML, Baumgartner MR, McCandless SE, Seminara J, Summar ML, Hoffmann GF, Kölker S, Burgard P; on behalf of the UCDC and the E-IMD consortium. Transatlantic Combined and Comparative Data Analysis of 1095 Patients with Urea Cycle Cisorders—a Successful Strategy for Clinical Research of Rare Diseases. J Inherit Metab Dis. 2018 Jul 4. doi.org/10.1007/s10545-018-0222-z.

Key Point: North American and European UCD patients share similarities in disease onset and diagnosis. The study results suggest further investigation is needed on how UCDs are diagnosed and treated.

The Urea Cycle Disorders Consortium (UCDC) and the European Registry and Network for Intoxication Type Metabolic Disorders (E-IMD) collaborated to compare the prevalence and characteristics of urea cycle disorders (UCDs) in North America and Europe. The UCDC Longitudinal Study data was used for North America. Combining registries from both consortia, researchers looked at the medical histories of over 1,000 patients with UCD for information such as UCD type, late disease onset (clinical symptoms at more than 28 days old) or early disease onset (clinical symptoms at 28 days old or less), and age of diagnosis. In North America and Europe, Ornithine Transcarbamylase Deficiency (OTCD) and late disease onset were the most common; however, the lack of early onset reports may have been caused by the voluntary nature of the registries, as the severe symptoms associated with early onset UCDs could make participation more difficult. The data also showed that the delay between age of clinical symptoms and age of diagnosis was shorter for early onset patients, which was likely due to the higher visibility of symptoms such as seizures.

Detailed Summary of Results:

The proportion of females with OTCD (fOTCD), particularly asymptomatic females (asfOTCD), was higher in North America than in Europe. However, the exclusion of asfOTCD data points resulted in similar proportions of UCD subgroups in North America and Europe.

The mean age at first symptoms was higher in North America compared to Europe for the late disease onset subgroup (> 28 days), but similar for those in the early disease onset subgroup (≤ 28 days).

The mean age at diagnosis and the mean of diagnostic delay (the difference between age at diagnosis and age at first symptoms) for early disease onset and late disease onset subgroups were similar in North America and Europe.

In most patients (including fOTCD), diagnosis was made after the onset of symptoms (59.9%) or by high-risk family screening (24.7%), and less often by newborn screening (8.9%) and prenatal testing (3.7%) (Figure 3).

Early disease onset patients presented with more symptoms than late disease onset patients, but that number of symptoms correlated (connected) with plasma ammonia levels in early disease onset patients only.

Liver transplantation was reported for 90 North American patients and 25 European patients.

The insights from this study and the availability of an extensive data source have encouraged further investigation into diagnosis programs and treatment plans.

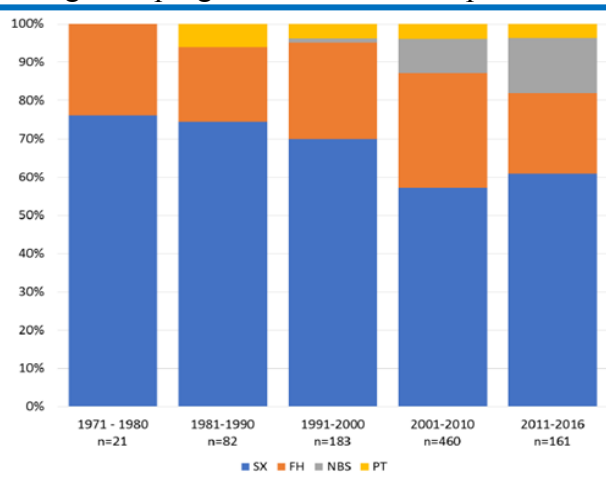


Figure 3. Modes of diagnosis and their distributions from 1971 until 2016.

This graph presents the percentages of participants who were diagnosed either by investigation after onset of symptoms (SX - blue), investigation due to family history (FH – orange), newborn screening (NBS – gray), or prenatal testing (PT – yellow) between 1971-1980 (bar 1), 1981-1990 (bar 2), 1991-2000 (bar 3), 2001-2010 (bar 4) and 2011-2016 (bar 5).

Cognition in OTC Deficiency

Buerger C, Garbade SF, Dietrich Alber F, Waisbren SE, McCarter R, Kölker S, and Peter Burgard on behalf of the Urea Cycle Disorders Consortium. Impairment of cognitive function in ornithine transcarbamylase deficiency is global rather than domain-specific and is associated with disease onset, sex, peak ammonium, and number of hyperammonemic events. J Inherit Metab Dis. 2018 Dec 27.

Key Point: Research shows OTCD-related complications impact IQ for both early and late disease onset patients. This suggests OTCD has a global impact on cognitive function (global intelligence rather than a specific effect on distinct cognitive domains (executive function, memory, visual-motion integration, visual perception)).

Ornithine transcarbamylase deficiency (OTCD) is the most common urea cycle disorder (UCD). OTCD presents with a wide range of symptom severity. In this study, the number of hyperammonaemic events, clinical findings, and cognitive functioning domains—intelligence (IQ), executive function, memory, visuomotor integration and visual perception—were compared across groups. The groups were separated according to disease onset type—

late disease onset (clinical symptoms at more than 28 days old), early disease onset (clinical symptoms at 28 days old or less), or asymptomatic—sex, and age. Participant information collected by the Urea Cycle Disorders Consortium (UCDC) through the Longitudinal Study between 2006 and 2014 served as the data source, to include 300 OTCD participants who underwent psychological evaluations. Although mean scores of late onset and asymptomatic individuals were within 1 SD of the population norm (IQ = 85-115, which is the normal range for individuals without UCD, as well), asymptomatic participants attained significantly higher scores than late onset participants and males scored higher than females. Intelligence scores proved to correlate with overall cognitive functioning. The correlation between maximum ammonia concentration and intelligence correlated significantly higher in early onset than in late onset participants (i.e. higher ammonia levels correlated with lower IQ). Correlation between the number of hyperammonemic events and intelligence scores were similar for early onset and late onset individuals. The number of clinical symptoms was significantly associated with intelligence, but not with scores in other domains. Results suggest that OTCD has a global impact on cognitive functioning rather than a specific effect on distinct cognitive domains (executive function, memory, visual-motor integration, visual perception).

Metabolomic Profiling in UCDs

Burrage LC, Thistlethwaite L, Stroup BM, Sun Q, Miller MJ, Nagamani SCS, Craigen W, Scaglia F, Sutton VR, Graham B, Kennedy AD, Members of the UCDC, Milosavljevic A, Lee BH, Elsea SH. Untargeted metabolomic profiling reveals multiple pathway perturbations and new clinical biomarkers in urea cycle disorders. Genetics in Medicine. 2019 Jan 23.

Key Point: Untargeted metabolomic profiling (the chemical analysis of small particles in cells, tissue and bodily fluid) is a possible tool for UCD diagnosis and management.

Urea cycle disorders (UCDs) are screened and diagnosed through the use of biochemical and molecular testing. If biomarkers of UCDs, such as elevated (higher) levels of ammonia and arginine, are present in a patient's screening tests, an analysis of their genetic composition (makeup) will confirm the disorder. However, this may not be a reliable method of diagnosis in all cases, as ornithine transcarbamylase deficiency (OTCD) can be difficult to identify due to the nature of the disorder. Untargeted metabolomic profiling has become an important tool in diagnosis and management of UCDs because it is able to detect biomarkers of UCDs that might not be captured by the traditional methods of diagnosis. In this study, samples from 48 subjects that were collected during routine clinical visits and samples from the Urea Cycle Disorders Consortium (UCDC) Longitudinal Study were analyzed with metabolomic profiling. Results from the analyses found known biomarkers of UCDs.

Arginase-1-Deficient Mice

Sin YY, Ballantyne LL, Richmond CR, Funk CD. Transplantation of Gene-Edited Hepatocyte-like Cells Modestly Improves Survival of Arginase-1-Deficient Mice. Mol Ther Nucleic Acids. 2018 Mar 2; 10: 122–130.

Key Point: Arginase-deficient (ARGD) mice implanted with healthy liver cells showed low rate of new liver tissue growth. Despite minimal improvements, research suggests there might be a place for such therapies in the future.

This study, funded by a Urea Cycle Disorders Consortium (UCDC) training grant, focused on the possibility of incorporating gene editing in a preclinical animal model for treatment of urea cycle disorder (UCD), specifically arginase deficiency. Induced pluripotent stem cells (iPSCs), or cells that can become any type of cell in the body, were turned into hepatocyte-like cells (HLCs), or liver cells. The HLCs were transplanted into mice with arginase deficiency, and investigators monitored the mice for signs that the HLCs were producing arginase enzyme. The results showed 5% liver regeneration, low productions of arginase, and a maximum lifespan of 22 days compared to 14 days in arginase-deficient mice that were not transplanted. Although the initial proof of concept study showed minimal improvements, the research suggests that there might be a place for such therapies in the future.

ASLD and Hypertension

Kho J, Tian X, Wong WT, Bertin T, Jiang MM, Chen S, Jin Z, Shchelochkov OA, Burrage LC, Reddy AK, Jiang H, Abo-Zahrah R, Ma S, Zhang P, Bissig KD, Kim JJ, Devaraj S, Rodney GG, Erez A, Bryan NS, Nagamani SCS, Lee BH. Argininosuccinate Lyase Deficiency Causes an Endothelial-Dependent Form of Hypertension. Am J Hum Genet. 2018 Aug 2;103(2):276-287.

Key Point: A chemical deficiency associated with ASLD often leads to high blood pressure. Nitric oxide supplementation is currently being investigated in clinical trials as a treatment option for individuals with ASLD.

In this study, the investigators used mice with argininosuccinate lyase deficiency (ASLD) and cells from patients with ASLD to study the mechanisms involved in causing high blood pressure in this disorder, which is the second most common UCD (Figure 4). The investigators show that loss of the urea cycle enzyme ASL in the lining cells of the blood vessels leads to reduction of a chemical called nitric oxide (NO) and an increase in oxidative stress that lead to vascular dysfunction. Using data from a human trial that was funded by the Urea Cycle Disorders Consortium (UCDC), they also show that the blood pressure in individuals with ASLD can be elevated.

The results of this study can have potential treatment implications. Currently, nitric oxide supplementation is being investigated in clinical trials as a treatment option for individuals with ASLD.

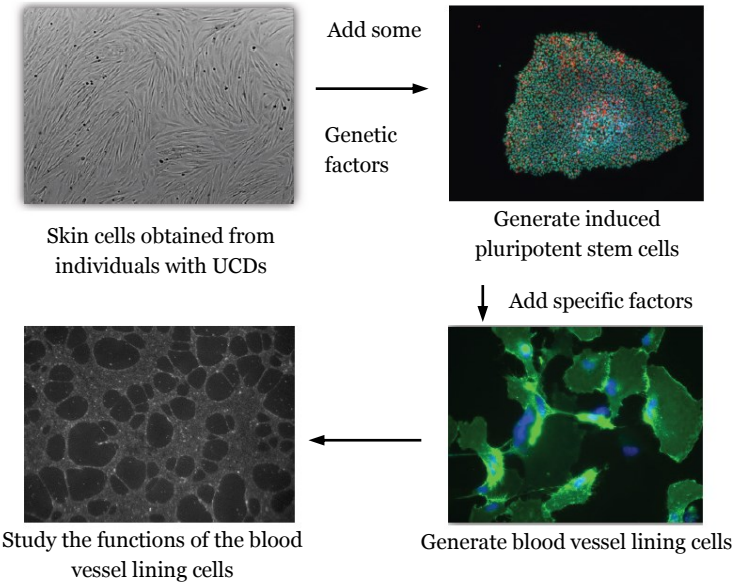


Figure 4. Skin cells were transformed into blood vessel lining cells to study the causes of high blood pressure in patients with ASLD.

EEG Monitoring of Infant Seizures

Wiwattanadittakul N, Prust M, Gaillard WD, Massaro A, Vezina G, Tsuchida TN, Gropman AL. The utility of EEG monitoring in neonates with hyperammonemia due to inborn errors of metabolism. Mol Genet Metab. 2018 Nov;125(3):235-240.

Key Point: Continuous video electroencephalogram (cvEEG) may measure effects of hyperammonemia on brain activity that are not directly related to common clinical symptoms of inborn errors of metabolism.

Continuous video electroencephalogram (cvEEG) is the long-term video monitoring of electrical activity in the brain. The typical length of an electroencephalogram (EEG) test is between 30 to 60 minutes, where as a cvEEG test can last for days at a time. Most infant seizures do not have clinical symptoms and can only be detected by an EEG, making cvEEG the preferred method for monitoring seizures associated with acute brain injury; however, the benefits of using cvEEG on infants with inborn errors of metabolism have not been determined. In this study, researchers analyzed the medical records of eight infants who experienced hyperammonemia due to inborn errors of metabolism and received prolonged EEG tests at Children's National Medical Center, Washington, D.C., between January 2009 and March 2017. The results showed that seven of the infants had seizures, and six had seizures that could only be detected by EEG.

Although there was evidence that elevated levels of blood ammonia and glutamine—common symptoms of urea cycle disorders—had a possible connection to seizure activity on EEG, it is important to note that some of the infants, after receiving medication that normalized their blood ammonia and glutamine levels, continued to have seizures (Figure 5). Seizures were also detected by EEG before blood ammonia levels spiked and occurred within 24 to 36 hours of clinical symptoms. These and other findings documented in the study suggest the importance of long-term cvEEG in the evaluation of inborn errors of metabolism.

Plasma amino acids tested at 17:41. Significantly elevated glutamine (4.222 umol/L) and citrul-

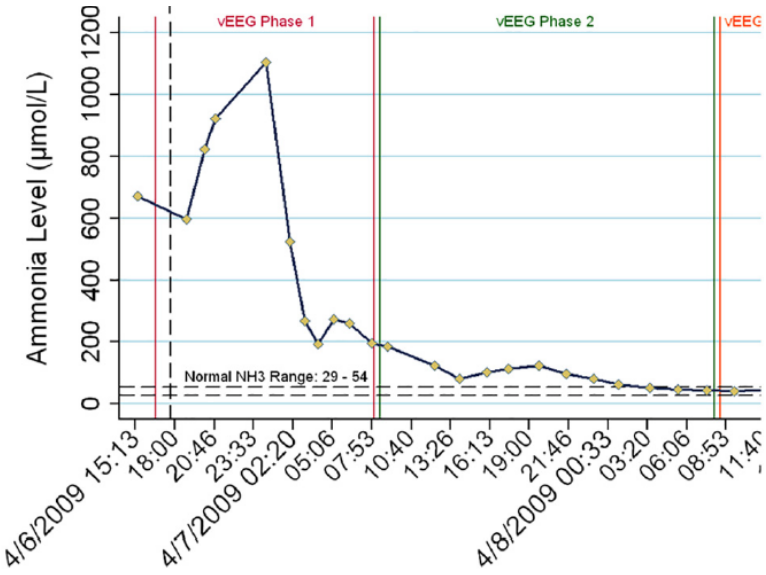


Figure 5: Shows the daily changes in the ammonia level of a single participant, captured by cvEEG. The time is displayed in 24-hour format.

UCDC Studies Currently Recruiting Participants and Contact Information

All Studies

General Information:
Jennifer Seminara
Clinical Research Program Manager
Phone: 202-306-6489
jseminar@childrensnational.org

5101: Longitudinal Study of Urea Cycle Disorders
All 16 UCDC sites (see map on the next page) participate in this study. Please visit our website (https://www.rarediseasesnetwork.org/cms/ucdc) to find a site near you or contact Jennifer Seminara at 202-306-6489 or jseminar@childrensnational.org.

5105: N-carbamylglutamate in the Treatment of Hyperammonemia
Leslie Atley
Clinical Research Coordinator II
Phone: 202-476-6137
LATLEY@childrensnational.org

5111: Orphan Europe Carbaglu® Surveillance Protocol In Collaboration with the Longitudinal Study of Urea Cycle Disorders
Kara Simpson, MS, CGC
Genetic Counselor/Research Study Coordinator
Phone: 202-476-6216
ksimpson@childrensnational.org

5113: Biomarkers of Neurological Injury and Recovery in Urea Cycle Disorders
Andrea Gropman, M.D., FAAP, FACMG
Principal Investigator
Phone: 202-476-2120
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5114: Effect of Nitric Oxide (NO) Supplementation on Neurocognitive Measures in Argininosuccinate Lyase Deficiency (ASLD)
Mary Mullins, RN, BSN
Clinical Research Coordinator
Phone: 832-822-4263
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5115: Manipulating the Gut Microbiome in Urea Cycle Disorders
Leslie Atley
Clinical Research Coordinator II
Phone: 202-476-6137
LATLEY@childrensnational.org

5118: Prospective Cross-Sectional Non-invasive Assessment of Chronic Liver Disease in Urea Cycle Disorders
Mary Mullins, RN, BSN
Clinical Research Coordinator
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Studies Categorized by Disorder

All Urea Cycle Disorders
5101

Arginase 1 Deficiency (ARG1D)
5118

Argininosuccinate Synthase Deficiency (Citrullinemia I)
5113, 5115

Argininosuccinate Lyase (ASL) Deficiency (Argininosuccinic Aciduria)
5113, 5114, 5115, 5118

Carbamyl Phosphate Synthetase Deficiency (CPSD)
5105, 5115

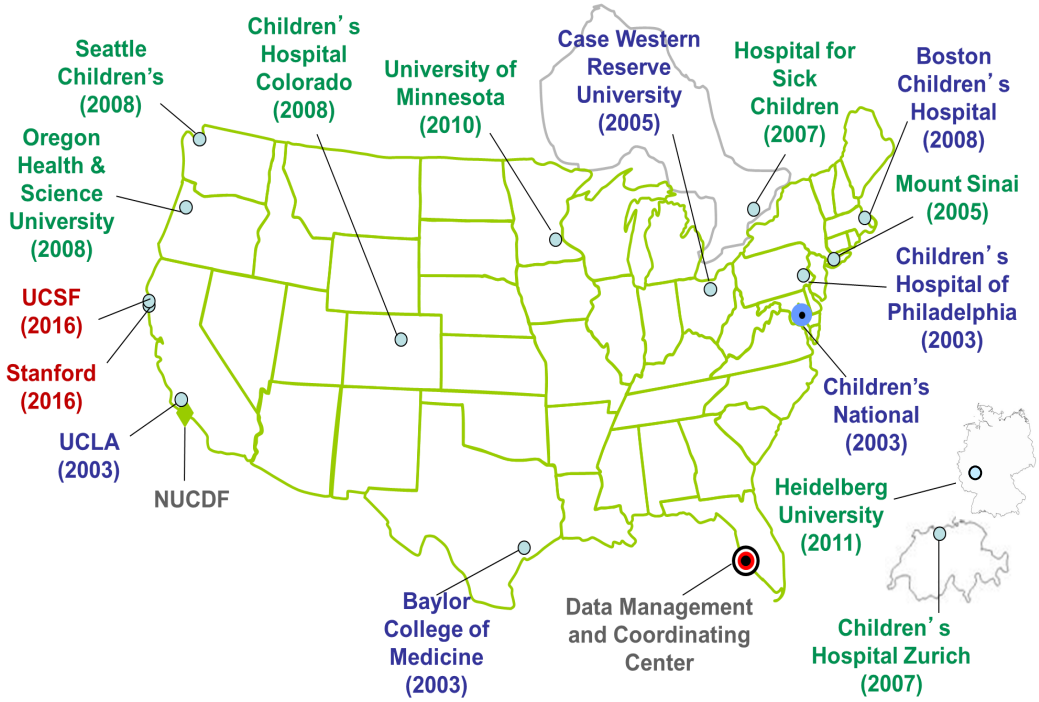
N-Acetylglutamate Synthase (NAGS) Deficiency
5111

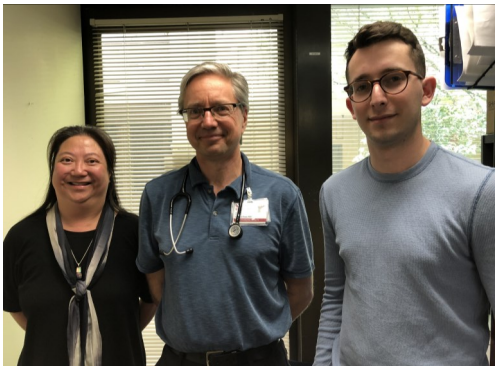
Ornithine Transcarbamylase (OTC) Deficiency
5105, 5113, 5115, 5118

Please visit the UCDC website (https://www.rarediseasesnetwork.org/cms/ucdc) for a full list of UCDC studies, sites and contact information. Watch our website, as we will be initiating new UCD studies in the next grant cycle.

Participating Urea Cycle Disorders Consortium Sites and Team Photographs

The year each site joined is shown in parentheses on the map.





Stanford University Medical Center
Stanford, CA
Thu Quan, MBA/HCM, Greg Enns, MD, Elijah Kravets

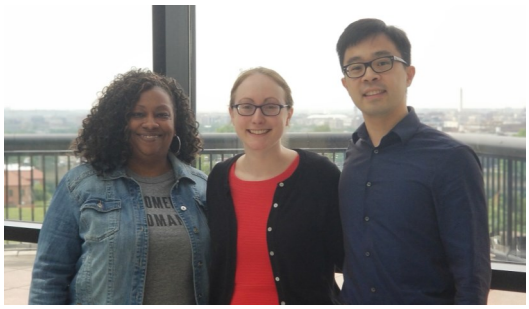
Neuropsychology Conference 2019
New York City, NY
Greta Wilkening, Psy.D. (Colorado), Jacqueline Sanz, PhD (Children's National) and Talin Babikian, PhD (UCLA) , David Schwartz PhD (Texas), Arianna Stefanatos, PhD (Philadelphia), Rachel Tangen, PhD (Case Western)

Society for Inherited Metabolic Disorders (SIMD)
Seattle, Washington
Danuta Krotoski, Ph.D. (NIH), Andrea Gropman, M.D., FAAP, FACMG (Children's National), Cynthia Le Mons (NUCDF)

Baylor College of Medicine
Houston, TX
Sandesh C S Nagamani, MD, FACMG, Saima Ali, RN, FNP-C, Lindsay Burrage, MD, PhD

Icahn School of Medicine at Mount Sinai
New York, NY
Kimihiko Oishi, MD, Angela Kurtz (dietician), Lauren "Mackenzie" Mason, MPAS, PA-C, George Diaz, MD PhD

The Hospital for Sick Children
University of Toronto, Toronto, Ontario
Haneen Ali, Ilona Tkachyova, Liora Caspi, Andreas Schulze, MD, PhD, FRCPC, Ashley Wilson



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