PROGRAM AND ABSTRACTS

ATP1A3 IN DISEASE

SYMPOSIUM 7TH MEETING

October 13-14 2018 CHICAGO, ILLINOIS, USA

Welcome to the 7th ATP1A3 in Disease Symposium!

On behalf of the Organizing Committee, thank you for attending the 7th ATP1A3 in Disease Symposium, and welcome to the Northwestern University Feinberg School of Medicine. This is the second time the ATP1A3 symposium has been held in the United States, and we are proud that the 'windy city' was selected for this event.

As regular attendees know, this recurring scientific conference commenced in 2012 following the discovery of ATP1A3 mutations in alternating hemiplegia of childhood (AHC). Although AHC was the driver for the first symposium, there has been important recognition these past few years that ATP1A3 mutations are associated with an expanding range of clinical disorders. This year, in addition to providing a forum to provide updates on advances in the genetics and pathogenesis of AHC and related neurological disorders, we chose to highlight ATP1A3-associated diseases with principally non-motor symptoms including rare cases of congenital psychosis. Appreciating the full spectrum of ATP1A3-associated diseases will lead to expanded indications for genetic testing and greater opportunities to correlate genotype with phenotype. Furthermore, given the substantial progress in determining the fundamental molecular and cellular defects responsible for ATP1A3-associated diseases, it is time that we focus more attention on developing strategies to improve treatment. This year, we specifically wanted to highlight emerging work being done to develop new strategies including gene and molecular therapies. Finally, we have emphasized the need to engage new investigators in the field and have provided expanded speaking opportunities for trainees and young scientists.

The Organizing Committee is proud that we secured a Conference Grant (R13-NS108697) from the National Institute for Neurological Diseases and Stroke. We are pleased that Dr. Nina Schor (Deputy Director, NINDS) will address the Symposium on the importance of rare disease research to the mission of NINDS. Following the Symposium, the 47th annual meeting of the Child Neurology Society (CNS) will be held across town, and we are grateful that Dr. Jon Mink (President, CNS) can join us for welcoming comments.

Planning for the 7th ATP1A3 in Disease Symposium was a team effort! The Organizing Committee members contributed their thoughtful input to the design of the conference, selected speakers, reviewed abstracts, and set the meeting agenda. The Standing Committee provided valuable direction and guidance. The host family organization, the AHC Foundation provided financial support for the conference and raised additional funds. We give hearty thanks to the Conference Organizers – Liz Murphy and Lexi Nash – without whom we could not have planned and executed the meeting. We also thank Michelle Mohney for help assembling the program book. Last, but certainly not least, we thank the generosity of our conference sponsors including the Lurie Children's Hospital of Chicago.

Organizing Committee

Allison Brashear
Kevin Ess
Al George
Kenneth Silver
Kathleen Sweadner

Host Organization

AHC Foundation – ahckids.org
Sharon Ciccodicola
Lynn Egan
Joshua Marszalek

Conference Organizers

Liz Murphy
Lexi Nash

Standing Committee

Karin Lykke-Hartmann
Mohamad Mikati
Hendrik Rosewich
Tsveta Schyns
Jeff Wuchich

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Oral Presentation Guidelines

Please bring your presentation as a PowerPoint file on a PC-compatible USB drive that has been checked for viruses.

If you prefer to connect your own PC/Mac laptop, please bring a USB drive as a back-up. The projector has laptop connections for HDMI, VGA, and mini-display port. Remember to bring a power cable and any necessary adapters.

A slide advancer/laser pointer and power strip will be provided.

All presenters should check-in with Lexi Nash during the break prior to their session to load their PowerPoint onto the computer or connect their laptop.

Poster Guidelines

All poster presenters are required to check-in at the registration desk to receive their poster number.

Poster presenters are required to stand by their own poster at the presentation time. Posters will not be attended by volunteers.

Poster size maximum dimensions: 68 inches (173 cm) wide by 44 inches (112 cm) high

Schedule: Sunday, October 14

Installation: 7:00 – 8:00 a.m.
Viewing: 10:00 – 10:30 a.m.
Presentation: 12:30 – 2:30 p.m.
Removal: 4:30 – 6:00 p.m.

ATP1A3 in Disease Symposium Program

Northwestern University Feinberg School of Medicine
Baldwin Auditorium & Ryan Family Atrium, Robert H. Lurie Medical Research Center
303 E. Superior Street, Chicago, IL 60611

Saturday, October 13, 2018

11:00am– 12:30pm	**Registration**
12:30 – 1:00pm	**Opening and Welcome** **Drs. Brashear, Ess, George, Silver, and Sweadner** The Importance of Family Organizations: Lynn Egan

Session 1: Basic Science of ATP1A3

Chair: Kathleen Sweadner

1:00 – 1:30pm	**Kathleen Sweadner** Conservation, structural biology and cell biology of ATP1A3 mutations
1:30 – 2:00pm	**Miguel Holmgren** Extracellular Na^+ interactions with the human ATP1A3
2:00 – 2:30pm	**Bente Vilsen** Functional effects of Na^+,K^+-ATPase mutations in health and disease
2:30 – 3:00pm	**Keiko Ikeda** Sodium pump and secondary active transporters
3:00 – 3:05pm	**Welcome by Dr. Jonathan Mink** **President of the Child Neurology Society**
3:05 – 3:30pm	**Coffee Break**

ATP1A3 in Disease Symposium Program

Saturday, October 13, 2018

Session 2: Non-motor Symptoms of ATP1A3

Chair: Hendrik Rosewich

3:30 – 3:50pm
Hendrik Rosewich
Clinical presentation of ATP1A3: psychiatric and cognitive

3:50 – 4:10pm
Diane Doummar
Early onset encephalopathy with paroxysmal movement disorders or epileptic seizures without hemiplegic attacks: about 4 children with novel ATP1A3 mutations

4:10 – 4:30pm
Catherine Brownstein
ATP1A3 in rare and orphan diseases at a tertiary children's hospital

4:30 – 5:30pm
Keynote Address - Christopher Gomez
Lessons from spinocerebellar ataxia

5:30 – 5:45pm
Thomas Holm
Pharmacological approaches to alleviate schizophrenia-like symptoms in a mouse model for alternating hemiplegia of childhood

5:45 – 6:00pm
Richard Smith
Generation of a cell-based assay for modeling childhood-onset schizophrenia

6:00 – 6:15pm
Christopher Thompson
Altered pump function and network activity in ATP1A3 associated childhood-onset schizophrenia

6:30 – 8:30pm
Cocktails & Dinner, Tip Top Tap Ballroom
Warwick Allerton Chicago, 23rd Floor
701 N. Michigan Avenue, Chicago, IL 60611

8:30 – 9:00pm
AHC Standing Committee
Opening Remarks: Next steps in ATP1A3 research

Invited Keynote Speaker - Joan M. Anzia
Beyond Survival: Starting to address the drivers of burnout in neurology

ATP1A3 in Disease Symposium Program

Sunday, October 14, 2018

7:30 – 8:30am — Breakfast, Registration, and Poster Set-Up

Session 3: Clinical Symptoms Pharmacologic Treatment: Dystonia and Parkinsonism

Chair: Allison Brashear

8:30 – 9:00am — **Ihtsham Haq**
Analysis of phenotype in a large RDP cohort updates indications for testing for ATP1A3

9:00 – 9:30am — **Harrison Walker**
Dystonia associated with parkinsonism – adaptable brain stimulation strategies for complex motor symptoms

9:30 – 9:45am — **John Snow**
An iPSC-derived neuronal model to investigate alternating hemiplegia of childhood

9:45 – 10:00am — **Linh Tran**
Novel combination and partial phenotypes of ATP1A3 related disease

10:00 – 10:30am — Coffee Break and Poster Viewing

Session 4: Epilepsy in ATP1A3 Diseases

Chair: Kevin Ess

10:30 – 11:00am — **Erin Heinzen**
Genetic bases of epilepsy disorders and relevance to AHC

11:00 – 11:30am — **Mohamad Mikati**
The epileptologist's view of epilepsy in AHC, management and possible treatments

11:30 – 11:50am — **Nina Schor**
The importance of rare diseases for neuroscience research and NIH

11:50am – 12:05pm — **Elena Arystarkhova**
ATP1A3 mutations that impair biosynthesis and trafficking

ATP1A3 in Disease Symposium Program

Sunday, October 14, 2018

12:05 – 12:20pm	**Simona Balestrini** Cardiac phenotype in ATP1A3 related-syndromes: an extended study
12:20 – 2:30pm	**Lunch and Poster Session**

Session 5: Biomarkers and New Therapeutic Approaches

Chair: Al George

2:30 – 3:00pm	**Christopher Whitlow** Clinical imaging biomarkers in ATP1A3 diseases
3:00 – 3:30pm	**Alan Lewis** Nicotinic receptors as a potential therapeutic target for challenging behaviors in neurodevelopmental disorders
3:30 – 4:00pm	**Steven Gray** Adeno-associated virus-based gene transfer to the nervous system
4:00 – 4:30pm	**Barry Ticho** Targeted augmentation of nuclear gene output (TANGO) as a novel treatment for genetic nervous system diseases
4:30 – 5:00pm	**Wrap up and Looking Forward** **ATP1A3 organizing and standing committee** Scientist perspective, families and patients

Invited Speakers

Joan M. Anzia, M.D., Northwestern University Feinberg School of Medicine

Catherine Brownstein, Ph.D., Boston Children's Hospital, Harvard Medical School

Diane Doummar, M.D., Armand-Trousseau Hospital

Christopher M. Gomez, M.D., Ph.D., University of Chicago

Steven J. Gray, Ph.D., University of Texas Southwestern Medical Center

Ihtsham ul Haq, M.D., Wake Forest School of Medicine

Erin Heinzen, Ph.D., Columbia University Irving Medical Center

Miguel Holmgren, Ph.D., National Institutes of Health, National Institute of Neurological Disorders and Stroke

Keiko Ikeda, M.D., Ph.D., International University of Health and Welfare, Jichi Medical University

Alan Lewis, M.D., Ph.D., Vanderbilt University School of Medicine

Mohamad Mikati, M.D., Duke University School of Medicine

Jonathan W. Mink, M.D., Ph.D., University of Rochester Medical Center, President of Child Neurology Society

Hendrik Rosewich, M.D., University Medical Center Göttingen, Georg August University Göttingen

Nina Schor, M.D., Ph.D., National Institutes of Health, National Institute of Neurological Disorders and Stroke

Kathleen Sweadner, Ph.D., Massachusetts General Hospital, Harvard Medical School

Barry Ticho, M.D., Ph.D., Stoke Therapeutics

Bente Vilsen, DMSci., Aarhus University

Harrison Walker, M.D., University of Alabama School of Medicine

Christopher T. Whitlow, M.D., Ph.D., Wake Forest School of Medicine, Wake Forest Baptist Medical Center

Extracellular Na^+ interactions with the human ATP1A3

Miguel Holmgren, Ph.D.
National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA

The human α subunit of the Na^+/K^+-ATPase (ATP1A3) is mostly expressed in neurons and it can associate with any isoform of the auxiliary β subunit (β1, β2 or β3) to form a functional unit. The protein complex transports $3Na^+$ from the cell and $2K^+$ into the cell against their electrochemical gradients, so it uses the energy derived from the hydrolysis of ATP. Many mutations of the ATP1A3 linked to Alternating Hemiplegia of Childhood (AHC) reside in the vicinity of the ion binding sites within the transmembrane core of the protein. Once $3Na^+$ are loaded from the intracellular side of the ATP1A3 and trapped within the permeation pathway, the ions are released to the extracellular bulk solution. This process occurs through a series of events than can be monitored using electrophysiological approaches. Here, we will discuss how we study these events, their kinetics, how different β subunit isoforms influence them, and preliminary results on the functional alterations of them by the AHC mutation D923N.

Functional effects of Na^+,K^+-ATPase mutations in health and disease

Bente Vilsen, DMSci
Aarhus University, Aarhus, Denmark

As a basis for trying to understand the relation between Na^+,K^+-ATPase structure and mechanism in health and disease we are lucky to have high resolution crystal structures representing intermediates of the reaction cycle of the Na^+,K^+-ATPase. In addition, the effects of mutations on Na^+,K^+-ATPase function throws light on mechanistic features and in the case of disease mutations helps understanding the pathophysiology. We have developed techniques for studying the effects of mutations on the individual partial reactions of Na^+,K^+-ATPase expressed in cell culture. In several disease mutants the compromised function can be traced to disturbance of the ion binding sites. I will discuss disease mutations as well as mechanistic information derived from mutational studies in the light of the structural features of the Na^+,K^+-ATPase.

Sodium pump and secondary active transporters

Keiko Ikeda, M.D., Ph.D.
International University of Health and Welfare, Narita, Japan
Jichi Medical University, Shimotsuke, Tochigi, Japan

Sodium pump (Na^+/K^+ ATPase) is the enzyme that plays a critical role in the maintenance of electrochemical Na^+ and K^+ gradients across the animal plasma membrane. The Na^+ and K^+ gradients across the membrane formed by this pump are utilized as energy source for uptake or release of various nutrients, ions, and neurotransmitters. The minimum constellation for an active pump is one α and one β subunit. There are four α and three β isoforms have been identified in mammals. The α2 is mainly expressed in glial cells, and the α3 in neurons in the adult brain, whereas the former is expressed in neurons in the fetal brain. Mutations of ATP1A2 and ATP1A3 which encode the human α2 subunit and α3 subunit, respectively, have been identified in various neurological disorders; FHM2 for α2 and RDP, AHC, and CAPOS for α3. To gain insight into the pathophysiology of these neurological disorders and to understand the functional roles of the pump, we electro physiologically and histologically examined neural network of cerebellum and brainstem using the Atp1a2 and Atp1a3 knockout mice. We found that deficient of Atp1a2 or Atp1a3 caused a remarkable reduction of the various transporter activity both in the brainstem and the cerebellum. Interestingly, some of the transporter activity was robustly increased, probably by compensatory mechanism, resulting in the dysfunctional of neural network plasticity. The present findings would provide a novel clue for understanding of cellular and molecular mechanisms underlying FHM2, RDP, and AHC.

Clinical presentation of ATP1A3: psychiatric and cognitive symptoms

Hendrik Rosewich, M.D.
University Medical Center Göttingen, Georg-August-University Göttingen, Germany

More than 40 years ago the first disease known to be caused by heterozygous *de novo* mutations in the ATP1A3 gene was described clinically – alternating hemiplegia of childhood. The definition of rapid-onset dystonia-parkinsonism (DYT12) followed in 1993 and CAPOS syndrome was designated in 1996. Since than it was shown that these well-defined complex clinical entities are part of a clinical continuum sharing key neurological symptoms. On the basis of a movement disorder of different type, severity and time course as defining element of ATP1A3 related disorders most of the research and clinical care focused on these symptoms. However, non-motor symptoms - psychiatric and cognitive – are crucial characteristic of these entities and have not been addressed comprehensively. The reasons for that are diverse: it is almost impossible to distinguish the degree of psychomotor delay arising from the movement disorder affecting learning and development of skills from the pure cognitive and psychiatric impairment as cause of disturbed neuronal networks of higher functions. Moreover, due to the abrupt change in the state of consciousness through different triggers as integral part of ATP1A3 related disorders, the application of standard IQ testing or other standardized psychological tests are not usable. The exact and comprehensive description of the capabilities and skills of every individual patient suffering from an ATP1A3 related disorder is crucial to define the best psychological support to reach the highest degree of life quality and independence. Moreover, we need to recognize behavioral disorders like depression or anxiety in more detail to provide adequate psychological and drug therapy. Thus, we started a comprehensive analyses of psychiatric and cognitive symptoms first focused on daily life skills in our German patient cohort that will be presented.

Early onset encephalopathy with paroxysmal movement disorders or epileptic seizures without hemiplegic attacks: about 4 children with novel ATP1A3 mutations

Diane Doummar, M.D.
Armand-Trousseau Hospital, Paris, France

Heterozygous mutations in the ATP1A3 gene are responsible for various neurological disorders, ranging from early-onset alternating hemiplegia of childhood to adult-onset dystonia-parkinsonism. Next generation sequencing allowed the description of other phenotypes, including early-onset epileptic encephalopathy in two patients. We report on four more patients carrying ATP1A3 mutations with a close phenotype and discuss the relationship of this phenotype to alternating hemiplegia of childhood. A retrospective analysis of clinical case records is reported.

Three patients had an unreported heterozygous de novo sequence variant in ATP1A3. These patients shared a similar phenotype characterized by early-onset attacks of movement disorders, some of which proved to be epileptic associated with moderate to severe developmental delay with autistic features in three patients. (Hemi)plegic attacks have never been considered before genetic testing.

Significance: Together with the two previously reported cases, our patients confirm ATP1A3 mutations are associated with a phenotype combining features of early-onset encephalopathy, epilepsy and dystonic fits, as in the most severe forms of alternating hemiplegia of childhood but in which (hemi)plegic attacks are absent or only suspected retrospectively.

ATP1A3 in rare and orphan diseases at a tertiary children's hospital

Catherine A. Brownstein, Ph.D.
Boston Children's Hospital, Harvard Medical School, Boston, USA

Complex phenotypes may be new syndromes, or may be a combination of several genetic factors. ATP1A3 is predominantly linked to Alternating Hemiplegia of Childhood and Rapid-onset Dystonia Parkinsonism, but new evidence suggests a higher incidence of behavioral symptoms in individuals. We have identified two patients with ATP1A3 *de novo* variants and elements of marked behavioral disorders. The first patient presented with onset of command auditory hallucinations and behavioral regression at age 6 in the context of longer standing selective mutism, aggression, and mild motor delays. WES revealed a previously unreported heterozygous *de novo* variant at c.385G>A (V129M) in ATP1A3. We are currently pursuing functional work to confirm and further investigate the role of ATP1A3 in very early onset psychosis. A second patient with autism has also been identified and published. Additionally, at Boston Children's Hospital we have encountered patients with ATP1A3 variants we are unsure of how to interpret. One fascinating case is of a paternally inherited ATP1A3 premature stop codon (p.W393X) in a child with a complex medical history including transposition of the great arteries s/p repair, global developmental delays, developmental regression, dystonic quadraparesis, truncal hypotonia, undescended testes, and seizures. The chief concern was developmental regression in the setting of diffuse infarcts on brain MRI. He passed away at 2 years of age. Father is asymptomatic. This and additional cases from Boston Children's Hospital will be presented.

Beyond Survival: Starting to address the drivers of burnout in neurology

Joan M. Anzia, M.D.
Northwestern University Feinberg School of Medicine, Chicago, USA

The corporatization of American medicine in combination with some aspects of the longstanding culture of medicine have resulted in a “perfect storm” of non-aligned values and goals in healthcare settings. Strategies to improve the individual wellbeing of physicians are fine, but are not going to yield the most benefit in improving physicians’ satisfaction and joy within their work. Neurology is one of the specialties that appears to be most affected by burnout, and a more in-depth look at the drivers at the team, institutional, and national levels is in order. Using Shanafelt’s table of drivers of burnout at each level, participants will identify “low-hanging fruit” in their home teams, divisions, and departments and discuss possible strategies to address these.

Dystonia associated with parkinsonism – adaptable brain stimulation strategies for complex motor symptoms

Harrison Walker, M.D.
University of Alabama School of Medicine, Birmingham, USA

Dystonia is a relatively neglected motor symptom of Parkinson's disease (PD) that can respond favorably to deep brain stimulation (DBS). Here we provide background on the phenomenology of dystonia associated with sporadic PD its response to both pharmacotherapy and DBS. We will also summarize early evidence on how DBS impacts dystonia/parkinsonism from DYT12-ATP1A3 dystonia. Recent advances in DBS technology such as a directional and adaptive stimulation provide new physiological insights and increased flexibility, promising to advance therapy for patients with dystonia from various etiologies.

Genetic bases of epilepsy disorders and relevance to AHC

Erin L. Heinzen, Ph.D.
Columbia University Irving Medical Center, New York, USA

Over the past ten years significant progress has been made identifying genes responsible for severe pediatric epilepsies and other neurodevelopmental disorders where seizures are a common feature. The vast majority of these genes, including ATP1A3, are believed to cause seizures by altering how neurons function through a wide range of mechanisms. In this presentation I will give an overview of our current knowledge of the genetic bases of epilepsy. In addition, I will share the results of an on-going collaborative study seeking to identify genetic variants responsible for the small subset of children with AHC who do not have a genetic variant in ATP1A3. In several of the ATP1A3 negative AHC cases we have found variants in genes previously associated with severe pediatric epilepsies, suggesting possible overlapping disease mechanisms in both AHC and severe pediatric epilepsies.

The importance of rare diseases for neuroscience research and NIH

Nina F. Schor, M.D., Ph.D.
National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, USA

The motto of the National Institutes of Health (NIH) is “Turning discovery into health”. NIH supports and performs research across the topical and methodological spectrum in service of advancing the health and well-being of the public in the U.S. and around the world. The National Institute of Neurological Disorders and Stroke (NINDS) is one institute of NIH. Its mission is to seek fundamental knowledge about the brain and nervous system and to use that knowledge to reduce the burden of neurological disease. NIH and NINDS are interested in the study of rare diseases to improve the health and well-being of individuals who suffer with them; to counsel families who manifest them; to learn from them the function of molecules, organelles, cells, circuits, brain loci, and pathways that are aberrant in them; to improve the health and well-being of people affected by common disorders through this newfound understanding; and to improve and safeguard the health and well-being of individuals not yet affected by neurological disorders. For example, the study of ATP1a3 mutation-related disorders, with their protean manifestations, stands to improve the lives of patients and families with these disorders; helps us understand the functions of ATP1a3 in the nervous system and throughout the body; and holds the promise of improving our understanding of more common disorders including migraine, hemiplegia, developmental delay, dystonia, parkinsonism, *pes cavus*, optic atrophy, and cerebellar ataxia.

Nicotinic receptors as a potential therapeutic target for challenging behaviors in neurodevelopmental disorders

Alan Lewis, M.D., Ph.D.
Vanderbilt University School of Medicine, Nashville, USA

Challenging behaviors such as irritability and aggression may be observed in individuals with neurodevelopmental disorders and lead to impairment in functioning. For many, current treatments are not fully effective or confer significant side effects, necessitating novel treatment avenues. In this talk, I will discuss the potential utility of targeting nicotinic acetylcholine receptors (nAChRs), a diverse family of ion channels that serve key neuromodulatory functions, all activated by nicotine. Interestingly, studies from over 50 years ago reported that purified nicotine reduced aggressive behavior across a variety of animal models. Using mouse models of aggression, our laboratory extended these studies to identify the alpha-7 nAChR as a critical target of nicotine, and the dentate gyrus/hippocampus as a key brain region regulating aggressive behavior. Given these findings, we conducted an exploratory, placebo-controlled, double blind clinical trial of nicotine, delivered by skin patch, for young adults with autism spectrum disorder. Compared to placebo patch, we found that 7 mg transdermal nicotine for one week was well tolerated, and improved scores on the Aberrant Behavior Checklist - Irritability subscale, the study's primary outcome. Surprisingly, caregiver ratings of subject's sleep were also consistently improved by transdermal nicotine. These studies encourage further investigation into the nAChR system to modulate neural circuitry influencing aggression and related behaviors, including trialing pharmacological agents with greater selectivity for the alpha-7 nAChR.

Adeno-associated virus-based transfer to the nervous system

Steven J. Gray, Ph.D.
University of Texas Southwestern Medical Center, Dallas, 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. However, several details hampered the clinical translation of this approach including: high vector doses (manufacturing burden), presence of natural anti-AAV neutralizing antibodies in some individuals, and high peripheral organ gene transfer potentially causing gene-dependent toxicity. Nonetheless, at least 3 clinical trials have been initiated using AAV9 vectors injected intravenously, for Spinal Muscular Atrophy, MPS IIIA, and MPS IIIB. In lieu of an intravenous route of administration, intrathecal administration of AAV9 also leads to broad CNS gene transfer in mice, pigs, and non-human primates, but overcomes some of the limitations associated with intravenous administration.

Using AAV9-mediated gene transfer as a general approach to treat an inherited CNS disease, we have initiated preclinical studies for Giant Axonal Neuropathy (GAN), Batten Disease, Aspartylglucosaminuria, Tay-Sachs disease, Krabbe disease, Rett syndrome, and others. Based on encouraging preclinical data showing reduce pathology and increased rotarod function in GAN mice, along with favorable safety data, a clinical trial was initiated in May 2015 at the NIH Clinical Center to test intrathecal administration of scAAV9/JeT-GAN in GAN patients. Using the same approach, our group has generated safety and efficacy data supporting the start of 6 additional clinical trials for other diseases in 2019. The studies of our lab and others are establishing intravenous or intrathecal AAV9 administration as a platform approach to treat many inherited neurological disorders.

Targeted augmentation of nuclear gene output (TANGO) as a novel treatment for genetic nervous system diseases

Barry Ticho, M.D., Ph.D.
Stoke Therapeutics, Bedford, USA

Stoke is developing antisense-oligonucleotide (ASO) medicines to treat genetic diseases. Stoke's technology, TANGO, exploits naturally-occurring, non-productive splicing events to increase gene expression via modulation of splicing. We have built a proprietary bioinformatics pipeline that has identified nearly 3,700 disease-associated genes that are potentially amenable to our technology. ASO screening of prioritized disease targets has yielded potent and gene-specific up-regulation for multiple targets. One of our programs is Dravet syndrome (DS), a childhood severe epileptic encephalopathy characterized by high seizure frequency, cognitive and motor impairments and increased risk of sudden death. DS is caused by mutations in the SCN1A gene leading to haploinsufficiency of the voltage-gated sodium channel alpha subunit (Na_v 1.1). We have identified ASOs that significantly increase SCN1A mRNA and reduce the target non-productive splicing event in human neural-progenitor cells. Injection of the TANGO ASO in wild-type mice yielded a significant and dose-dependent increase in SCN1A mRNA as well as Na_v 1.1 protein. Timecourse experiments showed a sustained increase in SCN1A expression 90 days post single, bolus ICV injection of the TANGO ASO. ICV injection of the TANGO ASO in a SCN1A +/- mouse model that recapitulates a number of DS manifestations leads to restoration of SCN1A mRNA and Na_v 1.1 levels. Efficacy studies are underway. These results indicate that Stoke's technology could provide the first gene-specific, disease-modifying approach to restore physiological Na_v 1.1 levels to prevent seizures. Stoke's technology offers a pioneering strategy to treat diseases, such as ATP1A3-related diseases, that result from reduced expression or insufficient activity of a gene that contains a non-productive splicing event.

Poster Listing

Number	Name	Title
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2	Ryan Vaden	Directional differentiation of beta oscillations within the basal ganglia in Parkinson's disease dystonia
3	Caroline Wilson	Associations between gray matter volume and executive function in patients with ATP1A3 mutations: a structural brain MRI investigation
4	Simona Balestrini	Cardiac phenotype in ATP1A3 related-syndromes: an extended study
5	Linh Tran	Novel combination and partial phenotypes of ATP1A3 related disease
6	Alejandro De La Torre Ribadeneira	Epilepsy masquerades: alternating hemiplegia of childhood with a novel mutation in the ATP1A3 gene, a case report with literature review
7	Fiorella Gurrieri	Familial occurrence of alternating hemiplegia of childhood with variable expressivity: the search for new gene(s) and the possible role of genetic landscape
8	Francesco Danilo Tiziano	Unusual clinical presentation of familial hemiplegic attacks in two first cousins: possible oligogenic inheritance of AHC-related phenotypes?
9	Elisa De Grandis	Alternating hemiplegia of childhood in a child harboring a novel TBC1D24 mutation
10	Thomas Holm	Pharmacological approaches to alleviate schizophrenia-like symptoms in a mouse model for alternating hemiplegia of childhood
11	Thomas Holm	A platform for induction, monitoring and quantifying neurological attacks in the $\alpha3^{+/D801Y}$ mouse
12	Vladimir Ferrafiat	Recurrent missense variants in ATP1A3 and its interactor FXYD6 are associated with childhood-onset schizophrenia
13	Christopher Thompson	Altered pump function and network activity in ATP1A3 associated childhood-onset schizophrenia
14	Richard Smith	Generation of a cell-based assay for modeling childhood-onset schizophrenia (COS)
15	Christine Simmons	Consequences of ATP1A3 mutations in patient-derived inhibitory GABAeric neurons
16	John Snow	An iPSC-derived neuronal model to investigate alternating hemiplegia of childhood
17	Emanuela Abiusi	Human neuroblastoma model of AHC: towards a medium throughput screening of candidate therapeutic compounds
18	Lorenzo Antonini	ATP1A3 wild type and mutated isoforms molecular dynamics simulations in a lipidic membrane bilayer. Insights on ion movement.
19	Cristina Moreno	Beta-dependent modulation of the binding/release of Na^+ in the WT and D923N-AHC hNa^+/K^+ATPase-A3
20	Christopher Hopkins	Functionalized ClinPhen: humanized animal models for detecting pathogenicity, interrogating mechanism of action, and enabling targeted drug screening in clinical variants
21	Elena Arystarkhova	ATP1A3 mutations that impair biosynthesis and trafficking

Poster 1

The studies and the projects of the IAHCRC consortium, an effective organizational model, for the progress of the collaborative research on the ATP1A3 rare diseases and of the care for the affected patients

Rosaria Vavassori[1]; Giosuè Lo Bosco[1,7], Andrey Megvinov[1], Alexis Arzimanoglou[2], Eleni Panagiotakaki[2], Mohamad Mikati[3], Lyndsey Prange[3], Arn van den Maagdenberg[4], Erin Heinzen[5], Sanjay Sisodiya[6], Simona Balestrini[6], and all the Centers of the IAHCRC Consortium[8]

[1]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy
[2]Epilepsy, Sleep and Pediatric Neurophysiology Dpt. University Hospitals of Lyon, France
[3]Division of Pediatric Neurology - AHC and Related Disorders Program, Duke University, School of Medicine, Durham, NC, USA
[4]Department of Human Genetics, Leiden University Medical Centre, Leiden, Netherlands
[5]Institute for Genomic Medicine - IGM, Columbia University, New York City, NY, USA
[6]Institute of Neurology, University College London, London, UK
[7]Department of Mathematics and Computer Science, University of Palermo, Italy
[8]www.iahcrc.net/consortium/members.html

The International Consortium IAHCRC was formed in 2012 to carry out a collaborative research that led to the identification of the ATP1A3 gene as the main cause of AHC (Heinzen et al., Nature Genetics, July 2012).

The official Charter of the Consortium was definitively approved by its 15 founding members on November 2014, with the objective to develop the collaborative clinical and basic science research in the field of AHC and of all the ATP1A3 related diseases, and to improve the quality of life of the affected patients and of their families.

The Consortium involves clinicians, geneticists and researchers working at University centers in Europe, USA and Australia; it works in close collaboration with health professionals and patient and mixed organizations, most of whom were already collaborating in the EU-funded projects “ENRAH for SMEs” (2005-2007) and nEUroped (2008-2011).

In 2013, the Consortium launched further collaborative studies, including the largest international cohort of AHC patients to date, whose results have already been published: the study of the genotype-phenotype correlations (Orphanet Journal of Rare Diseases, September 2015) and the study on some cardiac conduction abnormalities of AHC (Brain, August 2015).

The study for the identification of the secondary gene(s) for AHC (Project GEN2-AHC), the second study of the heart disturbances in the ATP1A3 diseases (Project ECG2_ATP1A3) and the expansion of the IAHCRC Common Data Elements (Workgroup EVCAL_AHC) are all in progress; the observational Study OBSERV-AHC is currently in the pre-launch phase.

In our poster presentation, we will provide an overview of all the current studies and projects of the Consortium in terms of objectives and expected results.

We will give a particular emphasis to the longitudinal Study OBSERV-AHC, based on the use of the IAHCRC-CLOUD Platform, a service for the data collection and sharing for the Studies of the Consortium. The Platform has been developed using the REDCap© Electronic Data Capture Tool by Vanderbilt University.

The aim of the OBSERV-AHC Study is to collect data regarding the natural history of the disease and regarding various therapies that are being attempted, in order to analyze the efficacy of specific agents and the predictors for long-term outcome.

The patients themselves can contribute positively to the Study, by entering their own part of data through the IAHCRC-CLOUD Platform Website or through the REDCap© Mobile App.

Poster 2

Directional differentiation of beta oscillations within the basal ganglia in Parkinson's disease dystonia

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Background: Dystonia is a common manifestation of Parkinson's disease, yet it is relatively neglected in research. Here we contrast cortical and subcortical local field potentials in patients with Parkinson's disease with and without dystonia using simultaneous recording from a novel directional deep brain stimulation (DBS) lead and electrocorticography (ECOG) over primary sensorimotor cortex.

Methods: During DBS surgery, we measured local field potentials from an 8 contact directional DBS lead in the subthalamic region and 6 contact ECOG strip over primary sensorimotor cortex, at rest and during simple hand, mouth, and leg movements. We calculated spectrograms using wavelet transforms and contrasted responses in participants with and without historical report of off dystonia with mixed effects linear models. Exploratory analyses compared the beta band and other frequencies across recording sites (cortical versus subcortical), within and across directional DBS contact segments in individuals, and during the different behavioral tasks.

Results: In this ongoing prospective study, 4 of 7 (57%) of the initial participants reported dystonia as a manifestation of their motor fluctuations. Among these, only 1 experienced "off" dystonia during the surgical procedure. Beta power varied significantly by directional DBS contact within and across individuals regardless of dystonia status (2.39 μV^2 ± 1.42, 19Hz). The individual who manifested foot dystonia during surgery displayed robust alpha power (6.45 μV^2, 10Hz).

Conclusions: Directional DBS contacts demonstrate substantial variation in beta power in the subthalamic region within individuals. Greater knowledge of the fast dynamics of local field potentials has promise to personalize therapy for dystonia and other motor manifestations of advanced Parkinson's disease.

Poster 4

Cardiac phenotype in ATP1A3 related-syndromes: an extended study

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Poster 4

Cardiac phenotype in ATP1A3 related-syndromes: an extended study

Mutations in the ATP1A3 gene are associated with a spectrum of neurologic disorders representing a clinical continuum in which at least three distinct, although overlapping, phenotypes have been delineated: rapid-onset dystonia-parkinsonism (RDP), alternating hemiplegia of childhood (ACH), and cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS). These conditions include paroxysmal events and chronic severely disabling neurological deficits. Sudden unexpected death in epilepsy (SUDEP) is increasingly reported in patients with AHC, and in an ATP1A3 knock-in mouse model a higher incidence of sudden death was observed. We previously showed electrocardiogram (ECG) abnormalities in AHC, reflecting those of inherited cardiac channelopathies, with mostly dynamic alteration of the repolarization phase. We have now designed the present observational study with the aim of further understanding cardiac dysfunction and preventing sudden death in AHC and other ATP1A3-related neurologic syndromes. A total of 112 cases with ATP1A3-related disorders were recruited, including 97 AHC, 9 RDP, 3 CAPOS, and 3 undefined syndromes. The mean age at inclusion was 16 years (standard deviation, SD; ±13), with 64 patients under the age of 16 years, and 48 aged 16 or over. The first 12-lead ECG available for the cohort was performed at an average age of 18 years (SD 15) and showed abnormalities in 61 patients (61%). These included: T wave abnormalities, anterior (n=33, 33%), lateral (n=23, 23%), and inferior (n=42, 42%); IVCD, anterior (n=15, 15%) and inferior (n=10, 10%); incomplete right bundle branch block (n=23, 23%); J-point elevation (n=3, 3%). The ECG was repeated in 18 patients one or more times, and showed dynamic changes in 10. The first Holter ECG was performed at an average age of 16 years (SD 13) and showed abnormalities in 25 (38%), either conduction disease (n=4) or T wave abnormalities (n=21). Atrial ectopics were present in 33 (50%), junctional ectopics in 1 (2%), ventricular ectopics in 19 (29%). Echocardiography was performed in 81 patients and did not show evidence of structural abnormalities. Evidence of conduction disease was seen in all ATP1A3-related syndromes, including AHC, CAPOS, RDP and undefined syndromes, requiring the insertion of a pacemaker or implantable cardioverter-defibrillator in 3 patients. Our findings confirm the presence of dynamic cardiac dysfunction in ATP1A3-related syndromes. This may account for some of the unexplained premature mortality including ‘SUDEP’. There are relevant implications in terms of treatment and prevention strategies. Our findings also suggest a broader phenotype of the ATP1A3-syndromes, with the need for systematic evaluation of all organs expressing ATP1A3.

Poster 6

Epilepsy masquerades: alternating hemiplegia of childhood with a novel mutation in the ATP1A3 gene, a case report with literature review

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Rationale: During early childhood, a number of conditions present with paroxysmal symptoms concerning for seizures. Most patients get extensive diagnostic work-up including labs, imaging and exhaustive genetic testing. This often delays the diagnosis and treatment in rare genetic conditions that mimic childhood epilepsy syndromes. Alternating hemiplegia of childhood (AHC) is a rare and complex neurological disorder characterized by repeated attacks of hemiplegia/tetraplegia, paroxysmal disturbances (tonic/dystonic spells and monocular nystagmus), epileptic seizures, developmental delay and episodes of autonomic dysfunction with the onset of symptoms prior to 18 months of age and characteristic improvement of plegic attacks with sleep. A focus on detailed history with knowledge of diagnostic criteria allows for early identification and management of this complex disorder.

Methods: Case of AHC with a previously unreported variant in ATP1A3, a gene encoding a Na/K-ATPase pump. Chart review and literature review of PubMed using the search terms: "Alternating Hemiplegia of Childhood" AND "ATP1A3".

Results: A 19 month-old male presented with quadriparesis, drooling and paroxysmal dystonic posturing with no electroencephalographic (EEG) correlation of paroxysmal events but abnormal baseline EEG. Initially diagnosed with epilepsy for abnormal eye movements and treated with increasing doses of antiepileptic medications with poor symptom control but significant adverse effects. A detailed clinical history and targeted genetic work up revealed AHC. The child is now improving on flunarizine. Genetic testing revealed a novelc. **2393t>C (p. Leu798Pro)** variant in the ATP1A3 gene previously not described in literature. Mutations in the ATP1A3 gene are identified as the primary cause of AHC, composing the majority of cases. Our search identified more than 85 different ATP1A3 mutations with a wide array of phenotypic presentation.

Conclusions: AHC is a rare autosomal dominant condition that should be strongly considered for any child presenting with paroxysmal attacks of hemiparesis/dystonia on alternating sides, early onset seizures and monocular nystagmus without an EEG correlate. This condition often presents with seizures initially but subsequent neurological symptoms are commonly misdiagnosed leading to poor outcome. Awareness and early identification of AHC allows for targeted diagnostic work up, prompt treatment, appropriate genetic counselling and improved prognosis; offering patients a better quality of life overall.

Poster 13

Altered pump function and network activity in ATP1A3 associated childhood-onset schizophrenia

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Mutations in ATP1A3 have been associated with alternating hemiplegia of childhood and a spectrum of other neurodevelopmental disorders. Recently, a nonsynonymous ATP1A3 variant (p.V129M) was discovered in a subject with childhood-onset schizophrenia (COS), a severe and rare form of schizophrenia with onset before age 13 years. In this study, we investigated the electrophysiological properties of neurons generated from COS patient-specific induced pluripotent stem cells (iPSCs), heterozygous for the variant, to determine cellular mechanisms underlying the disease. Cortical excitatory neurons were differentiated from patient and control iPSCs by neurogenin-2 (NGN2) induction followed by co-culture with mouse glia. Whole-cell voltage clamp recording of currents mediated by the Na/K-ATPase showed that while the overall "pump" current was unaltered, the ratio of ouabain (10 μM) sensitive current to total pump current was greater in patient-derived neurons compared to control neurons, suggesting a possible relative down-regulation of ATP1A1. Additionally, ATP1A3 mediated currents in patient-derived neurons exhibited greater sensitivity to extracellular K^+, suggesting that the p.V129M variant may confer a gain-of-function phenotype at low extracellular K^+ concentrations. Consistent with the gain-of-function hypothesis, COS neurons exhibited smaller veratridine-induced membrane depolarization than control neurons. Whole-cell current clamp recording showed no difference in resting membrane potential or evoked action potential firing between COS neurons and control cells. However, afterhyperpolarization following a burst of action potentials had both smaller amplitude and shorter duration in COS neurons compared to control neurons. Finally, patient-derived neurons exhibited less synaptic activity than control neurons as evidenced by smaller spontaneous excitatory current amplitude. Our findings suggest that altered neuronal Na/K-ATPase ion transport activity associated with ATP1A3 variant p.V129M in human COS neurons affects cellular and network activity, which may be critical factors in the development of this disease.

Poster 14

Generation of a cell-based assay for modeling childhood-onset schizophrenia (COS)

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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 differentiated these cells into excitatory cortical neurons via Neurogenin-2 for downstream analysis. 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 enhanced ATP1A3 pump function could help stabilize membrane potential. Transcriptome analysis of proband cortical neurons also revealed an enhancement of transcriptional programs related to psychiatric diseases. These results suggest the p.V129M ATP1A3 line may offer a valid cell-based platform for mechanistic modeling and drug screens of COS.

Poster 15

Consequences of ATP1A3 mutations in patient-derived inhibitory GABAeric neurons

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Mutations in ATP1A3 cause alternating hemiplegia of childhood (AHC) and other neurodevelopmental disorders. However, the fundamental consequence of these mutations in human neurons has not been investigated fully. Our approach to determining the disease mechanism uses human neurons generated from AHC patient-derived induced pluripotent stem cells (iPSCs). Previously, we reported the electrophysiological properties of two distinct AHC-associated mutations in differentiated cortical excitatory neurons. One missense mutation, G947R, exhibited an impaired Na/K-ATPase pump activity in excitatory neurons and was associated with an altered potassium ion gradient, depolarized resting membrane potential, functionally reduced sodium channel availability, and blunted neuronal excitability. A second mutation involves disruption of a splice site (c.2542+2T>C) leading to an in-frame retention of 66 nucleotides from the adjacent intron and absence of the mutant protein in excitatory neurons, which suggests ATP1A3 haploinsufficiency. In our current study, we focus on investigating these two mutations in patient-specific, iPSC-derived GABAergic neurons. Neuronal differentiation was induced by transcription factors Ascl1 and Dlx2, and the cells converted to mature human GABAergic neurons by co-culturing with mouse glial cells. The neurons were then compared with those differentiated from either iPSCs from a healthy control or an isogenic control iPSC created from the patient-specific line by CRISPR/Cas9 genome editing. Electrophysiology and biochemistry studies are underway to assess the molecular and cellular consequences in the inhibitory GABAeric neurons.

Poster 16

An iPSC-derived neuronal model to investigate alternating hemiplegia of childhood

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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 is 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 on the most phenotypically severe ATP1A3 mutation, E815K.

AHC patient-specific iPSCs have been differentiated to cortical glutamatergic neurons and GABAergic interneurons to test that hypotheses that E815K mutant α3 protein decreases neuronal Na,K-ATPase function in a dominant-negative manner, and that mutant α3 protein perturbs normal neurodevelopment and results in neuronal depolarization exacerbated during cellular stress. Results indicate that iPSC-derived control and ATP1A3 mutant neurons display similar temporal patterns of α3 protein expression during neuronal differentiation. Ongoing studies involve multielectrode array electrophysiological (MEA) analyses, transcriptome profiling of neural development in AHC, and testing for lineage-specific consequences of mutant α3 expression in GABAergic interneurons using newly published differentiation protocols. We are increasing the potential impact of our findings and probing the dominant negative disease hypothesis by generating isogenic cell lines. Utilizing CRISPR/Cas9 genetic editing techniques, we have corrected ATP1A3 mutations in AHC patient iPSCs, and are creating heterozygous and homozygous ATP1A3 knockout lines. This approach will allow for the mechanistic interrogation of disease pathogenesis and neural dysfunction in the presence of AHC-causing ATP1A3 mutations, while also providing a route toward therapeutic discovery in a human disease model.

Poster 18

ATP1A3 wild type and mutated isoforms molecular dynamics simulations in a lipidic membrane bilayer. Insights on ion movement

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Alternating hemiplegia of childhood (AHC) is an extremely rare neurological disorder primarily caused by mutations on the ATP1A3 gene which codes the Na^+/K^+-ATPase subunit alpha-3 (NKA α3), an essential cation pump protein responsible for the maintenance of the sodium and potassium gradients across the plasma membrane. The mutations mainly involved in the AHC occurrences are D801N and E815K. The aim of this study is to gain structural and functional insights on this Na^+/K^+ pump protein and to inspect how sequence point mutations affect structural arrangement and functioning of ion flow through the pump. Since no experimental structure is available, homology modeling techniques were applied to build three-dimensional structures of the wild type (wt) NKA α3 and D801N and E815K mutants. Being both the mutation in the transmembrane region, attention was first focused on protein E2 state. The three structures were embedded into a DOPC bilayer and then submitted to molecular dynamics simulations (MD) in presence of water and KCl at the concentration of 0.15 M. For each one of the three systems submitted to MD, a total simulation time of 500 ns was achieved. Analysis of the simulations data lead to identify the likely more stable conformations for the three proteins, to define the location of the cation binding sites in the transmembrane region and to get some insights in capability of K^+ ions binding for wt and mutants forms. The results indicated that the mutations reduces the protein affinity for K^+ ions during the MD. Further simulations, also on the E1 conformation, are ongoing. This study can be a solid basement for rational drug design on this target.

Financial support provided by AISEA is gratefully acknowledged.

Poster 19

Beta-dependent modulation of the binding/release of Na^+ in the WT and D923N-AHC hNa^+/K^+ATPase-A3

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The Na^+/K^+ATPase uses the energy of ATP hydrolysis to export 3Na^+ from the cell and import 2K^+. The transport cycle creates an electrochemical gradient across the membrane that is used by many processes. A functional ATPase is composed by the transporter alpha- and the modulatory beta-subunit. Four alpha (A1-4) and three beta (B1-3) isoforms have been found differentially expressed across the human body suggesting tissue-specific functions.

Mutations in ATP1A3 (A3-isoform) gene, are linked to Alternating Hemiplegia of Childhood (AHC), a neurological disorder in which transient attacks of hemiplegia occur. The molecular mechanism of AHC is not well understood. Based on the protein structure, it was proposed that AHC-causing mutations alter the ATPase function by affecting the ion binding site. However, a functional validation is needed. Here we aim to study 1) the beta-dependent modulation and 2) the effect of AHC-mutations on the binding/release of individual Na^+ of the hNa^+/K^+ATPase-A3 complex.

Xenopus Oocytes were injected with human Na^+/K^+ ATPase-A3 mRNA plus B1, B2 or B3 in a 1:1 molar ratio. The binding/release of three Na^+: slow, medium and fast components were evaluated by the Cut-Open Vaseline Gap technique.

In all A3-B combinations we found a temporal correlation between the slow, medium and fast components indicating that the three Na^+ bind to the protein one by one in a sequential manner: Fast-medium-slow. B2 appears to provide speed to both the medium and the slow component. B3 shifted the steady-state charge distribution of the slow component towards more positive potentials suggesting an increased Na^+ affinity compared to B1 and B2.

D923N mutation impairs the protein function by affecting Na^+ binding. D923N diminished the contribution of the slow component and decreased its transition rate indicating that the mutation targets primarily the binding of the last Na^+.

Poster 20

Functionalized ClinPhen: humanized animal models for detecting pathogenicity, interrogating mechanism of action, and enabling targeted drug screening in clinical variants

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There is growing demand for functional data on the clinical variants revealed in patient genome sequencing. Of the top 20 genes in ClinVar, 15.4% of variants are assigned to pathogenic/likely-pathogenic status and 8.9% are identified as benign/likely-benign. Remaining variants are either unreported (54%) or categorized as Variants of Uncertain Significance (22.1%). As a result, a large fraction (76%) are in need of better pathogenicity assessment. Our team at Nemametrix is developing humanized animal models ("Functionalized ClinPhen" platform) for robust functional assessment of variant pathogenicity. Animal models utilized insertion of a human cDNA as gene replacement for native ortholog coding sequence in C. elegans. When cDNA sequence exhibits a capacity to restore normal function, the platform is ready for variant installation. In the STXBP1 epilepsy gene, human cDNA was replaced for the unc-18 coding sequence and the result was near complete rescue of function in various assays. Next, the installation of pathogenic variants (R292H, R406H and R388X) in the humanized backbone yielded a series of detectable change-of-function phenotypes, as detected by assays for electrophysiology (pharynx pump frequency), locomotion (liquid thrashing) and behavior (chemotaxis to food source). The platform is expected to be broadly applicable because recent gene-swap humanized KCNQ2 and CACNB4 strains were found to also exhibit rescue-of-function activity. The Functionalized ClinPhen platform will be further expanded to address variant profiles for ATP1A3 and other neurological targets. In a precision medicine approach, drug screening is planned for pathogenic variant strains to finding new or existing AEDs with a likeliness to be therapeutic for a given patient variant. In other future work, the Functionalized ClinPhen will be adapted to 80% of the 7000 Rare Disease genes to help detect variant pathogenicity, find/repurpose drugs, and select inclusion/exclusion criteria for patients in clinical trials.

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