8th ATP1A3 Symposium in Disease
Reykjavik, Iceland October 3-4, 2019

The following is a report of the 8th ATP1A3 Symposium in Disease held in Reykjavik, Iceland. The conference was hosted by the AHC Association of Iceland (AHCAI) led by Sigurður 'Sigi' Jóhannesson (President of AHCAI) and Ragnheidur (Ragga) Hjaltadottir (Manager of AHCAI). Sigi and Ragga provided exceptional hospitality with a creative and entertaining program including a tour of the Perlan Museum complete with an indoor ice cave and planetarium showing images of the Northern Lights. A group dinner on the first day of the conference was highlighted by a live performance by Icelandic signer Særún Harðardóttir.

The conference was opened with the showing of an excerpt from the video Human Timebombs https://humantimebombs.com/. This was followed by welcoming remarks by the **President of Iceland** Guðni Th. Jóhannesson who explain that Iceland has kept extensive genealogical records on the entire population (350,000 people). This enabled deCode Genetics to build and extensive population genetics resource, which led to mapping of genes responsible for hundreds of genetic traits. He further emphasized the importance of working together.

- **Keynote Presentations**

There were two keynote presentations. On the first day of the conference, Dr. **David Goldstein** from Columbia University spoke on the '*Road to Precision Medicine*'. Dr. Goldstein, who led the team that first discovered *ATP1A3* mutations in AHC, emphasized the success of genetic approaches to finding disease-causing gene mutations. In some cases, discovery of a disease-causing gene leads directly to curative therapy citing the example of a rare disorder called Brown-Vialetto-Van Laere syndrome in which dietary supplementation with a vitamin (riboflavin) has dramatic effects. However, this is the exception rather than the rule. Dr. Goldstein also highlighted his ongoing studies to study neuron function in one of the AHC mouse models, then emphasized the importance of making mouse and other disease models more reliable and exchangeable. A second keynote address was given by Dr. **Hreinin Stefansson** from deCode Genetics. Dr. Stefansson, who leads the CNS division of deCode, presented an overview of population genetics in Iceland, and efforts by deCode to discover genes responsible for both rare and common neurological disorders. These discoveries have led to several ongoing drug discovery programs.

Over the two-day meeting, conference speakers covered several topics representing the most active areas of ongoing ATP1A3 research. The following summary of these presentations is organized by these topics.

- **Refining the clinical features of ATP1A3 disorders**

Several presentations focused on the clinical features and spectrum of ATP1A3-associated disorders. Dr. **Allison Brashear** (University of California at Davis) discussed what is new in rapid onset dystonia parkinsonism (RDP) emphasizing that there may be many undiagnosed ATP1A3 associated disorders particularly in older individuals. Referring to a recently published article from her former group at Wake Forest University (Haq, et all, Mov Disord. 2019 July 30), she discussed the observations that not everyone has rapid onset symptoms, and that the 'parkinson' features are not consistent (e.g., few have tremor). Additionally, cognitive problems are more common than originally thought.

Dr. **Eleni Panagiotakaki** (Centre Hospitalier Universitaire de Lyon) presented new data on brain MRI abnormalities in 22 *ATP1A3*-mutant AHC cases. Among the study participants, who were between age 27 months and 31 years old, nearly half had normal MRIs, whereas others showed variable features including cortical atrophy or cerebellar atrophy. There was no correlation with the specific ATP1A3 mutation, and she did not discuss whether age at the time of the imaging study was a factor. Dr. Agathe Roubertie (Centre Hospitalier Universitaire de Montpellier) gave a short talk on non-paroxysmal (i.e., permanent or sustained) movement disorders in a new study of 28 AHC patients. None of the study participants had normal muscle tone, and about two-thirds exhibited either dystonia or other abnormal movements (e.g., chorea). Those with dystonia tended to have a younger age at onset, more pronounced hypotonia, and more severe neurological impairments. A few cases in this study showed sustained neurological deficits following an acute episode.

Two additional short presentations focused on the challenges in diagnosing AHC given by Dr. **Yr Sigurdardotttir** from Iceland, and a parent's perspective of caring for a child with AHC made by **Laura Heimgartner** from the United States. Finally, Dr. **Hendrick Rosewich** (Georg August University, Göttingen, Germany) led a discussion on the quality of life affecting people with AHC, and presented his goal of developing a database of answers to frequently asked questions. This database was discussed further at the end of the conference and there was unanimous enthusiasm for the idea. Dr. Rosewich will lead this effort. There was also discussion of how best to create an annotated and curated database of videos of patients to illustrate clinical features of AHC as a resource for physicians and families. A suggestion was made to create a web site linking to existing online videos and provide expert descriptions of the clinical findings.

- **New information on other AHC genes**

While *ATP1A3* mutations are found in a majority of AHC patients, there remain many who do not have a positive genetic test. Dr. **Arn van den Maagdenberg** (Leiden University) presented

an update on efforts to determine if there are other genes responsible for AHC. He summarized the study design involving 40 patients who did not have *ATP1A3* mutations. There are three genes that appear associated with AHC: *RHOBTB2*, *ATP1A2*, and *SCN2A*. The genetic evidence appears strong for *RHOBTB2* in 3 families. Evidence supporting *ATP1A2* and *SCN2A* (presented by Dr. **Al George**) as potential AHC genes was discussed. Most of the families in this study still do not have a genetic diagnosis.

- **Structure and function of ATP1A3**

Understanding the structure of the ATP1A3 protein is the focus of work by Dr. **Poul Nissen** (Aarhus University, Denmark). He presented his group's efforts to use X-ray crystallography and cryo-electron microscopy to solve the atomic-level structures for various P-type ATPases including ATP1A3. They have discovered an approach to produce large quantities of purified ATP1A3 protein and have developed functional assays to study specific mutations. They are just starting to collect data to determine the structure of the protein. Dr. **Lorenzo Antonini** (Sapienza University, Rome) discussed his complementary approach to understand the structure and function of ATP1A3 using a method called molecular dynamics. This method uses powerful computer to simulate each atom in the ATP1A3 protein that results in a movie showing the microscopic motions during functional activities. Using this approach, they have investigated the structural mobility of ATP1A3 in different functional states, then simulated the impact of two mutations (D801N, E815K) on these properties. In their preliminary studies, the two mutations behave differently at the atomic level.

The function of ATP1A3 in neurons may be modulated by other protein including enzymes that add phosphate groups. Dr. **Marisol Castaneda** (Francis Crick Institute, London) is studying one such enzyme called GAK kinase (also called auxillin-2). This protein has been shown to have a connection with Parkinson's disease. Dr. Castaneda presented her biochemical studies showing that GAK kinase can modify specific amino acids in the ATP1A3 protein and change its functional activity. She has extended this work into mice to show the importance of this protein modification for neuron activity. Dr. **Elena Arystarkhova** (Massachusetts General Hospital) discussed her work showing how certain mutations cause misfolding of ATP1A3 and impair delivery of the protein to the correct location in cells. She highlighted a particular process in cells called the unfolded protein response (UPR) that servers to clear improperly folded proteins and may be important for understanding differences in the behavior of various *ATP1A3* mutations.

- **Cell and animal models of ATP1A3 mutations**

Various cellular and animal models have been used to understand how ATP1A3 mutations affect brain and neuron function. **Eveny Akkuratov** (Royal Institute of Technology, Stockholm) presented her study of a new RDP mouse model engineered with *ATP1A3* mutation T613M. The mice have considerable abnormalities in overall activity, movement and coordination with infrequent spontaneous attacks of weakness or dystonia. **John Snow** (Vanderbilt University) presented his work on patient-derived induced pluripotent stem cells (iPSC) he is using to

generate AHC neurons in the laboratory. His work is comparing the electrical activity of different types of neurons generated from these cells with the goal of determining which neuronal lineage contributes most to the pathogenesis of AHC. Additional experiments use temperature changes of the cultured neurons to assess the mechanism of triggers in AHC.

- **Strategies for finding new therapies and understanding existing treatments**

Several talks were devoted to complementary approaches to developing new treatment strategies. Dr. **Mohamed Mikati** (Duke University) gave an overview of the current therapeutic state-of-the-art for AHC with an emphasis on multidisciplinary evaluation and treatment. He highlighted recent publications from his group on the importance of physical therapy (Pediatr Phys Ther.) and neuropsychological interventions (Dev Med Child Neurol.). Dr. Mikati also provided an overview of drug therapies for AHC, dystonia, disruptive behaviors, sleep, and epilepsy. With regard to epilepsy, he emphasized that the EEG can be normal in the beginning but then evolve over time, and therefore in-hospital video-EEG monitoring can be very helpful in determining if seizures are occurring.

Dr. **Steven Gray** (University of Texas Southwestern) gave an update on the AAV gene therapy project. He began with an overview of the different types of AAV vectors and illustrations of success using this approach for other genetic neurological disorders. The ATP1A3 targeted AAV vectors have been prepared and are being tested in mouse models of AHC. He emphasized that in the best case scenario, there will take 5-6 years to go from design of the vectors to clinical trials.

Two speakers discussed strategies for identifying novel drug candidates. Dr. **Peter Vangheluwe** (University of Leuven) presented his drug screening platform for P-type ATPases, which has been successful for ATP12A2 (a Parkinson disease gene) and SERCA. They are not currently working with ATP1A3. Dr. **Francesco Danilo Tiziano** (Catholic University of Sacred Heart, Rome) reported their work using a human neuroblastoma cell model of AHC for use in screening compounds. A pilot screen of 551 compounds was completed, and they have plans to expand to a larger library.

The most widely used treatment of AHC is flunarizine, but the mechanism by which this drug works is unknown. Dr. **Al George** (Northwestern University) presented results of a study comparing the effects of flunarizine on neurons derived from iPSC lines generated from two girls each with the same *ATP1A3* mutation (G947R) but who exhibited divergent responses to the drug. In preliminary studies, flunarizine was observed to suppress the generation of action potentials by neurons from both patients, but cells from the non-responder showed less potent effects of the drug. These findings suggest that there are intrinsic differences at the cellular level that might determine flunarizine response in AHC.

The 9th ATP1A3 in Disease Symposium will be held in **Stockholm, Sweden** in 2020 (date to be announced later).