STEP UP 4 AHC

FUNDING THE AAV PROJECT AND AHC RELATED RESEARCH

AHC Foundation Newsletter Fall 2019

EXCITING NEWS FROM OUR PRESIDENT
– Josh Marszalek

I am pleased to announce the funding of important research at Northwestern and Vanderbilt Universities. A continuation of important work supported by the AHCF, Dr. George and Dr. Ess will lead their teams in collaboration toward NIH funding.

There remains much more work needed to fully understand the fundamental defects responsible for AHC at the molecular and cellular levels. Researchers at Vanderbilt and Northwestern Universities are using induced pluripotent stem cells (iPSCs) derived from children with AHC to generate neurons. These AHC patient neurons are being used to answer two important unanswered questions about the disease:

1) Is the fundamental mechanism in AHC that of haploinsufficiency or involve dominant-negative effects?

2) Do ATP1A3 mutations associated with distinct clinical disorders exhibit functional differences at the molecular and cellular level?

The answers to these two questions will guide how future therapies, including gene therapy, need to work in order to correct the underlying molecular and cellular defects in AHC. The unique iPSC models developed by this research team will also enable testing of therapies for both common and less common ATP1A3 mutations to ensure that new therapies will benefit all persons with AHC.

The Blockbuster Summer Sequel Busted The Box Office!

The Wedum Family Foundation challenge was to raise $25,000 during the summer.
You not only met the challenge, but exceeded it.
Over $41,000 was raised. Together, that is over $66,000 for AHC research!
Our thanks go out to the Wedum Family Foundation and the entire AHC community!

AHCF
Serving the International AHC community since 1993

AHCF Newsletter
Sharing information for advocates, caregivers, professionals and families.

Thank you to everyone who helped raise funds during the months of Aug-Sep-Oct.

You are doing the most important work for AHC research and your efforts are greatly appreciated.

THANKS!

IMPORTANT MEETING SUMMARY

Dr. George Reports Back to Our Families About Latest ATP1A3 Research

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).

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.

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.

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The AHCF Board of Directors extend warm wishes to our community for a healthy and happy holiday season.

We hope all families can celebrate the holidays surrounded by your loved ones with the peace and joy of an episode free celebration.

Enjoy!

MEETING SUMMARY continued

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.

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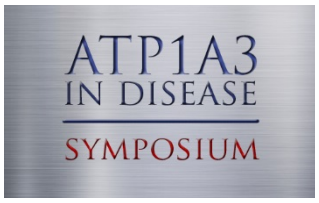
MEETING SUMMARY continued

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 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 (PediatrPhys 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. 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.

MEETING SUMMARY continued

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).

The AHCF extends are deepest appreciation and thanks to Dr. George for providing a thorough and helpful summary of the meeting. The was a very generous gift of his time to the AHC community.

GENE THERAPY 101 FOR THE AAV PROJECT

Some Basic Principles of Gene Therapy

We are happy to share with you some basic principles of gene therapy. **This month we'll cover is how long has gene therapy been around and how does it work.**

Gene therapy has been studied for more than 40 years and can help stop or slow the effects of disease on the most basic level of the human body—our genes.

Gene therapy is the introduction, removal or change in genetic material—specifically DNA or RNA—into the cells of a patient to treat a specific disease. The transferred genetic material changes how a protein—or group of proteins—is produced by the cell.

Gene therapy targets the cause of the disease and is not provided in the form of a pill, inhalation or surgery. It is provided through an injection or IV.

IMPORTANT RESEARCH ON EPILEPSY AND AHC JUST PUBLISHED…

51 AHC Patients Studied By Team at Duke University

In September, an article titled, **"The Epileptology of Alternating Hemiplegia of Childhood,"** was published in the journal *Neurology*. The paper looked at five questions: (1) multiple types of epileptic seizures occur in AHC, and these can be the initial presentation; (2) epileptiform abnormalities often appear well after clinical seizures; (3) nonepileptic reduced awareness spells (RAS) occur frequently; (4) epilepsy is commonly drug resistant but may respond to vagal nerve stimulation (VNS); and (5) status epilepticus (SE) is common and is usually refractory and recurrent.

They found, **32 of 51 patients had epilepsy**: **18 focal seizures, frontal more frequently than temporal**, and then posterior. **Eleven had primary generalized seizures** (tonic-clonic, myoclonic, and/or absence). **Epileptic seizures preceded other AHC paroxysmal events** in 8 (lag 5.63 ± 6.55 months; $p = 0.0365$). In 7 of 32, **initial EEGs were normal,** with the first epileptiform EEG lagging behind by 3.53 ± 4.65 years ($p = 0.0484$). **RAS occurred equally in patients with epilepsy** (16 of 32) and patients without epilepsy (10 of 19, $p = 1.0$). Twenty-eight patients had video-EEG; captured RAS showed no concomitant EEG changes. **Nineteen patients (59%) were drug resistant.** VNS resulted in >50% reduction in seizures in 5 of 6 ($p < 0.04$). Twelve patients (38%) had SE (9 of 12 multiple episodes), refractory/superrefractory in all ($p < 0.001$), and 4 of 12 had regression after SE.

The researchers concluded that epilepsy in AHC can be focal or generalized. Epileptic seizures may be the first paroxysmal symptom. EEG may become epileptiform only on follow-up. Epilepsy, although frequently drug resistant, can respond to VNS. RAS are frequent and nonepileptic. SE often recurs and is usually refractory/superrefractory. The observations are consistent with current data on AHC-ATP1A3 pathophysiology.

Neurology®

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MORE IMPORTANT RESEARCH ON EPILEPSY

Seizures in Babies: Sheds Light on Why They Have Lifelong Effects

A doctor at the University of Virginia is using a new approach to mapping brain activity to shed light on what happens during seizures in newborns that can lead to behavioral issues and learning disabilities much later. The research suggests that the brain's learning and memory centers are among the regions most affected by seizures caused by inadequate oxygen and blood flow.

Researchers are working with mice because there's no good way to map brain activity in infants suffering seizures. As doctors better understand what is occurring in the brains of infants and older children, they will be better able to determine how to treat them and ensure they have the best outcomes.

The research was published in the September 2019 issue of Annals of Neurology under the title, "Neuronal Circuit Activity during Neonatal Hypoxic-Ischemic Seizures in Mice." An abstract of the article can be found at:

https://onlinelibrary.wiley.com/doi/abs/10.1002/ana.25601

OUR CONGRATULATIONS TO AHCF ADVISOR
Dr. Laurie Ozelius and Colleagues on New Article

On August 16, 2019, the article, **"Factors in the Disease Severity of ATP1A3 Mutations: Impairment, Misfolding, and Allele Competition,"** was published in the journal, *Neurobiology of Disease.*

One of the authors, Dr. Laurie Ozelius, is a member of the AHCF Medical Advisory Board. She authored the article with 12 fellow research. Two of whom you may recognize; Dr. Alison Brashear and Dr. Kathleen Sweadner

The paper discusses how mutations of ATP1A3 cause neurological disorders with an exceptionally wide range of severity.

Their results suggest that a heterozygous mutation that only impairs Na,K-ATPase activity will produce relatively mild disease, while one that activates the unfolded protein response could produce severe disease and may result in death of neurons independently of ion pump inactivation.

Our thanks to the entire team for this great work.

LIGHTS – CAMERA – ACTION
Don't Miss Your Big Break By Not Being in the Next Family Meeting Picture

If you live with someone with AHC, or love someone who does, the AHCF 2020 Family Meeting is for you.

The **AHCF hosts a variety of events** throughout the year for the AHC community. Patients, families, and caregivers can find support from these events and **be a part of the progress to End AHC!**

The AHCF Family Meetings is the single best opportunity for learning about AHC from the experts.

AHC experts from around the world travel to AHCF events to help our community. You can sit next to doctors, researchers, and specialists and share your story.

Programming will be shared in the new year, but start planning now to join us in Los Angeles, California for an exciting, educational, and exceptional weekend of created just for you.

Keep an eye out for more details to come!

Indianapolis Meeting 2016

It is time to join the movement.
●
Be part of the team making the AAV Project a reality.
●
Contact Josh Marszalek for more info
joshua@ahckids.org

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