
ATP1A3 SYMPOSIUM IN DISEASE
- TOWARDS THE LIGHT
3 – 4 OCTOBER
2019
GRAND HOTEL REYKJAVIK
www.atp1a3symposium2019.org

Programme
&
Book of speaker abstracts

The 8th Annual Symposium on *ATP1A3* in Disease

Moving towards the light

3-4 October 2019
Reykjavík, Iceland

Programme

The 8th Annual Symposium on *ATP1A3* in Disease

Moving towards the light

Wednesday 2 October 2019

16:00 - 18:00 Parents meeting

18:00 - 20:00 Registration, poster hanging & welcome reception

Thursday 3 October 2019

08:30 - 08:40 Welcome and remarks by Sigurður Jóhannesson (organising committee member)

08:45 - 08:55 Opening remarks by **Guðni Th. Jóhannesson, the President of Iceland** (if his schedule permits)

SESSION 1 - Examples of how research move towards understanding of disease and progress towards a cure

Moderators: Hendrik Rosewich and Mohamad Mikati

08:55 - 09:00 Introduction by chair Hendrik Rosewich (organising committee member)

09:00 - 09:30 **David Goldstein**:
"The long road toward precision medicine in neurological diseases" (invited speaker)

09:30 - 09:55 **Peter Vangheluwe**:
"A screening platform for P-type ATPase drug discovery" (invited speaker)

09:55 - 10:20 **Mohamad Mikati**:
"Mechanisms of AHC: From Molecules to Networks" (invited speaker)

10:20 - 10:50 Coffee break and free poster time

SESSION 2 - Current knowledge and treatment of ATP1A3 diseases

Moderators: David Goldstein and Hendrik Rosewich

10:50 - 10:55 Introduction by chair Hendrik Rosewich (organising committee member)

10:55 - 11:20 **Allison Brashear**:
"RDP phenotypes: The Tip of the Iceberg?" (invited speaker)

11:20 - 11:45 **Mohamad Mikati:**
"Therapy of AHC: State of the Art" (invited speaker)

11:45 - 11:55 **Eleni Panagiotakaki**:
"Brain MRI abnormalities in a French cohort of 22 ATP1A3 *– positive AHC patients"*
(selected oral presentation)

11:55 - 12:05 **John P. Snow**:
"An iPSC-Derived Model to Investigate Neural Lineage Contributions to Alternating Hemiplegia of Childhood"
(selected oral presentation)

12:05 - 12:15 Round the table discussion and questions for the box

12:20 - 13:15 Lunch

SESSION 3 - New coming treatment of ATP1A3 diseases

Moderators: Sigurður Hólmar Jóhannesson and Karin Lykke-Hartmann

13:15 - 13:20 Introduction by chair Sigurður Hólmar Jóhannesson (organising committee member)

13:20 - 13:45 **Steven Gray**:
"Steps Toward Gene Therapy for ATP1A3*"* (invited speaker)

13:45 - 14:10 **Alfred L. George & Arn van den Maagdenberg**:
"SCN2A: an AHC gene?" (invited speaker & organizing committee member)

14:10 - 14:20 **Evgeny E. Akkuratov**:
"Abnormal gait control in a rapid-onset dystonia-parkinsonismmice mode"
(selected oral presentation)

14:20 - 14:30 **Agathe Roubertie**:
"Non-paroxysmal movement disorders in patients with Alternating Hemiplegia of Childhood: "soft" and "stiff" " (selected oral presentation)

14:30 - 14:40 Round the table discussion and questions for the box

14:40 - 15:20 Coffee break and **poster session** (poster presentors are available at their posters)

SESSION 4 - Quality of life and education

Moderators: Sigurður Hólmar Jóhannesson and Kevin Ess

15:20 - 15:25 Introduction by chair Sigurður Hólmar Jóhannesson (organising committee member)

15:25 - 15:45 **Hendrik Rosewich** – lecture title to be announced (organising committee member)

15:45 - 16:05 **Yr Sigurdardottir**:
"I met a zebra" – A talk about the difficulty in diagnosing rare diseases and the challenges of being the least knowledgeable person in the room
(invited speaker)

16:05 - 16:20 **Laura Darick Heimgartner**:
"How do we manage this - AHC and the Quality of Life" - It's our story of where we began with AHC, and how we find our Hope and Relief in the daily struggles of AHC
(invited speaker)

16:20 – 16:45 Coffee break

16:45 - 18:45 Tour to the Perlan Museum
Group photo will be taken at the museum
The museum entrance and bus transport is included in symposium reg. fee

20:00 - 22:30 Dinner
At the hotel restaurant
Badge will serve as entrance

21:30 - 21:50 **KEYNOTE LECTURE** by **Helga Birgisdottir**:
"We Can Do It!"

22:00 - 22:15 Entertainment by singer **Særún Harðardóttir**

22:30 - 23:30 Social interactions

Friday 4 October 2019

SESSION 5 - Molecular mechanisms of Na^+K^+-ATPases

Moderators: Hanne Poulsen and Poul Nissen

08:30 - 08:35 Introduction by chair Hanne Poulsen (organising committee member)

08:35 - 09:00 **Poul Nissen**:
"Electron microscopy studies of membrane proteins - towards structures of ATP1A3"
(organising committee member)

09:00 - 09:25 **Marisol Sampedro Castaneda**:
*"*ATP1A3 *phosphorylation by GAK kinases: a role in disease?"* (invited speaker)

09:25 - 09:35 **Elena Arystarkhova**:
"Misfolding mutations in ATP1A3*: cell biological approaches to overcome impaired biosynthesis"* (selected oral presentation)

09:35 - 09:45 **Lorenzo Antonini**:
*"*ATP1A3 *wild type and mutated isoforms molecular dynamics simulations in a lipid membrane bilayer. Insights on protein structure and ion interactions"*
(selected oral presentation)

09:45 - 10:30 Coffee break and **poster session** (poster presentors are available at their posters)

SESSION 6 - Towards new therapies

Moderators: Karin Lykke-Hartmann and Arn Van den Maagdenberg

10:30 - 10:35 Introduction by chair Karin Lykke-Hartmann (organising committee member)

10:35 - 11:00 **Guangping Gao**:
"Gene Therapy for CNS disorders – history, principles, challenges and approaches"
(invited speaker)

11:00 - 11:25 **Francesco Danilo Tiziano**:
"Human neuroblastoma model of AHC: towards a medium throughput screening of candidate therapeutic compounds" (invited speaker)

11:25 - 11:35 **Alfred L. George**:
"Effects of Flunarizine on iPSC-derived Neurons from AHC Patients Exhibiting Divergent Clinical Responses" (selected oral presentation)

11:35 - 11:45 **Catherine Brownstein**:
*"*ATP1A3 *variants in a Sudden Infant Death Syndrome cohort"*
(selected oral presentation)

11:45 - 12:00 Round the table discussion and questions for the box

12:00 - 13:00 Lunch

SESSION 7 - deCODE Genetics

13:00 - 13:05 Introduction by Sigurður Hólmar Jóhannesson (organising committee member)

13:05 - 13:45 **KEYNOTE LECTURE** by **Hreinn Stefánsson**:
"From gene discovery to therapeutic advances"

13:45 - 14:00 Short break

SESSION 8 - Moving towards the light

Moderators: Arn van den Maagdenberg and Karin Lykke-Hartmann

Introduction by chair Arn van den Maagdenberg (organising committee member)

14:00 - 14:10 **Poul Nissen and Hanne Poulsen** (organising committee members)

14:10 - 14:20 **Hendrik Rosewich** (organising committee member)

14:20 - 14:45 **Arn van den Maagdenberg** (organising committee member)

14:45 - 15:00 **Karin Lykke-Hartmann** (organising committee member)

Organising committee

The symposium host this year is the AHC Association of Iceland supported by an organizing committee that consist of European scientists that have been working on *ATP1A3* related diseases for many years.

Sigurður Holmar Jóhannesson
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The Standing Committee members of the ATP1A3 in Disease organisation:

Kevin C. Ess
Karin Lykke-Hartmann
Mohamad Mikati
Hendrik Rosewich
Tsveta Schyns-Liharska
Jeff Wuchich

The Standing Committee ensures that the Mission and the Vision are implemented. The current members of the Standing Committee were elected by the ATP1A3disease General Assembly at the London 2016 Symposium. The Standing Committee operates permanently to ensure meetings are continuously held at the highest scientific quality.

Main tasks include:

- select the Organisers of each annual meeting
- review and advising the Organisers(committee) on the meeting program, budget and organisation, according to the developed Guidelines
- ensure that dedicated funds are gathered and passed from one annual host to the next
- maintain and update a dedicated web site
- collect, store and publish information on the meetings
- communicate the outcomes of the meeting and promote and advertise the meeting and the cause of ATP1A3-related diseases community

Find more information here: http://www.atp1a3disease.org/sc.html

Book of speaker abstracts

Speaker abstracts are listed
according to the programme

A screening platform for P-type ATPase drug discovery

Peter Vangheluwe[1], Mujahid Azfar[1], Veronick Benoy[1], Patrick Chaltin[2], Jialin Chen[1], Jan Eggermont[1], Norin Hamouda[1], Rongjie Li[1], Shaun Martin[1], Marleen Schuermans[1], Sarah van Veen[1], Matthias Versele[2], Stephanie Vrijsen[1]

[1] Laboratory of Cellular Transport Systems, [2] Center for Drug Design and Discovery, KU Leuven, Leuven, Belgium

ATP1A3 belongs to the large family of P-type ATPases, which are transporters that establish vital gradients of ions or lipids in cells. Humans express 36 P-type ATPase genes of which many are critically involved in human diseases. Several P-type ATPases are established drug targets, such as H^+/K^+-ATPase with proton pump inhibitors and Na^+/K^+-ATPase with cardiac glycosides. In our lab, we study the molecular properties and cellular function of new candidate P-type ATPase drug targets. Our research feeds a pipeline of drug discovery that runs in collaboration with the Center for Drug Design and Discovery (CD3), a drug screening and development platform at KU Leuven. Via a structure function approach we study the mechanism of P-type ATPase regulation, which leads to rational design strategies for drug development. In addition, we develop and optimize new biochemical assays suitable for high throughput screening. Via hit screening and early hit to lead development programs we aim to design potent and selective modulators of new P-type ATPase drug candidates.

Invited speaker

Mechanisms of AHC: From Molecules to Networks

Mohamad Mikati, MD

The mechanisms of AHC involve pathophysiological abnormalities at multiple levels. The first is at the level of the Na+/K+ pump: Mutations in ATP1A3 that cause AHC reduce enzymatic ATPase function and the pump function of Na+ and K+ exchange, thus leading to abnormal transport of these ions. They also result in abnormal folding of the protein and abnormal cellular localization. Abnormalities in calcium ion concentrations and in signal transduction factors could potentially follow may also, potentially, be important. Abnormalities at the downstream network levels involve abnormal firing of cerebellar and hippocampal neurons, abnormal firing of fast spiking GABAergic interneurons and predisposition to spreading depression. Further research needs to concentrate not only on the effects of mutations on ion transport and intracellular trafficking of ATP1A3 but also on downstream effects on signal transduction factors and various motor control and related neural networks.

Invited speaker

RDP phenotypes: The Tip of the Iceberg?

Allison Brashear, MD, MBA

Dean, School of Medicine, University of California, Davis

We report a revised profile of the RDP phenotype and propose that hyperacuity to the possibility of ATP1A3 playing a role in dystonic disease should be considered by adult and pediatric neurologists and psychiatrists.

The expanding phenotype of ATP1A3 includes over 100 clinical paper in the last ten years. These reports range from isolated cases of ataxia, to larger reports of Rapid-Onset of Dystonia Parkinsonism (RDP) and Alternating Hemiplegia of Childhood (AHC) to the implication of a role in autism and childhood onset schizophrenia.

Our detailed analysis of our large co-hort of 50 mutation positive individuals with RDP compared to 44 familial and age matched controls. Of the gene positive cases, bulbar and rapid onset were not uniformly present. Unlike previously reported a rostral caudal gradient was present in only 7% of patients, while arm dystonia was most common. Triggers were present in 77% of cases and more than half of the cases were de novo.

We propose that given the breadth of findings in the literature and our detailed phenotypic analysis that ATP1A3 be considered a possibility in any case of dystonia, especially those presenting over weeks to months. Bulbar finding, positive family history and a rostral caudal gradient are not a requirement to consider ATP1A3 testing.

More broadly, with the implications of ATP1A3 as a potential role in more central nervous system diseases, including neurodegeneration, the concept of the known ATP1A3 phenotypes to be the “tip of the iceberg” will be discussed. This reinforces the importance of symposiums, such as this meeting in Iceland, to continue to push the field forward with the focus on identification of disease, prevention and treatments.

Disclosure: Dr. Brashear is the PI of RO1NS058949.

Invited speaker

Therapy of AHC: State of the Art

Mohamad Mikati, MD, Lyndsey Prange, PNP

Therapy of AHC involves addressing all the needs of the patients and is rapidly evolving with many interventions that are best addressed in a multi-disciplinary approach. This presentation will review the state of the art of the status of therapies of AHC. The multidisciplinary approach needs to address and prescribe therapies for problems in the multiple specialties: Neurology (hemiplegia, dystonia, seizures), Cardiology, Child Behavioral Health, Medical Genetics, Neurodevelopment, Neuropsychology, Nursing, Physical and Occupational Therapies, Psychiatry, Sleep Medicine, Respiration, and Speech/Language Pathology. The presentation will provide specific guidelines based on studies and on clinical experience in each of these areas.

Invited speaker

Steps Toward Gene Therapy for ATP1A3

Qinglan Ling and **Steven J. Gray**

Department of Pediatrics, UT Southwestern Medical Center, Dallas, TX, USA

Gene therapy for central nervous system (CNS) disorders has seen a recent resurgence with the discovery of adeno-associated virus (AAV) vectors that are capable of crossing the blood-brain barrier (BBB), such as AAV9. The Gray lab has been focused on examining the translational potential of AAV9 to treat inherited CNS disorders. Initial studies demonstrated that AAV9 can achieve dose-dependent, widespread gene transfer to neurons and astrocytes in mice as well as in non-human primates, when injected intravenously or intrathecally. Using AAV9-mediated gene transfer as a platform approach to treat an inherited CNS disease, in 2015 Dr. Gray and colleagues at the NIH initiated a Phase I clinical to test intrathecal administration of scAAV9/JeT-GAN in patients with Giant Axonal Neuropathy. Using the same technology, clinical trials from Dr. Gray's group are pending for Batten Disease (CLN1, CLN5, CLN7), Aspartylglucosaminuria, Tay-Sachs disease, Krabbe disease, Charcot-Marie-Tooth disease type 4J, and Austin disease.

ATP1A3 gene transfer to treat Alternating Hemiplegia of Childhood (AHC) has unique challenges that are likely to make gene therapy more complicated, but we are undertaking initial steps to assess the feasibility of ATP1A3 gene therapy. An update on the progress of this project will be provided.

Invited speaker

An iPSC-Derived Model to Investigate Neural Lineage Contributions to Alternating Hemiplegia of Childhood

Snow JP, Westlake GMP, and Ess KC

Vanderbilt University Medical Center, Department of Pediatrics, Nashville TN

Alternating Hemiplegia of Childhood (AHC) is a rare neurodevelopmental disease caused by heterozygous missense mutations in the *ATP1A3* gene, which encodes the neuronal specific α3 subunit of the Na,K-ATPase pump. AHC patients display unique symptoms beginning in early childhood, including episodes of weakness or paralysis often triggered by stress, abnormal eye movements, seizures, painful dystonia, and developmental delay. The majority of AHC cases are caused by one of three missense mutations in the *ATP1A3* gene: D801N, E815K, or G947R. Mechanisms that underlie patient symptoms remain poorly understood and there are no empirically proven treatments for AHC. We have generated induced pluripotent stem cells (iPSCs) from patients with the three most common mutations in AHC, and focus here on the most phenotypically severe *ATP1A3* mutation, E815K.

To clarify the contribution of distinct cortical lineages to disease pathogenesis, AHC *ATP1A3*$^{+/E815K}$ iPSCs, isogenic wildtype iPSCs, and unrelated control iPSCs have been differentiated to neurons using two protocols that generate cultures primarily comprised of either glutamatergic or GABAergic neurons. This patient-specific cell model was used to test the hypothesis that expression of E815K mutant α3 protein decreases Na,K-ATPase function, perturbs normal neurodevelopment, and results in altered neuronal function that is exacerbated by cellular stress. Our results indicate that iPSC-derived wildtype and *ATP1A3* mutant neurons from excitatory or inhibitory differentiation protocols display similar temporal patterns of α1 and α3 subunit protein expression during neuronal differentiation. RNA expression levels of all alpha and beta Na,K-ATPase subunits change as expected during differentiation, but are consistent between genotypes and differentiation method. Multielectrode array analyses demonstrate that in cultures dominated by iPSC-derived cortical glutamatergic neurons, *ATP1A3*$^{+/E815K}$ neurons display less overall activity than their wildtype counterparts. Current experiments involve stressing cultures with elevated temperature to analyze changes in overall activity and firing rates between genotypes. Additionally, we are using this model system to investigate the impact of flunarizine treatment on AHC patient neurons during heat stress and recovery. Characterization of synaptic density and neuronal morphology during *in vitro* differentiation is also underway to determine if the observed functional differences may manifest on a neurodevelopmental level. This approach can be modified for use with evolving neuronal differentiation methods and will allow for the mechanistic interrogation of disease pathogenesis in AHC, while providing a route toward therapeutic discovery in a human disease model.

Selected oral presentation

Brain MRI abnormalities in a French cohort of 22 *ATP1A3* – positive AHC patients

1) **Rebecca Moré**, interne, Department of Pediatric Neurology outpatient clinic / Neonatal pediatrics and Intensive care, CHU de Rouen, France rebecca.more@etu.univ-rouen.fr
2) Dr Gustavo Soto Ares, department of neuro-radiology, Hôpital Salengro, CHRU de Lille, France
3) Dr Gaëtan Lesca, Hospices Civils de Lyon, Department of Medical Genetics, Centre de Biologie Est, Lyon University Hospital, Member of the ERN EpiCARE, Lyon, France
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5) Dr Jacques Boulloche, Department of Pediatric Neurology outpatient clinic, Groupe hospitalier du Havre, France
6) Pr Arzimanoglou Alexis, Department of Paediatric Clinical Epileptology, sleep disorders and Functional Neurology, University Hospitals of Lyon; Member of the ERN EpiCARE, Lyon, France
7) **Dr Panagiotakaki Eleni**, Department of Paediatric Clinical Epileptology, sleep disorders and Functional Neurology, University Hospitals of Lyon; Member of the ERN EpiCARE, Lyon, France
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Background: Alternating hemiplegia of childhood (AHC) is a rare neurological disorder, characterized by bouts of unilateral or bilateral hemiplegia or paroxysmal dystonia, epileptic seizures and other events like abnormal ocular movements and episodes of autonomic dysfunction. Patients also present hypotonia and movement disorders like dystonia and/or chorea, and global neurological impairment (intellectual disability and behavioral or communication disorders). Onset occurs before the age of 18 months.

Diagnosis is based on Aicardi's criteria, and since 2012, supplemented by genetic testing for *ATP1A3* mutations. Nevertheless, diagnostic approach requires further exams, like metabolic testing, EEG or brain imagery in order to eliminate other potential diagnoses. Most of brain MRIs of patients are normal. Some studies found nonspecific abnormalities like cerebellar atrophy, progressive frontal cerebral atrophy, loss of white matter tract and spectroscopic abnormalities, but detailed data on neuroradiological findings are lacking.

Aim: To assess the prevalence and nature of MRI abnormalities in *ATP1A3* mutation-positive patients with AHC.

Methods: We performed a standardized review of brain MRIs by a single neuro-radiologist in order to describe in detail structural congenital or acquired abnormalities in a cohort of 22 French AHC patients. A questionnaire was completed by the first author by examination and direct interviews of patients and families including: clinical symptoms, treatment used and living conditions. Severity of the disease was calculated using paroxysmal and non-paroxysmal disability indices (Panagiotakaki et al., 2010).

Results: Twenty two patients (14 males, 8 females) were included. Seven had the p.Asp801Asn mutation, and 5 the p.Glu815Lys. Age at inclusion varied from 27 months to 31 years. Four patients had abnormal perinatal history. Half of the patients were epileptic (2 with status epilepticus). Abnormal MRIs were found in 12 patients and included: Bilateral fronto parietal (+/- occipital) polymicrogyria or bilateral fronto-parietal diffuse cortical abnormality (3 patients), dilated subarachnoid spaces, hypoplasia of the right fronto-parietal operculum associated with bi fronto parietal atrophy, thick corpus callosum, bilateral hippocampal sclerosis and progressive cortico-cortical atrophy. We searched for correlations between MRI abnormalities, and type of mutation as well as clinical presentation and degree of severity.

At last we will illustrate our findings by specifically presenting two clinical cases of Normand French patients with the two most frequent mutations, one with fronto parietal polymicrogyria (pGlu815Lys mutation) and the second with frontal cortical atrophy (p.Asp801Asn mutation). Their clinical symptoms and neurological impairment were very different.

Selected oral presentation

Abnormal gait control in a rapid-onset dystonia-parkinsonism mice model

Evgeny E. Akkuratov[1], Daniel C. Jans[1], Vasco Sousa[2], Laurence Picton[3], Xiaoqun Zhang[2], Per Svenningsson[2], Hjalmar Brismar[1, 4] and Anita Aperia[4]

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[2] - Department of Neurology and Clinical Neuroscience, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden
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[4] - Science for Life Laboratory, Department of Women and Children's Health, Karolinska Institutet, Stockholm, Sweden

Rapid-onset dystonia-parkinsonism (RDP) is characterized by abrupt onset of several symptoms including generalized dystonia, severe bradykinesia and gait instability. We have created a knock-in mice model with the most common mutation observed in RDP patients, T613M, located in ATP1A3 gene encoding alpha3 subunit of Na,K-ATPase, where we have specifically evaluated the gait. The motor cortex and other brain areas including basal ganglia and cerebellum are involved in planning and decision making for the initiation of movement, but the quality of gait control relies on the spinal neuronal network that directly control the rhythmic control of forelimbs and hindlimbs in mammals.

Homozygote mice were embryonically lethal, but Heterozygous (Het) mice were born normally and the Mendelian distribution of WT and Het crossing was 151 WT: 132 Het. We found 10 spontaneous death cases where eight animals were Het. Three Het animals had such severely disturbed gait that they had to be sacrificed for ethical reasons. One of these animals could hardly move and had signs of dystonia and ataxia. One Het animal was found trembling and hunching but did recover to normal state.

Several behavior tests demonstrated significant differences between WT and Het. During beam test Het made more mistakes than WT and needed more time and more steps on the way to home cage which indicates difficulties with motor coordination. During an open-field test Het moved with higher velocity and the total travelled distance was 50% larger than for WT. During elevated plus maze test Het animals also moved with higher velocity and spent twice more time in open arms and less time in closed arms compared to WT. During forced swim test Het animals were found in passive floating state almost twice less time compare to WT. Two memory tests revealed no difference between het and WT. Different triggers including ethanol administration and immobilization did not provoke additional symptoms.

Taken together, the behavior tests as well as the sporadic observation indicate that the ATP1A3 T613M mutation is associated with disturbance of quality and rhythmicity of gait control, and point to a pathological function of the neurons in the spinal cord. There is however very little information about the role of the Na,K-ATPase and the expression of the alpha3 catalytic subunit in spinal cord neurons, but in ongoing studies, using IHC and *in situ* techniques, we have found that the alpha3 subunit of Na,K-ATPase is widely expressed in a spinal cord. We are now identifying cell types which express alpha3 the most and looking for the pathologies in these cell types in Het animals.

Selected oral presentation

Non-paroxysmal movement disorders in patients with Alternating Hemiplegia of Childhood: "soft" and "stiff"

Eleni Panagiotakaki, MD[1*], Diane Doummar, MD[2*], Erika Nogue, MSc[3], Nicolas Nagot, MD, PhD[3], Gaetan Lesca MD, PhD[4], Florence Riant, PhD[5], Sophie Nicole, PhD[6], Alexis Arzimanoglou, MD[1], **Agathe Roubertie**, MD, PhD[7], and the AHC-movement disorder Study Group[♯]

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1. **OBJECTIVE:** To assess non-paroxysmal movement disorders in *ATP1A3* mutation-positive patients with alternating hemiplegia of childhood.
2. **METHODS:** Twenty-eight patients underwent neurological examination with particular focus on movement phenomenology by a specialist in movement disorders. Video recordings were reviewed by another movement disorders specialist, and data were correlated to patients' characteristics.
3. **RESULTS:** Ten patients were diagnosed with chorea, 16 with dystonia, four with myoclonus, and two with ataxia. Nine patients had more than one movement disorder and eight patients had none. The degree of movement disorder was moderate to severe in 12/28 patients. At inclusion, dystonic patients (n=16) were older (p=0.007) than non-dystonic patients. Moreover, patients (n=18) with dystonia and/or chorea had earlier disease onset (p=0.042) and a more severe neurological impairment (p=0.012), but this did not correlate with genotype. All patients presented with hypotonia, which was moderate or severe in 16/28. Patients with dystonia and/or chorea (n=18) had more pronounced hypotonia (p=0.011). Bradykinesia (n=16) was associated with an early age at assessment (p<0.01). Significant dysarthria was diagnosed in 11/25 cases. A history of acute neurological deterioration and further regression of motor function, typically after a stressful event, was reported in seven patients.
4. **CONCLUSION:** This is the first categorisation of movement disorders in AHC patients which may offer valuable insight into their precise characterization.

[♯]AHC-movement disorder Study Group:

Charlene Delaygue[1], Marie Anne Barthez[2], Marie Cécile Nassogne[3], Anne Dusser[4], Louis Vallée[5], Thierry Billette[6], Marie Bourgeois[7], Christine Ioos[8], Cyril Gitiaux[9], Cécile Laroche[10], Mathieu Milh[11], Vincent Desportes[12]

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Selected oral presentation

We Can Do It!

Helga Birgisdóttir - Gegga

Artist, CEO and Creator of SMILER, Iceland

When we choose the right fuel for our dreams anything is possible. When we open our minds and hearts and connect to others, even though other peoples ideas seem exotic to us, we can accomplish so much more and in certainty we can do miracles. Yes, there is no doubt in my mind by combining the best of both worlds; science and spirituality, we can move mountains. Let’s enjoy it together!

Invited speaker

ATP1A3 phosphorylation by GAK kinases: a role in disease?

Lin AW[1], Gill KK[1], **Sampedro Castaneda M[1]**, Matucci I[1], Eder N[1,2], Claxton S[1], Flynn H[2], Snijders AP[2], George R[3], Ultanir SK[1]

1 Kinases and Brain Development Lab, The Francis Crick Institute, London, UK
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Cyclin G-associated kinase is ubiquitously expressed in mammalian tissues. As demonstrated by global and conditional knock out mouse models, the serine/threonine kinase activity of GAK is essential for survival. GAK is well known for its role in vesicle trafficking in non-neuronal cells, but its function in neurons has remained poorly understood, partly due to the technical difficulties associated with the identification of direct kinase substrates. In recent years, independent GWAS studies have identified *GAK* as a candidate gene in Parkinson's disease aetiology, highlighting the importance of research in neuronal GAK signalling. Using a chemical genetics and mass spectrometry approach, our lab has identified and validated novel GAK substrates relevant for neuronal function, including the α3 subunit of the Na+,K+ ATPase. We have investigated the functional importance of this interaction in heterologous expression systems and neurons using phosphomutant variants of α3. Our results suggest that GAK-mediated phosphorylation is critical for α3 subcellular localization and this could be relevant in the context of human *ATP1A3*-related diseases.

Invited speaker

Misfolding mutations in ATP1A3: cell biological approaches to overcome impaired biosynthesis

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There is a wide range of severity in neurological disorders associated with *ATP1A3*. This implies distinct cellular consequences caused by mutations: inactivation, changes in the kinetics of the enzyme, or incorrect folding and trafficking of the neuronal α3 isoform during biosynthesis. As reported last year, isogenic cell lines expressing different mutations of *ATP1A3* in HEK293 cells allowed us to identify at least two mutants, L924P and D742Y, characterized by reduced level of α3 expression, manifestation of the immature ER form of the beta subunit, and aberrant trafficking of αβ complex to Golgi apparatus and plasma membrane. This implicates impaired biosynthesis. To investigate further, we performed thorough characterization of the L924P mutant. Phenotypically, L924P mutation was associated with the most severe manifestations of EEIE, including uncontrolled seizures, apnea, severe hypotonia, microcephaly, very poor development, and early death. In cellular fractionation experiments we identified a significant amount of the α3 and immature beta subunit retained in the ER fractions in the L924P mutant. This contrasted with control α3-WT cells where practically no α3 was seen in the ER. In eukaryotic cells, the ER compartment is involved in quality control of translation, and it disposes of misfolded proteins through ERAD (ER-associated degradation). This was confirmed using a proteasome inhibitor, lactacystin, suggesting that a majority of translated α3 in the L924P mutant was misfolded and went through the proteasome degradation pathway. In parallel, we demonstrated that at least one arm of the Unfolded Protein Response (UPR), the adaptive mechanism elicited to overcome cellular stress, is utilized in the L924P mutants: there was accumulation of PERK kinase with phosphorylation of its substrate, the eIF2α transcription factor. The data suggest that in parallel with removal of misfolded protein through ERAD, UPR down-regulates the protein translation rate to limit the amount of misfolded proteins entering the ER. It is possible to overcome impaired biosynthesis in the L924P mutant. We grew cells at a lower temperature (33ºC vs 35ºC). As a result, improved biosynthesis of α3 was achieved after 4 days in culture. Similarly, improved biosynthesis was obtained by utilizing a small chemical chaperone 4-phenylbutyric acid, 4-PBA. The results with 4-PBA-assisted folding correction in the L924P mutant supports the development of future therapeutics based on chaperone-assisted or drug-assisted correction of misfolding. Supported by NS058949 to A. Brashear.

HEK293	a basic human cell line
ER	endoplasmic reticulum, where membrane proteins are synthesized
EIEE	early infantile epileptic encephalopathy (many genes, not just ATP1A3)
UPR	a set of programs that a cell can use to protect itself from mutation-caused misfolding
PERK, eIF2	a pathway that reduces normal protein translation but increases defensive proteins

Selected oral presentation

ATP1A3 wild type and mutated isoforms molecular dynamics simulations in a lipid membrane bilayer. Insights on protein structure and ion interactions.

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Alternating hemiplegia of childhood (AHC) is an extremely rare neurological disorder primarily caused by mutations on the ATP1A3 gene which codes the Na^+/K^+-ATPase subunit alpha-3 (NKA α3), an essential cation pump protein responsible for the maintenance of the sodium and potassium gradients across the plasma membrane.[1] The mutations mainly involved in the AHC occurrences are D801N and E815K.[2] This study aims to gain structural and functional insights on this Na^+/K^+ pump protein and to inspect how sequence mutations can affect the structural arrangement and the functioning of the ion flow through the pump. The attention was focused on both on the E1 and the E2 conformational states of the enzyme, where the channel is accessible by Na^+ ions from the intracellular environment and by the K^+ ions from the outside of the cell respectively. Since no experimental structure is available, homology modelling techniques were applied in order to build three-dimensional structures of the wild type (wt) NKA α3 and the two mutants D801N and E815K. The six structures were embedded into a DOPC bilayer and then submitted to molecular dynamics simulations (MD) in presence of water and KCl or NaCl, depending on the conformational state, at the concentration of 0.15 M. For each one of the six systems submitted to MD, a simulation time of 200 ns was achieved, for 1.2 μs in total. Analysis of the simulations allowed us to understand how the two mutations affect the structural stability and the affinity for ions in both the E1 and E2 states. The results showed that both mutations impair the protein affinity for the ions and alter the structural stability in at least one conformational state.

[1] Roswich H, Thiele H, Ohlenbusch A, et al. (2012). "Heterozygous de-novo mutations in ATP1A3 in patients with alternating hemiplegia of childhood: a whole-exome sequencing gene-identification study". *Lancet Neurol.* 11 (9): 764–73. doi:10.1016/S1474-4422(12)70182-5.

[2] Heinzen EL, Swoboda KJ, Hitomi Y, et al. (2012). "*De novo* mutations in ATP1A3 cause alternating hemiplegia of childhood". *Nature Genet*ics 44 (9): 1030–4. doi:10.1038/ng.2358

Selected oral presentation

Human neuroblastoma model of AHC: towards a medium throughput screening of candidate therapeutic compounds

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Over the last few years, we have developed and characterized a cellular model of AHC, based on a human neuroblastoma cell line (SH-SY5Y). For the construction of the AHC model, SH-SY5Y have been stably transfected with the pcDNA3.1 vector, expressing the wild type form or four different ATP1A3 variants: E815K, D801N, D801Y, G947R. Mixed cell population underwent to clonal selection. The levels of expression of endogenous and mutated *ATP1A3* mRNA have been determined by absolute real time PCR, and clones expressing higher levels of mutated cDNAs were prioritized.

The electrophysiological characterization showed the accumulation of Na^+ and Ca^{2+} in both E815K and D801N, as well as the reduction of the membrane resting potential.

Our model provide us with a striking phenotype that can be easily tracked and evaluated by high-content screening platforms. We have analyzed about 600 safe-in-men compounds for their ability to restore Na^+ and Ca^{2+} levels in cells expressing the D801N variant. After two tiers of screening, 27 compounds were prioritized for their ability to reduce Na^+ levels specifically in mutated cells, leaving intact the ion concentration in the naive. These molecules are currently under further characterization and preclinical evaluation.

This work was supported by AISEA and AESHA.

Invited speaker

Effects of Flunarizine on iPSC-derived Neurons from AHC Patients Exhibiting Divergent Clinical Responses

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Flunarizine, a lipophilic diphenylpiperazine derivative and ion channel blocker, is used widely to treat AHC but the response is highly variable and the neurophysiological mechanisms responsible for its efficacy are unknown. We investigated the cellular effects of flunarizine on human neurons derived from human induced pluripotent stem cells (iPSCs) and compared effects on hiPSC-derived neurons from two AHC patients with divergent clinical responses to the drug. Both patients were females who are heterozygous for the same *de novo* ATP1A3 mutation (p.G947R). Patient 1 (described in Simmons, et al., *Neurobiol Dis*, 115:29-38, 2018) began having hemiplegic attacks at age 10 weeks and was diagnosed with AHC at age 11 months. She exhibited a clinical response to flunarizine (i.e., reduced frequency and duration of hemiplegic attacks). Patient 2 began having hemiplegic attacks at age 18 months and showed no clinical benefit with flunarizine treatment (described in Delorme, et al., *Ped Neurol*, 68:79-80, 2017). Induced pluripotent stem cells (iPSC) were generated from each patient, and cortical excitatory neurons were differentiated from iPSC lines using NGN2 induction followed by maturation on primary mouse glial cells. To investigate the action of flunarizine, we used whole cell patch clamp recording in current clamp mode to quantify action potential firing frequency evoked by a range of current stimuli. In initial experiments using iPSC-derived neurons from the flunarizine-responsive patient (Patient 1), acute application of flunarizine at concentrations approximating the therapeutic range in human brain tissue (0.1 – 0.5 μM) caused a reversible suppression of action potential firing with complete suppression of excitability at 5 μM (10-fold higher than the therapeutic range). In neurons (28-33 days post-induction) from both patients, acute application of flunarizine exhibited a concentration-dependent suppression of action potential firing frequency. However, the efficacy of flunarizine to suppress action potential firing was greater in neurons from Proband 1 (n = 14) than Proband 2 (n = 20) when quantified at a stimulus of 150 pA (holding potential -80 mV). These preliminary findings suggest that cell autonomous mechanisms may contribute to differences in flunarizine clinical responsiveness.

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Selected oral presentation

ATP1A3 variants in a Sudden Infant Death Syndrome cohort

By **Catherine Brownstein**, Christine Keywan, Ingrid Holm, Annapurna Poduri, and Richard Goldstein

Sudden infant death syndrome (SIDS), the death of an infant less than 1 year of age that remains unexplained after complete autopsy and death scene investigation, is the leading cause of post-neonatal infant mortality in the United States. SIDS is hypothesized to result from the interaction of intrinsic vulnerabilities in the infant, a critical developmental period, and exogenous stressors in what has been called the 'triple-risk' model of SIDS. In one study, the majority of SIDS infants (57%) had at least two extrinsic risks and one intrinsic risk factor.

We have an active investigation into potential mechanisms and genetic syndrome-related genes implicated in apnea and epilepsy, including *ATP1A3*, in the predisposition of some children to sudden death. We performed whole exome sequencing to evaluate a SIDS cohort with this hypothesis in mind. Among the findings, we report the discovery of a previously unreported, extremely conserved, and predicted pathogenic *ATP1A3* variant in a SIDS case. Further investigation into the association between *ATP1A3* and SIDS may extend the spectrum of *ATP1A3* to sudden death.

Selected oral presentation