

Project Updates – October 2018

Study of Neuronal Structure and Function Changes in Cystinosis, John Foxe, PhD and Krishnan Padmanabhan, PhD

Department of Neuroscience Ernst J. Del Monte Institute for Neuromedicine, University of Rochester School of Medicine and Dentistry, Rochester, NY

Year One: \$110,000 Total Grant: \$110,000

Investigators at the Department of Neuroscience Ernst J. Del Monte Institute for Neuromedicine, University of Rochester School of Medicine and Dentistry Rochester, NY have outlined a research proposal taking a novel systems neuroscience strategy to address the link between molecular/cellular pathology of the lysosomal system in neurons and the resultant changes in the structure and function of neuronal circuits using new electrophysiology, imaging and computational methods to understand the effect of cystinosis on neural circuits. By identifying the mechanisms underlying the neuropathology of the disease at the basic science level, this research program will provide important biomarkers for tracking disease progression, could identify new sites/targets for intervention and guide in the development of strategies for treatment. Following is a brief update on their project:

The aim of our research study is to understand how changes at the level of cells in the brain due to lysosomal storage dysfunction translate to changes in cognitive function and behavior. To study the neurobiological underpinnings of cystinosis, the lab uses a *Ctns* ^{-/-} mouse line which has a cystinosis, nephropathic, targeted mutation 1 in the gene. To do this, we have developed new technologies from recording the electrical activity of the brain in this model. Specifically, we have deployed a method for studying the physiology of individual neurons throughout the brain in awake behaving animals. This cutting edge research approach will allow us to identify the neurophysiological changes that arise from the *Ctns* ^{-/-} mouse and relate this to alterations in global brain dynamics. The ultimate goal being to discovery biomarkers of the disease and identify potential targets for subsequent intervention. In the first 6 months of this research project, we have successfully recruited and trained an MD/PhD student with interests in Neurology and development to perform surgeries and record from control mice to characterize baseline patterns of activity throughout the brain for comparison with the *Ctns* ^{-/-} mouse.

Altered protein kinase signaling as a cause of reduced adhesion and increased motility of renal epithelial cells in Cystinosis – E. Ivanova, L. van den Heuvel, E Levtchenko (Principal Investigator)
Katholieke Universiteit Leuven, Belgium

Year Two: \$88,493 Total Grant: \$165,494

Cystinosis is a genetic disease manifesting early in life (\approx 6-12 months) with progressive kidney disease resulting in renal failure early during childhood if not treated. In cystinosis the metabolism of the amino acid cystine is defective leading to its accumulation in the kidney and other organs. This cystine accumulation results in cellular damage and death, but the direct mechanisms beyond this phenomenon are largely unknown. Some harmful cellular events in cystinosis might not be directly related to cystine accumulation and are the subject of our research project. Based on our previous work we hypothesized that the loss of highly specified renal cells like glomerular podocytes and renal proximal tubular cells in urine is a major mechanism causing renal pathology of cystinosis. Increased rate of cellular abundance in urine can be explained by either the decreased adhesion of renal cells to their matrix or their increased motility or by a combination of both mechanisms. Indeed we demonstrated that both events occur in cultured human renal cells derived from cystinosis patients. We further tried to explore the mechanisms

beyond this cellular loss. It has been reported in other diseases that increased cell motility and defective adhesion can be associated with the altered protein kinase signaling. In cystinotic podocytes we found an increased expression of activated or phosphorylated Akt kinases compared to control cells. This could explain, at least partially, the abnormal phenotype. We are currently testing other protein kinases that might contribute to this mechanism. In addition we tested the gene expression of several integrin in podocytes, as podocytes adhere to the extracellular matrix using integrin receptors. Although only minor differences were found between cystinotic and control cells, cell surface expression of these proteins still has to be studied. So far most of our experiments were done in podocytes. We recently started to investigate proximal tubular epithelial cells which also showed an increased expression of phosphorylated Akt kinases unifying the concept of the hypothesis over different renal cell types. Our future plan includes also the experimentation with different kinase inhibitors to explore if they can reverse abnormal renal phenotype.

Mechanisms Underlying Neurocognitive Changes in Cystinosis, John Foxe, PhD Co-Principal Investigator, Sophie Molholm, PhD Co-Principal Investigator, Steven U. Walkley, DVM, PhD Co-Principal Investigator Departments of Neuroscience and Pediatrics, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY

Year three: \$100,980 Total Grant: \$467,950
Travel Addendum: \$38,352

The goal of this proposal continues to focus on brain-related changes in the lysosomal disease, cystinosis, through the use of complementary state-of-the-art neurocognitive studies (in cystinosis patients, Aim 1), cell biological analyses and possible treatment strategies (in the mouse model, Aims 2-3). Progress through year 02 of the proposal are outlined below.

AIM 1: To explore sensory processing and multisensory integration as potential biomarkers using high-density electrophysiological mapping techniques in individuals with cystinosis.

This section of the report details progress in the human arm of the CRN project entitled - "Mechanisms Underlying Neurocognitive Changes in Cystinosis". Under this arm of the project, our main aim was to "explore sensory processing and multisensory integration as potential biomarkers using high-density electrophysiological mapping techniques in individuals with Cystinosis". Therefore, initially, we recorded high-quality data from 7 patients with Cystinosis while they responded to a sensory processing task. We performed preliminary analyses of those data, compared the outcomes to similar recordings in another lysosomal disorder (Niemann-Pick-C (NPC); N=17) and to an already collected extensive normative dataset recorded from a cohort of matched neurotypical control participants (N=84). The most surprising aspect of those results was the strikingly "normal" patterns of multisensory behavior and neurophysiological responses that we obtained in Cystinosis, in stark contrast to those obtained in the NPC population. As a consequence of this finding, we decided to apply additional paradigms that tap into sensory processing and executive functions, which, based on the clinical phenotype of individuals with Cystinosis, are likely to provide sensitive brain measures of neural function/dysfunction in the Cystinosis population. In this second phase, we have collected data from 32 patients with Cystinosis and from 37 neurotypical control participants and performed analyses of part of the behavioral and the electrophysiological data. In this cumulative report, we indicate major progress over the past six months, since our last report, with blue font.

Scientific communications: We presented preliminary analyses of the data at the Cystinosis Research Network Family Conference in Utah. We also presented these data at the Pediatrics Research Day and at the Lysosomal Rounds at the Albert Einstein College of Medicine and to our Lysosomal Storage Disorders Grand Rounds at Einstein/Montefiore group (April 2018), and will present them at the International

Meeting of the Psychonomic Society taking place in the Netherlands (May 2018). We have started preparing the data from two of the datasets for publication, and are currently working on the first manuscript.

Recruitment Efforts: We engaged in extensive recruitment efforts through social media and during the Cystinosis Research Network Family Conference. Furthermore, enrollment capacity was greatly increased through the addition of funds from the CRN to fly families in for two days of data collection. We have met our new recruitment targets and have completed data collection. In the past year and a half, we collected data from 32 individuals diagnosed with cystinosis (19 children, 6 adolescents, and 5 adults) and from 37 neurotypical controls (16 children, 12 adolescents, and 9 adults). We propose to collect data from 9 more adults and 8 more adolescents with Cystinosis (plus healthy age matched controls) so that we may assess how perceptual and cognitive functions change over development in Cystinosis.