Second Symposium on ATP1A3 in disease:

Genotype/phenotype correlations, modelling and identification of potential targets for treatment

Catholic University School of Medicine, Rome, Italy, 23 - 24 September 2013

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The aim of the Symposium was to present the further progress of the research on Alternating Hemiplegia of Childhood (AHC), after the finding of the ATP1A3 gene as the primary cause of this rare neurological disease, to promote the international collaboration and to recruit new teams of researchers.

Lay Summary of Meeting Highlights

- ATP1A3 mutation E815K is associated with the most severe forms of AHC
- Mouse models have been, or are being, developed to mimic human AHC and RDP
- Structural and functional consequences of ATP1A3 mutations are beginning to reveal the main molecular basis of AHC and RDP.
- Many challenges must be overcome before conceiving of therapeutic trials in AHC and RDP
- Slow progress has been made in identifying strategies to pharmacologically treat AHC and RDP.
- Advocacy groups continue to have a very important role in enabling clinical research, promoting basic research and providing family support.

Day 1

Session 1: Clinical and genetic aspects of AHC

Clinical outcome measures and biomarkers for AHC patients

Dr. Mohamed Mikati (Duke University) discussed the complexity of spells including those with mixtures of hemiplegia and dystonic attacks then emphasized how this complexity will make determining treatment outcomes challenging. He further emphasized that outcome measures need to be carefully defined for the success of future clinical trials. He further reviewed the available measurements of disease severity that either consider features of spells or the residual disability evident between spells. Dr. Mikati further reviewed available information about biomarkers that he defined as

objective measures that serve as surrogates for clinical events or end points. New biomarkers are needed.

International collaborative group on genotype/phenotype correlations
Drs. Alexis Arzimanoglou and Eleni Panagiotakaki from Lyon, France presented preliminary findings on genotype-phenotype correlation based on a questionnaire study of 151 patients. Although preliminary, the data suggested that mutation E815K was associated with more frequent attacks, more severe dystonia, higher frequency of speech difficulties, more severe mental disability and a greater risk of becoming wheelchair bound. There was also twice the incidence of epilepsy in children with E815K than other mutations.

Session 2: Clinical aspects of AHC in different patients' groups

Catastrophic outcomes in AHC
Dr. Kathy Swoboda (University of Utah) presented her findings on catastrophic outcomes culled from the U.S/International AHCF database. She highlighted 10 cases that resulted in death including 4 who died suddenly in the setting of epilepsy (sudden unexplained death in epilepsy, SUDEP). Four of the cases she described had the E815K mutation. There was a female predominance among the 10 cases.

Genotype/phenotype correlation in Japanese patients with AHC
Dr. Masayuki Sasaki (Tokyo, Japan) presented findings on 35 Japanese AHC cases, of which 33 had ATP1A3 mutations. One third of his cases presented in early infancy with abnormal eye movements, half of the cases were never able to walk, whereas half were never able to speak in complete sentences. Carriers of E815K appeared to have more severe disease. Cases could be classified by the severity of their motor dysfunction. The D801N mutation was more often associated with the group with better motor function (80% able to walk or run). His study will be published soon in the journal Neurology. Dr. Sasaki reported that a small number of cases appeared to deteriorate after stopping flunarizine treatment, but this notion was vigorously challenged during the discussion period.

Natural history of the disease
Dr. Giuseppe Gobbi (Bologna, Italy) presented the Italian experience with 29 AHC patients, of which 21 had ATP1A3 mutations. He wished to emphasize that AHC patients do not have true ataxia but rather a gait disturbance caused by a mixture of dystonic and myoclonic movements. He further emphasized that hypotonia is very common in early infancy whereas dystonia and spasticity are uncommon. By contrast, dystonia and dyskinesias become the main movement disorders later in childhood.

A brief genetic update on AHC and RDP
Dr. Hendrik Rosewich (Göttingen, Germany) provided an overview of the ATP1A3 mutation spectrum in AHC and RDP. He emphasized that most AHC associated

mutations (73%) are found in just two exons and this might be a consideration for implementing rapid genetic testing.

Imaging results in RDP provide potential insights
Dr. Allison Brashear (Wake Forest University) presented clinical findings in 26 RDP cases, then discussed preliminary magnetic resonance imagining (MRI) findings in 3 patients and autopsy findings in one patient. Given the limited number of subjects, there were no obvious conclusions about common findings in brain images or brain pathology.

Session 3: Functional studies of ATPase

Genetic variation in ATP1A3 in neurological, developmental and neuropsychiatric diseases
Dr. Erin Heinzen (Duke University) focused her presentation on efforts to identify ATP1A3 mutations in various patient groups. She nicely summarized all known mutations in AHC, RDP and healthy populations. There is a striking clustering of mutations associated with AHC in one broad region of ATP1A3, but no such clustering of mutations was evident for RDP or for variants found in healthy persons.

Structural and biochemical studies addressing the AHC mutations
Dr. Paul Nissen (Aarhus University, Denmark) discussed the structural aspects of Na/K-ATPase. He presented a protein map of human AHC mutations superimposed on the structure of a related protein, Ca-ATPase, to illustrate that the three most common mutations cluster near ion binding sites or near the ATPase catalytic domain (i.e., regions of the protein important for function). Based on these observations, Dr. Nissen hypothesized that small molecules or drugs that potentially stabilize the ion binding geometry could rescue the functional defect caused by mutations. To illustrate this point, he present their findings of a theoretic computer-based survey of 30,000 candidate molecules to find compounds that may dock near the ion binding pocket. Some suggestive results were discussed, but no specific drugs or compounds were identified in his presentation.

Electrophysiological studies of sodium pump mutants
Dr. Hanne Poulsen (Aarhus University, Denmark) presented preliminary studies of mutations in ATP1A1 (different gene from ATP1A3) to illustrate the potential types of functional defects. Experiments were performed in frog (Xenopus) oocytes using electrophysiological recording methods. Her main observations were of ‘gain-of-function’ changes brought about by specific mutations. Specifically, some mutations caused a proton leak current whereas others caused a sodium ion leak. Dr. Poulsen did not present any data on AHC mutations but she is working on these mutations as well.

Session 4: The role of Institutions and Patient Associations in the support to the Collaborative Research on AHC

Telethon Italy and the alliance with the patients in the support to the research on rare genetic diseases

Francesca Sofia presented an overview of the Telethon mission and emphasized their interest in funding AHC research although so far no grants targeting this disease have been awarded. She discussed the rigorous peer review system they have in place to ensure that the best science is funded, and their history of moving projects from basic research to clinical trials. She further emphasized the cooperative model used by Telethon to engage patient associations, public institutions and the pharmaceutical industry.

The Message from the AHC Families Worldwide

Jeff Wuchich emphasized parent groups that have performed 'ad hoc' clinical trials on web forums such as RareConnect. He made a plea to researchers to educate families about what information would be most valuable to track in their children, and the desire to know more about the ‘road map ‘ for research. He finished by proposing new alliances with RDP families.

The International Patient Alliance AHCIA

Lynn Egan presented an overview of this organization and its purpose. She further reviewed features of the new web site and emphasized that more than 800 AHC cases are known worldwide due in part to efforts of patient organizations.

The European Federation AHCFE

Sigurdur Hólmar Johannesson presented the mission and purpose of AHCFE and emphasized their strategic alliances with other rare disease advocacy groups such as EURORDIS

The European Network for Research on Alternating Hemiplegia, ENRAH

Tsveta Schyns, founder of ENRAH, discussed the 10 year history and milestones of this organization emphasizing how they have been facilitating clinical and basic science research on AHC.

Breaking News from posters

Researchers from Paris presented their observation that the ATP1A3 D923N mutation causes both AHC and RDP. These findings were made in a single family with RDP in which the index case also had hemiplegic attacks, while other mutation carriers in the family appeared to be affected by an atypical form of AHC. This same mutation has been found in typical RDP affecting other families.

Dr. Akkuratov from Dr. Anita Aperia’s group at the Karolinski Institute presented their findings on the effects of ATP1A3 mutations associated with RDP on the ability of cultured neurons to regulate intracellular sodium concentration. Using rat hippocampal

neurons in culture, they observed impaired restoration of intracellular sodium concentrations when various RDP mutations were introduced.

An Italian group analyzed genotype-phenotype correlations in 33 Italian AHC patients of whom 63% had one of the two common ATP1A3 mutations and 6/33 had no detected ATP1A3 mutation despite having typical AHC. Patients with E815K had more severe features.

Dr. Petrucci from Rome presented MRI findings from a single AHC patient with the D801N mutation. The subject was 41 years old at the time of the study. Evidence was presented suggesting abnormal brain metabolism but it was uncertain if these changes were specific to AHC or could have been due to secondary events such as seizures.

A Japanese group presented a poster on ATP1A3 knockout mice. Surprisingly, the mice with one entire copy of the gene deleted had very minor abnormalities. These were preliminary findings.

Day 2

Session 5: ATP1A3opathies modeling

The *myshkin* mouse

Dr. Steven Clapcote (University of Leeds) presented findings on the Myshkin mouse, which has the I810N ATP1A3 mutation (analogous to I810S in AHC). He emphasized the prevalence of seizures in the animals and presented results from a genetic rescue experiment. The seizures in the mice are triggered by vestibular stress (tilting their cage) and become much worse when the animals are bred to different genetic backgrounds. The later observation points to the importance of genetic factors other than the ATP1A3 mutation in influencing or modifying the seizure phenotype, and this observation suggests that this mouse model of AHC could be used to genetically map modifier genes. He also presented preliminary finding of a genetic rescue experiment. In one line of mice in which the normal ATP1A3 gene was introduced, there were measurable improvements in dystonia and motor control.

A knock-in mouse model for RDP/AHC

Dr. Karin Lykke Hartmann (Aarhus University) presented her work on creating an RDP mouse by introducing the D801Y mutation. The animals had normal muscle strength but exhibit a peculiar hyperactivity. Additional studies were presented in a later talk by Dr. Kathy Sweadner (see below).

Oocyte modeling of ATP1A3 mutations

Dr. Steve Petrou (University of Melbourne) presented his group's experimental strategies to defining the functional consequences of ATP1A3 mutations. His group is using an electrophysiological approach in which frog oocytes are injected with messenger RNA coding for either the normal or mutant forms of the ATP1A3 gene. They have observed that the two most common AHC mutations have greatly reduced

functional activity. Similar findings were made for two RDP mutations. They found no evidence of a proton leak current, but did find evidence of a dominant-negative effect of the mutations (e.g., mutant pump inhibits the normal pump). He also gave an overview of future work planned in collaboration with Dr. Goldstein in which human stem cells will be engineered to have various ATP1A3 mutations, then neurons derived from these cells will be studied by means of multi-electrode arrays capable of recording electrical signals from groups of cells.

The knock-in mouse model

Dr. Mikati returned to present the ongoing work at Duke University to develop AHC mouse models. Progress is being made but no preliminary findings were available.

Structure-function studies and symptoms in a mutant mouse

Dr. Kathy Sweadner (Massachusetts General Hospital) presented on two topics. First, she described computer modeling of various mutations to illustrate potential structural perturbations caused by different amino acid substitutions in the protein. Position 801 where AHC and RDP mutations occur, was sensitive to amino acid substitutions as evidenced by specific structural perturbations including altered geometries of ion binding pockets. She also presented findings of the D801Y mouse (from Dr. Hartmann). Her group also observed the peculiar hyperactivity of the mice, which in some tests of motor coordination the mutant mice performed better than genetically normal mice. However, there were very subtle motor abnormalities evident including abnormal posture. Further, following physical exertion, the animals showed evidence of weakness and tremor, but no dystonia. Most impressive was the response of the animals to pharmacological stress with the anesthetic agent ketamine. In normal mice, ketamine was able to induce sleep from which the animals awoke quickly without any motor abnormalities. By contrast, the mutant mice did not fully sleep with even high doses of ketamine, then upon recovery from the effects of the drug had notable weakness.

Session 6: Pharmacologic modulation of ATPases activity

The presentation by Dr. Paolo Manunta on ‘Endogenous ouabain and ATPase: possible implications for rostafuroxin’ was canceled.

AMPK activators as potential candidates to the treatment of AHC

Dr. Alexander Chibalin (Karolinska Institute) presented his work on a class of drugs that modulate a class of enzymes (AMP-activated kinases) in skeletal muscle. The focus of his research is diabetes. This work was presented to highlight potential pharmacological strategies to increase the cell surface expression of Na-K-ATPases. He showed experimental findings demonstrating that insulin increases Na-K-ATPase cell surface expression and this could be mimicked by AICAR or A-769662, two compounds that turn on AMP-activated kinase.

Binding of digitalis-like compounds to Na,K-ATPase

Dr. Jan Koenderink (Nijmegen, The Netherlands) presented protein studies of Na-K-ATPases including several mutations associated with AHC and RDP. His laboratory generates proteins using insect cells infected with a virus (baculovirus), a standard system for generating large quantities of proteins. Measurements of ATPase enzymatic activity was found to be very low for all mutations tested except D220N, which exhibited activity of normal ATP1A3 suggesting that this might have been misclassified as a mutation. Additional enzyme studies revealed specific information about why ATP1A3 mutations lack activity. Additional experiments probed the relationship between the chemical structure of ouabain, a known Na-K-ATPase inhibitor) and the activity of ATP1A3 mutants.

<u>Session 7</u>: Round Table Discussion of Clinical Trials

This discussion session began with Dr. Rosaria Vavassori describing the Global Alliance. This was followed by a general description of the requirements for clinical trials by Dr. Tiaiana Granata. Dr. Mikati presented his rough sample size calculations and suggested that for a clinical trial in AHC, 150 patients (75 patients per arm) would be required for a strict randomized double blind clinical trial. But, only 75 patients would be needed for a cross-over study design. Dr. Swoboda emphasized challenges in quantifying spells based on her experience with the sodium oxybate trial. Dr. Brashear discussed current treatments for RDP and summarized efforts to find new treatments. There currently no clinical trials for RDP.