
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
Representative of the AHC Association of Iceland
Email: ahc@ahc.is

Karin Lykke-Hartmann
Associate professor
Department of Biomedicine
Aarhus University, Denmark
Email: kly@biomed.au.dk

Hanne Poulsen
Associate professor
Department of Molecular Biology and Genetics
The Danish Research Institute of Translational Neuroscience - DANDRITE
Aarhus University, Denmark
Email: hp@mbg.au.dk

Arn M.J.M van den Maagdenberg
Professor Molecular and functional neurogenetics
Dept. Neurology & Dept. Human Genetics
Leiden University Medical Center, The Netherlands
Email: A.M.J.M.van_den_Maagdenberg@lumc.nl

Hendrik Rosewich
Assistant Professor
Department of Pediatrics and Adolescent Medicine
University Medical Center Goettingen, Georg August University, Germany
Email: hendrik.rosewich@med.uni-goettingen.de

Poul Nissen
Professor of Protein Biochemistry
Department of Molecular Biology and Genetics
The Danish Research Institute of Translational Neuroscience - DANDRITE
Aarhus University, Denmark
Email: pn@mbg.au.dk

Administrative Assistant:

Karen Bech-Pedersen
Department of Molecular Biology and Genetics
The Danish Research Institute of Translational Neuroscience - DANDRITE
Aarhus University, Denmark
Email: karenb@mbg.au.dk

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
4) Pr Stéphane Marret, Department of Pediatric Neurology outpatient clinic / Neonatal pediatrics and Intensive care, CHU de Rouen, France
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
eleni.panagiotakaki@chu-lyon.fr

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]

[1] - Science for Life Laboratory, Department of Applied Physics, Royal Institute of Technology, Stockholm, Sweden
[2] - Department of Neurology and Clinical Neuroscience, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden
[3] - Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden
[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[♯]

(1) Department of Paediatric Clinical Epileptology, sleep disorders and Functional Neurology, University Hospitals of Lyon; Member of the ERN EpiCARE, Lyon, France (2) Service de Neurologie Pédiatrique, Hôpital Trousseau, APHP, Paris, France (3) Centre d'Investigation Clinique, CHU Montpellier, Montpellier, France (4) Hospices Civils de Lyon, Department of Medical Genetics, Centre de Biologie Est, Lyon University Hospital, Member of the ERN EpiCARE, Lyon, France (5) Laboratoire de Génétique, Groupe hospitalier Lariboisière-Fernand Widal AP-HP, Paris, France (6) IGF, Univ. Montpellier, CNRS, INSERM, Montpellier, France (7) Département de Neuropédiatrie, CHU Gui de Chauliac, INSERM U 1051, Institut des Neurosciences de Montpellier Montpellier, France

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]

(1) Département de Neuropédiatrie, CHU Gui de Chauliac, Montpellier, France (2) Service de Neuropédiatrie et Handicaps, Hôpital Gatien de Clocheville, CHU Tours, France (3) Pediatric Neurology Unit, Cliniques Universitaires Saint-Luc, UCLouvain, Brussels, Belgium (4) Service de Neuropédiatrie, CHU de Bicêtre, Kremlin-Bicêtre, France (5) Service de Neuropédiatrie, CHU Lille, Lille, France (6) Service de Neurologie Pédiatrique, Hôpital Trousseau, APHP, Paris, France (7)ervice de Neurochirurgie pédiatrique, Hôpital Necker-Enfants Malades, APHP, Paris, France (8)nUniversity Sorbonne Paris Cité, UFR SMBH Bobigny INSERM, Université Diderot, Paris, France (9) Service de Neurophysiologie, Hôpital Necker, AP-HP, Paris, France (10) Département de Pédiatrie, CHU Limoges, Limoges, France (11) Service de Neurologie Pédiatrique, CHU Timone Enfants, Marseille, France (12) Département de Neurologie Pédiatrique, Hospices Civils de Lyon, Bron, France

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
2 Mass Spectrometry Platform, The Francis Crick Institute, London, UK
3 Protein Purifcation Facility, The Francis Crick Institute, London UK

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

Elena Arystarkhova[1] and Kathleen J. Sweadner

Dept. of Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, USA

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.

Lorenzo Antonini,[a,b] Alexandros Patsilinakos,[a,b] Rino Ragno[a,b]
[a] *Department of Drug Chemistry and Technology, Sapienza University, P.le Aldo Moro 5, 00185 Rome, Italy*
[b] *Alchemical Dynamics s.r.l., 00125 Rome, Italy*
E-mail: lorenzo.antonini@uniroma1.it

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

Novelli A. [1*], Abiusi E.[1*], De Billy De Crispin E.[2], Spartano S. [1], Piacentini R. [3], Diano F. [1], Cocco S. [3], Di Pietro L[1], Ripoli C. [3], Gurrieri F [1,4], **Tiziano FD[1]**,

[1]: Institute of Genomic Medicine, Catholic University of Rome, Italy; [2]: Department of Hematology/Oncology and Stem Cells Transplantation, Bambino Gesù Children Hospital, Rome, Italy; [3]: Institute of Human Physiology, Catholic University of Rome, Italy; [4]: Fondazione Policlinico Universitario IRCCS "A. Gemelli", Roma. *: these authors contributed equally

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

Christine Q. Simmons,[1] Christopher H. Thompson,[1] Cecilia Bonnet,[2] Emmanuel Roze,[2] Kevin C. Ess,[3] and **Alfred L. George, Jr.[1]**

[1]*Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, USA*

[2]*Département de Neurologie, APHP, Hôpital Pitié Salpêtrière, Paris, France*

[3]*Department of Pediatrics, Vanderbilt University, Nashville, USA*

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.

Funding: Association Française Hémiplégie Alternante, and AHC Foundation (USA)

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

Poster list

Submitted poster abstracts

Agnese Novelli
Genomic Medicine Institute, Catholic University of Sacred Heart, Rome

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

Christine Q. Simmons,[1] Christopher H. Thompson,[1] Cecilia Bonnet,[2] Emmanuel Roze,[2] Kevin C. Ess,[3] and Alfred L. George, Jr.[1]
[1]Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, USA,
[2]Département de Neurologie, APHP, Hôpital Pitié Salpêtrière, Paris, France,
[3]Department of Pediatrics, Vanderbilt University, Nashville, USA

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

Snow JP, Westlake GMP, and Ess KC
Vanderbilt University Medical Center, Department of Pediatrics, Nashville TN

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

Richard S Smith[1,2,3], Catherine A. Brownstein[1,2,3], Chrystal Mavros[1,2,3], Elizabeth Buttermore[4],Christopher Thompson[4], Christine Q. Simmons[4], Alfred George[4], Christopher A Walsh[1,2,3], **Joseph Gonzalez-Heydrich**[5,6]
[1]Division of Genetics and Genomics, Boston Children's Hospital, Boston, Massachusetts 02115, USA;
[2]The Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, Massachusetts 02115, USA;
[3]Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, USA;
[4]Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60208, USA;
[5]Department of Psychiatry, Harvard Medical School, Boston, Massachusetts 02115, USA;
[6]Developmental Neuropsychiatry Research Program, Department of Psychiatry, Boston Children's Hospital, Boston, Massachusetts

Cellular modeling of a de novo mutation in Na^+/K^+ ATPase α-subunit involved in childhood-onset schizophrenia(COS)

Kate Vezyroglou[a,b], M. Kurian[a,b], C. Eltze[b], L. Carr[b], P. Prabhakar[b], R. Robinson[b], C. Hemingway[b], J. Helen Cross[ab]
[a]Institute of Child Health, Great Ormond Street BRC, University College London, London, UK [b]Great Ormond Street Hospital, London, UK

Genotype-Phenotype Correlation in ATP1A3-related disease. A literature review and 5 new mutations resulting in 5 separate phenotypes.

Katherine Behl[1], Dr Don Urquhart[2], Dr Catherine McDougall[3], Dr Richard Chin[4], Dr K Kamath Tallur[5]
[1] Parent of AHC child, Edinburgh; [2]Consultant Paediatric Respiratory Medicine, Royal Hospital for Sick Children, Edinburgh; [3]Consultant Paediatric Intensive Care and Respiratory Medicine, Royal Hospital for Sick Children, Edinburgh; [4]Senior Clinical Lecturer Paediatric Neurology, Director Muir Maxwell Epilepsy Centre, Child Life & Health, University of Edinburgh;[5]Consultant Paediatric Neurologist Royal Hospital for Sick Children, Edinburgh

Hypopnoea/Apnoea in Alternating Hemiplegia of Childhood

Kiyoshi Kawakami[1], Shin'Ichiro Satake[2,3], Keiko Ikeda[1,4]
[1]Jichi Medical University, Shimotsuke, Japan; [2]National Institute for Physiological Sciences, Okazaki, Japan; [3]The Graduate University for Advanced Studies, Okazaki, Japan; [4]International University of Health and Welfare, Narita, Japan

Weakened glutamate uptake in cerebellar Purkinje cells in Atp1a3 heterozygous knockout mice: glial compensation and its impacts on long-term depression

Livia Pisciotta[1 2], Ramona Cordani[1], Michela Stagnaro[1], Marcella Gherzi[2], I.B.AHC Consortium, Lino Nobili [1 2], Elisa De Grandis.[1 2]
1 Child Neuropsychiatry Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy.
2 DINOGMI Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics and Maternal and Children's Sciences, University of Genoa, Genoa, Italy.

Neuropsychological, cognitive, adaptive, and psychiatric features in 11 alternating hemiplegia of childhood Italian patients

Lyndsey Prange, Julie Uchitel, Melissa McLean, and Mohamad A. Mikati
Duke University Health System, Division of Pediatric Neurology, Durham, NC

Reduced Awareness Spells: A Newly Recognized Type of Spells in Alternating Hemiplegia of Childhood.

Mette Ozol and Hanne Poulsen
Dept. Molecular Biology and Genetics, DANDRITE – The Danish Research Institute of Translational Neuroscience, Aarhus University

Voltage clamp fluorometry of the sodium/potassium ATPase

Natalia V. Akkuratova, Igor Adameyko
Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden;

Distribution of α subunits of Na,K-ATPase during early development

Nikolay Kefilev
MC "Laser Med" - Sofia

Pathogenic renin secretion - Suppression Development Mechanism by the kidneys JGA

Cristina Moreno Vadillo, PhD, **Sho Yano**, MD PhD (Miguel Holmgren laboratory)
National Institutes of Health, Bethesda, MD, USA

Characterizing the effects of disease-causing variants on Na+/K+-ATPase function

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

Christine Q. Simmons,[1] Christopher H. Thompson,[1] Cecilia Bonnet,[2] Emmanuel Roze,[2] Kevin C. Ess,[3] and Alfred L. George, Jr.[1]

[1]*Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, USA*

[2]*Département de Neurologie, APHP, Hôpital Pitié Salpêtrière, Paris, France*

[3]*Department of Pediatrics, Vanderbilt University, Nashville, USA*

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.

Funding: Association Française Hémiplégie Alternante, and AHC Foundation (USA)

Title: Cellular modeling of a *de novo* mutation in Na^+/K^+ ATPase α-subunit involved in childhood-onset schizophrenia(COS)

Authors: Richard S Smith[1,2,3], Catherine A. Brownstein[1,2,3], Chrystal Mavros[1,2,3], Elizabeth Buttermore[4],Christopher Thompson[4], Christine Q. Simmons[4], Alfred George[4], Christopher A Walsh[1,2,3], Joseph Gonzalez-Heydrich[5,6]

[1]Division of Genetics and Genomics, Boston Children's Hospital, Boston, Massachusetts 02115, USA;
[2]The Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, Massachusetts 02115, USA;
[3]Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115, USA;
[4]Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60208, USA;
[5]Department of Psychiatry, Harvard Medical School, Boston, Massachusetts 02115, USA;
[6]Developmental Neuropsychiatry Research Program, Department of Psychiatry, Boston Children's Hospital, Boston, Massachusetts 02115, USA;

Abstract:
The role of Na^+/K^+ ATPase α-subunit *ATP1A3* in human disease is varied, causing rapid-onset dystonia-parkinsonism (RDP); alternating hemiplegia of childhood (ACH); cerebellar ataxia, areflexia, *pes cavus*, optic atrophy, and sensorineural hearing loss (CAPOS); and recently childhood onset schizophrenia (COS). How point mutations at different *ATP1A3* alleles generate these vastly different patient phenotypes remains an open question. Here we present an individual with COS resulting from a heterozygous *de novo* mutation c.385G>A (p.V129M) in *ATP1A3*. We derived induced pluripotent stem cells (iPSC) from the p.V129M proband and a parental control, and use Cas9 genome editing to generate an isogenic control from the proband IPSCs. We differentiated these cells into excitatory cortical neurons via Neurogenin-2 and GABAergic cells via iGABA protocols. Excitatory neurons plated on multi-electrode arrays demonstrated limited electrophysiological differences comparing p.V129M proband to parental control; including comparable resting spontaneous activity and electrode evoked network activity. However, rates of neuronal survival was improved in the proband during these experiments, suggesting altered *ATP1A3* pump function could help stabilize early growth periods. RNAseq based transcriptome analysis of proband excitatory and inhibitory neurons also revealed an enhancement of transcriptional programs related to psychiatric diseases. These results demonstrate p.V129M *ATP1A3* has significant cellular deficits and offers a valid cell-based platform for mechanistic modeling of COS and future drug screens.

Genotype-Phenotype Correlation in ATP1A3-related disease. A literature review and 5 new mutations resulting in 5 separate phenotypes.

K. Vezyroglou[a,b], M. Kurian[a,b], C. Eltze[b], L. Carr[b], P. Prabhakar[b], R. Robinson[b], C. Hemingway[b], J. Helen Cross[ab]

[a]*Institute of Child Health, Great Ormond Street BRC, University College London, London, UK*
[b]*Great Ormond Street Hospital, London, UK*

k.vezyroglou@ucl.ac.uk

Aim: To review the mutation specific phenotypic information available in the literature and compare it to our patient cohort.

Methods: We performed a pubmed search with the keyword ATP1A3 and reviewed specific phenotypic features associated with each *ATP1A3* mutation. Genotype and phenotype of our cohort at Great Ormond Street Hospital (GOSH) was compared to available published information.

Results: 110 *ATP1A3* mutations have been published since 2004. Of those, 72 were associated with Alternating Hemiplegia of Childhood (AHC), 10 with Rapid-Onset Dystonia Parkinsonism (RDP), 4 with either AHC or RDP, 1 with CAPOS, 7 with Early Infantile Epileptic Encephalopathy, 2 with Childhood Onset Schizophrenia and 11 with intermediate phenotypes. For 97 mutations a more detailed phenotypic description was available. Of the 677 patients known to us (literature + GOSH cohort), 430 (63.5%) carried 1 of 7 most common mutations. Those were 3 AHC mutations (D801N(24.5%), E815K(13.7%), G947R(7.2%)), an RDP mutation T613M(4.3%), the CAPOS mutation E818K(7.8%) and 2 mutations at residue 756 (R756C(2%) and R756H(3.8%)) associated with the emerging intermediate phenotype RECA. The phenotypic features of our patients are consistent with the information published on their mutation. Five of our patients carry previously unreported de novo mutations. Patient 1 (c.990_993+2del/ splice site) presents with mild AHC. In patient 2 (c.2213T>G/ p.M738R) the phenotype is dominated by early onset drug resistant epilepsy characterized by apneic seizures. Patient 3 (c. 958G>A/ p.A320T) presented with distal arthrogriposis. In the first year of life he started having abnormal eye movements, dystonic, apneic episodes and epileptic seizures. He has never had hemiplegic events. Patient 4 (c.964G>A/ p.V322I) presents with episodes of hemidystonia and ataxia. Patient 5 (c.1006C>T/ p.P336S) is microcephalic and predominantly presents with frequent episodes of generalized dystonia that resolve in sleep.

Conclusion: Mutations in *ATP1A3* result in a vast variability of phenotypes and novel mutations continue to emerge. Most patients share the commonest mutations linked to specific phenotypes, thus facilitating more accurate prognosis. It seems likely that collecting consistent information also on the rarer mutations will help define their associated phenotype in more detail. We propose an internet-based database to collect anonymized mutation specific phenotypic features with the view of developing a prognostic tool.

Hypopnoea/Apnoea in Alternating Hemiplegia of Childhood

Mrs Katherine Behl[1], Dr Don Urquhart[2], Dr Catherine McDougall[3], Dr Richard Chin[4], Dr K Kamath Tallur[5]

[1] Parent of AHC child, Edinburgh; [2]Consultant Paediatric Respiratory Medicine, Royal Hospital for Sick Children, Edinburgh; [3]Consultant Paediatric Intensive Care and Respiratory Medicine, Royal Hospital for Sick Children, Edinburgh; [4]Senior Clinical Lecturer Paediatric Neurology, Director Muir Maxwell Epilepsy Centre, Child Life & Health, University of Edinburgh;[5]Consultant Paediatric Neurologist Royal Hospital for Sick Children, Edinburgh

Background: Alternating Hemiplegia of childhood (AHC) is a rare neurological condition affecting children and is associated with episodes of dystonia, nystagmus, weakness, and autonomic disturbances and in some case, epileptic seizures. Whilst limited data are available on the association of AHC with sleep-disordered breathing, information on prevalence and management of respiratory abnormalities such as hypopnoea/apnoea when awake are not yet well described.

Aims: To describe a case of AHC with apnoea and a review of the literature.

Methods: We present a review of literature and describe a patient with AHC who presents with frequent episodes of paralysis/dystonia in conjunction with prolonged epileptic seizures. We further describe episodes of apnoea and hypopnoea, including some requiring intubation and ventilation that she has experienced.

Results: The literature is sparse on the association of AHC with hypopnoea/apnoea. We further present the challenges of compiling a clinical deterioration management plan that encompasses situations that include apnoea/hypopnoea, seizure and dystonic episodes, with the roles of oxygen, bag-valve-mask ventilation and rescue medications all requiring clear guidance for use within a treatment algorithm.

Conclusion: This case highlights the rare but life threatening nature of varied clinical manifestations of AHC. It is important to collate the information on AHC children to develop clear clinical guidance to help children and their families which may prevent morbidity and mortality in the longer term. Standardisation of data collection may allow understanding of the prevalence of breathing control issue in AHC, and collective experience may help guide management for those with this rare condition.

Weakened glutamate uptake in cerebellar Purkinje cells in *Atp1a3* heterozygous knockout mice: glial compensation and its impacts on long-term depression

Kiyoshi Kawakami[1], Shin'Ichiro Satake[2,3], Keiko Ikeda[1,4]

[1]Jichi Medical University, Shimotsuke, Japan; [2]National Institute for Physiological Sciences, Okazaki, Japan; [3]The Graduate University for Advanced Studies, Okazaki, Japan; [4]International University of Health and Welfare, Narita, Japan

Excitatory amino acid transporters (EAATs) are responsible for cellular uptake of neurotransmitter glutamate utilizing the electrochemical Na^+/K^+ gradients across the cell membrane. Sodium pump plays a critical role in the maintenance of the gradient and mutations in the human Na pump α3 subunit gene, *ATP1A3*, have been identified as the cause for rapid-onset dystonia with Parkinsonism (RDP), alternating hemiplegia of childhood (AHC) and Cerebellar ataxia - areflexia - pes cavus - optic atrophy - sensorineural hearing loss (CAPOS) syndrome. However, it is unknown if EAATs contribute to the sodium pump neurological disorders. Considerable evidence suggests a possible involvement of the cerebellum in dystonia, although the basal ganglia have been proposed as the primary responsible region. The α3 subunit protein is abundantly expressed in Purkinje cells (PCs), the sole output neuron of the cerebellar cortex. Therefore, we examined the EAAT activity in the cerebellum in *Atp1a3* heterozygous knock out mice (*Atp1a3*$^{+/-}$). We found a remarkable reduction of the glutamate uptake-coupled currents mediated by the EAAT4 subtype in PCs in *Atp1a3*$^{+/-}$ compared with those of wild type littermates. On the contrary, the amplitude of EAAT currents in the astrocyte Bergmann glia (BG) was profoundly higher in *Atp1a3*$^{+/-}$. Consistently, the protein levels of the glia-specific EAAT1 subtype in the cerebellum were increased. Furthermore in *Atp1a3*$^{+/-}$, long-term depression (LTD, postsynaptic origin) was diminished at the excitatory synapses from parallel fibers (PFs) to PCs. The impaired LTD was rescued by application of the EAAT1 inhibitor UCPH102 and the mGluR1 agonist DHPG. Taken together, it is suggested that the enhanced glutamate uptake by BG submerged the weakened neuronal EAAT activity in *Atp1a3*$^{+/-}$, thereby attenuating the extracellular diffusion of the glutamate spilled out of the PF-PC synaptic clefts and reducing the activation of the perisynaptically distributed mGluR1s on PC dendritic spines. It is quite likely that these events interfere with LTD. Our findings would provide underlying mechanisms for the onset of dystonia symptoms in RDP and AHC.

Neuropsychological, cognitive, adaptive, and psychiatric features in 11 alternating hemiplegia of childhood Italian patients.

Livia Pisciotta[1 2], Ramona Cordani[1], Michela Stagnaro[1], Marcella Gherzi[2], I.B.AHC Consortium, Lino Nobili [1 2], Elisa De Grandis.[1 2]

1 Child Neuropsychiatry Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy.

2 DINOGMI Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics and Maternal and Children's Sciences, University of Genoa, Genoa, Italy.

Objective:
It is known that developmental delay, intellectual disability and behavioral disturbances occur in Alternating Hemiplegia in Childhood (AHC). Moreover, many AHC patients, in particular adults, may present psychopathological disorders, although these features are frequently poorly considered and not treated by specialists. Due to the rarity of the disease, large and systematic neuropsychological and psychopathological studies are lacking. We aim to characterize a cohort of patients with Alternating Hemiplegia of Childhood in terms of intellectual disability (Intelligent Quotient), adaptive impairment and psychiatric features.

Methods: we studied 11 Italian patients with AHC, aged from 17,2 to 48,9 years, 9 M, 2 F. Intelligent Quotient and adaptive skills were tested with age appropriate Wechsler Scale of Intelligence and Vineland Adaptive Behavior Scales Second Edition (VABS-II) questionnaires. Psychiatric features and behavioral abnormalities were evaluated clinically, according to DSM 5 criteria.

Results:
Intellectual Disability (ID) was detected in all cases. In particular 5/11 patients (50%) presented severe ID, 5/11 (42%) showed moderate ID and only 1 case had a mild cognitive impairment. In all cases Working Memory Index and Processing Speed Index were at the lowest scores.
As far as the adaptive level is concerned, this was moderately low or low in most domains, however communication and daily living skills (excluding the written and community subdomains) were the less impaired scales.
Four patients showed anxious features, functionally compromising, in 2 of them a depressive disorder was associated. In the other patients we found: non-self-directed aggressivity and oppositional traits (2/11) psychotic symptoms (2/11), oppositional-defiant disorder DSM 5 defined (1/11). The remaining 18% (2/11) did not present any particular psychopathological problem.

Conclusions:
AHC is a complex disorder in which cognitive, adaptive and psychopathological profile may be the consequence of several aspects, including paroxysmal and chronic motor dysfunctions. Our preliminary results suggest that the majority of AHC adult patients presents a moderate-severe ID. In spite of this, adaptive level is better, with daily living skills and communication being the less impaired domains. Psychopathological disturbances are common (82%). They seem to be “non-specific” and difficult to characterize in certain psychiatric disorders according to the most used criteria (DSM 5), similarly to those found in most diseases with cognitive delay. Other studies on larger cohorts, with the use of standardized tools and specific tests, seem necessary to possibly underline a typical psychiatric pattern, planning a more appropriate follow-up and a targeted treatment.

Voltage clamp fluorometry of the sodium/potassium ATPase

Mette Ozol and Hanne Poulsen

We use voltage clamp fluorometry (VCF) to study the sodium/potassium ATPase. VCF is a method which couples functional information about the ion transport to structural features of the ATPase. An environmental sensitive fluorophore is incorporated into the sodium/potassium ATPase and the ATPase is investigated using electrophysiology, which provides information of how the ions are transported by the ATPase. Simultaneously with the electrophysiological recording, the fluorescence is recorded. A change in fluorescence indicates that a conformational change in the ATPase has occurred (Figure 1). We use an unnatural amino acid (called Anap), which can be genetically incorporated into the sodium/potassium ATPase. In contrast to the common used labeling of cysteines this method enables labeling of all sites of the protein and not only extracellular sites. We study the conformational changes in the transmembrane region of the ATPase in response to sodium ion release. Initially this study will be used to provide information about details in the mechanism of the sodium/potassium ATPase, but the method also has the potential to elaborate on how disease mutations impair the function of the ATPase.

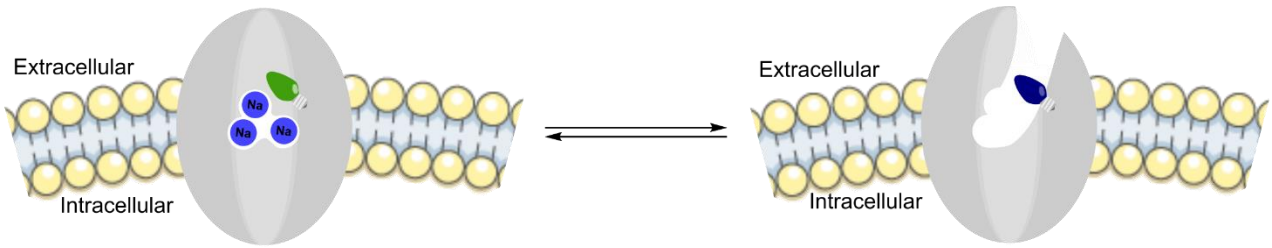

Figure 1. Release of three sodium ions from the sodium/potassium ATPase (grey oval) results in conformational changes. These can be observed by a strategically incorporated fluorophore (light bulb).

Distribution of α subunits of Na,K-ATPase during early development

Natalia V. Akkuratova[1], Igor Adameyko[1]

1 - Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden;

While it is commonly accepted that in adult organisms the α3 isoform of α subunit Na,K-ATPase is expressed only in neurons, it appears to have different expression patterns during embryonic development. We would like to compare distribution of α3 subunit to other alpha subunits during different embryonic stages in order to reveal a function of alpha3 during development.
We found unexpected patterns of expression of the α3 isoform on WT embryonic mouse slices. At the early embryonic stages the α3 isoform was expressed only in the heart while at later stages it was found in the heart, neurons and muscles. Interesting fact that the α1 subunit has the highest expression pattern in the heart at the same early stages too, while at the later stages it doesn't expressed in specific organs or tissues.
In the ongoing study we are performing *in situ* hybridization to validate IHC results and also to increase sensitivity since *in situ* is more accurate method than IHC. Using in situ probes we can finally show patterns of expression of the α2 subunit what was not possible applying IHC because there are no specific α2 antibodies.
Previously together with Mohamad Mikati and Karin Lykke-Hartmann we found phenotypical differences between mutants having AHC disease and WT embryos. Knowing the function of the α3 subunit will help us to find early pathology in this disease.

Pathogenic Renin Secretion Suppression Development Mechanism by the Kidneys' JGA in Alternating Hemiplegia of Childhood

Author: Nikolay Kefilev, MD

Due to the ubiquitous presence of the sodium-potassium ATPase in the human body, any impairment of its function leads to a pathological distribution of the sodium and potassium located on the sides of the cellular and mitochondrial membranes for the cells of all organs and systems. Under such conditions, there is a serious impairment of the body's homeostasis, including a significant shift in water and electrolyte balance. When the function of the sodium-potassium pump is insufficient, there is a higher intracellular accumulation of sodium ions. The presence of an abnormal quantity of these ions leads to intracellular hyperhydration and the development of edema within the cell. As such, its function is emphatically impeded.

Other than a base mechanism to support the water-electrolyte balance between the cells and the surrounding interstitium, the sodium-potassium pump is integrated into multiple executive and regulatory mechanisms that support the body's functional fitness.

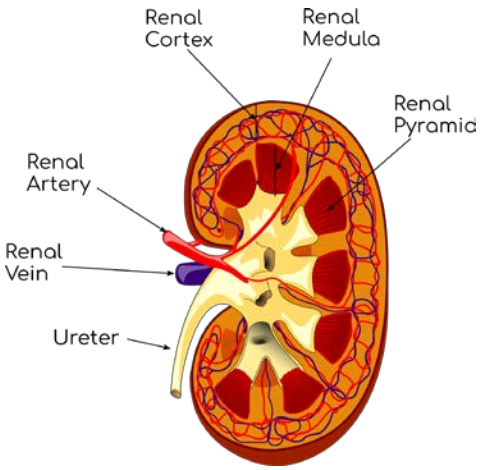

Figure 1: The kidney

A stark demonstration of this is the pathological change that occurs on a renal level when this condition is present.

The role of the sodium-potassium ATPase in sodium's reabsorption on the renal tubular system level can be classified as key. The physiological mechanism which enacts the transportation of the sodium ion through the tubular epithelial cells includes the availability of an apically located active transport system where the cells intracellularly exchange hydrogen cations for the sodium ions located in the tubular lumen. The proper function of the sodium-potassium ATPase located in the part of tubular epithelial cells neighboring the vascular capillary peritubular network (basal part) is essential for the transfer of these sodium ions from the cell's interior into the renal capillary system. Through this sodium-potassium pump, the tubular epithelial cells transfer intracellular sodium to the underlying capillary system, exchanging it for potassium ions. When reviewing this physiological mechanism, it is important to note that the number of active apical Na-H+ exchangers is variable. The number of these exchangers depends directly on the blood and tissue levels of Angiotensin II. Some authors (1, p. 119) note that parallel with the number of apical transport units, Angiotensin II also regulates the activity of basally-located Na-K-ATPase.

In the pathogenesis of the condition currently under review, considering this physiological mechanism that prevents the renal loss of sodium ions for the body, there is an impeded reabsorption of approximately 65% of the sodium filtered through the renal glomerulus. This reabsorption is performed alongside the tubular system, starting from the proximal nephron tubule and reaching to the juxtaglomerular apparatus (JGA)'s macula densa. The impeded sodium reabsorption leads to elevated natriuria in the aforementioned sections of the nephron and ultimately leads to this a high concentration of sodium in the part of the urine which passes past the JGA's macula densa. Here I must remind that within the JGA's macula densa, there is a receptor system that links directly to the granular, renin-secreting cell located on the wall of the arteriole adducing to the renal glomerulus, neighboring the JGA. The receptor system in question, when registering a high sodium concentration in the urine filtrate (detects a combination of sodium concentration and urine discharge), induces suppression of the granular cell's renin secretion. This suppression leads to the development of basal reninemia or even hyporeninemia. As a result of this hyporeninemia, there is a chronic hypoangiotensin II-emia condition within the body. In turn, developing a hypoangiotensin II-emia condition reduces the amount of Na-H+ exchangers in the renal tubular epithelial cells and reduces the sodium-potassium ATPase activity in those cells. This process results in a vicious cycle down the chain: Low NA-K-ATPase activity – high natriuria levels in the nephron's proximal end – suppression of renin secretion by the JGA and developing hyporeninemia – maintaining hypoangiotensin II-emia – low Na-H+ exchanger count in renal tubular epithelial cells – low sodium-potassium

ATPase activity.

Aside from maintaining this vicious cycle which could lead to severe hyponatremia in the body, we should note the negative effect lowered blood and tissue levels of angiotensin II have over vascular spasm levels, their effect on renal microcirculation, over the entire body's interstitium, and the altered regulation of its dependent E-group prostaglandins. Before I proceed with describing the mineralocorticoid hormone (aldosterone)'s behavior, I should note that the altered state mentioned above matches the patient's clinical condition when they are not undergoing an episode, and there are no manifesting neurological symptoms of listlessness.

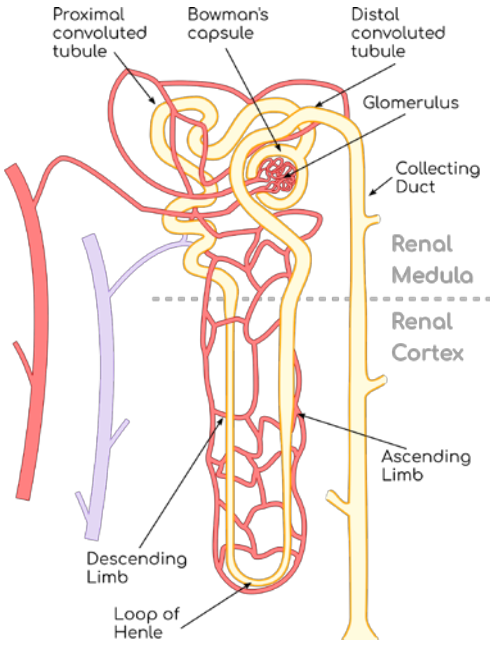

Figure 2: A nepron

Here is another question of high importance. How does the mineralocorticoid hormone aldosterone behave in a chronic suppression state of the renin-angiotensin II system?

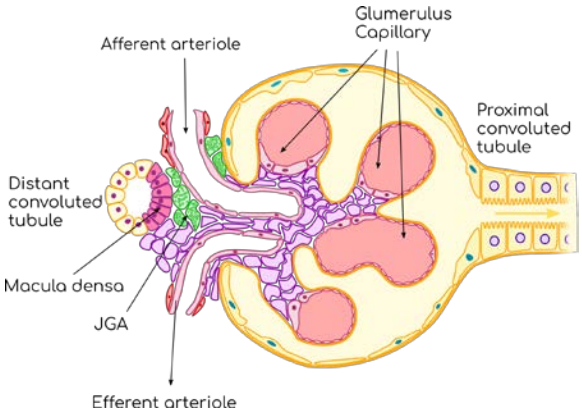

Figure 3: Bowman's capsule and JGA

The answer to this question is related to the matter of physiological mechanism(s) that regulate the secretion of aldosterone. The largest portion of members of the medical, and possibly scientific community accept that the base regulation of aldosterone secretion is dependent on blood levels of renin and angiotensin II. In scientific medical literature, the following term has been introduced:

Renin-angiotensin-aldosterone system (RAAS), indicative of an accepted and backed by facts statement that elevation in reninemia, and therefore angiotensin-II-emia, consecutively leads to an increase

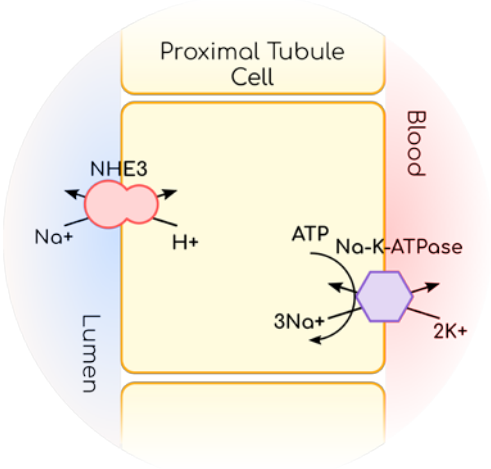

Figure 4: Proximal tubule cell

of aldosteronemia. Does this mean that a pathological reduction of reninemia and angiotensin-II-emia would cause a pathological decline in aldosteronemia?

Patient tests are a clear demonstration of this. Tests indicate hyporeninemia and levels of aldosteronemia that are higher than those expected for such reninemia levels. The clinical laboratory where the tests were made set a reference threshold for the aldosterone/renin value ratio. This threshold is set at 20. Any elevation to the aldosterone/renin ratio beyond 20 is classified as an autonomous aldosterone secretion process. In such cases, we accept that there is elevated secretion of aldosterone which has been developed via other mechanisms, regardless of reninemia (respectively angiotensin-II-emia) levels. In such a patient you should, therefore, exclude primary hyperaldosteronism (Conn's syndrome). In the case under review, the presence of normal aldosterone blood levels accompanied by basal natremia values and values matching hyponatremia rule out the presence of an autonomous process in the patient's adrenal gland. The patient's tests from the latency period are proof that base aldosterone regulation is not related to reninemia and angiotensin-II-emia.

I should note here that "reduction of sodium in the serum is the main stimulus for aldosterone secretion" (2, p. 548).

In this specific case: chronically elevated hypernatriuria values in the nephron's proximal end (next to the distal convoluted tubule) poses a risk for the body of developing severe hyponatremia. The body's response in the event of a pathological decrease of natremia is to elevate the aldosteronemia via its base physiological regulation mechanism which doesn't include the renin-angiotensin II system. By elevating aldosteronemia as a result of a pathological natremia decrease, hyponatremia manages to approximate the base reference value. Clinical laboratory test analysis for this patient indicates that aldosteronemia levels compensate for the renin and angiotensin insufficiency when it comes to the developed hypernatriuria condition in the nephron's proximal end.

The significance of this phenomenon is two-fold.

First: By using the aldosterone secretion's base regulation mechanism, the body manages to protect itself from severe hyponatremia should there be a pathological substance decrease on the renin-angiotensin II chain.

Second: Clinical laboratory testing of natremia, reninemia, and aldosteronemia can be used as a credible noninvasive test to discover medical and pathophysiological conditions where elevated glomerulus filtration and decreased sodium reabsorption are present in the renal tubular system. The combination of base natremia and hyponatremia with base reninemia levels or the presence of hyporeninemia and elevated aldosteronemia/reninemia ratio values prove the presence of renin-angiotensin II system suppression.

From a medical and clinical point of view, I should note that the chronic base natremia and hyponatremia condition, when combined with base angiotensin II (and hypoangiotensin-II-emia) levels, leads to a chronic lack of thirst. The lack of thirst is a symptom that should be actively sought when taking the medical history of the parents of patients with alternating hemiplegia of childhood.

Medical reference used:

1. *Проф. Д. Ийтън, Проф. Дж. Пулър. – Бъбречна физиология на Вандер. – 2011 г. Изд. „Парадигма", преводно издание, превод: д-р Васил Василев*

2. *Белоев, Й.Д. дмн, Илиев, И.Т. дмн, Колебинов, Н.Х. дмн, Попов, А.Т. проф, Цанев, А.Н. дмн. – Ръководство по вътрешни болести – том 3 – под редакцията на Акад. А. Малеев и доц. Св. Иванов. Издание: 1986г., Изд.: „Медицина и физкултура"*

Hypothesis we still need to research?

Statement #1

The neurogenic sympathicus (NS) initiates the crisis (it appears to be the triggering factor)

Factors supporting the statement

1. When sleeping, patients have no crisis periods
2. Provocation in changing the "mood" of patients
3. Delaying the crisis and good response to midazolam

Required tests to prove the statement

Monitoring the patient for a heart rate increase (peripheral arterial pulse), arterial blood pressure increases.

Statement #2

The activated neurogenic sympathicus leads to very strong humoral sympathicotonia with a rapid increase of the humoral catecholamines (adrenaline, noradrenaline), angiotensin II, vasopressin (ADH), leading to very increased vascular spasm of the brain vessels.

Increase of the aldosterone level due to the increase during the crisis of the angiotensin II-emia.

Factors supporting the statement

1. Increase of the natremia compared to the previous period and decrease of the kalemia (improvement of the work of the sodium-potassium ATPase in the presence of increased levels of adrenaline and angiotensin II or as a result of the increase of the aldosteronemia during a crisis)
2. Decrease of the diuresis during a crisis?
3. Therapeutic behavior: An attempt for a decrease in the level of the brain vascular spasm through vasodilators of the brain vessels: Flunarizine, alpha blockers, possibly: Calcium antagonist (Nimodipine)

Required tests to prove the statement

1. Electrolytes: sodium, potassium, calcium;
2. Humoral vasoconstrictors: noradrenaline, adrenaline, renin, angiotensin II, vasopressin (ADH), cortisol, aldosterone
3. Tests of a breakdown product of catecholamines in 24-hour diuresis (urine): Nephrin, metanephrine, vanilmandelic acid.

Comparison of the obtained results with results from tests during a non-crisis period.

Statement #3

The increase of the vascular spasm is not accompanied by physiological increase of prostaglandins of the E group.

Factors supporting the statement

There is no increase in body temperature up to 37,5 degrees Celsius, decreased diuresis is present, there is an increase of the arterial blood pressure (in preliminary sufficient fluid intake).

Required tests to prove the statement

Testing the blood levels of Prostaglandin E at the beginning of the development of the crisis. Multiple tests are required due to the transient presence of these substances.

Characterizing the effects of disease-causing variants on Na^+/K^+-ATPase function

Cristina Moreno Vadillo, PhD, **Sho Yano, MD PhD** (Miguel Holmgren laboratory)
National Institutes of Health, Bethesda, MD, USA

The genotype-phenotype correlations observed in *ATP1A3*-associated disorders suggest that different disease-causing mutations have differing effects on Na^+/K^+-ATPase function that go beyond simple loss of pump activity. While certain recurrent mutations are associated with more severe phenotypes, such as E815K in AHC, others cause distinct syndromes, such as the E818K mutation in CAPOS syndrome and R756 mutations associated with fever-triggered neurological deficits. Determining which specific impairments of Na^+/K^+-ATPase function are common to many disease mutations, and which are associated with more severe symptoms, could lead to targets for rationally designed drug therapy.

Since mutations closer to the ion binding sites in the protein structure tend to cause more severe disease, one potential explanation for phenotypic differences between mutations is that they have different effects on ion binding. In fact, characterization of Na^+ binding using transient Na^+-mediated currents indicates that severity of disease mutations may be related to which Na^+ ion binding site(s) are affected. Human α3/β1 Na^+/K^+-ATPases were expressed in *Xenopus* oocytes and the binding/release of individual Na^+ was measured using the cut-open vaseline gap method. While D923N primarily affected the binding of the last Na^+ ion, D801N targeted the binding of the second Na^+ ion, consistent with published crystal structures indicating that D923 coordinates the site III Na^+ ion and D801 coordinates the site I and II Na^+ ions. In contrast, E815K impaired binding of the first Na^+ ion, possibly by indirectly disrupting this binding site or affecting E1P-E2P conformational transitions of the Na^+/K^+ ATPase. Interference with sequential binding of the other two Na^+ ions might explain why E815K results in a more severe phenotype despite being located farther from the binding site. R756H, which is associated with a relatively milder phenotype, reduced the binding of the second Na^+ ion but, in contrast to D801N, did not abolish binding of the last Na^+ ion.

Other possibilities include dominant-negative effects on the function of wildtype Na^+/K^+-ATPases. To study the effects of variant Na^+/K^+-ATPases on the abundance, regulation and localization of wildtype Na^+/K^+-ATPases in the same cell, a mouse neuroblastoma model system is being developed using fluorescently tagged murine *Atp1a3* and *Atp1b1.* Finally, identifying and characterizing additional disease mutations in paralogous alpha subunit genes or in beta subunit genes could help identify common mechanisms of pathogenesis shared between different isoforms of the pump. Initial functional studies of candidate variants in these genes are being performed by two-electrode voltage-clamp measurements of pump currents from human Na^+/K^+-ATPases expressed in *Xenopus* oocytes.

Glossary ATP1A3 Symposium 2019, Iceland

This glossary aims to cover some key terms that will come up in the conference including both scientific and medical terms.

If there are some terms mentioned during the conference, please highlight these to the organising committee so we can make sure these are covered during the question and answer sessions.

The glossary is split up in sections to make following lectures around these topics easier.

Scientific terms

Cell structure (general cells):

Cell (plasma) membrane – the lipid layer surrounding a cell, which separates the inside from the outside. The membrane contains many lipids and enzymes, which control the movement of substances into and out of the cell.
Endoplasmic reticulum (ER) – a structure inside a cell. This is where membrane proteins are synthesized, and protein molecules folded. It is a confined part of the cell, surrounded by a membrane.
Nucleus – centre of the cell that contains almost all the genetic material. It is a confined part of the cell, surrounded by a membrane.
Mitochondria – part of the cell that produces energy (it is regarded the energy factory inside a cell). It generates most of the cell's supply of ATP. It is a confined part of the cell, surrounded by a membrane.
Transmembrane – crossing of the plasma membrane, so from inside to outside the cell, or the other way around.
Pluriopotent stem cell (PSC) - a sub-type of stem cell which can differentiate into any specialised cell and is found naturally early in development.
[see below] **iPSC-derived** – **i**nduced **p**luripotent **s**tem **c**ells. Produced in laboratories from specialised cells e.g. skin cells. These cells have the ability to differentiate into any specialised cell and potentially replace diseased cells.
Stem cell – a cell with a unique ability to differentiate into many types of cells. When the word pluripotent is added, this indicates that the stem cell can differentiate into any of the 200 plus specialised cell types within the body. Multipotent stem cells, however, can differentiate into a limited number of specialised cell types. Early embryos have the highest ability to differentiate into any cell.
Isogenic cell lines – cells from a single donor that are engineered and used in research to model a disease.
HEK293 – a basic human cell line derived from human embryonic kidney.
UPR – unfolded **p**rotein **r**esponse is a set of programmes that a cell can use to protect itself from mutation-caused misfolding.
PERK, eIF2α - a pathway that reduces normal protein translation but increases defensive proteins.

Other terms:

Cation – positively charged ion (molecule)
Anion – negatively charged ion (molecule)
Intrinsic – internal factor
Extrinsic – external factor
Exogenous – external cause, something originating from outside the cell or human body.
Endogenous – internal cause, something originating from within the cell or human body.

Nerve cells

Neuron/nerve cell– a cell of the nervous system and consists of several parts (axon, dendrites) and conducts nerve impulses through synapses.
Cortical - pertaining to the outer layer of the brain, the cerebral cortex. It plays a role in perception, though and consciousness.
Myelin – protective sheath/cover around the axon. Like insulation around an electric wire, it ensures that the electrical signals pass quickly.
Dendrites – short branch-like extensions of a neuron that connects to other neurons.
Axon – the long extension of a neuron. It conducts an electrical signal (action potential) to the synapse at the end, where it triggers the release of a neurotransmitter. The longest axons in the human body can be over a metre long.
Synapse – gap or space between brain cells. Chemical signals (neurotransmitters) are released from one neuron and cross this gap to get to the next cell/neuron to pass on the message/action.
Pre-synaptic – before the gap (some drugs work on this site).
Post-synaptic – after the gap (some drugs work on this site).
GABAergic neuron – neurons that make GABA (neurotransmitter)
Glutamatergic neuron – neurons that make glutamate (neurotransmitter)

Neurotransmitters – *these are a variety of chemicals that are produced in the body to transmit signals within the nervous system*

GABA – gamma-aminobutyric acid, is a main neurotransmitter. One of the main roles is reducing nerve cell excitability.
Ach (Acetylcholine) – a neurotransmitter released by nerve cells and transmits signals to muscle cells.
Glutamate – main neurotransmitter for exciting neurons.
Adrenaline (epinephrine) – hormone (and medication) that acts as a neurotransmitter to increase blood pressure, heart rate and other changes.
Noradrenaline (norepinephrine) – hormone and neurotransmitter that is involved like adrenaline in increasing blood pressure and heart rate. It is released continuously.

Serotonin – neurotransmitter, sometimes called the happy chemical. In addition to affecting mood, it has a complex biological function including affecting cognition, reward, learning, memory.
Dopamine – a neurotransmitter sending signals to other cells and is important for reward-motivation and movements (motor control). It is reduced in Parkinson's disease.

Genetic structures

DNA – deoxyribo**n**ucleic **a**cid (DNA) is a molecule consisting of two chains that are coiled together and form a double helix ("twisted ladder" structure). It carries the genetic material. It is essential for life.
RNA – ribo**n**ucleic **a**cid, a single chain of nucleotides (rather than the double chain like DNA). RNA is made by copying a piece of DNA and is the produce when a gene is expressed.
Nucleotides –These are the building block of DNA and RNA. There are four kinds, and they are connected together in a specific order to form long chains of DNA/RNA, which can be read as a manuscript written with four letters. When the RNA is decoded to make a protein, a row of three nucleotides specify one amino acid.
Base pairs – the four nucleotides fit together in two sets of base pairs. Guanine (G) is always paired with Cytosine (C), Adenine (A) is always paired with Thymine (T) (Uracil (U) in RNA). These bases pair together as building blocks and form the backbone of DNA as part of the double stranded helix of DNA.
Amino acid (a.a) –They are building blocks of proteins and involved in many other processes. There are 20 types of amino acids, which can be connected together in a specific order. When a specific sequence of amino acids is formed, the chain folds up into a protein. If one specific amino acid is changed to another, it may alter how the protein functions, or destroy the protein completely. Amino acids have a three-letter code and a one-letter code (e.g. Asp and D both refer to the amino acid called Aspartic acid).
Protein – a large molecule made up of amino acids and can have many different roles in the body. They are the 'functional units' in the cell – the machines, the building blocks etc. They have many different functions e.g. muscle contraction; cell structures; sending signals; transporting other chemicals.

Gene – an essential unit that is inherited and is made up of DNA. It is a sequence in the DNA that codes for a molecule with a function, e.g. a protein. There are ~20,000 protein-coding genes in the human genome. For each gene, there are two copies, one inherited from the father and one from the mother. (In men, genes on the X chromosome are only inherited from the mother.)

Gene therapy – term that encompasses several different types of therapy using genes to improve genetic conditions. Genetic material is delivered into a patient's cells for treatment of a disease.
AAV – Adeno-associated virus. A virus (not pathogenic) that is used as a vector (biological transporter or carrier) to carry a gene in gene therapy.

Vector – this can be considered a 'transporter' and is used in gene therapy to carry the good copy of the gene from outside to inside the human cells.
Wildtype (WT) – refers to the 'normal' gene
Knock-out – where a gene is removed (e.g. in a mouse) so that it is then missing e.g. a specific protein.
Knock-in – where a gene is altered (e.g. in a mouse) to reflect a genetic mutation by making the 'mouse-model' of the genetic condition seen in humans.

ATP1A3 and cell signalling

Na^+- sodium ion (molecule).
K^+- potassium ion (molecule).
ATP- **a**denosine **t**ri-**p**hosphate, the universal fuel of cells.
ADP- **a**denosine **d**i-**p**hosphate, a product formed when ATP is consumed.
***ATP1A3* gene** – this is the gene that codes for the sodium/potassium transporting ATPase enzyme. It encodes the alpha (α) 3 subunit that is the cause of most AHC/RDP patients.
ATP1A3 – sodium/potassium transporting ATPase subunit alpha 3 is an enzyme.
ATPase – a group of enzymes that break down (hydrolyses) ATP to ADP and uses the energy from this for another process.
Sodium/potassium ATPase (sodium/potassium pump) – cell membrane enzyme responsible for maintaining electrochemical gradients across the cell membrane. It is composed of two subunits (alpha and beta). For every ATP molecule that the pump uses, 3 sodium (Na^+) ions are pumped out of the cell and 2 potassium (K^+) are pumped into the cell. Nerve cells depend on the pump to transmit signals. It is found in practically all cells in animals, where it maintains the cell volume, resting potential and many other functions of the cell.
De novo – this means 'of new' and refers to a new mutation in the child that is not inherited from either parent but happened by chance in the egg or sperm.
Mutation – this refers to the abnormal gene.
Heterozygous (Het) – where one copy of the gene is normal, and one copy of the gene is abnormal (mutated) in a medical genetic condition.
Homozygous – where both copies of the gene are abnormal (mutations) in a medical genetic condition.
Autosomal dominant – Inheriting one abnormal copy of the gene which causes the disease.
Autosomal recessive – Inheriting one abnormal copy of the gene does not cause the disease, but two abnormal copies (one from each parent) does cause the disease.
Subunits – when two or more individual amino acid chains (subunits) are together in a complex to form a protein.
Alpha subunit – the catalytically active subunit in the sodium/potassium ATPase. It is the subunit involved in ATP1A3 gene conditions.
D801N – the commonest disease-causing mutation within the ATP1A3 gene. This mutation causes the amino acid aspartic acid (abbreviated to D or Asp), at position 801 within the ATP1A3 gene to be changed to a different amino acid, asparagine (abbreviated to N or Asn). D is the one letter code for Aspartic acid (an amino acid).

Asp801Asn – this is the same as above (D801N) but written in three letter amino acid code.
E815K – second commonest disease-causing mutation within the ATP1A3 gene. E, or Glutamic acid, is the amino acid at position 815 which has been changed to a different amino acid, lysine (abbreviated to K or Lys).
Genotype – genetic make-up of a cell (the genes).
Phenotype – how the symptoms of a condition present in a person.
Ion channel – a protein in the membrane that can open and close to allow specific ions to flow into or out of the cell. E.g. when a sodium channel opens, sodium flows into the cell because of the difference in sodium concentrations across the membrane created by the sodium/potassium ATPase.
Ion channel blocker – a drug that binds to an ion channel and hinders ions from flowing through it.
Resting potential – resting voltage of a cell. The voltage at which a cell rests and is not active. There is a difference across the cell membrane with the cell being negative on the inside versus the outside.
Action potential – in a nerve cell, an electrical impulse that travels down the axon and changes the membrane electrochemical gradient (potential). This results in the activation of neurotransmitters which are released across the synapse.

Laboratory tests/terms

***In vitro* –** this means within *'the glass'*, and refers to an artificial environment, not in a living organism.
In vivo – in the living organism.
Multielectrode array – device which connects neurones to an electronic measuring system
Patch clamp recording – a technique in electrophysiology to study the currents of ions in cells.
Current clamp method – detects the transmembrane voltage that results from the movement of ions.
Whole exome sequencing (WGS) – process of determining the complete DNA sequence of a person.
Molecular dynamics simulations – a computer simulation method. It is used to study the movements of molecules.

Brain Anatomy

Hemisphere – the brain is divided into two halves (left and right) and 4 lobes (parts). Hemisphere refers to one half of the brain. Right side of the brain controls left side of body and vice versa.
Cerebrum – biggest part of the brain made up of two halves. Responsible for motor function (body movement); thinking; behaviour; emotions; and sensory input e.g. hearing, seeing, touch, smell and taste.
Cerebral – referring to cerebrum.
Cerebellum – smaller part of the brain at the back of the head and top of neck. Helps to coordinate movement and balance.
Corpus callosum – this is a membrane separating the two halves of the brain.
Caudate nucleus – structure in the midbrain (and part of basal ganglia).
Putamen – round structure in the forebrain. It has many functions including regulating movements.
Thalamus – part of the brain that is involved with sensory and motor signals. It is also involved with sleep and awake/alertness.
Brainstem – is at the back (posterior) part of the brain and is connected to the spinal cord (the cord that leaves the brain and travels down the back. It includes the midbrain and other structures of the brain.
Basal ganglia – a collection of parts within the brain that are located in the midbrain. One of the function is regulation of movements.
Substantia nigra – part of the midbrain and source of the neurotransmitter dopamine.
Hippocampus – part of the brain that is involved in memory predominantly.
Occipital – a lobe (part) of the brain at the back. Where sight is based.
Temporal – a lobe (part) of the brain at the side. Where hearing is based. A type of epilepsy starts from here.
Parietal – a lobe (part) of the brain at the top towards the back. It has many roles including sensory and spatial awareness.
Frontal – the lobe (part) of the brain at the front of the head. Responsible for many functions including speech expression and emotions.
Fronto-parietal – part of the brain including both frontal and parietal lobes.
Ventricles – fluid-filled pockets inside the brain.
Subarachnoid spaces – space between different layers surrounding the brain.
White matter – part of the central nervous system that are made up of axons and tracts.
Grey matter – major part of the central nervous system made up of neurones/cell bodies.
Glial cells - these cells surround neurons and provide support/insulation
Atrophy – wasting of a part of the body/brain
Sclerosis – replacement of normal tissue with scar like tissue
Polymicrogyria – a condition that affects multiple (poly) parts of the brain.
Spinal cord – nervous tissue that starts at base of brain and travels down the spine/vertebra
CSF or Cerebral spinal fluid – fluid in the ventricles, around the brain, and around the spinal cord. Samples can be taken from the bottom of the spine.

BBB - Blood brain barrier that separates the brain from the rest of the body.
Intrathecal injection - Injection into the spinal canal or rarely into the brain.

Seizures

Focal – seizures that affect one hemisphere of the brain (previously referred to as partial).
Generalised – this affects both hemispheres of the brain. A common type of seizure is a generalised tonic clonic seizure (*grand mal*).
Absence – period of vacantness/absence associated with a seizure. The symptoms will vary between people. It is a type of generalised seizure.
Todd's paresis – focal weakness in part/parts of the body after a seizure. It typically affects one side of the body (hemiplegia) and resolves commonly 48 hours after the seizure.

Clinical terms – conditions all caused by ATP1A3 mutations

AHC – Alternating Hemiplegia of Childhood (in 70-80% of patients disease is caused by a mutation in the *ATP1A3* gene. In the remaining patients the clinical diagnosis may be different or further genes are still to be found)
CAPOS – Cerebellar ataxia (incoordination), Areflexia (no reflexes), Pes cavus (inward sloping chest wall), Optic atrophy (vision loss) and Sensorineural hearing (hearing loss due to neurological condition) loss.
RDP – Rapid onset Dystonia of Parkinsonism.
EEIE – Early Epileptic Infantile Encephalopathy.
FIPWE – Fever-Induced Paroxysmal Weakness and Encephalopathy.
RECA – Recurrent Episodes of Cerebellar Ataxia.

Clinical Symptoms

Orphan diseases - a rare disease that has not been adopted by the pharmaceutical industry because it provides negligible financial incentive to invest in research and therapeutic developments.
Clinical response – the change that can be seen by a doctor (clinician) in response to a treatment or event
Generalised – used in relation to symptoms happening all over the body/area
Episode – a range of neurological symptoms referred to within AHC event.
Paroxysmal – sudden recurrence of symptoms.
Hemiplegia/Hemiparesis – paralysis of one side (one half left or right) of the body
Unilateral – one side of body (left or right).
Bilateral – both sides (e.g. both arms or both legs).
Quadraplegia/Quadraparesis – paralysis of all four limbs.
Paroxysmal movement disorders – Involuntary abnormal movements that only occur during an episode, seizure or attack.
Dystonia – rigidity of one or many muscles (often extremely painful).

Bradykinesia – slowness of movement
Ataxia – a type of movement associated with incoordination.
Gait – medical term for walking and way a person walks.
Motor symptoms – movement symptoms
Encephalopathy – an altered mental state/confusion due to the brain.
Nystagmus - flickering of the eye (normally from side to side). There are many causes for this and can be abnormal (pathological) or normal (physiological).
Monocular – one eye.
Biocular – both eyes.
Choreathetosis – involuntary movements that involve chorea (irregular contractions) and athetosis (twisting and writhing).
Chorea – involuntary movements that are 'jerky' in nature.
Clonus– involuntary rhythmic muscular contractions. It is present in several neurological conditions.
Myoclonus – involuntary spasmodic contractions of the muscles that can be described as rhythmic or jerky.
Dyskinesia – group of movement disorders that cause involuntary muscle movements.

Action tremor – tremor that develops on doing something (action).
Resting tremor – tremor that is evident at rest.
Pill rolling tremor – a fine rolling subtle tremor (named as like rolling a pill in-between fingers).
Intention tremor – a tremor that is most obvious when trying to touch an object (on intention). It is normally due to damage in the cerebellum.

Apnoea – temporary stopping of breathing, often during sleep.
Sleep study – several different type of sleep studies but they ultimately study during sleep that looks at breathing, apnoea, heart rate, brain activity.
Respiratory arrest – when a person stops breathing or is breathing ineffectively.
Sudden infant death syndrome (SIDS) – is the unexplained death of a child less than one year old.
Sudden Unexplained Death in Epilepsy (SUDEP) – a fatal complication of epilepsy.

ECG/EKG – electrocardiogram (measures the heart rhythm).
Cardiac arrythmia – a disturbance of normal heart rhythm.
Tachyarrythmia – a fast abnormal heart rhythm.
Bradyarrythmia – a slow abnormal heart rhythm.
Tachycardia – a fast heartbeat.
Bradycardia – a slow heartbeat.
Syncope – a loss of consciousness/collapse due to a cardiac (heart) cause such as an abnormal heart rhythm.
Pauses – when the heart rhythm stops for a certain time and starts again.
Heart Block – where the heart rhythm is abnormal and has stops intermittently. (several different types of heart block and some need a pacemaker to correct).

QT interval – part of the ECG that relates to the contraction of part of the heart. muscle and is displayed on the ECG as an electrical movement called QT
QTc – this is the QT interval but 'c' stands for corrected (so it is corrected for the heart rate and is more accurate).

BP – blood pressure.
Sats – refers to oxygen saturations (sats).
Pulse oximeter – used to measure oxygen saturations (also referred to as a 'sats monitor') and normally attached to finger or foot.

Drugs

These are listed below with their generic, non-trade names, but some common trade names are included also. These will vary by country.

Flunarizine – a calcium channel blocker (calcium antagonist) used in AHC which blocks the calcium channels in the cell membrane reducing the calcium that comes into the cell, but it is unclear how it works in AHC. It is sometimes referred to as an ion channel blocker (calcium is an ion, molecule).
Diphenylpiperazine – a drug that enhances the serotonin receptor.
Benzodiazepine – several different types of drugs within this category. They are used for their sedating properties (for seizures and also AHC episodes). These include:
Midazolam, Diazepam, Clobazam, Clonazepam, Lorazepam

Acetaminophen/paracetamol – pain relief
Acetazolamide – carbonic anhydrase (found in red blood cells) inhibitor, which works in the first part of the kidney and results in bicarbonate, sodium and chloride being excreted from the kidney into urine.
Trihexiphendyl (artane) – is an anti-Parkinson drug and antimuscarinic (relaxes the muscles) and is used for dystonia.
Chloral hydrate – a sedative drug.
Gabapentin – is a gabapentinoid, which is an anti-seizure drug that also has properties for pain (neuropathic) relief and other symptoms including helping with sleep.
Cannabidiol – from the cannabis plant
THC – stands for tetrahydrocannabinol. This is the psychoactive part of the cannabis plant.
Epidiolex – cannabidiol medication licensed in some countries.
Sativex – cannabis-based medication licensed in some countries, contains THC and cannabidiol.

Lipophilic – combines/mixes with fats (used in relation to drugs as well as other mechanisms at the cell level)

Organisations/Abbreviations

IAHCRC – International Consortium for the Research on Alternating Hemiplegia of Childhood
OBSERV-AHC – Worldwide research study that has just started to collect information on the children and adults with AHC
Eurodis - European Organisation for Rare Diseases

This glossary was prepared by Katherine Behl