Summary of the 11th ATP1A3 in Disease Symposium

The 11th ATP1A3 in Disease Symposium was held in Chicago on October 27-28, 2023 at the Northwestern University Feinberg School of Medicine. The conference was jointly hosted by the four U.S. foundations (Alternating Hemiplegia of Childhood Foundation, Cure AHC, Hope for Annabel, and For Henry AHC). Additional support for the conference came from the AHC foundations of the United Kingdom, Spain, Germany and Canada. Local support for the conference was provided by the Northwestern University Department of Pharmacology. The meeting attracted 115 attendees from 14 countries and was presented in-person and through a virtual platform.

The theme for the conference, *Taking the Next Steps*, was intended to encourage speakers and attendees to think about the future. The conference program consisted of four scientific sessions and a poster session followed on the last day by a group dinner featuring live music. Attendees were engaged and enthusiastic and left with a feeling of immense progress in the field.

A brief high-level summary of the conference follows. Because many speakers presented unpublished results, the summary omits potentially sensitive details.

Session I – Disease Natural History

This session provided important updates by the International AHC Research Consortium (IAHCRC) and their ongoing OBSERV-AHC study with a focus on the natural history and clinical features of *ATP1A3*-related disorders. Dr. **Eleni Panagiotakaki** (University of Lyon) kicked off the session with an overall description of the consortium followed by a presentation of unpublished results from an ongoing single center study of EEG findings in AHC. The study analyzed 24-hour video and EEG recordings from 31 individuals including recordings during neurological events. Some study participants exhibited unusual EEG patterns that were difficult to classify, and so far there did not appear to be consistent EEG changes associated with hemiplegia or dystonia. Following this presentation, Dr. **Mohamad Mikati** (Duke University) described the design of the OBSERV-AHC study, which was just published (Dr. Mikati offered to provide an electronic copy of the paper upon request). In addition, Dr. Mikati presented early findings from a study of non-sleep related apnea in AHC patients. In preliminary analyses, there was a substantial fraction of patients who had some degree of apneic events with many being severe. There was an association with seizures and other triggers.

Three other presentations shared findings from the OBSERV-AHC study. **Lyndsey Prange** (Duke University) shared results from a multi-center study designed to train caregivers on classifying neurological events ('spells') in AHC. In this study, caregivers were trained using a predetermined set of videos illustrating plegic events, dystonic events, and abnormal eye movements. The accuracy of caregiver event classification appears to improve with training. She also described a related study in which caregivers were asked to document spells using a paper calendar record, which could be revised by a health care provider. There was a high accuracy rate in a pilot study of 5 patients. Another member of the Duke team, Dr. **Shital Patel**, presented a retrospective study of cannabidiol (CBD) treatment in AHC. Because of the retrospective

nature of this study, the dose and source of CBD was variable. The efficacy of CBD was compared with that of flunarizine using a clinical global impression score. Sixteen patients were enrolled at Duke and Lyon Universities. Dr. Patel explained that CBD and flunarizine were equally effective at preventing AHC spells (plegia, dystonia, etc.), but only CBD showed anti-seizure effects. The ketogenic diet was the focus of a presentation by Dr. **Carmen Fons** (Barcelona), who described a new combined retrospective and prospective study to assess efficacy of this diet in AHC. This study is in the enrollment phase.

The final two talks in Session I included one from Dr. **Andrew Landstrom**, a cardiologist from Duke University. Dr. Landstrom discussed the occurrence of abnormal heart rhythms in patients with ATP1A3 variants including a young child with status epilepticus who had a severe cardiac arrhythmia (ventricular fibrillation). This case motivated him to perform a retrospective analysis of several AHC cases that revealed the presence of short QT syndrome, a known predisposition to cardiac arrhythmia, mainly associated with the D801N mutation. His group investigated potential cellular mechanisms for this observation by making cardiomyocytes (heart muscle cells) from induced pluripotent stem cells (iPSC) obtained from the index case. Using this cell model, they identified abnormalities in calcium ion handling by the cells that were thought consistent with a predisposition to cardiac arrhythmia. The final talk in Session I was given by **Nina Frost** (Hope for Annabel) who presented results from a recent patient survey about priorities, and a strategic framework for research that emphasizes real-time discussion of findings with research teams and the need to dismantle research silos to enable more effective data sharing.

Session II – Basic Mechanisms / Neurophysiology

This session focused on basic science research on *ATP1A3*-related disorders. The first presentation was given by Dr. **Sho Yano** (University of Chicago) who described his recently published work on a novel *ATP1A3*-related disorder characterized by spastic paraparesis and associated with a novel mutation (P775L) in 9 patients. His investigation into the molecular mechanism of the disorder revealed an abnormal ion leak into cells, which was not detected with the recurrent D801N mutation. Investigations into the structural basis for ATP1A3 dysfunction were presented by Dr. **Hanne Poulsen** (Aarhus University). Dr. Poulsen discussed work to determine the atomic-level structure of the ATP1A3 protein and explained challenges in purifying some mutant ATP1A3 proteins. One mutation associated with the D-DEMO disorder was amenable to structural analysis, from which new information about abnormal folding of the protein was obtained. Importantly, Dr. Poulsen also explained that AlphaFold, a new computational method to determine protein structures, is unable to predict the effects of *ATP1A3* mutations. The topic of spreading depolarization as a pathophysiological mechanism in *ATP1A3*-related disorders was discussed by Dr. **Arn van den Maagdenberg** (Leiden University). Spreading depolarization refers to a slow-moving wave of suppressed neural activity in the brain that was first observed in migraine with aura. This neurophysiological phenomenon may also be responsible for the transient neurological symptoms of AHC. Dr. van den Maagdenberg discussed his work with mouse models of migraine and ischemic brain injury.

Session II continued with presentations on new models of *ATP1A3*-related disorders. Dr. **Hendrik Rosewich** (University of Tübingen) shared unpublished findings from using bioengineered neuronal organoids ('mini brains') made from human cells with the E815K mutation. Using a multi-electrode array method, Dr. Rosewich found altered neuronal network activity in organoids with the mutation compared to cells without the mutation. Dr. **Anne Hart** (Brown University) presented her studies using a genetic model organism (the round worm, *C. elegans*) to investigate functional consequences of *ATP1A3* mutations. The advantage of using *C. elegans* relates to the extensive knowledge about each neuron in the organism and advanced genetic tools. Dr. Hart described ongoing efforts to study the effects of specific disease-associated *ATP1A3* mutations into the worm genome. Preliminary finding from this work indicates that mutations cause defects in neural function. The final scheduled talk in Session II was given by Dr. **Matthew Campbell** (Trinity College Dublin) on the topic of the blood brain barrier. His presentation focused on plasma biomarkers of barrier dysfunction that could be informative in *ATP1A3*-related disorders. Dr. Campbell discussed his published work on a genetic disorder associated with mutations in the *CLDN5* gene that resembles AHC and is associated with dysfunction of the blood brain barrier.

Session II included two short presentations selected from the submitted abstracts. Dr. **Jennifer Kearney** (Northwestern University) presented preliminary findings from a study of a new AHC mouse model with the G947R mutation. Her work focused on evidence that these mice have a prominent seizure disorder in addition to abnormal motor function consistent with AHC. The second short presentation was given by Dr. **Kathleen Sweadner** (Massachusetts General Hospital) who reported new findings from cellular models of *ATP1A3*-related disorders. She reported discovery of abnormal post-translational processing of mutant proteins, especially altered glycosylation (addition of sugars) of the ATP1A3 protein and its accessory subunits.

Session III – Therapeutic Advances

Session III was devoted to the topic of therapeutic advances, which began with a recorded talk by Dr. **Anna Mingorance** (Dracaena Consulting) who outlined the critical steps needed to develop new therapeutic approaches for rare genetic diseases. She had encouraging words about the state of the AHC field and offered suggestions for what needs to be done next including a study to determine the reversibility of the disease. The second presentation was given by Dr. **Bryan Dickinson** (University of Chicago) who recently published a study of a novel RNA-based method to restore normal protein levels of the gene *SYNGAP1*, which is responsible for a severe neurodevelopmental disorder. His strategy uses translation-activating RNA (taRNA) to boost protein production of *SYNGAP1* in cultured cells and human neurons. This technology might have value for some *ATP1A3*-related disorders.

The next three presentations shared unpublished results from three different genetic therapeutic approaches for AHC. **Alex Sousa** (Broad Institute, MIT/Harvard) gave updates on his use of gene editing to correct *ATP1A3* mutations including the three most common AHC variants (D801N, E815K, G947R). He explained use of two related approaches: base editing and prime editing. Each system was optimized in cultured cells then tested either in mice or human neurons. Both approaches show promise with high degrees of mutation correction. Dr. **Al**

George (Northwestern University) gave an update on testing antisense oligonucleotides (ASO) to target the *ATP1A3* E815K mutation in human neurons. The goal of this work was to demonstrate that the mutant copy of the gene can be selectively suppressed at the RNA level without affecting the normal functional copy. Some promising findings were discussed. Finally, **Markus Terrey** from the Jackson Laboratory gave an update on characterizing two AHC mouse models with either the D801N or E815K mutation that are being used to test gene therapies. Progress has been made generating mice on a genetic background that can be bred easily and have a disease-relevant phenotype. The team at Jackson Laboratory is testing prime editing and AAV gene therapy with these mice.

Session IV – Quantifying Phenotypes & Clinical Trial Readiness

The final session of the symposium highlighted advances in measuring clinical features of *ATP1A3*-related disorders. The presentation by Dr. **Allison Brashear** (University of Buffalo) highlighted advances in performing detailed clinical evaluation of patients with rapid-onset dystonia parkinsonism (RDP) including the recent transition to telemedicine approaches. Dr. Brashear shared information about new methods to assess gait, speech, and cognitive abilities. Dr. **Maria (Marietta) Papadopoulou** (University of Lyon) discussed development and testing of a smart phone electronic diary to track events. The e-diary is available in four languages (French, English, Spanish, Italian). Her group is tracking usage and user preferences. The final speaker in Session IV was Dr. **Terry Jo Bichell** (CombinedBrain) who gave an inspiring talk about her personal experience as a parent of a child with a rare neurodevelopmental disorder (Angelman syndrome) and her amazing journey advocating for research to develop cures for the disease, which included earning a Ph.D. in molecular neuroscience. She emphasized the need for patient-centered outcomes, clear evidence for natural history, and a conceptual model. Above all, she stressed to not give up hope.