6th Symposium on *ATP1A3* in Disease (*ATP1A3* 2017)

September 21-22, 2017. Tokyo, Japan

PROGRAM & ABSTRACTS

by Miya

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Main Host: National Center of Neurology and Psychiatry (NCNP)
Co-Host: Japan AHC Family Association (JAFA)
Support: Japanese Society of Child Neurology

6th Symposium on *ATP1A3* in Disease (*ATP1A3* 2017)

September 21-22, 2017, Tokyo, Japan

PROGRAM & ABSTRACTS

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Venue: Palace Hotel Tachikawa

4F Rose room

2-4-15 Akebono-cho, Tachikawa city, Tokyo, Japan 190-0012

Organizer: Masayuki Sasaki, M.D.
Department of Child Neurology,
National Center of Neurology and Psychiatry

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Greetings from the Organizer of *ATP1A3* 2017

Dear Colleagues and Friends,

It is our great pleasure to host the **6th Symposium on *ATP1A3* in Disease** in Tokyo, Japan.

The 1st Symposium on *ATP1A3* in Disease was held in Brussels, Belgium in December 2012, when it was first discovered that alternating hemiplegia of childhood (AHC) was caused by *ATP1A3* mutations. Since then the symposiums have been held in Europe and the US. This is the first time that the symposium is held in Asia.

ATP1A3 encodes the Na^+/K^+-ATPase α3 subunit which is expressed mainly in neurons in the central nervous system. Na^+/K^+-ATPase is an essential transmembrane protein, which is ubiquitously expressed in all animal cell membranes. The Na^+/K^+-ATPase transports and exchanges $3Na^+$ and $2K^+$ through the plasma membrane using energy from hydrolysis of an ATP. This pump function was discovered 60 years ago (1957), and it is said that 30% of cellular ATPs are used by Na^+/K^+-ATPase in living creatures.

Thirteen years ago (2004), the first human disease caused by *ATP1A3* mutations was discovered. It was rapid-onset dystonia–parkinsonism (RDP), which mainly occurs in adulthood. Eight years later (2012), three independent research groups (Heinzen et al, Rosewich et al, and Ishii et al) reported that AHC was caused by *ATP1A3* mutations. AHC mainly occurs in early childhood. Since then several rare conditions other than RDP or AHC have been demonstrated to be caused by *ATP1A3* mutations. However, effective treatments for *ATP1A3*-related diseases have not yet been established. In this symposium, we would like to discuss the characteristics of *ATP1A3*-related diseases, the physiological functions of the Na^+/K^+-ATPase, and the possible new treatment methods for *ATP1A3*-related diseases.

We have invited experts on clinical research of *ATP1A3*-related diseases and basic research of the Na^+/K^+-ATPase from all over the world. In addition, some Japanese experts on the relevant and very exciting fields will give us great talk. We welcome many attendants who are interested in Na^+/K^+-ATPase and *ATP1A3*-related diseases. We hope that all attendants will enjoy this symposium and the pleasant autumn season of Japan.

Masayuki Sasaki, M.D.,
Organizer of the **6th Symposium on *ATP1A3* in Disease**

Organizing committee

Organizer: Masayuki Sasaki, M.D. Department of Child Neurology,
National Center of Neurology and Psychiatry (NCNP)

Organizing Committee:

Takao Takahashi, M.D., Professor, Department of Pediatrics, Keio University
Atsushi Araki, M.D., Director, Department of Pediatrics, Noe Hospital
Hitoshi Osaka, M.D., Professor, Department of Pediatrics, Jichi Medical University
Yoshiaki Saito, M.D. Associate Professor, Department of Child Neurology,
Tottori University
Shin-ichi Hirose, M.D. Professor, Department of Pediatrics, Fukuoka University
Takashi Shiihara M.D. Director, Department of Child Neurology,
Gunma Children's Hospital

Secretariats

Yuko Motohashi, M.D., Department of Child Neurology, NCNP
Eri Takeshita, M.D., Department of Child Neurology, NCNP
Noriko Nakamura, Department of Child Neurology, NCNP

Registration

Registration Fee

The registration fee covers the Symposium brochure (Program and abstracts), the coffee breaks and the lunches on 21 and 22 September 2017, and the social events on 21 September 2017.

Category	Standard Fee	Reduced Fee * (JPY 6,000 reduction)
First early bird April 1 – May 31	JPY 36,000	JPY 30,000
Second early bird June 1 – July 21	JPY 40,000	JPY 34,000
Standard registration July 22 – Sep 22	**JPY 44,000**	**JPY 38,000**
Special parents discount	If both parents attend,	1/2 discount for the Second person

* For those who register The 15th International Conference on Na,K-ATPase and Related Transport ATPases (Otsu, September 24-30, 2017), or The 23rd World Congress of Neurology (Kyoto, September 16-21, 2017)

On-site Registration

Registration desk will open at

Day 1: Thursday, Sep 21 8:30-18:00

Day 2: Friday, Sep 22 8:30-12:00

Social event

Conference Dinner

Date: Thursday, Sep 21 18:30-20:30

Venue: Meeting room, 4th floor, Palace Hotel Tachikawa

Instructions for presentations

(1) To all oral presenters
We prepare WINDOWS7 PC and one projector.
A single projection screen without sound is available for presentation.
Please use your own PC for the presentation.
Don’t forget to the following items; AC Adaptor & Connection cables.
Please ensure that your computer is equipped with the monitor connector of mini D-sub 15 pins.

If your computer does not have this connection, please bring an appropriate converter with you.

Please keep your presentation time.
For example, (20+5) means 20 minutes presentation and 5 minutes discussion time.
Please complete your presentation data in English.

(2) To all poster presenter

All poster presenters are requested to make a registration at this Symposium.

Poster area is prepared in front of the Symposium hall.

Poster discussion time is scheduled twice in the last 30 minutes in both lunch times.

Poster presentation is required to stand by your own poster at the presentation time.

No chairperson will stand by.

Installation: 8:30-9:00 Sep 21

Presentation 13:30-14:00 Sep 21, 12:30-13:00 Sep 22

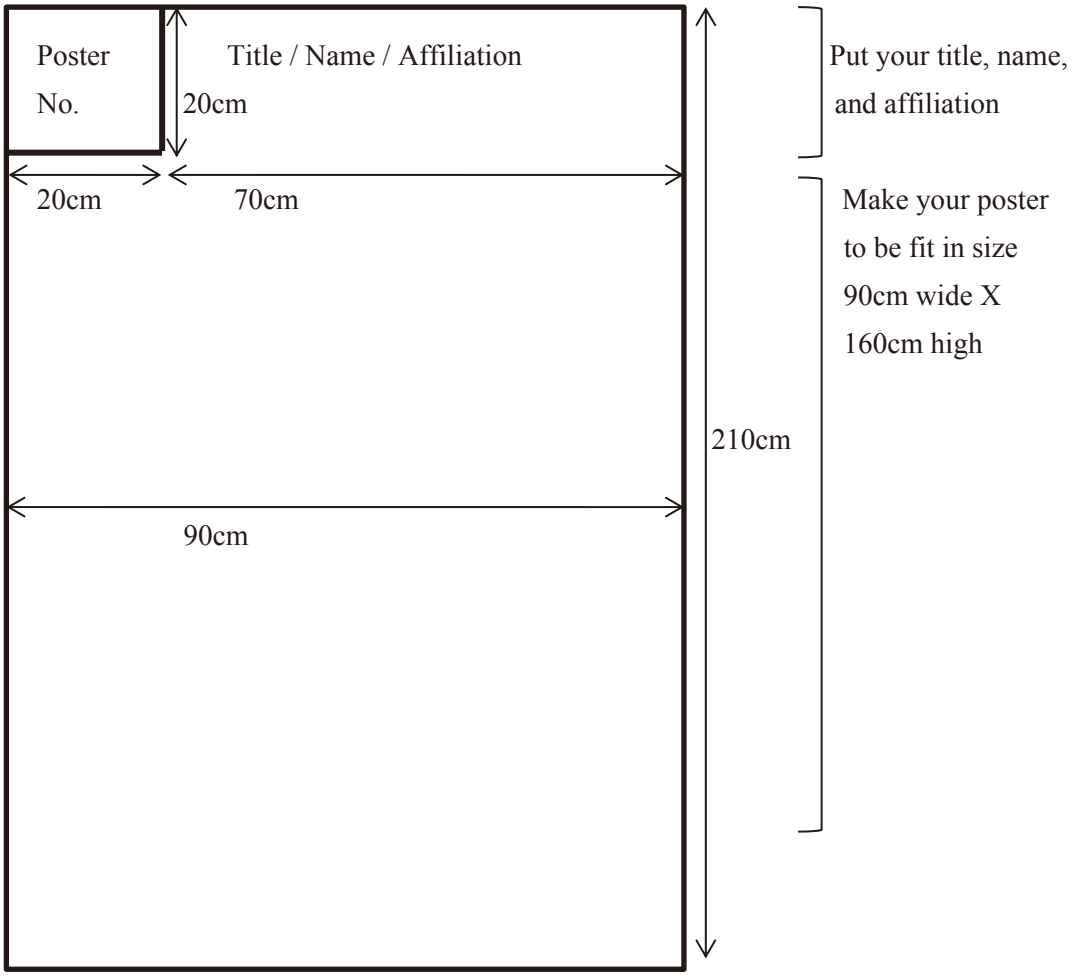
Removal 15:10-15:30 Sep 22 (All posters will be removed)

Poster panel size is 90cm wide X 210cm high.

For poster itself, recommendable size is 90cm X 160cm.

Poster number will be prepared with push pins for your poster in size 20cm X 20cm.

Please prepare a banner to show your Title, Name, and Affiliation in size 70cm X 20cm.



Program (invited speakers and titles)

September 21

8:30- Registration

9:00-9:20 Opening address

Masayuki Sasaki, National Center of Neurology and Psychiatry, Japan
Overview of Clinical Studies on Alternating Hemiplegia of Childhood

9:20-11:00 Session 1. Clinical Aspects and Genotype/Phenotype Correlations in *ATP1A3*-related Disease (Chair Mohamad Mikati and Allison Brashear)

1. **Hendrik Rosewich**, Georg August Medical University Göttingen, Germany. (20+5)
   Clinical and genetic spectrum of *ATP1A3*-related disorders
2. **Allison Brashear**, Wake Forest University School of Medicine, USA. (20+5)
   Rapid-onset dystonia-parkinsonism (RDP): Clinical and Genetic Updates
3. **Yasunari Sakai**, Kyushu University, Japan (10+5)
   Relapsing encephalopathy with cerebellar ataxia (RECA)
4. **Naoko Ishihara**, Fujita Health University, Japan (10+5)
   Catastrophic early life epilepsy with *ATP1A3* mutation
5. **Kathleen J. Sweadner**, Massachusetts General Hospital, USA (10+5)
   Genotype-Structure-Phenotype: what makes *ATP1A3* mutations different?

11:00-11:20 Coffee break

11:20-12:40 Session 2. Clinical Diversity in AHC and other *ATP1A3*-related Disease (Chair Kathy Swoboda, Hendrik Rosewich)

1. **Eleni Panagiotakaki**, University Hospitals of Lyon, France (20+5)
   Clinical profile of patients with *ATP1A3* mutations in AHC in an International Cohort
2. **Kathryn J. Swoboda**, Massachusetts General Hospital, USA (20+5)
   Transcriptome analysis and clinical database/biorepository of Alternating Hemiplegia of Childhood patients with *ATP1A3* mutations for potential therapeutic discovery
3. **Simona Balestrini,** UCL Institute of Neurology, UK (20+5)
   Cardiac phenotype in *ATP1A3* related-syndromes

12:40-14:00 Lunch
Restaurant (1F, iL PePe, Palace Hotel Tachikawa)

13:30-14:00 Poster presentation (1) 15 Posters for 2 days at the 4th floor

14:00-15:00 Special lecture 1

(Chair Takao Takahashi: Keio University School of Medicine, the President of Japan Pediatric Society and the President of Japanese Society of Child Neurology)

Hideyuki Okano, Keio University School of Medicine, Japan (50+α)

Modeling Human Diseases with iPS cells and Genetically Modified Non-Human Primates

15:00-16:00 Special lecture 2

(Chair Bente Vilsen)

Chikashi Toyoshima, The University of Tokyo, Japan (50+α)

Structural biology of Na^+,K^+-ATPase: towards understanding of mutations in *ATP1A3*

16:00-16:20 Coffee break

16:20-18:00 Session 3. Basic research topics on *ATP1A3* (Chair Jan Koenderink)

1. **Arn M.J.M. van den Maagdenberg**, Leiden University, The Netherlands (20+5)
   New causative gene(s) in AHC?
2. **Jan B. Koenderink**, Radboud University, the Netherland (20+5)
   Biochemical consequences of *ATP1A3* mutations
3. **Bente Vilsen**, Aarhus University, Denmark (20+5)
   ***ATP1A3* neurological disease mutations affecting Na^+ binding: Structural and functional perspectives and rescue of compromised function continued**

18:30- Conference Dinner (the same floor as the meeting)

Family talk

1. DVD. Human Timebomb
2. Talk from the Japanese AHC Family Association
3. Europe and the US Family

September 22

9:00-10:20 Session 4. Model animal studies in *ATP1A3*-related disease (Chair Mohamad Mikati)

1. **Steven Clapcote**, University of Leeds, UK (20+5)
   Development of new treatments in the *Myshkin* mouse model of AHC
2. **Karin Lykke-Hartmann**, Aarhus University, Denmark (20+5)
   Hypothermia-induced dystonia and abnormal cerebellar activity in a mouse model with a single disease-mutation in the sodium-potassium pump
3. **Mohamad A. Mikati**, Duke University, USA (20+5)
   Study of Knock-in model mouse; Development of new treatments

10:20-10:40 Coffee break

10:40-11:10 Special lecture 3 (Chair Hitoshi Osaka: Jichi Medical school)

Minako Hoshi, Institute of Biomedical Research and Innovation, Japan (25+5)
Na^+, K^+-ATPase α3 is a Death Target of Alzheimer Amyloid-β Assembly
What Shall We Do Next towards A Better Understanding of Na^+, K^+-ATPase α3's Role in Health and Disease?

11:10-11:40 Special lecture 4 (Chair Hitoshi Osaka)

Noriyuki Matsuda, Tokyo Metropolitan Institute of Medical Science, Japan (25+5)
Mitochondrial autophagy and familial Parkinson's disease

11:40-13:00 Lunch
Restaurant (1F iL PePe, Palace Hotel Tachikawa)

12:30-13:00 Poster Presentation (2) the same posters as the day 1

13:00-14:40 Session 5. Treatment trials (Chair Hendrik Rosewich, Kathy Swoboda)

1. **Atsushi Ishii**, Fukuoka University, Japan (20+5)
   Treatment with adenosine- 5'- triphosphate for AHC
2. **Steven Petrou,** University of Melbourne, Australia (20+5)
   Loss of function *ATP1A3* mutations differentiated by pre-steady-state analysis
3. **Kevin C. Ess**, Vanderbilt University, USA (20+5)
   Modeling Alternating Hemiplegia of Childhood using Patient Derived Stem Cells
4. **Alfred L. George, Jr**, Northwestern University, USA (20+5)
   Modeling Alternating Hemiplegia of Childhood in Induced Pluripotent Stem Cells

14:40-15:00 General discussion (Chair Allison, Mohamad, Tsveta)

The function of the Na^+/K^+-ATPase α3 isoform and the new treatment methods

15:00-15:10 Conclusions and future priorities (Hendrik Rosewich)

[Symposium closed]

15:30-17:30
Optional event: A stroll in the Showa Memorial Park (about 2 hour course)

Organizer Speech

Overview of Clinical Studies on Alternating Hemiplegia of Childhood

Masayuki Sasaki, M.D.
Department of Child Neurology, National Center of Neurology and Psychiatry

Alternating hemiplegia of childhood (AHC) was first reported as a complicated migraine beginning in infancy (Verret S and Steel JC, Pediatrics 1971). Diagnostic criteria were proposed (Aicardi J, Dev Med Child Neurol 1980) and have long been used. Flunarizine was first reported to be effective in one case (Casaer P, Lancet 1984); subsequently, several groups have observed that flunarizine is effective in reducing the duration and severity of hemiplegic attacks (Casaer P. Neuropediatrics 1987, Silver K, Neurology 1993, Bourgeois M, J Pediatr 1993, Sasaki M, Brain Dev 2001) but not in curing the disease.

Large patient studies have revealed clinical courses and no diagnostic clue in patients with AHC (Sakuragawa N, Brain Dev 1993, Mikati MA, Pediatr Neurol 2000, Sweney MT, Pediatrics 2009, Panagiotakaki E, Brain 2010). All studies showed that brain magnetic resonance imaging and electroencephalogram failed to show specific abnormalities in patients with AHC.

The pathophysiology of AHC in terms of mitochondrial function (Arnold DL, Ann Neurol 1993, Kemp GJ, Ann Neurol 1995), brain perfusion (Aminian A, Ann Neurol 1993, Siemes H, Dev Med Child Neurol 1993), brain glucose metabolism (de Silva EA, Ann Neurol 1996, Sasaki M, Brain Dev 2009), and small-vessel studies (Auvin S, Neurology 2006, Sasaki M, Brain Dev 2011) has been investigated by many groups. However, these studies could not reveal the pathophysiology of AHC.

A point mutation in *ATP1A2*, which encodes the Na^+/K^+-ATPase α2 subunit, was noted in only one autosomal dominant AHC family (Swoboda KJ, Ann Neurol 2004) but no follow-up studies have confirmed these results (Boileau S, Dev Med Child Neurol 2008).

Calcium and other ion channels have been studied, but only negative results have been reported (Haan J, Caphalalgia 2000, de Vries B, Cephalalgia 2008, Hirose S, Sasaki M, unpublished data).

In 2012, three study groups independently discovered the causative gene *ATP1A3* (Heinzen EL, Nat Genet 2012, Rosewich H, Lancet Neurol 2012, Ishii A, PLoS One 2013), which encodes the Na^+/K^+-ATPase α3 subunit. Rapid-onset dystonia–parkinsonism (RDP) had been known to be caused by the same gene abnormalities (de Carvalho AP, Brashear A, Neuron 2004). Therefore, AHC could be an allelic disorder of RDP. A genotype–phenotype correlation was seen in *ATP1A3*-related disorders (Heinzen EL, Lancet Neurol 2014, Rosewich H, Neurology 2014).

Before the causative gene of AHC was uncovered, some patients presented with sudden death or severe deterioration (Neville BG, Dev Med Child Neurol 2007, Saito Y, Epilepsy Res 2010), but the etiology of irreversible deterioration was not known. Our observation on the relation between clinical course and gene mutation type revealed that there could be genotype–phenotype correlations even in AHC (Sasaki M, Neurology 2014). Larger observational studies have reported the same tendencies (Viollet L, PLoS One 2015, Panagiotakaki E, Orphanet J Rare Dis 2015).

New phenotypes have been reported, and the concepts of *ATP1A3*-related neurological disorders are expanding (Demos MK, Orphanet J Rare Dis 2014, Dard R, Dev Med Child Neurol 2015, Paciorkowski AR, Epilepsia 2015).

Precise mechanisms underlying the symptoms and gene abnormalities have been studied using animal models (Clapcote SJ, Proc Natl Acad Sci USA 2009, Ikeda K, J Physiol 2013, Hunanyan AS, Epilepsia 2015) or cell models (Li M, Neurobiol Dis 2015) but have not yet been completely elucidated. Curable treatment methods are currently being investigated using new technologies.

Masayuki SASAKI

Present Position

Director, Department of Child Neurology,
National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan

Education

1983 MD, Niigata University School of Medicine, Niigata, Japan
1983-1988 Residency of Pediatrics, Niigata University Hospital
1988-1990 Residency of Child Neurology, Department of Child Neurology, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan
1990-1992 Fellow, Division of Inherited Metabolic Disorders, National Institute of Neuroscience, National Center of Neurology and Psychiatry
1992 PhD (Dr. of Medical Science), Toho University
1992-1994 Visiting fellow, Myelin Section, Laboratory of Molecular and Cellular Neurosciences, National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Health (NIH), Bethesda, Maryland, USA

Academic Appointments

1994-1996 Department of Child Neurology, National Center of Neurology and Psychiatry
1996-2002 Section Chief, Department of Child Neurology, National Center of Neurology and Psychiatry
2002-Present Director, Department of Child Neurology, National Center of Neurology and Psychiatry

Publications

On alternating hemiplegia of childhood (AHC)

1. Saito Y, Sakuragawa N, **Sasaki M**, Sugai K, Hashimoto T: A case of alternating hemiplegia of childhood with cerebellar atrophy. *Pediatr Neurol* 1998;19:65-68.
2. **Sasaki M**, Sakuragawa N, Osawa M: Long-term effect of flunarizine on patients with alternating hemiplegia of childhood. *Brain Dev* 2001;23:303-305.
3. **Sasaki M**, Sakuma H, Fukushima A, Yamada KI, Ohnishi T, Matsuda H. Abnormal cerebral glucose metabolism in alternating hemiplegia of childhood. *Brain Dev* 2009;31:20-26.
4. Saito Y, Inui T, Sakakibara T, Sugai K, Sakuma H, **Sasaki M**. Evolution of hemiplegic attacks and epileptic seizures in alternating hemiplegia of childhood. *Epilepsy Res* 2010;90:248-258.
5. **Sasaki M**, Matsufuji H, Inui T, Arima K. Absence of small-vessel abnormalities in alternating hemiplegia of childhood. *Brain Dev* 2011;33:390-393.
6. Inui T, Saito Y, Sakuma H, Hatakeyama H, Goto Y-I, Arai H, **Sasaki M**. Profiles of blood biomarkers in alternating hemiplegia of childhood -increased MMP-9 and decreased Substance P suggest its pathophysiology- *Brain Dev* 2012;34:196-200.
7. Ishii A, Saito Y, Mitsui J, Ishiura H, Yoshimura J, Arai H, Yamashita S, Kimura S, Oguni H, Morishima S, Tsuji S, **Sasaki M**, Hirose S. Identification of *ATP1A3* mutations by exome sequencing as the cause of alternating hemiplegia of childhood in Japanese patients. *PLoS One* 2013;8:e56120.
8. **Sasaki M**, Ishii A, Saito Y, Hirose S. Intermediate form between alternating hemiplegia of childhood and rapid-onset dystonia-parkinsonism. *Mov Disord* 2014;29:153-154.
9. **Sasaki M**, Ishii A, Saito Y, Morisada N, Iijima K, Takada S, Araki A, Tanabe Y, Arai H, Yamashita S, Ohashi T, Oda Y, Ichiseki H, Hirabayashi S, Yasuhara A, Kawawaki H, Kimura S, Shimono M, Narumiya S, Suzuki M,

Yoshida T, Oyazato Y, Tsuneishi S, Ozasa S, Yokochi K, Dejima S, Akiyama T, Kishi N, Kira R, Ikeda T, Oguni H, Zhang B, Tsuji S, Hirose S. Genotype-phenotype correlations in alternating hemiplegia of childhood. *Neurology* 2014;82:482-490.

10. Heinzen EL, Arzimanoglou A, Brashear A, Clapcote SJ, Gurrieri F, Goldstein DB, Johannesson SH, Mikati MA, Neville B, Nicole S, Ozelius L, Poulsen H, Schyns T, Sweadner KJ, van den Maagdenberg A, Vilsen B for **the ATP1A3 Working Group**. Distinct neurological disorders with ATP1A3 mutations. *Lancet Neurol* 2014 May;13(5):503-514.
11. Rosewich H, Sweney MT, DeBrosse S, Ess K, Ozelius L, Andermann E, Andermann F, Andrasco G, Belgrade A, Brashear A, Ciccodicola S, Egan L, George AL Jr, Lewelt A, Magelby J, Merida M, Newcomb T, Platt V, Poncelin D, Reyna S, **Sasaki M**, Sotero de Menezes M, Sweadner K, Viollet L, Zupanc M, Silver K, Swoboda K. Research conference summary from the 2014 International Task Force on *ATP1A3*-Related Disorders. *Neurol Genet* 2017;3: e139.
12. **Sasaki M**, Ishii A, Saito Y, Hirose S. Progressive brain atrophy in alternating hemiplegia of childhood. *Mov Disord Clin Prac* 2017 May/June;4(3):406-411.

Select publications other than AHC

1. Kurachi Y, Oka A, Muzuguchi M, Ohkoshi Y, **Sasaki M**, Itoh M, Hayashi M, Goto Y, Takashima S: Rapid immunologic diagnosis of classic late infantile neuronal ceroid-lipofuscinosis. *Neurology* 2000;25:1676-1680.
2. Ohnishi T, Matsuda H, Hashimoto T, Kunihiro T, Nishikawa M, Uema T, **Sasaki M**: Abnormal regional cerebral blood flow in childhood autism. *Brain* 2000;123:1838-1844.
3. Shimojo Y, Osawa Y, Fukumizu M, Hanaoka S, Tanaka H, Ogata F, **Sasaki M**, Sugai K: Severe infantile dentatorubral pallidoluysian atrophy with extreme expansion of CAG repeats. *Neurology* 2001;56:277-278.
4. Sudo A, **Sasaki M**, Sugai K, Matsuda H. Therapeutic effect and [123I]IMP SPECT findings of sodium dichloroacetate in a patient with MELAS. *Neurology* 2004;62:338-339.
5. **Sasaki M**, Takanashi J, Tada H, Sakuma H, Furushima W, Sato N. Diffuse cerebral hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum. *Brain Dev* 2009;31:582-587.
6. Saitsu H, Osaka H, **Sasaki M**, Takanashi J, Hamada K, Yamashita A, Shibayama H, Shiina M, Kondo Y, Nishiyama K, Tsurusaki Y, Miyake N, Doi H, Ogata K, Inoue K, Matsumoto N. Mutations in *POLR3A* and *POLR3B* encoding RNA polymerase III subunits cause an autosomal-recessive hypomyelinating leukoencephalopathy. *Am J Hum Genet* 2011Nov 11;89:644-651.
7. Maruyama S, Saito Y, Nakagawa E, Saito T, Komaki H, Sugai K, **Sasaki M**, Kumada S, Saito Y, Tanaka H, Minami N, Goto YI. Importance of CAG repeat length in childhood-onset dentatorubral pallidoluysian atrophy. *J Neurol* 2012;259:2329-2334.
8. Nagai S, Saito Y, Endo Y, Sugai K, Saito T, Ishiyama A, Komaki H, Nakagawa E, **Sasaki M**, Saito Y, Sukigara S, Ito M, Goto Y, Ito S, Matsuoka K. Hypoalbuminemia in dentatorubral pallydoluysian atrophy due to albumin leakage in multiple organs. *J Neurol* 2013;260:1263-1271.
9. Miyatake S, Osaka H, Shiina M, **Sasaki M**, Takanashi JI, Haginoya K, Wada T, Morimoto M, Ando N, Ikuta Y, Nakashima M, Tsurusaki Y, Miyake N, Ogata K, Matsumoto N, Saitsu H. Expanding the phenotypic spectrum of *TUBB4A*-associated hypomyelinating leukoencephalopathies. *Neurology* 2014; 82:2230-2237.
10. **Sasaki M**, Ohba C, Iai M, Hirabayashi S, Osaka H, Hiraide T, Saitsu H, Matsumoto N. Sporadic infantile-onset spinocerebellar ataxia caused by missense mutations of the inositol 1,4,5-triphosphate receptor type 1 gene. *J Neurol* 2015;262:1278-1284.
11. Kodera H, Ohba C, Kato M, Maeda T, Araki K, Tajima D, Matsuo M, Hino-Fukuyo N, Kohashi K, Ishiyama A, Takeshita S, Motoi H, Kitamura T, Kikuchi A, Tsurusaki Y, Nakashima M, Miyake N, **Sasaki M**, Kure S, Haginoya K, Saitsu H, Matsumoto N. De novo *GABRA1* mutations in Ohtahara and West syndromes. *Epilepsia* 2016;57:566-73.

Special Invited Speakers

Special lecture 1

Modeling Human Diseases with iPS cells and Genetically Modified Non-Human Primates

Hideyuki Okano
Keio University School of Medicine, Japan

For effective modelling of human psychiatric/psychiatric disorders, we took advantage of iPS cell technologies and transgenic non-human primates. So far, we have established iPS cells from the patients of about 40 human psychiatric/psychiatric disorders, including Alzheimer disease[1,2], Parkinson disease [3, 4, 5], ALS[6], Rett syndrome[7,8], Pelizaeus-Merzbacher disease[9] (Kuroiwa-Numasawa et all., Stem Cell Reports, 2014), Lissencephaly[10], retinitis pigmentosa[11] and Pendred Syndrome[12]. Furthermore, for faithfully modeling the human disorders *in vivo*, we developed transgenic non-human primates (common marmosets) with germline transmission[13]. In the present talk, we also wish to mention our recent data of generation of common marmoset transgenic models of neurodegenerative diseases, including Parkinson disease, Alzheimer disease and ALS. Furthermore, we could generate knock-out technologies of common marmoset using genome editing technologies[14], which can be applied for generation of models of autism and psychiatric disorders. At the end, I will mention about Brain Mapping Projects in Japan, in which investigation of common marmoset brains plays key roles[15, 16, 17].

References

1. Yagi T *et al*.: Modeling familial Alzheimer's disease with induced pluripotent stem cells. **Hum Mol Genet.** 20(23):4530-4539, 2011.
2. Imaizumi K *et al*.: Controlling regional identity of human pluripotent stem cell-derived neurons: a novel approach to uncover neuronal subtype specificity of neurological disease phenotypes. **Stem Cell Reports**, 5(6):1010-22, 2015.
3. Imaizumi Y *et al*.: Mitochondrial dysfunction associated with increased oxidative stress and alpha-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. **Mol Brain**. 5(1): 35, 2012.
4. Ohta E *et al*.: I2020T mutant LRRK2 iPSC-derived neurons in the Sagamihara family exhibit increased Tau phosphorylation through the AKT/GSK-3 **Hum Mol**

Genet. 24(17):4879-900, 2015.
5. Matsumoto T *et al*.: Functional neurons generated from T cell-derived iPSCs for neurological disease modeling **Stem Cell Reports,** 6(3):422-435, 2016.
6. Ichiyanagi N *et al*.: Establishment of in vitro FUS-associated familial amyotrophic lateral sclerosis model using human induced pluripotent stem cells. **Stem Cell Reports**, 6(4):496-510, 2016.
7. Andoh-Noda T *et al*.: Differentiation of multipotent neural stem cells derived from Rett syndrome patients is biased toward the astrocytic lineage. **Mol Brain.** 8:31, 2015.
8. Yasui T *et al*., Hypoxia epigenetically confer astrocyte differentiation potential on human pluripotent cell-derived neural precursor cells. **Stem Cell Reports**, 2017 (Accepted for Publication).
9. Numasawa-Kuroiwa Y *et al*.: Involvement of Endoplasmic Reticulum Stress in Dysmyelination of Pelizaeus-Merzbacher Disease with *PLP1* Missense Mutations Shown by Induced-Pluripotent-Stem Cell-Derived Oligodendrocytes. **Stem Cell Reports**, 2(5): 648-661, 2014.
10. Bamba Y et al.: *In vitro* characterization of neurite extension using induced pluripotent stem cells derived from lissencephaly patients with TUBA1A missense mutations. **Mol Brain** 9(1):70, 2016.
11. Yoshida T *et al*.: The use of induced pluripotent stem cells to reveal pathogenic gene mutations and explore treatments for retinitis pigmentosa. **Mol Brain.** 7 (1): 45, 2014.
12. Hosoya M *et al*.: Cochlear cell modeling using disease-specific iPSCs unveils a degenerative phenotype and suggests treatments for congenital progressive hearing loss. **Cell Reports**, 18(1):68-81, 2017.
13. Sasaki E *et al*.: Generation of transgenic non-human primates with germ line transmission. **Nature**, 459(7246): 523-527, 2009.
14. Sato K *et al*.: Generation of a Non-human primate model of severe combined immunodeficiency using highly efficient genome editing. **Cell Stem Cell**, 19: 127–138, 2016.
15. Okano H, Miyawaki A, Kasai K.: Brain/MINDS: Brain-Mapping Project in Japan. **Philos Trans R Soc Lond B Biol Sci.** 370: 20140310, 2015.
16. Okano H, Yamamori T: How can brain mapping initiatives cooperate to achieve the same goal? **Nat Rev Neurosci** 17, 733-734, 2016.
17. Okano H, Sasaki E, Yamamori T, Iriki A, Shimogori T, Yamaguchi Y, Kasai K and Miyawaki A.: Brain/MINDS: a Japanese National Brain Project for Marmoset Neuroscience, **Neuron**, 92 (3): 582-590, 2016.

Hideyuki Okano, M.D., Ph.D.

Position:

-Dean, Keio University School of Medicine, Tokyo, Japan.
-Professor, Department of Physiology, Keio University School of Medicine, Tokyo, Japan
-Team Leader, Laboratory for Marmoset Neural Architecture, Brain Science Institute RIKEN

Education:

1988 Ph.D. (Dr. of Medical Science), Keio University
1983 M.D. Keio University School of Medicine

Employment:

2015~Present	**Dean, Keio University School of Medicine**
2014~Present:	Team Leader, Laboratory for Marmoset Neural Architecture, Brain Science Institute RIKEN
2009~Present	University of New South Wales Visiting Professor, Australia
2008~Present	University of Queensland Honorary Professor in the Queenland Brain Institute, Australia
2001~Present	**Professor, Department of Physiology, Keio University School of Medicine.**
2007~ 2015	Dean, Graduate School of Medicine, Keio University.
1997-2001:	Professor, Division of Neuroanatomy, Department of Neuroscience, Osaka University Graduate School of Medicine
1994-1997:	Professor, Department of Molecular Neurobiology, Institute of Basic Medical Sciences, University of Tsukuba.
1992-1994:	Instructor, Department of Molecular Neurobiology, Institute of Medical Science, University of Tokyo.
1989-1993	Postdoctoral Research Fellow, Department of Biological Chemistry, The Johns Hopkins University School of Medicine.
1985-1989:	Instructor, Institute for Protein Research, Osaka University.
1983-1985:	Instructor, Department of Physiology, Keio University School of Medicine.

Board of Director

The International Society for Stem Cell Research (ISSCR)

The Japan Neuroscience Society (JNS) (Vice President)

The Japanese Society of Inflammation and Regeneration (JSIR)

The Japanese Society for Neurochemistry (JSN)

The Japanese Society for Regenerative Medicine (JSRM)

Editor and Editorial Boards

Inflammation and Regeneration **(Editor-in-Chief)**

Stem Cell Reports **(Associate Editor)**

eLife **(Board of Editor)**

Scientific Reports **(Editorial Board)**

Cell Stem Cell **(Editorial Board)**

Stem Cells **(Editorial Board)**

Genes to Cells **(Associate Editor)**

Journal of Neuroscience Research **(Associate Editor)**

Neuroscience Research **(Associate Editor)**

Stem Cells and Development **(Editorial Board)**

Development, Growth & Differentiation **(Editor)**

Developmental Neuroscience **(Editorial Board (past))**

Major Grants:

Project Leader, the Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) supported by MEXT

Principal Investigattor, Program for Intractable Disease Research Utilizing Disease-specific iPS Cells funded by the Japan Science and Technology Agency (JST)

Principal Investigattor, Research Center Network for Realization of Regenerative Medicine supported by JST

Awards:

2017 DGD Editor-in-Chief Prize
2016 Faculty Award for Internationalization: Impact Factor Most Outstanding Lab Award (from Keio University)
2016 Molecular Brain Award (from The Association for the Study of Neurons and Diseases (A.N.D.)
2014 Erwin von Bälz Award (from Boehringer Ingelheim GmbH)
2013 Stem Cell Innovator Award (from GeneExpression Systems & Apasani Research Conference USA)
2011 The Johnson & Johnson Innovation Award
2009 A Medal of Honor with Purple Ribbon (from Japanese Emperor)
2008 Inoue Prize for Science (from Inoue Foundation for Science)
2007 Lead Reviewer Award (from *Stem Cells*)
2006 Minister Award of Ministry of Education, Culture, Sports, Science and Technology
2004 Medical Award of The Japan Medical Association (from The Japan Medical Association)
2004 Distinguished Scientist Award (from University of Catania School of Pharmacy)
2004 Gold Medal, Tokyo Techno-Forum 21 Award（from Tokyo Techno Forum 21）
2001 Naka-akira Tsukahara Award (from Brain Science Foundation)
1998 Kitasato Award (from Sanshi-Kai, Keio University School of Medicine)
1995 Yoshihiro Kato Memorial Award (from Yoshihiro Kato Memorial Foundation)
1988 Sanshikai Award (from Sanshi-Kai, Keio University School of Medicine)

Special lecture 2

Structural biology of Na^+,K^+-ATPase: towards understanding of mutations in *ATP1A3*

Chikashi Toyoshima
Institute of Molecular and Cellular Biosciences
The University of Tokyo

We have been working on crystal structure studies of two P-type ion transporting ATPases, namely, Ca^{2+}-ATPase (SERCA) and Na^+,K^+-ATPase. As crystallization of Na^+,K^+-ATPase is much more difficult than that of SERCAs, structural and biochemical studies of Na^+,K^+-ATPase are far behind those of SERCA. Furthermore, Na^+,K^+-ATPase is a much more complex system than SERCA and finely regulated. As locations of regulatory proteins (i.e. FXYD with Na^+,K^+-ATPase and phospholamban / sarcolipin with SERCA) in their 3D structures are different, the mode of regulation is likely to be different. Yet similarity between the two ATPases is strong enough to provide us with insight into the structural and functional consequences of mutations identified in ATP1A3. In this talk, I will try to explain, in structure terms, the functional consequences of some of the disease-related mutations. It might be of particular interest that several compounds are now found with SERCA that increase the maximum ATPase activity, as it might be possible to find similar compounds that amend impaired Na^+,K^+-ATPase activity.

Chikashi TOYOSHIMA

・ Present Position

Professor, Institute of Molecular Celluar Biosciences, the University of Tokyo, Tokyo, Japan

・ Education

1978 BSc, Department of Physics, Faculty of Science, the University of Tokyo

1978-1983 DSc, Department of Physics, Division of Science, the University of Tokyo

・ Academic Appointments

1984-1986 Research Associate at the Department of physics, the University of Tokyo,

1986-1988 Postdoctoral fellow, Department of Cell Biology, Stanford University

1988-1989 Scientific staff, Medical Research Council Laborartory of Molecular Biology, Cambridge, UK

1989-1989 Research scientist, Frontier Research Program (RIKEN).

1990-1994 Associate professor, Department of Biological Sciences, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology

1994-present Professor, Institute of Molecular and Cellular Biosciences, the University of Tokyo

2005-present Foreign Associate of the National Academy of Science, USA.

2008 Hitchcock Professor, University of California, Berkeley, USA

2010-present Director of the Center for Challenging Proteins, Institute of Molecular and Cellular Biosciences, the University of Tokyo

・ Publication

1. Y. Norimatsu and C. Toyoshima, Protein-lipid interplay revealed with crystals of a calcium pump. *Nature* In press

2. C. Toyoshima, The road to understanding an ion pump, *Physica Scripta*, 91, 042501 (2016)

3. F. Cornelius, M. Habeck, R. Kanai, C. Toyoshima and S.J.D. Karlish: General and specific lipid–protein interactions in Na,K-ATPase. *Biochim. Biophys. Acta.* 1848, 1729-1743 (2015)

4. H. Ogawa, F. Cornelius, A. Hirata and C. Toyoshima: Sequential substitution of K^+ bound to Na^+,K^+-ATPase visualised by X-ray crystallography. *Nature Commun.***6**, 8004 (2015)

5. M. Habeck, H. Haviv, A. Katz, E. Kapri-Pardes, S. Ayciriex, A. Shevchenko, H. Ogawa, C. Toyoshima, and S.J.D. Karlish: Stimulation, inhibition or stabilization of Na,K-ATPase caused by specific lipid interactions at distinct sites. *J. Biol. Chem.* **290** 4829-4842 (2015)

6. R. Kanai, H. Ogawa, B. Vilsen, F. Cornelius and C. Toyoshima: Crystal structure of a Na^+-bound Na^+,K^+-ATPase preceding the E1P state. *Nature* **502**, 201-206 (2013)

7. C. Toyoshima, S. Iwasawa, H. Ogawa, A. Hirata, J. Tsueda and G. Inesi: Crystal structures of the calcium pump and sarcolipin in the·Mg^{2+}-bound E1 state. *Nature* **495**, 260-264 (2013)

8. F. Cornelius, R. Kanai, and C. Toyoshima: A structural view on the functional importance of the sugar moiety and steroid hydroxyls of cardiotonic steroids in binding to Na,K-ATPase. *J. Biol. Chem.* **288**, 6602-6616 (2013)

9. H. Ogawa, T. Shinoda, F. Cornelius and C. Toyoshima: Crystal structure of the sodium-potassium pump (Na^+,K^+-ATPase) with bound potassium and ouabain. *Proc. Nat. Acad. Sci. USA* **106**, 13742-13747 (2009)

10. T. Shinoda, H. Ogawa, F. Cornelius and C. Toyoshima: Crystal structure of the sodium-potassium pump at 2.4 Å resolution. *Nature* **459**, 446-450 (2009)

11. C. Toyoshima, Y. Norimatsu, S. Iwasawa, T. Tsuda and H. Ogawa: How processing of aspartylphosphate is coupled to lumenal gating of the ion pathway in the calcium pump. *Proc. Nat. Acad. Sci. USA*. **104**, 19831-19836 (2007)

12. C. Toyoshima, H. Nomura and T. Tsuda: Lumenal gating mechanism revealed in calcium pump crystal structures with phosphate analogues. *Nature* **432**, 361-368 (2004)

13. C. Toyoshima and T. Mizutani: Crystal structure of the calcium pump with a bound ATP analogue. *Nature* **430,** 529-535 (2004)

14. C. Toyoshima and H. Nomura: Structural changes in the calcium pump accompanying the dissociation of calcium. *Nature* **418,** 605-611 (2002)

15. C. Toyoshima, M. Nakasako, H. Nomura and H. Ogawa: Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature* **405,** 647-655 (2000)

Special lecture 3

Na^+, K^+-ATPase α3 Is a Death Target of Alzheimer Amyloid-β Assembly

What Shall We Do Next Towards A Better Understanding of Na^+, K^+-ATPase α3's Role In Health And Disease?

Minako Hoshi, Ph.D.
Director, Center for Brain and Neurodegenerative Disease Research, Institute of Biomedical Research and Innovation, Foundation for Biomedical Research and Innovation

Alzheimer's disease (AD) impairs a person's neural network involved in cognitive function by affecting neuronal synaptic connections and degenerating neurons themselves. Amyloid β-protein (Aβ), a small protein produced by proteolytic cleavages of amyloid precursor protein (APP) in a physiological pathway, has been considered to play a primary role in such loss of synapses and neurons. Recent studies have shown that Aβ has an ability to form structurally distinct assemblies, which exert different toxic functions through different targets. Our laboratory has long been focusing on understanding mechanisms of neurodegeneration in AD and has identified Aβ assemblies from AD patient brains, termed amylospheroids (ASPD), as responsible for neurodegeneration (Hoshi et al. PNAS2003, Noguchi et al. JBC2009). Recently, we discovered that the neuron-specific α3 subunit of the Na^+, K^+-ATPase pump (NAKα3), the catalytic subunit that is essential for neuronal excitability, is a toxic target for ASPD (Ohnishi et al. PNAS2015). This is a new system that involves pre-synaptic calcium hyperactivation, which is triggered by impairing NAKα3-derived NAK pump activity, leading to neurodegeneration (Figure 1). We also discovered that ASPD-binding tetrapeptides blocked the ASPD:NAKα3 interaction and protected mature neurons from ASPD neurotoxicity. Interestingly, a French group who have long worked on neurotoxicity of α-synuclein assemblies independently found that NAKα3 is a toxic target for α-synuclein assemblies and the α-synuclein: NAKα3 interaction is related to neurodegeneration in Parkinson's disease (Shrivastava et al. EMBO J 34, 2408-2423, 2015). Surprisingly, ASPD and α-synuclein share the essential binding region in the fourth extracellular loop of NAKα3. At the seminar, I would like to present how we identified first ASPD, and then NAKα3 as a toxic target of ASPD. I would be happy to discuss about what we shall do as a next to uncover distribution and function of NAKα3 in health and disease.

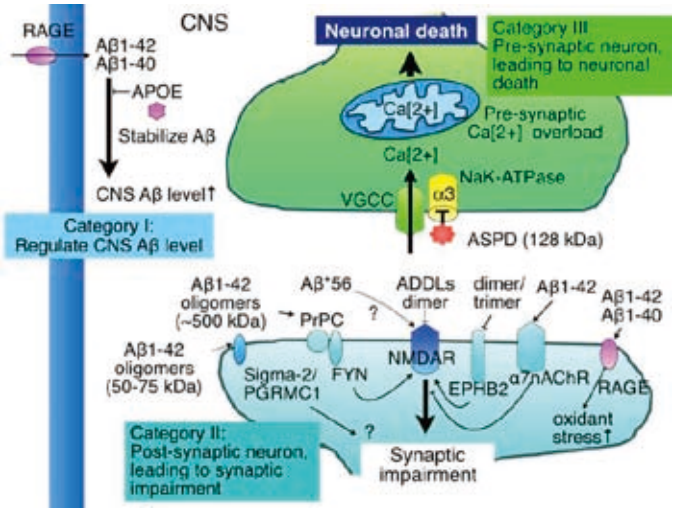


Figure 1. Aβ receptor/ligand systems.

MINAKO HOSHI

Degrees: Ph. D. (Biochemistry), The University of Tokyo (1991)
M. Sci. (Biochemistry), The University of Tokyo (1988)
B. Sci. (Biochemistry), The University of Tokyo (1986)

Present post: Director, Center for Brain and Neurodegenerative Disease Research
Institute of Biomedical Research and Innovation,
Foundation for Biomedical Research and Innovation (2017-)

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Previous posts:
Associate Professor, Department of Anatomy and Developmental Biology, Graduate School of Medicine, Kyoto University (2009-2017)
Principal Researcher, Mitsubishi Kagaku Institute of Life Science (2001-2009)
Associate Professor, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology (2004-2008)
Principal Investigator, a Proposal-Oriented Research Promotion Program from Japanease Science and Technology Agency (2000-2003)
Researcher (Project Head), Mitsubishi Kagaku Institute of Life Sciences (1997-2001)
Associate Scientist, Mitsubishi Kagaku Institute of Life Sciences (1996-1997)
Researcher, Neuropathology, Tokyo Metropolitan Institute of Gerontology (1996-1997)
Postdoctoral Fellow, Mitsubishi Kagaku Institute of Life Sciences (1994-1996)
National Institute Postdoctoral Fellow from Japanese Science and Technology Agency, National Institute of Neuroscience, National Center of Neurology and Psychiatry (1991-1994)
Research Fellow of Japanese Society for the Promotion of Science (1991)

Recent Selected Publications

1. Hoshi M, Sato M, Matsumoto S, Noguchi A, Yasutake K, Yoshida N, Sato K. Spherical aggregates of beta-amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3beta. Proc Natl Acad Sci USA. 2003 May 27;100(11):6370-6375.
2. Roychaudhuri R, Yang M, Hoshi MM, Teplow DB. Amyloid beta-protein assembly and Alzheimer disease. J Biol Chem. 2009 Feb 20;284(8):4749-4753.
3. Noguchi A, Matsumura S, Dezawa M, Tada M, Yanazawa M, Ito A, Akioka M, Kikuchi S, Sato M, Ideno S, Noda M, Fukunari A, Muramatsu S, Itokazu Y, Sato K, Takahashi H, Teplow DB, Nabeshima Y, Kakita A, Imahori K, Hoshi M. Isolation and characterization of patient-derived, toxic, high mass amyloid beta-protein (Abeta) assembly from Alzheimer disease brains. J Biol Chem. 2009 Nov 20;284(47):32895-32905.
4. Matsumura S, Shinoda K, Yamada M, Yokojima S, Inoue M, Ohnishi T, Shimada T, Kikuchi K, Masui D, Hashimoto S, Sato M, Ito A, Akioka M, Takagi S, Nakamura Y, Nemoto K, Hasegawa Y, Takamoto H, Inoue H, Nakamura S, Nabeshima Y, Teplow DB, Kinjo M, Hoshi M. Two distinct

amyloid beta-protein (Abeta) assembly pathways leading to oligomers and fibrils identified by combined fluorescence correlation spectroscopy, morphology, and toxicity analyses. J Biol Chem. 2011 Apr 1;286(13):11555-11562.
5. Xiao Y, Ma B, McElheny D, Parthasarathy S, Long F, Hoshi M, Nussinov R, Ishii Y. Aβ(1-42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer's disease. Nat Struct Mol Biol. 2015 Jun;22(6):499-505.
6. Parthasarathy S, Inoue M, Xiao Y, Matsumura Y, Nabeshima Y, Hoshi M, Ishii Y. Structural Insight into an Alzheimer's Brain-Derived Spherical Assembly of Amyloid β by Solid-State NMR. J Am Chem Soc. 2015 May 27;137(20):6480-6483.
7. Ohnishi T, Yanazawa M, Sasahara T, Kitamura Y, Hiroaki H, Fukazawa Y, Kii I, Nishiyama T, Kakita A, Takeda H, Takeuchi A, Arai Y, Ito A, Komura H, Hirao H, Satomura K, Inoue M, Muramatsu S, Matsui K, Tada M, Sato M, Saijo E, Shigemitsu Y, Sakai S, Umetsu Y, Goda N, Takino N, Takahashi H, Hagiwara M, Sawasaki T, Iwasaki G, Nakamura Y, Nabeshima Y, Teplow DB, Hoshi M. Na, K-ATPase α3 is a death target of Alzheimer patient amyloid-β assembly. Proc Natl Acad Sci USA. 2015 Aug 11;112(32):E4465-4474.

Mitochondrial autophagy and familial Parkinson's disease

Noriyuki Matsuda,
Tokyo Metropolitan Institute of Medical Science

Parkinson's disease (PD) is a common movement disorder characterized by dopaminergic neuronal loss. The majority of PD cases are sporadic, however, the discovery of the genes linked to hereditary forms of PD has provided important insights into the molecular mechanisms. For example, functional analysis of the recessive familial PD-related genes has revealed that the disease is relevant to mitochondrial quality control. This is consistent with the prior idea that several cases of sporadic and chemical-induced PDs have been associated with mitochondrial dysfunction.

We now focus on *PINK1* and *PARKIN*, responsible genes for hereditary recessive PD. *PINK1* and *PARKIN* encode Ser/Thr kinase and ubiquitin ligase (E3), respectively. We revealed that when the mitochondrial membrane potential decreased, PINK1 phosphorylates ubiquitin at Ser65, and the phosphorylated ubiquitin functions as an activator for E3 function of Parkin (Koyano, Nature 2014). Moreover, phosphorylated poly-ubiquitin chain on damaged mitochondria recruits Parkin to damaged mitochondria by functioning as a Parkin receptor (Okatsu, JCB 2015). Consequently, trio of PINK1, Parkin, and phospho-ubiquitin tag outer membrane proteins on depolarized mitochondria with ubiquitin. Damaged mitochondria are then targeted for selectively degradation, because ubiquitin functions as a signal for degradation by the proteasome and autophagy. Impairment of this process predisposes to familial PD. Summary of the latest knowledge for relationship between mitochondrial quality control, autophagy, and Parkinson's disease will be discussed.

Noriyuki MATSUDA

[Present Position]

Project Leader, Ubiquitin Project,
Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

[Education]

1991 - 1995 Bachelor of Science, University of Tokyo, Japan
1995 - 1997 Master of Science, University of Tokyo, Japan
1997 - 2001 Ph.D. (Biological Sciences), University of Tokyo, Japan

[Career]

2001 - 2002 Special Postdoctoral Researcher, Molecular Membrane Biology Laboratory, RIKEN, Japan.
2002 - 2007 Postdoctoral Fellow, Department of Molecular Oncology, Tokyo Metropolitan Institute of Medical Science (TMIMS), Japan.
2007 - 2008 Senior Scientist, Systems and Structural Biology Center of RIKEN, Japan.
2008 - Present Senior Researcher (2008-2011), Chief Researcher (2011-2013), Associate Director-Researcher (2013 - 2015), and Project Leader (2015 -) of Ubiquitin Project, TMIMS, Japan.

[Selected Publications]

1) Yamano, K., Queliconi, B.B., Koyano, F., Saeki, Y., Hirokawa, T., Tanaka, K., and **Matsuda, N**. Site-specific interaction mapping of phosphorylated ubiquitin to uncover Parkin activation. **J. Biol. Chem.,** 290(42), 25199-25211 (2015)

2) Okatsu, K., Koyano, F., Kimura, M., Kosako, H., Saeki, Y., Tanaka, K., and **Matsuda, N**. Phosphorylated ubiquitin chain is the genuine Parkin receptor. **Journal of Cell Biology**, 209(1), 111-128 (2015)

3) Koyano, F., Okatsu, K., Kosako, H., Tamura, Y., Go, E., Kimura, M., Kimura, Y., Tsuchiya, H., Yoshihara, H., Hirokawa, T., Endo, T., Fon, E-A., Trempe, J-F., Saeki, Y., Tanaka, K., and **Matsuda, N**. Ubiquitin is phosphorylated by PINK1 to activate Parkin. **Nature**, 510(7503), 162-166 (2014)

4) Okatsu, K., Oka, T., Iguchi, M., Imamura, K., Kosako, H., Tani, N., Kimura, M., Go, E., Koyano, F., Funayama, M., Shiba-Fukushima, K., Sato, S., Shimizu, H., Fukunaga, Y., Taniguchi, H., Komatsu, M., Hattori, N., Mihara, K., Tanaka, K., and **Matsuda, N**. PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. **Nature Commun**. 3, e1016 (2012)

5) **Matsuda, N**., Sato, S., Shiba, K., Okatsu, K., Saisho, K., Gautier, C., Sou, Y-S., Saiki, S., Kawajiri, S., Sato, F., Kimura, M., Komatsu, M., Hattori, N. and Tanaka, K. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. **Journal of Cell Biology,** 189(2), 211-221 (2010)

Invited Speakers

Session 1-1

Clinical and genetic spectrum of *ATP1A3*-related disorders

Hendrik Rosewich, MD, Assistant Professor
Department of Pediatrics and Adolescent Medicine, Division of Pediatric Neurology, University Medical Center Göttingen, Georg August University, Faculty of Medicine, Göttingen, Germany

P-type cation transport proteins establish and maintain electrochemical gradients for Na^+ and K^+ across the plasma membrane. Mutations in the α3 catalytic subunit, encoded by *ATP1A3*, almost exclusively expressed in neurons, are associated with at least 3 distinct, yet overlapping, neurologic syndromes: rapid-onset dystonia parkinsonism (RDP); alternating hemiplegia of childhood (AHC); and cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS). From the past descriptions of each entity, distinct phenotypes have emerged, demonstrating that classic AHC, classic RDP, and CAPOS syndrome constitute clinical prototypes in a continuous and still expanding phenotypic spectrum of ATP1A3 related disorders.
This still expanding phenotypic spectrum is an excellent example how modern sequencing techniques can help to diagnose a condition not primarily thought to be associated with a certain gene (reverse genetics). Moreover, it causes a diagnostic challenge, even for experienced clinicians. Reviews about the currently known broad phenotypic spectrum are needed to provide useful diagnostic guidelines to physicians to select the right patients amongst suspected phenotypes. For this purpose, core clinical features, that in combination make an ATP1A3 related disorder most likely, have to be extracted from the complex and wide-ranging known phenotypic spectrum.
Mutation updates of all patient mutations reported in the *ATP1A3* gene and clear assignability to a certain phenotype are needed for both, clinicians and basic scientists. Clinicians can discuss with the patients and parents the most likely clinical course, and researchers studying the cellular function of ATP1A3 can interpret their data on the basis of the cellular and clinical phenotype. The understanding of the pathomechanism in combination with the clinical phenotype is mandatory to establish future therapeutic trials. For those, natural history studies at least for the clinically well-defined entities (AHC/RDP/CAPOS) are an indispensable prerequisite to define study endpoints in pharmacological trials.

Hendrik ROSEWICH

- **Present Position**

Assistant Professor, Department of Pediatric and Pediatric Neurology, University Medical Center Göttingen, Georg August University Göttingen, Germany

- **Education and Academic Appointments**

1996 - 2003	Medicine, Heinrich Heine University, Düsseldorf, Germany, 2003 Medical Licensure
2002	Predoctoral Fellowship Howard Hughes Medical Institute and Department of Pediatrics, Neurology and Molecular Biology, Johns Hopkins Medical Institutions Baltimore, U.S.A. (Director: Professor David Valle)
2003 - 2016	Clinical and Research Fellow in Pediatrics, Department of Pediatrics and Pediatric Neurology; University Medicine Göttingen, Germany (Director: Professor Dr. Jutta Gärtner)
Since 2009	Clinical Board Certification for Pediatrics
2014	Habilitation and Venia legendi for Pediatrics, Georg August University, Göttingen, Germany
Since 2016	Clinical Board Certification for Pediatric Neurology
Since 2014	Assistant Professor, Senior Physician, Department of Pediatric and Pediatric Neurology, University Medical Center Göttingen, Georg August University Göttingen, Germany

- **Publication**

Rosewich H, Sweney MT, DeBrosse S, Ess K, Ozelius L, Andermann E, Andermann F, Andrasco G, Belgrade A, Brashear A, Ciccodicola S, Egan L, George AL Jr, Lewelt A, Magelby J, Merida M, Newcomb T, Platt V, Poncelin D, Reyna S, Sasaki M, Sotero de Menezes M, Sweadner K, Viollet L, Zupanc M, Silver K, Swoboda K. Research conference summary from the 2014 International Task Force on ATP1A3-Related Disorders. Neurol Genet. 2017 Mar 2;3(2):e139.

Rosewich H, Baethmann M, Ohlenbusch A, Gärtner J, Brockmann K.: A novel ATP1A3 mutation with unique clinical presentation. J Neurol Sci. 2014; 341(1-2):133-5.

Rosewich H, Ohlenbusch A, Huppke P, Schlotawa L, Baethmann M, Carrilho I, Fiori S, Lourenço CM, Sawyer S, Steinfeld R, Gärtner J, Brockmann K.: The expanding clinical and genetic spectrum of ATP1A3-related disorders. Neurology. 2014; 82(11):945-55.

Heinzen EL, Arzimanoglou A, Brashear A, Clapcote SJ, Gurrieri F, Goldstein DB, Jóhannesson SH, Mikati MA, Neville B, Nicole S, Ozelius LJ, Poulsen H, Schyns T, Sweadner KJ, van den Maagdenberg A, Vilsen B; ATP1A3 Working Group (**Rosewich H**).: Distinct neurological disorders with ATP1A3 mutations. Lancet Neurol. 2014; 13(5):503-14.

Rosewich H, Thiele H, Ohlenbusch A, Maschke U, Altmüller J, Frommolt P, Zirn B, Ebinger F, Siemes H, Nürnberg P, Brockmann K, Gärtner J.: Heterozygous de-novo mutations in ATP1A3 in patients with alternating hemiplegia of childhood: a whole-exome sequencing gene-identification study. Lancet Neurol. 2012; 11(9):764-73.

Session 1-2

Rapid-onset dystonia-parkinsonism (RDP): Clinical and Genetic Updates

Allison Brashear, MD, MBA
Professor & Chair, Department of Neurology Walter C. Teagle Endowed Chair, Wake Forest School of Medicine, Winston Salem, NC USA
Christopher T. Whitlow, MD, PhD, MHA
Associate Professor of Radiology Division Head, Neuroradiology, Wake Forest School of Medicine Winston Salem, NC USA

Since 1993, with the original description of RDP, ATP1A3-related disorders have broadened to include not only RDP, but CAPOS, ataxia, and AHC. Multiple lines of evidence, including imaging, clinical examinations and pathology suggest that ATP1A3 mutations contribute to a complex cascade of signs and symptoms indicating wide spread impact on the brain. Imaging and human pathology suggest that ATP1A3 mutations lead to impairment of the cerebello-thalamo-cortical (CbTC) pathways. Our findings are supported by human brain pathology in patients who were symptomatic over a life time, demonstrating cell death in areas including the globus pallidus, subthalamic nucleus, red nucleus, inferior olivary nucleus, cerebellar purkinje and granule cell layers and dentate. When and how cell death occurs, how this may change over time and whether a reproducible biomarker can be followed for treatment or prevention is part of our current body of work.

In our preliminary investigation, data were analyzed from thirteen participants with ATP1A3 mutations (age range 14-65; mean ± SD = 38 ± 14) who were enrolled in a larger ongoing Institutional Review Board approved study of brain structure/function at Wake Forest School of Medicine. All subjects were diagnosed using a standard battery of movement disorder assessments, including Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS), Unified Parkinson's Disease Rating Scale motor subscore (UPDRS), and International Cooperative Ataxia Rating Scale (ICARS). Brain MRI, including structural anatomic and arterial spin labeling cerebral blood flow images were acquired using standard techniques. Our results suggest that there were more associations between measures of dystonia and cerebral blood flow in components of the CbTC compared to gray matter volume, suggesting that changes in brain function may play an important role in phenotypes associated with *ATP1A3* gene mutations. Whether MRI measures of brain structure and function can be used as biomarkers to track progression and/ or treatment over time is a larger goal of the study.

Overall heightened awareness of the broad spectrum of *ATP1A3* diseases and an understanding of how the pump may be altered by specific mutations opens the door for potential prevention and treatment. The overlap of clinical, imaging, genetic and bench work in studying *ATP1A3* disease is a unique opportunity to develop a new line of therapies for neurologic diseases.

Supported by NINDS grant 5R01NS058949-08 (AB)

Allison Brashear, MD, MBA

Allison Brashear, MD, MBA is Professor and Chair of the Department of Neurology at Wake Forest School of Medicine in Winston-Salem, NC (2005-present). She holds the Walter C. Teagle Chair of Neurology. Dr. Brashear also served as Interim Chair of the Department of Psychiatry.

Dr. Brashear is the principal clinician to describe a unique genetic form of dystonia-parkinsonism, Rapid-Onset Dystonia-Parkinsonism (RDP). Her group reported the genetic mechanism responsible for RDP (mutations in the Na/K ATPase alpha 3 subunit) in Neuron in July 2004. In 2008, NINDS funded her group to study these mutations in the ATP1A3 gene in more depth (R01NS058949; PI: Brashear). This work was refunded in April, 2015 for five years. The results are published in Lancet Neurology, Brain, Neuron, Acta Neuropathologica, Neurology, Annals of Neurology and Movement Disorders. In 2016 Dr. Brashear was appointed a permanent member of the NINDS NST-1 (K awards) study section.

Dr. Brashear is the lead principal investigator in many multi-center trials for the treatment of cervical dystonia and spasticity. She is the lead author on the pivotal paper, “Intramuscular Injection of Botulinum Toxin for the Treatment of Wrist and Finger Spasticity after a Stroke”, published in The New England Journal of Medicine. Her work with botulinum toxin has been published in Lancet Neurology, Neurology, Movement Disorders and Archives of PMR.

Dr. Brashear served on the American Academy of Neurology (AAN) Board of Directors from 2013-15. In 2015 she completed six year terms on the AAN Education Committee and the Medical Economic Management Committee. She served on the Board of Directors of the American Neurological Association (ANA), United Council for Neurological Subspecialties (UCNS, vice chair) and currently serves of council of the American Board of University Professors of Neurology (AUPN). In August, 2014 Dr. Brashear was appointed to a renewable four year term as a Director of the American Board of Psychiatry and Neurology (ABPN).

In 2008 the Wake Forest School of Medicine and North Carolina Baptist Hospital integrated to form the Wake Forest Baptist Medical Center. Dr. Brashear served as the first faculty member appointed to the Wake Forest Baptist Medical Center Board of Directors (2007-2013). She also served on the following WFBMC Board of Directors committees: finance, audit, clinical and academic affairs committees. She also serves as one of two faculty members of the WFBMC Capital Campaign Cabinet.

Dr. Brashear completed the Harvard School of Public Health Leadership program for physicians in 2004, and in 2007 she finished the year-long national program for women leaders, Executive Leadership Academic Medicine (ELAM). In 2014 she was selected as an AAMC Council of Dean’s Fellow. Dr. Brashear earned an MBA from Fuqua School of Business at Duke University in 2012, with additional certification in Health Sector Management.

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BIBLIOGRAPHY

Brashear A, Elovic E, (eds). Spasticity Diagnosis & Management. New York: Demos Medical Publishing, 2010. ISBN: 978-1-933864-51-8 and second edition published in 2016. ISBN: 978-1-62070-072-3 (ISBN ebook: 978-1-61705-242-2)

Peer-Reviewed Journal Articles (select publications out of 88)

1. Rosewich H, Sweney MT, DeBrosse S, Ess K, Ozelius L, Andermann E, Andermann F, Andrasco G, Belgrade A, **Brashear A**, Ciccodicola S, Egan L, George AL Jr, Lewelt A, Magelby J, Merida M, Newcom T, Platt V, Poncelin D, Reyna S, Sasaki M, Sotero de Menezes M, Sweadner K, Viollet L, Zupanc M, Silver K, Swoboda K. Research conference summary from 2014 International Task Force on ATP1A3 Related Disorders. Neurol Genet. 2017 Mar 2;3(2):e139
2. Szuch E, Caress JB, Paudyal B, **Brashear A**, Cartwright MS, Strowd RE 3rd. Head drop after Botox: Electrodiagnostic evaluation of iatrogenic botulinum toxicity. Clin Neurol Neurosurg. 2017 Mar 5; 156:1-3

3. Sweadner KJ, Toro C, Whitlow CT, Snively BM, Cook JF, Ozelius LJ, Markello TC, **Brashear A**. ATP1A3 Mutation in Adult Rapid-Onset Ataxia. PLoS.One. 2016;11(3):e0151429.
4. Gracies JM, **Brashear A**, Jech R, McAllister P, Banach M, Valkovic P, Walker H, Marciniak C, Deltombe T, Skoromets A, et al. Safety and efficacy of abobotulinumtoxinA for hemiparesis in adults with upper limb spasticity after stroke or traumatic brain injury: a double-blind randomised controlled trial. Lancet Neurol. 2015 Oct;14(10):992-1001
5. Paciorkowski AR, McDaniel SS, Jansen LA, Tully H, Tuttle E, Ghoneim DH, Tupal S, Gunter SA, Vasta V, Zhang Q, Tran T, Liu YB, Ozelius LJ, **Brashear A**, Sweadner KJ, Dobyns WB, Hahn S. Novel mutations in ATP1A3 associated with catastrophic early life epilepsy, episodic prolonged apnea, and postnatal microcephaly. Epilepsia 2015 Mar;56(3):422-30.
6. Oblak AL, Hagen MC, Sweadner KJ, Haq I, Whitlow CT, Maldjian JA, Epperson F, Cook JF, Stacy M, Murrell JR, et al. Rapid-onset dystonia-parkinsonism associated with the I758S mutation of the ATP1A3 gene: a neuropathologic and neuroanatomical study of four siblings. Acta Neuropathol. 2014 Jul;128(1):81-98.
7. Heinzen EL, Arzimanoglou A, **Brashear A**, Clapcote SJ, Gurrieri F, Goldstein DB, Johannesson SH, Mikati MA, Neville B, Nicole S, et al. Distinct neurological disorders with ATP1A3 mutations. Lancet Neurol. 2014 May;13(5):503-14.
8. Saunders-Pullman R, Fuchs T, San LM, Raymond D, **Brashear A**, Ortega R, Deik A, Ozelius LJ, Bressman SB. Heterogeneity in primary dystonia: lessons from THAP1, GNAL, and TOR1A in Amish-Mennonites. Mov Disord. 2014 May;29(6):812-8.
9. Cook JF, Hill DF, Snively BM, Boggs N, Suerken CK, Haq I, Stacy M, McCall WV, Ozelius LJ, Sweadner KJ, **Brashear A**. Cognitive impairment in rapid-onset dystonia-parkinsonism. Mov Disord. 2014 Mar;29(3):344-50.
10. Tan AH, Ozelius L, **Brashear A**, Lang A, Ahmad-Annuar A, Tan CT, Lim, SY. Rapid-Onset Dystonia-Parkinsonism in a Chinese Girl with De Novo ATP1A3 c.2267G-A (p.R756H) Genetic Mutation.(case report) MDC 3, 12122, 8/12/14
11. **Brashear A**, Ozelius LJ, Sweadner KJ. ATP1A3 mutations: what is the phenotype? Neurology 2014 Feb 11;82(6):468-9
12. **Brashear A**, Bushong L. Coding for the use of botulinum toxin for movement disorders. Continuum (Minneap.Minn.) 2013 Oct;19(5 Movement Disorders):1406-15
13. **Brashear A**, Cook JF, Hill DF, Amponsah A, Snively BM, Light L, Boggs N, Suerken CK, Stacy M, Ozelius L, Sweadner KJ, McCall VW. Psychiatric disorders in rapid-onset dystonia parkinsonism. Neurology. 2012; 79(11): 1168-1173.
14. **Brashear A**, Cook JF, Hill DF, Amponsah A, Snively BM, Light L, Boggs N, Suerken CK, Stacy M, Ozelius L, Sweadner KJ, McCall WV. Psychiatric disorders in rapid-onset dystonia-parkinsonism. Neurology 2012 Sep 11;79(11):1168-73.
15. **Brashear A**, Mink JW, Hill DF, Boggs N, McCall WV, Stacy MA, Snively B, Light LS, Sweadner KJ, Ozelius LJ, Morrison L. ATP1A3 mutations in infants: a new rapid-onset dystonia-Parkinsonism phenotype characterized by motor delay and ataxia. Dev Med Child Neurol. 2012 Nov;54(11):1065-7.
16. Barbano RL, Hill DF, Snively BM, Light LS, Boggs N, McCall WV, Stacy M, Ozelius L, Sweadner KJ,

Brashear A. New triggers and non-motor findings in a family with rapid-onset dystonia-parkinsonism. Parkinsonism.Relat Disord. 2012 Jul;18(6):737-41.

17. **Brashear A**. Comment: The new mentoring and team leadership. Neurology 2011 Aug 16;77(7):685
18. Chen R, **Brashear A**. A diagnostic test to distinguish psychogenic dystonia from organic dystonia? Neurology 2011 Feb 15;76(7):590-1
19. Simpson DM, Blitzer A, **Brashear A**, Comella C, Dubinsky R, Hallett M, Jankovic J, Karp B, Ludlow CL, Miyasaki JM, et al. Assessment: Botulinum neurotoxin for the treatment of movement disorders (an evidence-based review): report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology 2008 May 6;70(19):1699-706
20. Brin MF, Comella CL, Jankovic J, Lai F, Naumann M; CD-017 BoNTA Study Group (including **Brashear A**. Long-term treatment with botulinum toxin type A in cervical dystonia has low immunogenicity by mouse protection assay. Mov Disord. 2008 Jul 30;23(10): 1353-1360.
21. **Brashear A**, Dobyns WB, de Carvalho AP, Borg M, Frijns CJ, Gollamudi S, Green A, Guimaraes J, Haake BC, Klein C, et al. The phenotypic spectrum of rapid-onset dystonia-parkinsonism (RDP) and mutations in the ATP1A3 gene. Brain 2007 Mar;130(Pt 3):828-35
22. Jankovic J, Hunter C, Dolimbek BZ, Dolimbek GS, Adler CH, **Brashear A**, Comella CL, Gordon M, Riley DE, Sethi K, et al. Clinico-immunologic aspects of botulinum toxin type B treatment of cervical dystonia. Neurology 2006 Dec 26;67(12):2233-5
23. **Brashear A**. The safety and tolerability of botulinum toxins for the treatment of cervical dystonia. Expert.Opin.Drug Saf 2005 Mar;4(2):241-9
24. Gordon MF, **Brashear A**, Elovic E, Kassicieh D, Marciniak C, Liu J, Turkel C, BOTOX Poststroke Spasticity Study Group. Repeated dosing of botulinum toxin type A for upper limb spasticity following stroke. Neurology 2004 Nov 23;63(10):1971-3
25. Zaremba J, Mierzewska H, Lysiak Z, Kramer P, Ozelius LJ, **Brashear A**. Rapid-onset dystonia-parkinsonism: a fourth family consistent with linkage to chromosome 19q13. Mov Disord. 2004 Dec;19(12):1506-10
26. de Carvalho AP, Sweadner KJ, Penniston JT, Zaremba J, Liu L, Caton M, Linazasoro G, Borg M, Tijssen MA, Bressman SB, Dobyns WB, **Brashear A**, Ozelius LJ. Mutations in the Na+/K+ -ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. Neuron 2004 Jul 22;43(2):169-75
27. Kamm C, Leung J, Joseph S, Dobyns WB, **Brashear A**, Breakefield XO, Ozelius LJ. Refined linkage to the RDP/DYT12 locus on 19q13.2 and evaluation of GRIK5 as a candidate gene. Mov Disord. 2004 Jul;19(7):845-7
28. **Brashear A**, Zafonte R, Corcoran M, Galvez-Jimenez N, Gracies JM, Gordon MF, McAfee A, Ruffing K, Thompson B, Williams M, et al. Inter- and intrarater reliability of the Ashworth Scale and the Disability Assessment Scale in patients with upper-limb poststroke spasticity. Arch.Phys.Med.Rehabil. 2002 Oct;83(10):1349-54
29. **Brashear A**, Gordon MF, Elovic E, Kassicieh VD, Marciniak C, Do M, Lee CH, Jenkins S, Turkel C, Botox Post-Stroke Spasticity Study Group. Intramuscular injection of botulinum toxin for the treatment of wrist and finger spasticity after a stroke. N.Engl.J.Med. 2002 Aug 8;347(6):395-400
30. Koman LA, **Brashear A**, Rosenfeld S, Chambers H, Russman B, Rang M, Root L, Ferrari E, Garcia de

Yebenes PJ, Smith BP, et al. Botulinum toxin type a neuromuscular blockade in the treatment of equinus foot deformity in cerebral palsy: a multicenter, open-label clinical trial. Pediatrics 2001 Nov;108(5):1062-71

31. **Brashear A**, Bergan K, Wojcieszek J, Siemers ER, Ambrosius W. Patients' perception of stopping or continuing treatment of cervical dystonia with botulinum toxin type A. Mov Disord. 2000 Jan;15(1):150-3
32. **Brashear A**, Lew MF, Dykstra DD, Comella CL, Factor SA, Rodnitzky RL, Trosch R, Singer C, Brin MF, Murray JJ, et al. Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A-responsive cervical dystonia. Neurology 1999 Oct 22;53(7):1439-46
33. **Brashear A**, Mulholland GK, Zheng QH, Farlow MR, Siemers ER, Hutchins GD. PET imaging of the pre-synaptic dopamine uptake sites in rapid-onset dystonia-parkinsonism (RDP). Mov Disord. 1999 Jan;14(1):132-7
34. **Brashear A**, Butler IJ, Ozelius LJ, Kramer PI, Farlow MR, Breakefield XO, Dobyns WB. Rapid-onset dystonia-parkinsonism: a report of clinical, biochemical, and genetic studies in two families. Adv.Neurol. 1998;78:335-9
35. **Brashear A**, Butler IJ, Hyland K, Farlow MR, Dobyns WB. Cerebrospinal fluid homovanillic acid levels in rapid-onset dystonia-parkinsonism. Ann.Neurol. 1998 Apr;43(4):521-6
36. **Brashear A**, Ambrosius WT, Eckert GJ, Siemers ER. Comparison of treatment of tardive dystonia and idiopathic cervical dystonia with botulinum toxin type A. Mov Disord. 1998 Jan;13(1):158-61
37. **Brashear A**, DeLeon D, Bressman SB, Thyagarajan D, Farlow MR, Dobyns WB. Rapid-onset dystonia-parkinsonism in a second family. Neurology 1997 Apr;48(4):1066-9
38. **Brashear A**, Farlow MR, Butler IJ, Kasarskis EJ, Dobyns WB. Variable phenotype of rapid-onset dystonia-parkinsonism. Mov Disord. 1996 Mar;11(2):151-6
39. Hendrie HC, Hall KD, Hui S, Unverzagt FW, Yu CE, Lahiri DK, Sahota A, Farlow MR, Musick B, Class CA, **Brashear A**, Burdine VE, Osuntokun BO, Ogunniyi AO, Gureje O, Baiyewu O, Schellenberg GD. Apolipoprotein E genotypes and Alzheimer disease in a community study of elderly African-Americans. Ann Neurol. 1995;37(1):118-120. PMID: 7818244
40. Dobyns WB, Ozelius LJ, Kramer PL, **Brashear A**, Farlow MR, Perry TR, Walsh LE, Kasarskis EJ, Butler IJ, Breakefield XO. Rapid-onset dystonia-parkinsonism. Neurology 1993 Dec;43(12):2596-602

Session 1-3

Relapsing encephalopathy with cerebellar ataxia (RECA)

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Abstract

Background*:* Alternating hemiplegia of childhood (AHC) is a rare neurological disorder characterized by recurrent hemiplegia, oculogyric and choreoathetotic involuntary movements. *De novo* mutations in *ATP1A3* cause AHC and its associated syndromes. It remains to be determined, however, whether rare variations in *ATP1A3* may lead to atypical neurological phenotypes.

Case*:* A 7-year-old Japanese boy started presenting with recurrent symptoms of generalized paralysis at 17 months of age. Hypotonia, rapidly bouncing or rotating ocular movements, dystonia and choreoathetosis persisted for more than a month after the onset. These symptoms recurred on febrile illness. Serial recordings of electroencephalogram and neuroimaging studies did not support the diagnosis of acute encephalopathy.

Results*:* Whole-exome sequencing identified a *de novo* mutation in *ATP1A3* (NM_152296.4: exon17: c.2266C>T: p.R756C). Previously reported cases carrying p.R756C or p.R756H mutations showed both overlapping and distinct phenotypes compared to those of our case. These symptoms were recently designated as relapsing encephalopathy with cerebellar ataxia (RECA). Molecular studies uncovering the pathogenic mechanisms of this disease are currently under progress.

Conclusion*:* This study confirmed that p.R756C mutation of *ATP1A3* caused atypical forms of AHC-associated disorders. The wide spectra of neurological phenotypes in AHC are linked to still unknown deficits in the molecular functions of *ATP1A3*.

Keywords:

Alternating hemiplegia of childhood (AHC), Whole-exome sequencing, relapsing encephalopathy with cerebellar ataxia (RECA), and *ATP1A3*

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• Publication

1. **Sakai Y**, Shaw CA, Dawson BC, Dugas DV, Al-Mohtaseb Z, Hill DE, Zoghbi HY. Protein interactome reveals converging molecular pathways among autism disorders. *Science Translational Medicine* 3, 86ra49 (2011)
2. Kanemasa H, **Sakai Y***, Fukai R, Torio M, Miyake N, Lee S, Ono H, Akamine S, Nishiyama K, Sanefuji M, Ishizaki Y, Torisu H, Saitsu H, Matsumoto N, Hara T. De novo p.Arg756Cys mutation of ATP1A3 causes an atypical form of alternating hemiplegia of childhood with prolonged paralysis and choreoathetosis. *BMC Neurology* 16:174 (2016)

3. Matsushita Y, **Sakai Y**, Shimmura M, Shigeto H, Nishio M, Akamine S, Sanefuji M, Ishizaki Y, Torisu H, Nakabeppu Y, Suzuki A, Takada H, Hara T. Hyperactive mTOR signals in the proopiomelanocortin-expressing hippocampal neurons cause age-dependent epilepsy and premature death in mice. *Scientific Reports* 6: 22991 (2016)
4. Yamaguchi Y, Torisu H, Kira R, Ishizaki Y, **Sakai Y**, Sanefuji M, Aiba H, Ichiyama T, Oka A, Kishi T, Kimura S, Kubota M, Takanashi J, Takahashi Y, Tamai H, Natsume J, Hamano S, Hirabayashi S, Maegaki Y, Mizuguchi M, Minagawa K, Yoshikawa H, Kira J, Kusunoki S, Takada H, Hara T. A nationwide survey of pediatric acquired demyelinating syndromes in Japan. *Neurology* 87:2006-2015 (2016)
5. **Sakai Y**, Fukai R, Matsushita Y, Miyake N, Saitsu H, Akamine S, Torio M, Sasazuki M, Ishizaki Y, Sanefuji M, Torisu H, Shaw CA, Matsumoto N, Hara T. De novo truncating mutation of TRIM8 causes an atypical form of epileptic encephalopathy. *Annals of Human Genetics* 80:235-240 (2016)

Session 1-4

Catastrophic early life epilepsy with *ATP1A3* mutation

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【Introduction】Mutations of *ATP1A3* have been associated with various phenotypes such as Rapid Onset Dystonia-Parkinsonism (RDP), Alternating Hemiplegia of Childhood (AHC), and Cerebellar ataxia with Areflexia, Pes cavus, Optic atrophy, and Sensorineural hearing loss (CAPOS). Recently, two cases of catastrophic early life epilepsy with heterozygous mutations in *ATP1A3* were reported (Paciorkowski et al. *Epilepsia*. 2015), which indicates their mutations cause most severe phenotypes of *ATP1A3*-related disorder spectrum. However, gene functions and genotype-phenotype correlations were remained unclear. Here we report one child with catastrophic early life epilepsy, respiratory failure, postnatal microcephaly, and severe developmental disability, with a novel heterozygous mutation of *ATP1A3*.

【Subject】A 2-year-old boy who was born to nonconsanguineous parents with normal delivery, and transferred to NICU because of respiratory failure at second day of birth. He also showed extreme hypotonia, episodic oculomotor abnormality and tachycardia, and frequent epileptic seizures. Mechanical ventilation was required to support his respiration. Epileptic seizures were intractable with treatment of multiple antiepileptic drugs, including extremely high dose Phenobarbital. In spite of many examinations including chromosomal analysis and muscle biopsy, etiology of his symptoms remained unknown.

【Methods and Results】Whole exome sequencing analysis was performed for subject and his parents. First we filtered the data with the known gene list for severe brain abnormality but no candidate mutation was found. Then we filtered for de novo mutation and found one heterozygous mutation in *ATP1A3* (c.2736_2738CTTdel, p.Phe913del).

【Discussion】Phe913 is a residue which has been highly conserved among vertebrates. There is a report that p.Val919del located in a same transmembrane domain reduces the activity of ATP1α3, suggesting that the current mutation also impairs the function. Two previously reported cases of catastrophic early life epilepsy had heterozygous mutations localized to the P domain of ATP1α3 (p.Gly358Val and p.Ile363Asn), and the observed mutations resulted in significant reduction of ATP1α3 activity in vitro. As allelic disorders of *ATP1A3*, e.g. RDP and AHC, appeared significant difference in phenotype, the cases with catastrophic early life epilepsy showed profound outcome and required much intensive care. Further functional studies are recommended to clarify the relationship between the mutation and such distinct phenotype.

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1. **Ishihara N**, Armsen W, Papadopoulos T, Betz H, Eulenburg V. Generation of a mouse line expressing Cre recombinase in glycinergic interneurons. Genesis. 2010 Jul;48(7):437-45.
2. **Ishihara N**, Shimada A, Kato J, Niimi N, Tanaka S, Miura K, Suzuki T, Wakamatsu N, Nagaya M. Variations in aganglionic segment length of the enteric neural plexus in Mowat-Wilson syndrome. J Pediatr Surg. 2005 Sep;40(9):1411-9.
3. **Ishihara N**, Yamada K, Yamada Y, Miura K, Kato J, Kuwabara N, Hara Y, Kobayashi Y, Hoshino K, Nomura Y, Mimaki M, Ohya K, Matsushima M, Nitta H, Tanaka K, Segawa M, Ohki T, Ezoe T, Kumagai T, Onuma A, Kuroda T, Yoneda M, Yamanaka T, Saeki M, Segawa M, Saji T, Nagaya M, Wakamatsu N. Clinical and molecular analysis of Mowat-Wilson syndrome associated with ZFHX1B mutations and deletions at 2q22-q24.1. J Med Genet. 2004 May;41(5):387-93.

4. Takeuchi T, Natsume J, Kidokoro H, **Ishihara N**, Yamamoto H, Azuma Y, Ito Y, Kurahashi N, Tsuji T, Suzuki M, Itomi K, Yamada K, Kurahashi H, Abe S, Okumura A, Maruyama K, Negoro T, Watanabe K, Kojima S. The effects of co-medications on lamotrigine clearance in Japanese children with epilepsy. Brain Dev. 2016 Sep;38(8):723-30.
5. Ogawa C, Natsume J, Yamamoto H, **Ishihara N**, Tashiro A, Kidokoro H, Negoro T, Yoshida M, Watanabe K. Autopsy findings of a patient with acute encephalitis and refractory, repetitive partial seizures. Seizure. 2016 Feb;35:80-2.
6. Natsume J, Ogawa C, Fukasawa T, Yamamoto H, **Ishihara N**, Sakaguchi Y, Ito Y, Takeuchi T, Azuma Y, Ando N, Kubota T, Tsuji T, Kawai H, Naganawa S, Kidokoro H. White Matter Abnormality Correlates with Developmental and Seizure Outcomes in West Syndrome of Unknown Etiology. AJNR Am J Neuroradiol. 2016 Apr;37(4):698-705.
7. Yokoi S, **Ishihara N**, Miya F, Tsutsumi M, Yanagihara I, Fujita N, Yamamoto H, Kato M, Okamoto N, Tsunoda T, Yamasaki M, Kanemura Y, Kosaki K, Kojima S, Saitoh S, Kurahashi H, Natsume J. TUBA1A mutation can cause a hydranencephaly-like severe form of cortical dysgenesis. Sci Rep. 2015 Oct 23;5:15165.
8. Yamamoto H, Natsume J, Kidokoro H, **Ishihara N**, Suzuki M, Tsuji T, Kubota T, Yamada A, Ozeki M, Kato Z, Kawamura Y, Yoshikawa T, Okumura A, Ando N, Saitoh S, Takahashi Y, Watanabe K, Kojima S. Clinical and neuroimaging findings in children with posterior reversible encephalopathy syndrome. Eur J Paediatr Neurol. 2015 Nov;19(6):672-8.
9. Ito Y, Natsume J, Kidokoro H, **Ishihara N**, Azuma Y, Tsuji T, Okumura A, Kubota T, Ando N, Saitoh S, Miura K, Negoro T, Watanabe K, Kojima S. Seizure characteristics of epilepsy in childhood after acute encephalopathy with biphasic seizures and late reduced diffusion. Epilepsia. 2015 Aug;56(8):1286-93.
10. Kawamura Y, Nakayama A, Kato T, Miura H, **Ishihara N**, Ihira M, Takahashi Y, Matsuda K, Yoshikawa T. Pathogenic Role of Human Herpesvirus 6B Infection in Mesial Temporal Lobe Epilepsy. J Infect Dis. 2015 Oct 1;212(7):1014-21
11. Natsume J, Maeda N, Itomi K, Kidokoro H, **Ishihara N**, Takada H, Okumura A, Kubota T, Miura K, Aso K, Morikawa T, Kato K, Negoro T, Watanabe K. PET in infancy predicts long-term outcome during adolescence in cryptogenic West syndrome. AJNR Am J Neuroradiol. 2014 Aug;35(8):1580-5.

Session 1-5

Genotype-Structure-Phenotype: what makes *ATP1A3* mutations different?

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ATP1A3 is in the P2 family of ion transport ATPases with similar structures and mechanisms. There is strong sequence homology, and it is logical to expect that homologous DNA variants that are pathogenic in one gene would be pathogenic in all. Programs that predict the pathogenicity of VUS (variants of unknown significance) in patients are based on this. It is surprising, then, that the mutations actually found in patients differ very significantly between ATPases, comparing *ATP1A3* (AHC, RDP, etc.), *ATP1A2* (FHM2), and *ATP2A2* (a calcium ATPase, Darier disease, dominant inheritance). In Darier disease, mutations are distributed broadly in the protein structure, and can be truncations or frameshifts as well as missense. This is consistent with haploinsufficiency. For the Na,K-ATPases this is not the case. Almost all mutations are missense, indicating that the damaged protein must be present to cause the phenotypes.

The question is whether there is a genotype-phenotype relationship that underlies the specific symptoms that people exhibit. We analyzed the genetic changes (both random variants and human disease mutations) in all four of the Na,K-ATPase alpha subunit genes, and compared the locations of the disease mutations in crystal structures in E1-Na and E2-K conformations. The results are striking. The frequency of random variants differs among the genes, and shows the effects of negative selection. The specific patterns of disease mutations are equally different: highly restricted somatic mutations for *ATP1A1*, broadly distributed for *ATP1A2*, largely clustered for *ATP1A3*, and no mutations of *ATP1A4*. By examining the positions of mutations in the crystal structures, a genotype-phenotype relationship emerges. *ATP1A3* mutations that produce milder and more severe symptoms have different distributions in three-dimensional space. Finally, no mutations have been found in certain parts of the Na,K-ATPases, predicting that entirely new phenotypes will be discovered.

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- **Publications**

1. **Sweadner, KJ**. Enzymatic properties of separated isozymes of the Na,K- ATPase: substrate affinities, kinetic cooperativity, and ion transport stoichiometry. *J. Biol. Chem*. 1985; 260: 11508-11513.

2. McGrail, KM, Phillips, JM, and **Sweadner, KJ**. Immunofluorescent localization of three Na,K-ATPase isozymes in the rat central nervous system: Both neurons and glia can express more than one Na,K-ATPase. *J. Neurosci*. 1991; 11: 381-391.

3. de Carvalho Aguiar, P, **Sweadner, KJ**, Penniston, JT, Zaremba, J, Liu, L, Caton, M, Linazasoro, G, Borg, M, Tijssen, MAJ, Bressman, SB, Dobyns, WB, Brashear, A, Ozelius, LJ. Mutations in the Na^+,K^+-ATPase α3 gene (*ATP1A3*) are associated with rapid onset dystonia parkinsonism. *Neuron* 2004, *43*: 169-175.

4. Liu,YB, Tewari, A, Salemeh, J, Arystarkhova, E, Hampton, T, Brashear, A, Ozelius, L, Khodakhah, K, **Sweadner, KJ**. A dystonia-like movement disorder with brain and spinal defects caused by mutation of the mouse laminin β1 subunit, *Lamb1*. eLIFE 2015 doi: 10.7554/eLife.11102.

5. **Sweadner, KJ**, Toro,C, Whitlow, CT, Snively,BM, Cook, JF, Ozelius, LJ, Markello, TC, Brashear A. *ATP1A3* mutation in adult rapid-onset ataxia. PLOS One 2016 11(3):e0151429. Doi 10.1371/journal.pone.00151429.

Session 2-1

Clinical profile of patients with *ATP1A3* mutations in AHC in an International Cohort

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In 2012, mutations in the *ATP1A3* gene (MIM 182350) were identified as the primary cause of AHC (AHC2, MIM 614820). Mutations in *ATP1A3* are found in approximately 75 % of cases and the disease is transmitted as an autosomal dominant trait. The mutations are usually de novo, but some have been found to be transmitted to offspring. Alternating hemiplegia of childhood (AHC) is a rare neurological disorder characterized by transient episodes of alternating hemiplegia/hemiparesis, dystonic attacks, paroxysmal abnormal ocular movements, epileptic seizures and episodes of autonomic dysfunction. The disease usually starts before 18 months of life and in the majority of patients before the age of 6 months. Plegic and tonic attacks disappear with sleep. Between attacks patients have an abnormal neurological examination often presenting ataxia, dystonia and other involuntary abnormal movements, and almost all present an intellectual disability. AHC has a prevalence of 1:100,000 children. There is a significant variability of the disease course between individuals and some genotype-phenotype correlations exist.

Clinical data from an international cohort of 155 AHC patients (84 females, 71 males; between 3 months and 52 years) were gathered using a specifically formulated questionnaire and analyzed relative to the mutational *ATP1A3* gene data for each patient.

RESULTS:

In total, 34 different *ATP1A3* mutations were detected in 85 % (132/155) patients. In general, mutations were found to cluster into five different regions. The most frequent mutations included: p.Asp801Asn (43 %; 57/132), p.Glu815Lys (16 %; 22/132), and p.Gly947Arg (11 %; 15/132). Of these, p.Glu815Lys was associated with a severe phenotype, with more severe intellectual and motor disability. p.Asp801Asn appeared to confer a milder phenotypic expression, and p.Gly947Arg appeared to correlate with the most favorable prognosis, compared to the other two frequent mutations. Overall, the comparison of the clinical profiles suggested a gradient of severity between the three major mutations with differences in intellectual ($p = 0.029$) and motor ($p = 0.039$) disabilities being statistically significant. For patients with epilepsy, age at onset of seizures was earlier for patients with either p.Glu815Lys or p.Gly947Arg mutation, compared to those with p.Asp801Asn mutation ($p < 0.001$). With regards to the five mutation clusters, some clusters appeared to correlate with certain clinical phenotypes. No statistically significant clinical correlations were found between patients with and without *ATP1A3* mutations.

CONCLUSIONS:

Our results, demonstrate a highly variable clinical phenotype in patients with AHC that correlates with certain mutations and possibly clusters within the *ATP1A3* gene. Our description of the clinical profile of patients with the most frequent mutations and the clinical picture of those with less common mutations confirms the results from previous studies, and further expands the spectrum of genotype-phenotype correlations.

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Academic Qualifications (most current date first)

Degree/Certification

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French inter-University Diploma of Epileptology. 2005 University Henri Poincaré, Nancy 1, France.
Board certified in Paediatrics. 2003 Faculty of Medicine, Athens, Greece.
Doctorate of Medicine. 1993 Faculty of Medicine, Patras, Greece.

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From January 2004 to February 2008. Senior Consultant in Pediatric Neurology. Hospital Robert Debré, Department of Pediatric Neurology, Paris, France.
From March 2003 to January 2004. Paediatric Hospital of Aghia Sophia, Athens, Greece.
From 2000 to 2002. Resident in paediatrics Paediatric hospital of Aghia Sophia, Athens, Greece.
From May 1996 to October 1998. Resident in paediatrics General Hospital of Kalamata, Greece.
From October 1993 to October 1995. General Practitioner. Following Greek legislation, all doctors are obliged, after their degree, to work for at least one year in a rural area. Public Medical Center of Areopolis, Greece.

Peer Reviewed Publications

1: Rheims S, Herbillon V, Villeneuve N, Auvin S, Napuri S, Cances C, Berquin P, Castelneau P, Nguyen The Tich S, Villega F, Isnard H, Nabbout R, Gaillard S, Mercier C, Kassai B, Arzimanoglou A; investigators of the Paediatric Epilepsy REsearch NEtwork (PERENE). ADHD in childhood epilepsy: Clinical determinants of severity and of the response to methylphenidate. Epilepsia. 2016 Jul;57(7):1069-77.

2: Mignot C, von Stülpnagel C, Nava C, Ville D, Sanlaville D, Lesca G, Rastetter A, Gachet B, Marie Y, Korenke GC, Borggraefe I, Hoffmann-Zacharska D, Szczepanik E, Rudzka-Dybała M, Yiş U, Çağlayan H, Isapof A, Marey I, **Panagiotakaki E**, Korff C, Rossier E, Riess A, Beck-Woedl S, Rauch A, Zweier C, Hoyer J, Reis A, Mironov M, Bobylova M, Mukhin K, Hernandez-Hernandez L, Maher B, Sisodiya S, Kuhn M, Glaeser D, Weckhuysen S, Myers CT, Mefford HC, Hörtnagel K, Biskup S; EuroEPINOMICS-RES MAE working group, Lemke JR, Héron D, Kluger G, Depienne C. Genetic and neurodevelopmental spectrum of SYNGAP1-associated intellectual disability and epilepsy. J Med Genet. 2016 Aug;53(8):511-22.

3: **Panagiotakaki E**, De Grandis E, Stagnaro M, Heinzen EL, Fons C, Sisodiya S, de Vries B, Goubau C,

Weckhuysen S, Kemlink D, Scheffer I, Lesca G, Rabilloud M, Klich A, Ramirez-Camacho A, Ulate-Campos A, Campistol J, Giannotta M, Moutard ML, Doummar D, Hubsch-Bonneaud C, Jaffer F, Cross H, Gurrieri F, Tiziano D, Nevsimalova S, Nicole S, Neville B, van den Maagdenberg AM, Mikati M, Goldstein DB, Vavassori R, Arzimanoglou A; Italian IBAHC Consortium; French AHC Consortium; International AHC Consortium. Clinical profile of patients with ATP1A3 mutations in Alternating Hemiplegia of Childhood-a study of 155 patients. Orphanet J Rare Dis. 2015 Sep 26;10:123.

4: Lepais L, Cheillan D, Frachon SC, Hays S, Matthijs G, **Panagiotakaki E**, Abel C, Edery P, Rossi M. ALG3-CDG: Report of two siblings with antenatal features carrying homozygous p.Gly96Arg mutation. Am J Med Genet A. 2015 Nov;167A(11):2748-54.

5: Arzimanoglou A, **Panagiotakaki E**, Bouveyron S. [Clinical presentations, etiologies and prognosis of epilepsy in children]. Soins Pediatr Pueric. 2015 Mar-Apr;(283):14-8.

6: Boshuisen K, van Schooneveld MM, Uiterwaal CS, Cross JH, Harrison S, Polster T, Daehn M, Djimjadi S, Yalnizoglu D, Turanli G, Sassen R, Hoppe C, Kuczaty S, Barba C, Kahane P, Schubert-Bast S, Reuner G, Bast T, Strobl K, Mayer H, de Saint-Martin A, Seegmuller C, Laurent A, Arzimanoglou A, Braun KP; TimeToStop cognitive outcome study group. Intelligence quotient improves after antiepileptic drug withdrawal following pediatric epilepsy surgery. Ann Neurol. 2015 Jul;78(1):104-14.

7: Laurent A, Arzimanoglou A, **Panagiotakaki E**, Sfaello I, Kahane P, Ryvlin P, Hirsch E, de Schonen S. Visual and auditory socio-cognitive perception in unilateral temporal lobe epilepsy in children and adolescents: a prospective controlled study. Epileptic Disord. 2014 Dec;16(4):456-70.

8: Boshuisen K, Schmidt D, Uiterwaal CS, Arzimanoglou A, Braun KP, Study Group T; TimeToStop Study Group. Time to relapse after epilepsy surgery in children: AED withdrawal policies are a contributing factor. Epileptic Disord. 2014 Sep;16(3):305-11. doi: 10.1684/epd.2014.0681. PubMed PMID: 25204012.

9: Heinzen EL, Arzimanoglou A, Brashear A, Clapcote SJ, Gurrieri F, Goldstein DB, Jóhannesson SH, Mikati MA, Neville B, Nicole S, Ozelius LJ, Poulsen H, Schyns T, Sweadner KJ, van den Maagdenberg A, Vilsen B; ATP1A3 Working Group. Distinct neurological disorders with ATP1A3 mutations. Lancet Neurol. 2014 May;13(5):503-14.

10: Ciumas C, Saignavongs M, Ilski F, Herbillon V, Laurent A, Lothe A, Heckemann RA, de Bellescize J, **Panagiotakaki E**, Hannoun S, Marinier DS, Montavont A, Ostrowsky-Coste K, Bedoin N, Ryvlin P. White matter development in children with benign childhood epilepsy with centro-temporal spikes. Brain. 2014 Apr;137(Pt 4):1095-106. doi: 10.1093/brain/awu039. Epub 2014 Mar 4. PubMed PMID: 24598359.

11: Bizec CL, Nicole S, **Panagiotakaki E**, Seta N, Vuillaumier-Barrot S. No Mutation in the SLC2A3 Gene in Cohorts of GLUT1 Deficiency Syndrome-Like Patients Negative for SLC2A1 and in Patients with AHC Negative for ATP1A3. JIMD Rep. 2014;12:115-20.

12: Heinzen EL, Swoboda KJ, Hitomi Y, Gurrieri F, Nicole S, de Vries B, Tiziano FD, Fontaine B, Walley NM, Heavin S, **Panagiotakaki E**; European Alternating Hemiplegia of Childhood (AHC) Genetics Consortium; Biobanca e Registro Clinico per l'Emiplegia Alternante (I.B.AHC) Consortium; European Network for Research on Alternating Hemiplegia (ENRAH) for Small and Medium-sized Enterpriese (SMEs) Consortium, Fiori S, Abiusi E, Di Pietro L, Sweney MT, Newcomb TM, Viollet L, Huff C, Jorde LB, Reyna SP, Murphy KJ, Shianna KV, Gumbs CE, Little L, Silver K, Ptáček LJ, Haan J, Ferrari MD, Bye AM, Herkes GK, Whitelaw CM, Webb D, Lynch BJ, Uldall P, King MD, Scheffer IE, Neri G, Arzimanoglou A, van den Maagdenberg AM, Sisodiya SM, Mikati MA, Goldstein DB. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nat Genet. 2012 Sep;44(9):1030-4.

13: Boshuisen K, Arzimanoglou A, Cross JH, Uiterwaal CS, Polster T, van Nieuwenhuizen O, Braun KP; TimeToStop study group. Timing of antiepileptic drug withdrawal and long-term seizure outcome after paediatric epilepsy surgery (TimeToStop): a retrospective observational study. Lancet Neurol. 2012 Sep;11(9):784-91.

14: Fons C, Campistol J, **Panagiotakaki E**, Giannotta M, Arzimanoglou A, Gobbi G, Neville B, Ebinger F, Nevšímalová S, Laan L, Casaer P, Spiel G, Ninan M, Sange G, Artuch R, Schyns T, Vavassori R, Poncelin D; ENRAH Consortium. Alternating hemiplegia of childhood: metabolic studies in the largest European series of patients. Eur J Paediatr Neurol. 2012 Jan;16(1):10-4.

15: Vuillaumier-Barrot S, **Panagiotakaki E**, Le Bizec C, El Baba C; ENRAHs for SME Consortium, Fontaine B, Arzimanoglou A, Seta N, Nicole S. Absence of mutation in the SLC2A1 gene in a cohort of patients with alternating hemiplegia of childhood (AHC). Neuropediatrics. 2010 Dec;41(6):267-9.

16: **Panagiotakaki E**, Gobbi G, Neville B, Ebinger F, Campistol J, Nevsímalová S, Laan L, Casaer P, Spiel G, Giannotta M, Fons C, Ninan M, Sange G, Schyns T, Vavassori R, Poncelin D; ENRAH Consortium, Arzimanoglou A. Evidence of a non-progressive course of alternating hemiplegia of childhood: study of a large cohort of children and adults. Brain. 2010 Dec;133(Pt 12):3598-610..

17: Manolaki N, Nikolopoulou G, Daikos GL, **Panagiotakaki E**, Tzetis M, Roma E, Kanavakis E, Syriopoulou VP. Wilson disease in children: analysis of 57 cases. J Pediatr Gastroenterol Nutr. 2009 Jan;48(1):72-7.

18**: Panagiotakaki E**, Tzetis M, Manolaki N, Loudianos G, Papatheodorou A, Manesis E, Nousia-Arvanitakis S, Syriopoulou V, Kanavakis E. Genotype-phenotype correlations for a wide spectrum of mutations in the Wilson disease gene (ATP7B). Am J Med Genet A. 2004 Dec 1;131(2):168-73.

19: Loudianos G, Lovicu M, Solinas P, Kanavakis E, Tzetis M, Manolaki N, **Panagiotakaki E**, Karpathios T, Cao A. Delineation of the spectrum of Wilson disease mutations in the Greek population and the identification of six novel mutations. Genet Test. 2000;4(4):399-402.

Session 2-2

Transcriptome analysis and clinical database/biorepository of Alternating Hemiplegia of Childhood patients with *ATP1A3* mutations for potential therapeutic discovery

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Mutations in the *ATP1A3* gene have been identified to be prevalent in patients with alternating hemiplegia of childhood (AHC). Over the past twenty years, we have made important contributions towards understanding AHC by establishing a clinical database and linked biorepository. This resource now contains data from approximately 300 affected probands. Our biorepository contains ~350 biological samples from probands including DNA, RNA, plasma, serum, whole blood, and cell lines, as well as CNS tissues from autopsy in 5 patients. Of 300 patients with an AHC phenotype, ~55% (164/300) have confirmed *ATP1A3* mutations with a total of 40 unique mutations. The majority of mutations are in exons 17, 18, and 21 with the most frequent mutations being: p.Asp801Asn (D801N) 20% (61/300), p.Glu815Lys (E815K) 14% (42/300), and p.Gly947Arg (G947R) 3.7% (11/300). Other *ATP1A3* mutations are present in 17% (51/300) and no *ATP1A3* mutations are present in ~12% (35/300) of affected individuals.

To date, no large scale transcriptomic data exist for human-derived AHC samples. The Connectivity Map (CMap) is a database of transcriptional signatures from cell lines treated with chemical and genetic reagents. When paired with the high-throughput L1000 by using a pattern-matching algorithm, the CMap links compounds with disease phenotypes. These CMAP/L1000 tools have helped identify several drug classes or compounds with potential activity for other treatment indications. Examples include the antihelminthic drug parbendazole as an inducer of osteoclast differentiation, the triterpene celastrol as a leptin sensitizer, compounds targeting COX2 and ADRA2A as potential treatments for diabetes, and small molecule therapeutics for skeletal muscular atrophy and spinal muscular atrophy. These tools have also helped to generate new therapeutic hypotheses for the treatment of inflammatory bowel disease and cancer. The present work leverages the rich tissue and phenotypic resources in our database/repository and expertise of the microarray analysis and deep sequencing resources at the Broad Institute/MIT. Using the CMap/L1000 tools, we performed a pilot experiment with 31 whole blood RNA samples from AHC patients and 89 healthy control samples. Our preliminary results revealed differences in the dynamic range of fold-expression achieved per gene in AHC samples when compared to controls. The gene ontology pathways most represented in the AHC gene expression profiles included genes involved in the circulatory system, vascular and embryonic development, as well as in cell proliferation pathways.

Following completion of testing and analyses of the current dataset, we hope to generate hypotheses and perform additional studies in patient derived samples including blood, cell lines, and brain. Identification of unique gene profiles in AHC could help identify novel pathways involved in disease pathogenesis and compounds that could ameliorate the disease phenotype.

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07/91-06/94	Resident	Neurology	Harvard Longwood Neurology Training Program, Brigham and Women's Hospital, Boston, MA
11/94-10/97	Fellow	Genetics	Harvard Medical School Genetics Training Program, Children's Hospital, Boston, MA
07/95-06/96	Fellow	Neurophysiology	EMG/Neuromuscular Fellowship Program, Lahey Hitchcock Clinic and Boston Children's Hospital, Boston, MA

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04/98-06/03	Assistant Professor	Department of Neurology	University of Utah School of Medicine, Salt Lake City, UT
04/98-06/03	Adjunct Assistant Professor	Division of Neurology, Department of Pediatrics	University of Utah School of Medicine, Salt Lake City, UT
07/03-03/15	Associate Professor	Department of Neurology	University of Utah School of Medicine, Salt Lake City, UT
07/03-03/15	Adjunct Associate Professor	Division of Neurology, Department of Pediatrics	University of Utah School of Medicine, Salt Lake City, UT
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04/98-03/15	Neurology Faculty	University of Utah Health Sciences Center	Salt Lake City, Utah
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Publications

1. Heinzen EL, **Swoboda KJ**, Hitomi Y, Gurrieri F, Nicole S, de Vries B, Tiziano FD, Fontaine B, Walley MN Heavin S, Panagiotakaki E, European Alternating Hemiplegia of Childhood, Genetics Consortium, Biobanca e Registro Clinico per l'Emiplegia Alternate (I.A.AHC) Consortium, European Network for

Research on Alternating Hemiplegia (ENRAH) for small and medium size enterprises (SMEs) Consortium, Fiori S, Abiusi E, DiPietro L, Sweney MT, Newcomb TM, Viollet L, Huff C, Jorde LB, Reyna SP, Murphy KJ, Shianna KV, Gumbs CE, Little L, Silver K, Ptacek LJ, Haan J, Ferrari MD, Bye AM, Herkes GK, Whitelaw CM, Webb D, Lynch BJ, Uldall P, King MD, Scheffer IE, Neri G, Arzimanoglou A, Van den Maagdenberg AM, Sisodiya SM, Mikati MA, Goldstein DB. (07/29/2012). De Novo mutations in ATP1A3 cause alternating hemiplegia of Childhood. *Nat Genet*, 2012; *44*(9), 1030-1034.

2. Flanigan KM, Ceco E, Lamar KM, Kaminoh Y, Dunn DM, Mendell JR, King WM, Pestronk A, Florence JM, Mathews KD, Finkel RS, **Swoboda KJ**, Gappmaier E, Howard MT, Day JW, McDonald C, McNally EM, Weiss RB; United Dystrophinopathy Project. LTBP4 genotype predicts age of ambulatory loss in Duchenne Muscular Dystrophy. *Ann Neurol.* 2012 Nov 26. doi: 10.1002/ana.23819. [Epub ahead of print] PMID: 23440719
3. Rothwell E, Anderson RA, **Swoboda KJ**, Stark L, Botkin JR. Public attitudes regarding a pilot study of newborn screening for spinal muscular atrophy. *Am J Med Genet A*. 2013 Apr; 161(4): 679-86. doi: 10.1002/ajmg.a.35756. Epub 2013 Feb 26. PMID: 23443997
4. Kobayashi DT, Shi J, Stephen L, Ballard KL, Dewey R, Mapes J, Chung B, McCarthy K, **Swoboda KJ**, Crawford TO, Li R, Plasterer T, Joyce C; Biomarkers for Spinal Muscular Atrophy Study Group, Chung WK, Kaufmann P, Darras BT, Finkel RS, Sproule DM, Martens WB, McDermott MP, De Vivo DC; Pediatric Neuromuscular Clinical Research Network, Walker MG, Chen KS. SMA-MAP: a plasma protein panel for spinal muscular atrophy. *PLoS One.* 2013;8(4): e60113. doi: 10.1371/journal.pone.0060113. Epub 2013 Apr 2. PMID: 23565191
5. Rice GI, Reijns MA, Coffin SR, Forte GM, Anderson BH, Szynkiewicz M, Gornall H, Gent D, Leitch A, Botella MP, Fazzi E, Gener B, Lagae L, Olivieri I, Orcesi S, **Swoboda KJ**, Perrino FW, Jackson AP, Crow YJ. Synonymous Mutations in RNASEH2A Create Cryptic Splice Sites Impairing RNase H2 Enzyme Function in Aicardi-Goutières Syndrome. *Hum Mutat*. 2013 Apr 16. doi: 10.1002/humu.22336. [Epub ahead of print] PMID: 23592335
6. Cano SJ, Mayhew A, Glanzman AM, Krosschell KJ, **Swoboda KJ**, Main M, Steffensen BF, Bérard C, Girardot F, Payan CA, Mercuri E, Mazzone E, Elsheikh B, Florence J, Hynan LS, Iannaccone ST, Nelson LL, Pandya S, Rose M, Scott C, Sadjadi R, Yore MA, Joyce C, Kissel JT; International Coordinating Committee for SMA Clinical Trials Rasch Task Force. Rasch Analysis of Clinical Outcome Measures in Spinal Muscular Atrophy. *Muscle Nerve*. 2013 Jul 8. PMID: 23836324
7. Rice GI, Forte GM, Szynkiewicz M, Chase DS, Aeby A, Abdel-Hamid MS, Ackroyd S, Allcock R, Bailey KM, Balottin U, Barnerias C, Bernard G, Bodemer C, Botella MP, Cereda C, Chandler KE, Dabydeen L, Dale RC, De Laet C, De Goede CG, Del Toro M, Effat L, Enamorado NN, Fazzi E, Gener B, Haldre M, Lin JP, Livingston JH, Lourenco CM, Marques W Jr, Oades P, Peterson P, Rasmussen M, Roubertie A, Schmidt JL, Shalev SA, Simon R, Spiegel R, **Swoboda KJ**, Temtamy SA, Vassallo G, Vilain CN, Vogt J, Wermenbol V, Whitehouse WP, Soler D, Olivieri I, Orcesi S, Aglan MS, Zaki MS, Abdel-Salam GM, Vanderver A, Kisand K, Rozenberg F, Lebon P, Crow YJ. "Assessment of interferon-related biomarkers in Aicardi-Goutières syndrome associated with mutations in TREX1,RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: a case-control study. *Lancet Neurol.* 2013 Dec;12(12):1159-69. Epub 2013 Oct 30. PMID:24183309
8. **Swoboda KJ**, Margraf RL, Carey JC, Zhou H, Newcomb TM, Coonrod E, Durtschi J, Mallempati K, Kumanovics A, Katz BE, Voelkerding KV, Opitz JM. "A Novel Germline PIGA Mutation in Ferro-Cerebro-Cutaneous Syndrome: A Neurodegenerative X-linked Epileptic Encephalopathy with Systemic Iron-Overload." *Am J Med Genet A*. 2013 Nov 20 PMID: 24259288
9. Butterfield RJ, Foley AR, Dastgir J, Asman S, Dunn DM, Zou Y, Hu Y, Donkervoort S, Flanigan KM, **Swoboda KJ**, Winder TL, Weiss RB, Bönnemann CG. "Position of glycine substitutions in the triple helix of COL6A1, COL6A2, and COL6A3 is correlated with severity and mode of inheritance in collagen VI myopathies." *Hum Mutat.* 2013 Nov;34(11):1558-67. PMID: 24038877
10. Davis RH, Godshall BJ, Seffrood E, Marcus M, Lasalle BA, Wong B, Schroth MK, **Swoboda KJ**. Nutritional Practices at a Glance: Spinal Muscular Atrophy type I Nutrition Survey Findings. *J Child Neurol*. 2013 Oct 4. [Epub ahead of print] PMID: 24097849
11. Botkin JR, Lewis MH, Watson MS, **Swoboda KJ**, Anderson R, Berry SA, Bonhomme N, Brosco JP, Comeau AM, Goldenberg A, Goldman E, Therrell B, Levy-Fisch J, Tarini B, Wilfond B; on behalf of the Bioethics and Legal Work Group of the Newborn Screening Translational Research Network. Parental

Permission for Pilot Newborn Screening Research: Guidelines From the NBSTRN. *Pediatrics* 2014 Jan 6. [Epub ahead of print] PMID: 24394680
12. Botkin JR, Lewis MH, Watson MS, **Swoboda KJ**, Anderson R, Berry SA, Bonhomme N, Brosco JP, Comeau AM, Goldenberg A, Goldman E, Therrell B, Levy-Fisch J, Tarini B, Wilfond B; Bioethics and Legal Work Group of the Newborn Screening Translational Research Network. Parental Permission for Pilot Newborn Screening Research: Guidelines From the NBSTRN. *Pediatrics* 2014 Feb;133(2):e410-7. PMID:24394680
13. Anderson RL, Murray K, Chong JX, Ouwenga R, Antillon M, Chen P, Diaz de Leon L, **Swoboda KJ**, Lester LA, Das S, Ober C, Waggoner DJ. Disclosure of Genetic Research Results to Members of a Founder Population. J Genet Couns. 2014 Apr 29. PMID:24777552
14. Butterfield RJ, Stevenson TJ, Xing L, Newcomb TM, Nelson B, Zeng W, Li X, Lu H-M, Lu H, Gonzalez KDF, Wei, J-P, Chao EC, Prior TW, Snyder PJ, Bonkowsky JL, **Swoboda KJ**. Congenital Lethal Motor Neuron Disease with a Novel Defect in Ribosome Biogenesis. *Neurology* 2014 Apr 15;82(15):1322-30. PMID:24647030
15. Heinzen EL, Arzimanoglou A, Brashear A, Clapcote SJ, Gurrieri F, Goldstein DB, Johannesson SH, Mikati MA, Neville B, Nicole S, Ozelius L, Poulsen H, Schyns T, Sweadner KJ, van den Maagdenberg A, Vilsen B for **the ATP1A3 Working Group**. Distinct neurological disorders with ATP1A3 mutations. *Lancet Neurol* 2014 May;13(5):503-514. PMID: 24739246
16. Hunt D, Leventer RJ, Simons C, Taft R, **Swoboda KJ**, Gawne-Cain M; the DDD study, Magee AC, Turnpenny PD, Baralle D. Whole exome sequencing in family trios reveals de novo mutations in PURA as a cause of severe neurodevelopmental delay and learning disability**.** *J Med Genet* 2014 Oct 23 doi: 10.1136/jmedgenet-2014-102798. PMID: 25342064
17. Findlay AR, Wein N, Kaminoh Y, Taylor LE, Dunn DM, Mendell JR, King WM, Pestronk A, Florence JM, Mathews KD, Finkel RS, **Swoboda KJ**, Howard MT, Day JW, McDonald C, Nicolas A, Le Rumeur E, Weiss RB, Flanigan KM; United Dystrophinopathy Project. Clinical phenotypes as predictors of the outcome of skipping around DMD exon 45. Ann Neurol. 2015 Apr;77(4):668-74. doi: 10.1002/ana.24365. Epub 2015 Mar 2. PMID:25612243
18. Sweney MT, Newcomb TM, **Swoboda KJ**. The expanding spectrum of neurological phentoypes in children with ATP1A3 mutations: Alternating Hemiplegia of Childhood, Rapid-onset Dystonia-Parkinsonism, CAPOS and beyond. Pediatr Neurol. 2015 Jan;52(1):56-64. doi: 10.1016/j.pediatrneurol.2014.09.015. Epub 2014 Oct 13.PMID:25447930
19. Rindt H, Feng Z, Mazzasette C, Glascock JJ, Valdivia D, Pyles N, Crawford TO, **Swoboda KJ**, Patitucci TN, Ebert AD, Sumner CJ, Ko CP, Lorson CL. Astrocytes Influence the severity of spinal muscular atrophy. *Hum Mol Genet*. 2015 Apr 24. PMID 25911676
20. Viollet L, Glusman G, Murphy KJ, Newcomb TM, Reyna SP, Sweney M, Nelson B, Andermann F, Andermann E, Acsadi G, Barbano RL, Brown C, Brunkow ME, Chugani HT, Cheyette SR, Collins A, DeBrosse SD, Galas D, Friedman J, Hood L, Huff C, Jorde LB, King MD, LaSalle B, Leventer RJ, Lewelt AJ, Massart MB, Mérida MR 2nd, Ptáček LJ, Roach JC, Rust RS, Renault F, Sanger TD, Sotero de Menezes MA, Tennyson R, Uldall P, Zhang Y, Zupanc M, Xin W, Silver K, **Swoboda KJ**. Alternating Hemiplegia of Childhood: Retrospective Genetic Study and Genotype-Phenotype Correlations in 187 Subjects from the US AHCF Registry. PLoS One. 2015 May 21;10(5):e0127045. PMID: 25996915
21. Roosing S, Hofree M, Kim S, Scott E, Copeland B, Romani M, Silhavy JL, Rosti RO, Schroth J, Mazza T, Miccinilli E, Zaki MS, **Swoboda KJ**, Milisa-Drautz J, Dobyns WB, Mikati M, İncecik F, Azam M, Borgatti R, Romaniello R, Boustany RM, Clericuzio CL, D'Arrigo S, Strømme P, Boltshauser E, Stanzial F, Mirabelli-Badenier M, Moroni I, Bertini E, Emma F, Steinlin M, Hildebrandt F, Johnson CA, Freilinger M, Vaux KK, Gabriel SB, Aza-Blanc P, Heynen-Genel S, Ideker T, Dynlacht BD, Lee JE, Valente EM, Kim J, Gleeson JG. Functional genome-wide siRNA screen identifies KIAA0586 as mutated in Joubert syndrome. Elife. 2015 May 30;4. PMID:26026149

Session 2-3

Cardiac phenotype in *ATP1A3* related-syndromes

S Balestrini, M Mikati, R Vavassori, M McLean, A Brashear, C Miller, R Samoes, J Novy, E de Grandis, E Veneselli, M Stagnaro, F Ragona, T Granata, N Nardocci, C Zucca, C Fons, J Campistol, I Scheffer, G Hollingsworth, E Pangiotakaki, A Arzimanoglou, I Carrilho, S Groppa, A Potic, QS Padiath, V Brankovic, R Pons, K Dzieżyc , M Mazurkiewicz-Bełdzińska, J Pilch, K Vezyroglou, H Cross, JP Kaski, SM Sisodiya

Alternating hemiplegia of childhood (AHC) is a rare neurodevelopmental disorder with significant phenotypic diversity, caused in ~80% of cases by mutations in the ATP1A3 gene. This gene encodes the catalytic alpha-3 subunit of the Na+/K+ ATPase exchange pump. Known outcomes range from life into adulthood, with comparatively little disability, to premature mortality from sudden death, including sudden unexpected death in epilepsy (SUDEP). We recently demonstrated that many patients with AHC have ECG changes, varying from repolarisation abnormalities, J-wave or J-point changes, to periods of asystole, with an increasing prevalence with age. The ECG abnormalities were dynamic, reflecting characteristics of inherited cardiac channelopathies, and suggesting, along with the paroxysmal neurological features, periodic systemic decompensation in ATP1A3-expressing organs. Given these findings, systematic cardiac investigation is warranted in this condition, as cardiac arrhythmic morbidity and mortality are potentially preventable (with implantation of a cardiac pacemaker or defibrillator). Also, certain drugs should be avoided in view of the increased risk of precipitating serious arrhythmias. We are now carrying out a second study enlarging the cohort (113 cases in total so far) and including cases with other *ATP1A3*-related phenotypes: cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS); and rapid-onset dystonia–parkinsonism (RDP). In this study we are investigating the cardiac phenotype further, collecting data also on prolonged ECG monitoring, echocardiography and other cardiological evaluation tests. This will validate our previous findings and give more insight on the phenotype-genotype correlation in AHC and other ATP1A3-associated syndromes.

Simona BALESTRINI

WORK EXPERIENCE

From 1.8.2014 ongoing. Clinical Research Associate at the Department of Clinical and Experimental Epilepsy, University College of London (UCL). Main topic of research: genetics of epilepsies, exome and genome analysis, genotype-phenotype association studies, transcranial magnetic stimulation.

From 23.2.2017 ongoing. Honorary Consultant Neurologist, at University College London Hospital (UCLH) NHS Foundation Trust.

From 1.7.2016 to 30.11. 2016. Locum Consultant Neurologist – Special Interest in Epilepsy, at University College London Hospital (UCLH) NHS Foundation Trust.

From 30.6.2009 to 4.7.2014. Residence in Neurology at Polytechnic University of Marche (Ancona, Italy), with focus on epilepsy, neuro-oncology, cerebrovascular disease, epidemiological research.

EDUCATION AND TRAINING

From 1.11.2013 to 1.3.2017. Phd student at Polytechnic University of Marche (Ancona, Italy). Main topic of research: genetics of drug-resistant epilepsy. Final thesis on 'Biomarkers of Sudden Unexpected Death in Epilepsy (SUDEP)'.

On 4th Jul 2014. Completion of Medical Residency in Neurology at Polytechnic University of Marche (Ancona, Italy), with thesis on "Electrical Stimulations of Parietal Lobe: Stereo-EEG Study in Patients with Drug-Resistant Focal Epilepsy" (50/50 with honour).

From Jan to Dec 2013. Clinical Research Associate at the Department of clinical and experimental Epilepsy, University College of London (UCL) Main topics: clinical research projects about genetic basis of epilepsy, phenotype-genotype correlation, genetic mechanisms of SUDEP.

On 24th Jul 2008. Degree in Medicine and Surgery at Polytechnic University of Marche with thesis on "Progression of Carotid Atherosclerosis and Zinc Omeosthasis" (110/110 with honour).

PUBLICATIONS

1. **Balestrini S**, Sisodiya SM. Pharmacogenomics in epilepsy. Neurosci Lett. 2017 Jan 10. pii: S0304-3940(17)30024-1. doi: 10.1016/j.neulet.2017.01.014.
2. Lippiello P, **Balestrini S**, Leo A, Coppola A, Citraro R, Elia M, Russo E, De Sarro G. From cannabis to cannabidiol to treat epilepsy, where are we? Curr Pharm Des. 2016 Aug 31
3. de Lange IM, Helbig KL, Weckhuysen S, Møller RS, Velinov M, Dolzhanskaya N, Marsh E, Helbig I, Devinsky O, Tang S, Mefford HC, Myers CT, van Paesschen W, Striano P, van Gassen K, van Kempen M, de Kovel CG, Piard J, Minassian BA, Nezarati MM, Pessoa A, Jacquette A, Maher B, **Balestrini S**, Sisodiya S, Warde MT, De St Martin A, Chelly J; EuroEPINOMICS-RES MAE working group, van 't Slot R, Van

4. Maldergem L, Brilstra EH, Koeleman BP. De novo mutations of KIAA2022 in females cause intellectual disability and intractable epilepsy. J Med Genet. 2016 Jun 29. pii: jmedgenet-2016-103909
5. **Balestrini S**, Sisodiya SM. Audit of use of stiripentol in adults with Dravet syndrome. Acta Neurol Scand. 2016 May 27. doi: 10.1111/ane.12611.
6. **Balestrini S**, Milh M, Castiglioni C, Lüthy K, Finelli MJ, Verstreken P, Cardon A, Stražišar BG, Holder JL Jr, Lesca G, Mancardi MM, Poulat AL, Repetto GM, Banka S, Bilo L, Birkeland LE, Bosch F, Brockmann K, Cross JH, Doummar D, Félix TM, Giuliano F, Hori M, Hüning I, Kayserili H, Kini U, Lees MM, Meenakshi G, Mewasingh L, Pagnamenta AT, Peluso S, Mey A, Rice GM, Rosenfeld JA, Taylor JC, Troester MM, Stanley CM, Ville D, Walkiewicz M, Falace A, Fassio A, Lemke JR, Biskup S, Tardif J, Ajeawung NF, Tolun A, Corbett M, Gecz J, Afawi Z, Howell KB, Oliver KL, Berkovic SF, Scheffer IE, de Falco FA, Oliver PL, Striano P, Zara F, Campeau PM, Sisodiya SM. TBC1D24 genotype-phenotype correlation: Epilepsies and other neurologic features. Neurology. 2016 Jun 8. pii: 10.1212/WNL.0000000000002807.
7. Santulli L, Coppola A, **Balestrini S**, Striano S. The challenges of treating epilepsy with 25 antiepileptic drugs. Pharmacol Res. 2016 Mar 16;107:211-219.
8. **Balestrini S**, Hart Y, Thunell S, Sisodiya SM. Safe use of perampanel in a carrier of variegate porphyria. Pract Neurol. 2016 Feb 2. pii: practneurol-2015-001305. doi:10.1136/practneurol-2015-001305.
9. Coppola A, Caccavale C, Santulli L, **Balestrini S**, Cagnetti C, Licchetta L, Esposito M, Bisulli F, Tinuper P, Provinciali L, Minetti C, Zara F, Striano P, Striano S. Psychiatric comorbidities in patients from seven families with autosomal dominant cortical tremor, myoclonus, and epilepsy. Epilepsy Behav. 2016 Jan 28;56:38-43. doi:10.1016/j.yebeh.2015.12.038.
10. **Balestrini S**, Francione S, Mai R, Castana L, Casaceli G, Marino D, Provinciali L, Cardinale F, Tassi L. Reply: The dorsal cingulate cortex as a critical gateway in the network supporting conscious awareness. Brain. 2015 Dec 30. pii: awv382.
11. Leu C, **Balestrini S**, Maher B, Hernández-Hernández L, Gormley P, Hämäläinen E, Heggeli K, Schoeler N, Novy J, Willis J, Plagnol V, Ellis R, Reavey E, O'Regan M, Pickrell WO, Thomas RH, Chung SK, Delanty N, McMahon JM, Malone S, Sadleir LG, Berkovic SF, Nashef L, Zuberi SM, Rees MI, Cavalleri GL, Sander JW, Hughes E, Helen Cross J, Scheffer IE, Palotie A, Sisodiya SM. Genome-wide Polygenic Burden of Rare Deleterious Variants in Sudden Unexpected Death in Epilepsy. EBioMedicine. 2015 Jul 10;2(9):1063-70.
12. Wandschneider B, Koepp M, Scott C, Micallef C, **Balestrini S**, Sisodiya SM, Thom M, Harper RM, Sander JW, Vos SB, Duncan JS, Lhatoo S, Diehl B. Structural imaging biomarkers of sudden unexpected death in epilepsy. Brain. 2015 Oct;138(Pt 10):2907-19. doi: 10.1093/brain/awv233.
13. Cerqua R, **Balestrini S**, Perozzi C, Cameriere V, Renzi S, Lagalla G, Mancini G, Montanari M, Leoni P, Scerrati M, Iacoangeli M, Silvestrini M, Luzzi S, Provinciali L. Diagnostic delay and prognosis in primary central nervous system lymphoma compared with glioblastoma multiforme. Neurol Sci. 2016 Jan;37(1):23-9. doi: 10.1007/s10072-015-2353-4.

14. Chapman SC, Horne R, Eade R, **Balestrini S**, Rush J, Sisodiya SM.Applying a perceptions and practicalities approach to understanding nonadherence to antiepileptic drugs. Epilepsia. 2015 Sep;56(9):1398-407. doi: 10.1111/epi.13097.
15. **Balestrini S**, Francione S, Mai R, Castana L, Casaceli G, Marino D, Provinciali L, Cardinale F, Tassi L. Multimodal responses induced by cortical stimulation of the parietal lobe: a stereo-electroencephalography study. Brain. 2015 Sep;138(Pt 9):2596-607. doi: 10.1093/brain/awv187.
16. **Balestrini S**, Clayton LM, Bartmann AP, Chinthapalli K, Novy J, Coppola A, Wandschneider B, Stern WM, Acheson J, Bell GS, Sander JW, Sisodiya SM. Retinal nerve fibre layer thinning is associated with drug resistance in epilepsy. J Neurol Neurosurg Psychiatry. 2015 Apr 17. pii: jnnp-2015-310521. doi: 10.1136/jnnp-2015-310521
17. Cagnetti C, Buratti L, Foschi N, **Balestrini S**, Provinciali L Generalized epilepsy in a patient with myotonic dystrophy type 2. Neurol Sci. 2013 Nov 26
18. **Balestrini S**, Cagnetti C, Foschi N, Buratti L, Petrelli C, Luzzi S, Silvestrini M, Provinciali L. Emergency room access for recurring seizures: when and why. Eur J Neurol. 2013 Oct; 20(10):1411-6.
19. **Balestrini S**, Provinciali L, Benbadis SR. Burden of uncontrolled epilepsy in patients requiring an emergency room visit or hospitalization. Neurology. 2013 Jun 4;80(23):2170.
20. Buratti L, **Balestrini S**, Altamura C, Viticchi G, Falsetti L, Luzzi S, Provinciali L, Vernieri F, Silvestrini M.Markers for the Risk of Progression from Mild Cognitive Impairment to Alzheimer's Disease. J Alzheimers Dis. 2015 Jan 29.
21. Buratti L, **Balestrini S**, Avitabile E, Altamura C, Vernieri F, Viticchi G, Falsetti L, Provinciali L, Silvestrini M. Sex-associated differences in the modulation of vascular risk in patients with asymptomatic carotid stenosis. J Cereb Blood Flow Metab. 2015 Jan 14. doi: 10.1038/jcbfm.2014.248.
22. Diomedi M, Scacciatelli D, Misaggi G, **Balestrini S**, Balucani C, Sallustio F, Di Legge S, Stanzione P, Silvestrini M. Increased common carotid artery wall thickness is associated with rapid progression of asymptomatic carotid stenosis. J Neuroimaging. 2014 Sep-Oct;24(5):473-8.
23. Zagaglia S, **Balestrini S**, Perticaroli E, Danni MC, Luzzi S, Silvestrini M, Provinciali L. Percutaneous transluminal angioplasty for chronic cerebrospinal venous insufficiency in multiple sclerosis: dichotomy between subjective and objective outcome scores. Neurol Sci. 2013 May 10
24. **Balestrini S**, Perozzi C, Altamura C, Vernieri F, Luzzi S, Bartolini M, Provinciali L, Silvestrini M. Severe carotid stenosis and impaired cerebral hemodynamics can influence cognitive deterioration. Neurology. 2013 Jun 4;80(23):2145-50.
25. **Balestrini S**, Lupidi F, Balucani C, Altamura C, Vernieri F, Provinciali L, Silvestrini M. One-year progression of moderate asymptomatic carotid stenosis predicts the risk of vascular events. Stroke. 2013 Mar;44(3):792-4.
26. Talebi Chahvar S, Zosmer A, Caragia A, **Balestrini S**, Sabatini L, Tranquilli AL, Al-Shawaf T. Coasting, embryo development and outcomes of blastocyst transfer: a case-control study. Reprod Biomed Online 2014;29(2):231-8.

Session 3-1

New causative gene(s) in AHC?

Arn M.J.M. van den Maagdenberg, PhD

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As not all patients with alternating hemiplegia of childhood (AHC) carry a mutation in the coding regions of the *ATP1A3* gene, a gene hunt was initiated aimed at identifying a second gene for this disabling disorder. Data of exome sequencing on genomic DNA of over 25 trio's (that is DNA of the patient and both parents) was performed to search primarily for *de novo* pathogenic mutations. Even with this large number of samples, the search for additional AHC genes is challenging, not in the least because of clinical heterogeneity among *ATP1A3*-negative cases, many being classified as atypical. The status of the gene hunt will be discussed on behalf of the Consortium. Approaches to screen for possible mutations in *ATP1A3* in presumed *ATP1A3*-negative cases will also be discussed. The gene hunt suggests that there may not be a second major gene after all. To unravel the (molecular) pathophysiology of AHC, it may be fruitful to learn from developments, with respect to disease pathways involved and functional assays to assess brain dysfunction, in disorders with resemblance to AHC.

Arn M.J.M. VAN DEN MAAGDENBERG

- **Present position**

Professor, Departments of Human Genetics and Neurology,
Leiden University Medical Centre, Leiden, The Netherlands

- **Education**

1981 - 1988 Biology, Catholic University of Nijmegen, the Netherlands
1989 - 1993 Fellow in Human Genetics, Leiden University Medical Centre, the Netherlands
1993 PhD, Leiden University Medical Centre, the Netherlands

- **Academic appointments**

1993 - 1998 Postdoctoral Fellow, Department of Cell Biology & Histology, University of Nijmegen, The Netherlands
1998 - 2005 Postdoctoral Fellow, Department of Human Genetics, Leiden University Medical Centre, The Netherlands
2005 - 2011 Associate Professor, Departments of Human Genetics & Neurology, Leiden University Medical Centre
2011 - Full professor, Departments of Human Genetics & Neurology (LUMC). Chair: "Molecular and Functional Neurogenetics"

- **Publications** (relevant selection of in total 240 peer-reviewed publications)

1. Heinzen E, Swoboda K, Hitomi Y, …. **van den Maagdenberg A**, Sisodiya S, Mikati M, Goldstein D. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nature Genetics 2012;44:1030-1034.
2. Freilinger T, Anttila V, de Vries B, ….. **van den Maagdenberg AM**; International Headache Genetics Consortium. Genome-wide association analysis identifies susceptibility loci for migraine without aura. Nature Genetics 2012;44:777-782.
3. Heinzen E, Arzimanoglou A, Brashear A, …. **van den Maagdenberg A**, Vilsen B; ATP1A3 Working Group. Distinct neurological disorders with ATP1A3 mutations. Lancet Neurology 2014;13:503-514.
4. Ferrari M*, Klever R*, Terwindt G, Ayata C, **van den Maagdenberg A**. Migraine pathophysiology: lessons from mouse models and human genetics. Lancet Neurology 2015;14:65-80.
5. Eising E, Shyti R, 't Hoen PAC, …. **van den Maagdenberg AM**. Cortical Spreading Depression Causes Unique Dysregulation of Inflammatory Pathways in a Transgenic Mouse Model of Migraine. Molecular Neurobiology 2017;54:2986-2996.

Session 3-2

Biochemical consequences of *ATP1A3* mutations

Jan B. Koenderink

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Alternating Hemiplegia of Childhood (AHC) is a neurodevelopmental disorder caused by de novo mutations in *ATP1A3*, the gene encoding the α3 subunit of Na,K-ATPase. Na,K-ATPase regulates electrogenic transport by exporting three Na^+ ions in exchange for two K^+ ions across the cellular membrane by hydrolysis of one adenosine triphosphate molecule. Maintaining these ionic gradients has been shown to be of great importance in a variety of cellular functions, including neuronal activity.
Depending on the location of the mutation in *ATP1A3* differences in AHC development have been observed. Moreover, some mutations in *ATP1A3* do not cause AHC, but rapid-onset dystonia-parkinsonism (RDP) and cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS).
The functional consequences of many AHC mutations have been characterized using over-expression in different mammalian cells, insect cells or *Xenopus laevis* oocytes. In addition, also several functional characteristics have been analyzed: Electrophysiological activity, ATPase activity, cell survival, ouabain binding, and phosphorylation capacity. A loss of function has been observed in most assays. A detailed analysis has been performed only for some of the many different mutations detected.
Many fundamental questions still remain, but what did we learn from these functional assays? Is it important to continue detailed functional assays for all mutations identified? Which knowledge gaps still exist and should be addressed?

Jan Koenderink

Jan is assistant professor in biochemical toxicology at the Department of Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, The Netherlands. His research interest is focussed on membrane transport proteins and their ligands (substrates and inhibitors). The interaction between membrane transporters (uptake and efflux) and novel and existing human drugs, as well as the multidrug resistance transport proteins (ABC transporters) of parasites (e.g. *Mycobacterium tuberculosis* and *Plasmodium falciparum*) are investigated. In addition, the molecular activity and interactions of the sodium pump (Na,K-ATPase) are studied. The studies include the expression of recombinant transport proteins and their molecular / biochemical and pharmacodynamic characterization.

Publications

Alternating Hemiplegia of Childhood mutations have a differential effect on Na(+),K(+)-ATPase activity and ouabain binding. Weigand KM, Messchaert M, Swarts HG, Russel FG, **Koenderink JB**. Biochim Biophys Acta. 2014 Jul;1842(7):1010-6.

Na(+),K(+)-ATPase isoform selectivity for digitalis-like compounds is determined by two amino acids in the first extracellular loop. Weigand KM, Laursen M, Swarts HG, Engwerda AH, Prüfert C, Sandrock J, Nissen P, Fedosova NU, Russel FG, **Koenderink JB**. Chem Res Toxicol. 2014 Dec 15;27(12):2082-92.

Vital and dispensable roles of Plasmodium multidrug resistance transporters during blood- and mosquito-stage development. Rijpma SR, van der Velden M, Annoura T, Matz JM, Kenthirapalan S, Kooij TW, Matuschewski K, van Gemert GJ, van de Vegte-Bolmer M, Siebelink-Stoter R, Graumans W, Ramesar J, Klop O, Russel FG, Sauerwein RW, Janse CJ, Franke-Fayard BM, **Koenderink JB**. Mol Microbiol. 2016 Jul;101(1):78-91.

Moxifloxacin Is a Potent In Vitro Inhibitor of OCT- and MATE-Mediated Transport of Metformin and Ethambutol. Te Brake LH, van den Heuvel JJ, Buaben AO, van Crevel R, Bilos A, Russel FG, Aarnoutse RE, **Koenderink JB**. Antimicrob Agents Chemother. 2016 Nov 21;60(12):7105-7114.

The role of efflux pumps in TB treatment and their promise as a target in drug development: unraveling the black box. Te Brake LH, de Knegt GJ, de Steenwinkel JE, van Dam TJ, Burger DM, Russel FG, van Crevel R, **Koenderink JB**, Aarnoutse RE. Annual Review of Pharmacology and Toxicology, 2017, *In press.*

Session 3-3

ATP1A3 neurological disease mutations affecting Na^+ binding: Structural and functional perspectives and rescue of compromised function continued

B. Vilsen[1], R. Holm[1], C.P. Rønn[1], L.E. Christiansen[1], M.S. Toustrup-Jensen[1], A.P. Einholm[1], V.R. Schack[1]

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Several neurological phenotypes derive from ATP1A3 mutations. The effects of several of these mutations on Na^+,K^+-ATPase function have been studied *in vitro* [1]. The high-resolution crystal structures of the Na^+,K^+-ATPase provide a solid basis for understanding functional defects of the ATP1A3 disease mutants and of the corresponding ATP1A1 mutants. Na^+ binds at three sites, I, II, and III, of which I and II overlap with the K^+ binding sites, whereas site III is unique and Na^+ specific [2]. In several ATP1A3 disease mutants the compromised function can be traced to disturbance of the Na^+ specific binding site III, although some ATP1A3 disease mutants show disruption of Na^+ binding at sites I and II. Furthermore a limited number of the ATP1A3 mutations occur in the cytoplasmic P domain near the catalytic site. We have recently characterized the functional disturbance caused by some of these mutations.
A secondary mutation was found to rescue the defective Na^+ binding at site III caused by RDP/AHC mutation D923N [1, 3]. A perspective is that it may be feasible to develop an efficient pharmaceutical mimicking the rescuing effect, which optimally would rescue the compromised function of a variety of ATP1A3 mutants. In our continued work on the rescuing effect we have studied combinations of the rescuing mutation with mutations in sites I and II and additional ATP1A3 mutations.

References:

1. Holm, R., Toustrup-Jensen, M.S., Einholm, A.P., Schack, V.R., Andersen, J.P., and **Vilsen, B**. (2016). Neurological disease mutations of α3 Na^+,K^+-ATPase: Structural and functional perspectives and rescue of compromised function. *Biochim. Biophys. Acta – Bioenerg.* 1857, 1807–1828.

2. Kanai, R., Ogawa, H., **Vilsen, B**., Cornelius, F., and Toyoshima, C. (2013). Crystal structure of a Na^+-bound Na^+,K^+-ATPase preceding the E1P state. *Nature* 502, 201–206.

3. Holm, R., Einholm, A.P., Andersen, J.P., and **Vilsen, B**. (2015). Rescue of Na^+ affinity in aspartate 928 mutants of Na^+,K^+-ATPase by secondary mutation of glutamate 314. *J. Biol. Chem.* 290, 9801–9811.

Bente VILSEN

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Professor, Department of Biomedicine, Faculty of Health, Aarhus University, Aarhus, Denmark

Education

1984	Masters degree (cand.scient.) in Biology, Aarhus University
1995	D.M.Sc. (dr.med.), Aarhus University

Academic Appointments

1986-1988	Research Assistant, Department of Physiology, Aarhus University
1988-1989	Visiting Scientist at Professor D.H. MacLennan, University of Toronto, Canada
1988-1992	Assistant Professor, Department of Physiology, Aarhus University
1992-2006	Associate Professor, Department of Physiology, Aarhus University
2006-Present	Full professor, Department of Biomedicine, Aarhus University

Publication (119 articles in international journals, H-index 41)

1. Rodacker V, Toustrup-Jensen M, **Vilsen B**. Mutations Phe^{785}Leu and Thr^{618}Met in Na^+,K^+-ATPase, associated with familial rapid-onset dystonia parkinsonism, interfere with Na^+ interaction by distinct mechanisms. The Journal of Biological Chemistry 2006; 281: 18539-18548.

2. Blanco-Arias P, Einholm AP, Mamsa H, Concheiro C, Gutiérrez-de-Terán H, Romero J, Toustrup-Jensen MS, Carracedo A, Jen JC, **Vilsen B**, Sobrido MJ. A C-terminal mutation of ATP1A3 underscores the crucial role of sodium affinity in the pathophysiology of rapid-onset dystonia-parkinsonism. Human Molecular Genetics 2009; 18: 2370-2377.

3. Clapcote SJ, Duffy S, Xie G, Kirshenbaum G, Bechard AR, Schack VR, Petersen J, Sinai L, Saab BJ, Lerche JP, Minassian BA, Ackerley CA, Sled JG, Cortez MA, Henderson JT, **Vilsen B**, Roder JC. Mutation I810N in the α3-isoform of Na^+,K^+-ATPase causes impairments in the sodium pump and hyperexcitability in the CNS. Proceedings of the National Academy of Sciences U.S.A. 2009; 106: 14085-14090.

4. Kanai, R., Ogawa, H., **Vilsen, B**., Cornelius, F., Toyoshima, C. Crystal structure of a Na^+-bound Na^+,K^+-ATPase preceding the E1P state. Nature 2013; 502: 201-206.

5. Toustrup-Jensen MS, Einholm AP, Schack VR, Nielsen HN, Holm R, Sobrido MJ, Andersen JP, Clausen T, **Vilsen B**. Relationship between intracellular Na^+ concentration and reduced Na^+ affinity in Na^+,K^+-ATPase mutants causing neurological disease. The Journal of Biological Chemistry 2014; 289: 3186-3197.

6. Holm R, Einholm AP, Andersen JP, **Vilsen, B**. Rescue of Na^+ Affinity in Aspartate-928 Mutants of Na^+,K^+-ATPase by Secondary Mutation of Glutamate-314. The Journal of Biological Chemistry 2015; 290: 9801-9811.

7. Holm R, Toustrup-Jensen MS, Einholm AP, Schack VR, Andersen JP, **Vilsen B**. Neurological disease mutations of α3 Na^+,K^+-ATPase: Structural and functional perspectives and rescue of compromised function. Biochimica et Biophysica Acta - Bioenergetics 2016; 1857: 1807-1828.

8. Holm R, Khandelwal J, Einholm AP, Andersen JP, Artigas, P, **Vilsen B**. Arginine substitution of a cysteine in transmembrane helix M8 converts Na^+,K^+-ATPase to an electroneutral pump similar to H^+,K^+-ATPase. Proceedings of the National Academy of Sciences U.S.A. 2017; 114: 316-321.

Session 4-1

Development of new treatments in the *Myshkin* mouse model of AHC

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Alternating hemiplegia of childhood (AHC; OMIM: 614820) is a rare neurodevelopmental disorder that manifests as episodic hemiplegia starting in the first 18 months of life, with a spectrum of persistent motor, movement and cognitive deficits that become progressively more apparent with age. Flunarizine reduces the severity, duration, or frequency of hemiplegic attacks in some patients, while two AHC patients have shown marked improvements in symptoms when treated with a ketogenic diet. However, the complexity and severity of AHC make it imperative that new therapeutic options be explored.

Heterozygous missense mutations of the *ATP1A3* gene, encoding the neurone-specific Na^+,K^+-ATPase (NKA) α3 subunit, have been identified as the primary cause of AHC as well as rapid-onset dystonia-parkinsonism (RDP) and CAPOS syndrome. Towards gaining a fuller understanding of the neurological effects of NKA α3 dysfunction, we developed the *Myshkin* mouse model, which has an I810N mutation identical to that present in AHC.

Heterozygous *Myshkin* mice move with a paretic, tremulous gait that becomes transiently more severe after stress. Other phenotypic abnormalities include decreased body mass, neuronal hyperexcitability, increased susceptibility to epileptic seizures, motor dysfunction and cognitive impairment. Genetic (*Atp1a3* transgene), pharmacological (rostafuroxin), and dietary (medium-chain fatty acids) approaches to the treatment of AHC-related phenotypes in the *Myshkin* model will be presented.

Myshkin mice carrying a wildtype *Atp1a3* transgene that confers a 16% increase in brain-specific total NKA activity show significant phenotypic improvements compared with non-transgenic *Myshkin* mice. Chronic treatment with rostafuroxin (PST-2238), a compound which selectively displaces endogenous ouabain from the NKA, reduces the risk-taking behaviour of *Myshkin* mice. The potential therapeutic effects of a diet rich in medium-chain fatty acids will also be discussed.

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Present Position

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Education

1993 BSc, University College of North Wales, University of Wales, UK

1994 MSc, Institute of Genetics, University of Edinburgh, UK

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1998-2002 Postdoctoral Fellow, Dept. of Zoology, University of Oxford, UK

2002-2007 Postdoctoral Fellow, Centre for Modeling Human Disease, Mount Sinai Hospital, Toronto, Canada

Academic Appointments

2007-2008 Neurogenetics Project Leader, School of Molecular and Clinical Medicine, University of Edinburgh, UK

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Relevant Publications

1. **CLAPCOTE, S.J.**, Duffy, S., Xie, G., Kirshenbaum, G., Bechard, A.R., Rodacker Schack, V., Petersen, J., Sinai, L., Saab, B.J., Lerch, J.P., Minassian, B.A., Ackerley, C.A., Sled, J.G., Cortez, M.G., Henderson, J.T., Vilsen, B. & Roder, J.C. (2009). Mutation I810N in the α3 isoform of Na+,K+-ATPase causes impairments in the sodium pump and hyperexcitability in the CNS. Proceedings of the National Academy of Sciences of the USA 106: 14085-14090.

2. Kirshenbaum, G.S., **CLAPCOTE, S.J.**, Duffy, S., Burgess, C.R., Petersen, J., Jarowek, K.J., Yücel, Y.H., Cortez, M.A., Snead III, O.C., Vilsen, B., Peever, J.H., Ralph, M.R. & Roder, J.C.(2011). Mania-like behavior induced by genetic dysfunction of the neuron-specific Na+,K+-ATPase α3 sodium pump. Proceedings of the National Academy of Sciences of the USA 108: 18144-18149.

3. Kirshenbaum, G.S., **CLAPCOTE, S.J.**, Petersen, J., Vilsen, B., Ralph, M.R. & Roder, J.C.(2012). Genetic suppression of agrin reduces mania-like behavior in Na+,K+-ATPase α3 mutant mice. Genes, Brain and Behavior 11: 436-443.

4. Kirshenbaum, G.S., Dawson, N., Mullins, J.G.L., Johnston, T.H., Drinkhill, M.J., Edwards, I.J., Fox, S.H., Pratt, J.A., Brotchie, J.M., Roder, J.C. & **CLAPCOTE, S.J.** (2013). Alternating hemiplegia of childhood-related neural and behavioural phenotypes in Na+,K+-ATPase α3 missense mutant mice. PLoS One 8: e60141.
5. Edwards, I.J., Bruce, G., Lawrenson, C., Howe, L., **CLAPCOTE, S.J.**, Deuchars, S.A. & Deuchars, J. (2013). Na+/K+ ATPase α1 and α3 isoforms are differentially expressed in α- and γ-motoneurons. Journal of Neuroscience 33: 9913-9919.
6. Heinzen, E.L., Arzimanoglou, A., Brashear, A., **CLAPCOTE, S.J.** et al. (2014). Distinct neurological disorders with ATP1A3 mutations. The Lancet Neurology 13: 503-514.
7. Kirshenbaum, G.S., Dachtler, J., Roder, J.C. & **CLAPCOTE, S.J.** (2015). Characterization of cognitive deficits in mice with an alternating hemiplegia-linked mutation. Behavioral Neuroscience 129: 822-831.
8. Kirshenbaum, G.S., Dachtler, J., Roder, J.C. & **CLAPCOTE, S.J.** (2016). Transgenic rescue of phenotypic deficits in a mouse model of alternating hemiplegia of childhood. Neurogenetics 17: 57-63.
9. Kirshenbaum, G.S., Idris, N.F., Dachtler, J., Roder, J.C. & **CLAPCOTE, S.J.** (2016). Deficits in social behavioral tests in a mouse model of alternating hemiplegia of childhood. Journal of Neurogenetics 30: 42-49.

Session 4-2

Hypothermia-induced dystonia and abnormal cerebellar activity in a mouse model with a single disease-mutation in the sodium-potassium pump

Isaksen TJ, Kros L, Vedovato N, Holm Th, Vitenzon A, Gadsby DC, Khodakhah K and **Lykke-Hartmann K**

The neurological spectrum associated with mutations in the *ATP1A3* gene, encoding the α_3 isoform of the Na^+/K^+-ATPase, is complex and still poorly understood. To elucidate the disease-specific pathophysiology, we examined a mouse model harboring the mutation D801Y, which was originally found in a patient with Rapid onset Dystonia Parkinsonism, but recently, also in a patient with Alternating Hemiplegia of Childhood. We found that this model exhibited motor deficits and developed dystonia when exposed to a drop in body temperature. Cerebellar *in vivo* recordings in awake mice revealed irregular firing of Purkinje cells and their synaptic targets, the deep cerebellar nuclei neurons, which was further exacerbated and evolved into abnormal high-frequency burst firing during dystonia. The development of specific neurological features within the *ATP1A3* mutation spectrum, such as dystonia, are thought to reflect the functional consequences of each mutation, thus to investigate the consequence of the D801Y mutations we characterized mutated D-to-Y Na^+/K^+-ATPases expressed in *Xenopus* oocytes. These *in vitro* studies showed that the D-to-Y mutation abolishes pump-mediated Na^+/K^+ exchange, but still allows the pumps to bind Na^+ and become phosphorylated, trapping them in conformations that instead support proton influx.

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Academic appointments
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Publications (selected)
*Isaksen, Kros, Vedovato, Holm, Gadsby, Khodakhah and **Lykke-Hartmann,** *Hypothermia-indiced dystonia in an RDP mouse model.*
2017. PLOS Genetics, May 4;13(5):e1006763. doi: 10.1371/journal.pgen.1006763. [Epub ahead of print]
*Ernst, Grøndahl, Grund, Heuck, Sunde, Andersen, Villesen, **Lykke-Hartmann.**
"*Dormancy and activation of human oocytes: Molecular clues to oocyte regulation*". **2017**. Human Reproduction, accepted
* Siupka, Hersom, **Lykke-Hartmann**, Johnsen, Thomsen, Andresen, Moos, Abbott, Brodin, Nielsen *Bidirectional apical-basal traffic of the cation-independent mannose-6-phosphate receptor in brain endothelial cells.*
2017. The Journal of Cerebral Blood Flow & Metabolism, DOI: 10.1177/0271678X17700665, *in press*
* Carvacho, Lee, Ardestani, McGarvey, Fissore, **Lykke-Hartmann**
TRPM7-like channels are functionally expressed in oocytes and modulate post-fertilization embryo development in mouse".
2016. Scientific Reports **6**: 34236 (2016) doi:10.1038/srep34236
***** Holm, Isaksen, Glerup, Heuck, Bøttger, Fuchtbauer, Nedergaard, Nyengaard, Andreasen, Nissen and **Lykke-Hartmann.** *Cognitive deficits caused by a disease-mutation in the α3 Na+/K+-ATPase isoform*
2016. Scientific Reports. *6. doi:10.1038/srep31972*
*Bøttger, Glerup, Gesslein, Illarianova, Isaksen, Heuck, Clausen, Füchtbauer, Gramsbergen, Gunnarson, Aperia, Lauritzen, Lambertsen, Nissen and **Lykke-Hartmann**.
"*Co-morbid Psychiatric Manifestations Couple to Glutamate Defects in a FHM2-mutation Mouse Model*".
2016. Scientific Reports, 6:22047
*Hilbers, Kopec, Isaksen, Holm, **Lykke-Hartmann**, Nissen, Khandelia, and Poulsen.
"*Tuning of the Na,K-ATPase by the beta subunit*"
2016. Scientific Reports. **6**, 20442; doi: 10.1038/srep20442.

Session 4-3

Study of Knock-in model mouse; Development of new treatments

Mohamad Mikati MD

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Alternating hemiplegia of childhood is a serious disorder that together with its related disorders is becoming increasingly recognized. Recent advances in animal models of AHC including the knock in mouse models of the two most common mutations have improved the understanding of the underlying pathophysiology of AHC and are suggesting possible therapeutic interventions and potential translational research. For example studies in the D801Y model showed improvement in cognitive deficits using clonazepam, in the E815K model showed that Flunarizine acutely decreased the number of hemiplegia and dystonia spells, but caused after its discontinuation no chronic deterioration or protection suggesting that the detrimental effects observed in some patients after Flunarizine discontinuation are due to withdrawal of its beneficial effects of the drug. Finally the D801N model is being used for screening of medications as related to the underlying physiology. In that model we have demonstrated increased excitability, increased predisposition to spreading depression, and decreased GABAergic inhibition as demonstrated in our lab. The Deepening understanding of the physiology is bound to continue to help in choosing future candidate drugs.

Mohamad A. Mikati M.D., is the Wilburt C. Davison Professor of Pediatrics, Professor of Neurobiology, and Chief of the Division of Pediatric Neurology. Dr. Mikati's clinical research has centered on characterization and therapy of pediatric epilepsy and neurology syndromes, describing several new pediatric neurological entities with two carrying his name (POSSUM syndromes # 3708 and 4468), developing novel therapeutic strategies for epilepsy and related disorders particularly Alternating Hemiplegia of Childhood, and applying cutting edge genetic and Magnetic Resonance Imaging techniques to drug resistant pediatric epilepsy. In the laboratory he has elucidated mechanisms of seizure related neuronal injury, particularly those related to the ceramide pathway, and demonstrated neuroprotective effects of several agents including erythropoietin. Most recently he has concentrated his laboratory research on the pathophysiology of ATP1A3 dysfunction in the brain as model for epilepsy and of Alternating Hemiplegia of Childhood.

He has more than 210 peer reviewed publications, 300 abstracts 40 chapters one book and two booklets. He also has more than 6,214 citations in the literature with an h-index of 46 and an i-10index of 127. Dr. Mikati has written chapters on epilepsy and related disorders in the major textbooks of Pediatrics and Pediatric Neurology including Swaiman's Pediatric Neurology and Nelson's Pediatrics.

Before joining Duke in 2008 he had completed his M.D. and Pediatric training at the American University of Beirut, his Neurology at the Massachusetts General Hospital, his Neurophysiology at Boston Children's Hospital and had been on the Faculty at Harvard as Director of Research in the Epilepsy Program at Boston Children's Hospital and then as Professor and Chairman, Department of Pediatrics, Founder and Director of the Adult and Pediatric Epilepsy Program at the American University of Beirut.

Dr. Mikati has had several international leadership roles including being President of the Union of the Middle Eastern and Mediterranean Pediatric Societies, on the Standing Committee of the International Pediatric Association, Officer of the International Child Neurology Association, Member of the Pediatric Content and Scientific Program Committees, Child Neurology Society International Affairs Committee Consultant to UNICEF, WHO, and the American Board of Pediatrics, and being one of only two Pediatric Neurologists, initially chosen worldwide, on the WHO advisory committee for the International Classification of Disease being the head of the neuromuscular group in that committee. He is also a co-founder of the International Alternating Hemiplegia of Childhood Research Consortium and Deputy Scientific Coordinator of the Consortium. He is also a member of the Planning Committee for the International ATP1A3 Disease Group. He has received several national and international honors including, among others, Merritt Putnam American Epilepsy Society Fellowship Award, Harvard Community Health Plan Peer Recognition Award, Debs Research Award, Hamdan Award for contributions to Medicine, Hans Zellweger Award for contributions to Pediatric Neurology, Patient Choice Award, American University of Beirut Alumni Achievement and Service Award, and the Michael Frank Award for research and lifetime contributions to the field of Pediatric Neurology. His name consistently appears in America's Top Doctors Series.

Mikat MA, authored and co-authored Alternating Hemiplegia of Childhood Publications

1. Masoud M, Lyndsey Prange L, CPNP, Wuchich J, Hunanyan A, **Mikati MA**. Diagnosis and Treatment of Alternating Hemiplegia of Childhood. Current Treatment Options in Neurology, 2017 Feb;19(2):8
2. Panagiotakaki E, De Grandis E, Stagnaro M, Heinzen EL, Fons C, Sisodiya S, de Vries B, Goubau C, Weckhuysen S, Kemlink D, Scheffer I, Lesca G, Rabilloud M, Klich A, Ramirez-Camacho A, Ulate-Campos A, Campistol J, Giannotta M, Moutard ML,Doummar D, Hubsch-Bonneaud C, Jaffer F, Cross H, Gurrieri F, Tiziano D, Nevsimalova S, Nicole S, Neville B, van den Maagdenberg AM, **Mikati M**, GoldsteinDB, Vavassori R, Arzimanoglou A; Italian IBAHC Consortium.; French AHC Consortium.; International AHC Consortium.. Clinical profile of patients withATP1A3 mutations in Alternating Hemiplegia of Childhood-a study of 155 patients. Orphanet J Rare Dis. 2015 Sep 26;10:123. doi: 10.1186/s13023-015-0335-5. PubMed PMID: 26410222; PubMed Central PMCID: PMC4583741.
3. Jaffer F, Avbersek A, Vavassori R, Fons C, Campistol J, Stagnaro M, De Grandis E, Veneselli E, Rosewich H, Gianotta M, Zucca C, Ragona F, Granata T, Nardocci N, **Mikati M**, Helseth AR, Boelman C, Minassian BA, Johns S, Garry SI, Scheffer IE, Gourfinkel-An I, Carrilho I, Aylett SE, Parton M, Hanna MG, Houlden H, Neville B, Kurian MA, Novy J, Sander JW, Lambiase PD, Behr ER, Schyns T, Arzimanoglou A, Cross JH, Kaski JP, Sisodiya SM. Faulty cardiac repolarization reserve in alternating hemiplegia of childhood broadens the phenotype. Brain. 2015 Oct;138(Pt 10):2859-74. doi: 10.1093/brain/awv243. PubMed PMID: 26297560; PubMed Central PMCID: PMC4671482.
4. Hunanyan AS, Fainberg NA, Linabarger M, Arehart E, Leonard AS, Adil SM, Helseth AR, Swearingen AK, Forbes SL, Rodriguiz RM, Rhodes T, Yao X, Kibbi N, Hochman DW, Wetsel WC, Hochgeschwender U, **Mikati MA**. Knock-in mouse model of alternating hemiplegia of childhood: behavioral and electrophysiologic characterization. Epilepsia. 2015 Jan;56(1):82-93. doi: 10.1111/epi.12878. PubMed PMID: 25523819.
5. Heinzen EL, Arzimanoglou A, Brashear A, Clapcote SJ, Gurrieri F, Goldstein DB, Jóhannesson SH, **Mikati MA**, Neville B, Nicole S, Ozelius LJ, Poulsen H, Schyns T, Sweadner KJ, van den Maagdenberg A, Vilsen B; ATP1A3 Working Group.. Distinct neurological disorders with ATP1A3 mutations. Lancet Neurol. 2014 May;13(5):503-14. doi: 10.1016/S1474-4422(14)70011-0. Review. PubMed PMID: 24739246; PubMed Central PMCID: PMC4238309.
6. Kansagra S, **Mikati MA**, Vigevano F. Alternating hemiplegia of childhood. Handb Clin Neurol. 2013;112:821-6. doi: 10.1016/B978-0-444-52910-7.00001-5. Review. PubMed PMID: 23622289.
7. Heinzen EL, Swoboda KJ, Hitomi Y, Gurrieri F, Nicole S, de Vries B, Tiziano FD, Fontaine B, Walley NM, Heavin S, Panagiotakaki E; European Alternating Hemiplegia of Childhood (AHC) Genetics Consortium.; Biobanca e Registro Clinico per l'Emiplegia Alternante (I.B.AHC) Consortium.; European Network for Research on Alternating Hemiplegia (ENRAH) for Small and Medium-sized Enterpriese (SMEs)Consortium., Fiori S, Abiusi E, Di Pietro L, Sweney MT, Newcomb TM, Viollet L, Huff C, Jorde LB, Reyna SP, Murphy KJ, Shianna KV, Gumbs CE, Little L, Silver K, Ptáček LJ, Haan J, Ferrari MD, Bye AM, Herkes GK, Whitelaw CM, Webb D, Lynch BJ, Uldall P, King MD, Scheffer IE, Neri G, Arzimanoglou A, van den Maagdenberg AM,Sisodiya SM, **Mikati MA**, Goldstein DB. De novo mutations in ATP1A3 cause alternating

hemiplegia of childhood. Nat Genet. 2012 Sep;44(9):1030-4. doi: 10.1038/ng.2358. PubMed PMID: 22842232; PubMed Central PMCID: PMC3442240.
8. **Mikati MA**, Kramer U, Zupanc ML, Shanahan RJ. Alternating hemiplegia of childhood: clinical manifestations and long-term outcome. Pediatr Neurol. 2000 Aug;23(2):134-41. PubMed PMID: 11020638.
9. **Mikati M**. Alternating hemiplegia of childhood. Pediatr Neurol. 1999 Oct;21(4):764. PubMed PMID: 10580894.
10. Dangond F, Garada B, Murawski BJ, Rey-Casserly C, Holman BL, **Mikati MA**. Focal brain dysfunction in a 41-year old man with familial alternating hemiplegia. Eur Arch Psychiatry Clin Neurosci. 1997;247(1):35-41. Review. PubMed PMID: 9088804.
11. **Mikati MA**, O'Tuama L, Dangond F. Autosomal Dominant Alternating Hemiplegia of Childhood. In: Alternating Hemiplegia of Childhood. Eds: Aicardi J, Andermann F. New York: Raven. 1995:125-34.
12. **Mikati MA**, Fischman A. PET Scan Findings in Alternating Hemiplegia of Childhood. In: Alternating Hemiplegia of Childhood. Eds: Aicardi J, Andermann F. New York: Raven. 1995:109-14.
13. **Mikati MA**, Maguire H, Barlow CF, Ozelius L, Breakefield XO, Klauck SM, Korf B, O'Tuama SL, Dangond F. A syndrome of autosomal dominant alternating hemiplegia: clinical presentation mimicking intractable epilepsy; chromosomal studies; and physiologic investigations. Neurology. 1992 Dec;42(12):2251-7. PubMed PMID: 1361034.

Session 5-1

Treatment with adenosine- 5'- triphosphate for AHC

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The primary treatment for AHC has been anti-epileptic drugs (AEDs) and while none of these have been reported effective on non-epileptic paroxysmal attacks, some reports indicate their success as prophylactics. Flunarizine, a non-selective calcium ion channel, is commonly used in long term treatment. It has been reported to be effective in reducing the frequency and intensity of plegic attacks. There is still no gold standard treatment for patients with AHC,

The principal cause of AHC is an abnormality of ATPase pump which is caused by mutation in the *ATP1A3* gene. ATPase pump is the catalytic component of the active enzyme, which catalyzes the hydrolysis of Adenosine-5'-triphosphate (ATP) coupled with the exchange of sodium and potassium ions across the plasma membrane. This action establishes and maintains the electrochemical gradients of sodium and potassium ions. These gradients are essential for osmoregulation, the sodium-coupled transport of a variety of organic and inorganic molecules, and the electrical excitability of nerves and muscles. Some of AHC causing mutations has been reported to reduce ATP pump activity. Therefore, this function of ATPase pump supports the hypothesis that supplementation of ATP may improve the dysfunction of Na+/K+ ATPase to alleviate symptoms.

In an innovative therapy done with the approval of the IRB commission, a 7-year-old boy with a de novo splicing mutation (c. 2542+1G>A) in ATP1A3 was treated for two years with oral ATP supplements. Therapy outcome was evaluated through the follow-up of improvement of hemiplegic episodes and psychomotor development. Side-effects were regularly monitored to insure patient safety. With the dosage of ATP administration increased, he showed significantly less frequency and shorter duration of hemiplegic episodes. Treatment with ATP was correlated with a marked amelioration of alternating hemiplegia of childhood episodes, and psychomotor development has improved. The maximum dose of oral administration of ATP reached 25 mg/kg per day. ATP therapy was well tolerated without complaint of discomfort and side effects. The 2-year follow-up outcome of ATP therapy for alternating hemiplegia of childhood was successful. This therapy is now considered one of the most useful treatments for AHC.

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Education

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2007 M.D. Fukuoka University, School of Medicine
2013 Ph.D. Fukuoka University, School of Medicine

Clinical training

Residencies:
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Fellowships:
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4/2010-3/2011 Clinical Fellow in Pediatrics, Kyusyu Kosei Nenkin Hospital
4/2011-8/2013 Clinical Fellow in Pediatrics, Fukuoka University Hospital

Academic appointments

7/2001-3/2007 Undergraduate research in molecular pathology, Pediatrics, Fukuoka University
4/2007-3/2010 Graduate research in the Research Center for the Molecular Pathomechanisms of Epilepsy, Fukuoka University
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4/2013-8/2013 Research Fellow in Central Research Institute for the Molecular Pathomechanisms of Epilepsy, Fukuoka University
9/2013-8/2014 Postdoctoral Scholar in Center for Human Genome Variation, School of Medicine, Duke University
9/2014-2/2016 Postdoctoral Scholar in University of Arizona Genetics Core, University of Arizona
4/2016-3/2017 Chief physician in Faculty of Pediatrics, Miyakonojo Medical Association Hospital
4/2017-Present Assistant Professor in Department of Pediatrics, Fukuoka University

Publications

1. **Ishii A.**, Watkins JC., Chen D., Hirose S., Hammer MF.. Clinical implications of SCN1A missense and truncation variants in a large Japanese cohort with Dravet syndrome. Epilepsia. 2016 Dec 1.
2. **Ishii A**, Kang JQ., Schornak CC., Hernandez CC., Shen W., Watkins JC., Macdonald RL., Hirose S.. A de novo missense mutation of GABRB2 causes early myoclonic encephalopathy. Journal of Medical Genetics. 2016 Oct 27:jmedgenet-2016.
3. Yamamoto T., Shimojima K., Sangu N., Komoike Y., **Ishii A**, Abe S., Yamashita S., Imai K., Kubota T., Fukasawa T., Okanishi T., Enoki H., Tanabe T., Saito A., Furukawa T., Shimizu T., Milligan C. J.,

Petrou S., Heron S. E., Dibbens L. M., Hirose S., Okumura A. Single Nucleotide Variations in CLCN6 Identified in Patients with Benign Partial Epilepsies in Infancy and/or Febrile Seizures. PLoS One. 2015;10(3):e0118946.
4. Saitoh M., Shinohara M., **Ishii A**, Ihara Y., Hirose S., Shiomi M., Kawawaki H., Kubota M., Yamagata T., Miyamoto A., Yamanaka G., Amemiya K., Kikuchi K., Kamei A., Akasaka M., Anzai Y., Mizuguchi M. Clinical and genetic features of acute encephalopathy in children taking theophylline. Brain Dev. 2015;37(5):463-470.
5. Kouga T., Shimbo H., Iai M., Yamashita S., **Ishii A**, Ihara Y., Hirose S., Yamakawa K., Osaka H. Effect of CYP2C19 polymorphisms on stiripentol administration in Japanese cases of Dravet syndrome. Brain Dev. 2015;37(2):243-249.
6. Sasaki M*., **Ishii A** *., Saito Y., Hirose S. Intermediate form between alternating hemiplegia of childhood and rapid-onset dystonia-parkinsonism. Mov Disord. 2014;29(1):153-154. *equal contribution
7. Sasaki M*., **Ishii A** *., Saito Y., Morisada N., Iijima K., Takada S., Araki A., Tanabe Y., Arai H., Yamashita S., Ohashi T., Oda Y., Ichiseki H., Hirabayashi S., Yasuhara A., Kawawaki H., Kimura S., Shimono M., Narumiya S., Suzuki M., Yoshida T., Oyazato Y., Tsuneishi S., Ozasa S., Yokochi K., Dejima S., Akiyama T., Kishi N., Kira R., Ikeda T., Oguni H., Zhang B., Tsuji S., Hirose S. Genotype-phenotype correlations in alternating hemiplegia of childhood. Neurology. 2014;82(6):482-490. * equal contribution
8. Maeda T., Shimizu M., Sekiguchi K., **Ishii A**., Ihara Y., Hirose S., Izumi T. Exacerbation of benign familial neonatal epilepsy induced by massive doses of phenobarbital and midazolam. Pediatr Neurol. 2014;51(2):259-261.
9. Jingami N., Matsumoto R., Ito H., **Ishii A**., Ihara Y., Hirose S., Ikeda A., Takahashi R. A novel SCN1A mutation in a cytoplasmic loop in intractable juvenile myoclonic epilepsy without febrile seizures. Epileptic Disord. 2014;16(2):227-231.
10. **Ishii A**., Kanaumi T., Sohda M., Misumi Y., Zhang B., Kakinuma N., Haga Y., Watanabe K., Takeda S., Okada M., Ueno S., Kaneko S., Takashima S., Hirose S. Association of nonsense mutation in GABRG2 with abnormal trafficking of GABAA receptors in severe epilepsy. Epilepsy Res. 2014;108(3):420-432.
11. Inoue S., **Ishii A**., Shirotani G., Tsutsumi M., Ohta E., Nakamura M., Mori T., Inoue T., Nishimura G., Ogawa A., Hirose S. Case of Desbuquois dysplasia type 1: potentially lethal skeletal dysplasia. Pediatr Int. 2014;56(4):e26-29.
12. Heinzen E. L., Arzimanoglou A., Brashear A., Clapcote S. J., Gurrieri F., Goldstein D. B., Johannesson S. H., Mikati M. A., Neville B., Nicole S., Ozelius L. J., Poulsen H., Schyns T., Sweadner K. J., van den Maagdenberg A., Vilsen B.,.. **Ishii A**., et al ., for the A. T. P. A. Working Group. Distinct neurological disorders with ATP1A3 mutations. Lancet Neurol. 2014;13(5):503-514.
13. Fukasawa T., Kubota T., Negoro T., Saitoh M., Mizuguchi M., Ihara Y., **Ishii A**., Hirose S. A case of recurrent encephalopathy with SCN2A missense mutation. Brain Dev. 2014;10.1016/j.braindev.2014.10.001.
14. **Ishii A**., Yoshimura K., Ideguchi H., Hirose S. A Pancreatic Solid Pseudo-Papillary Tumor Detected After Abdominal Injury. Gastroenterology Research. 2013;6(2):67-70.
15. **Ishii A**., Yasumoto S., Ihara Y., Inoue T., Fujita T., Nakamura N., Ohfu M., Yamashita Y., Takatsuka H., Taga T., Miyata R., Ito M., Tsuchiya H., Matsuoka T., Kitao T., Murakami K., Lee W. T., Kaneko

S., Hirose S. Genetic analysis of PRRT2 for benign infantile epilepsy, infantile convulsions with choreoathetosis syndrome, and benign convulsions with mild gastroenteritis. Brain Dev. 2013;35(6):524-530.
16. **Ishii A**., Shioda M., Okumura A., Kidokoro H., Sakauchi M., Shimada S., Shimizu T., Osawa M., Hirose S., Yamamoto T. A recurrent KCNT1 mutation in two sporadic cases with malignant migrating partial seizures in infancy. Gene. 2013;531(2):467-471.
17. **Ishii A**., Saito Y., Mitsui J., Ishiura H., Yoshimura J., Arai H., Yamashita S., Kimura S., Oguni H., Morishita S., Tsuji S., Sasaki M., Hirose S. Identification of ATP1A3 mutations by exome sequencing as the cause of alternating hemiplegia of childhood in Japanese patients. PLOS ONE. 2013;8(2):e56120.
18. Inoue T., Hattori K., Ihara K., **Ishii A**., Nakamura K., Hirose S. Newborn screening for Fabry disease in Japan: prevalence and genotypes of Fabry disease in a pilot study. J Hum Genet. 2013;58(8):548-552.
19. Wang J. W., Shi X. Y., Kurahashi H., Hwang S. K., **Ishii A**., Higurashi N., Kaneko S., Hirose S., Epilepsy Genetic Study Group Japan. Prevalence of SCN1A mutations in children with suspected Dravet syndrome and intractable childhood epilepsy. Epilepsy Res. 2012;102(3):195-200.
20. Shi X., Wang J., Kurahashi H., **Ishii A**., Higurashi N., Kaneko S., Hirose S. On the likelihood of SCN1A microdeletions or duplications in Dravet syndrome with missense mutation. Brain Dev. 2012;34(8):617-619.
21. **Ishii A**., Miyajima T., Kurahashi H., Wang J. W., Yasumoto S., Kaneko S., Hirose S. KCNQ2 abnormality in BECTS: Benign childhood epilepsy with centrotemporal spikes following benign neonatal seizures resulting from a mutation of KCNQ2. Epilepsy Res. 2012;102(1-2):122-125.
22. Shi X., Huang M. C., **Ishii A**., Yoshida S., Okada M., Morita K., Nagafuji H., Yasumoto S., Kaneko S., Kojima T., Hirose S. Mutational analysis of GABRG2 in a Japanese cohort with childhood epilepsies. J Hum Genet. 2010;55(6):375-378.
23. **Ishii A**., Zhang B., Kaneko S., Hirose S. Positive association between benign familial infantile convulsions and LGI4. Brain Dev. 2010;32(7):538-543.
24. Sugiura Y., Nakatsu F., Hiroyasu K., **Ishii A**., Hirose S., Okada M., Jibiki I., Ohno H., Kaneko S., Ugawa Y. Lack of potassium current in W309R mutant KCNQ3 channel causing benign familial neonatal convulsions (BFNC). Epilepsy Res. 2009;84(1):82- 85.
25. Kurahashi H., Wang J. W., **Ishii A**., Kojima T., Wakai S., Kizawa T., Fujimoto Y., Kikkawa K., Yoshimura K., Inoue T., Yasumoto S., Ogawa A., Kaneko S., Hirose S. Deletions involving both KCNQ2 and CHRNA4 present with benign familial neonatal seizures. Neurology. 2009;73(15):1214-1217.
26. **Ishii A**., Fukuma G., Uehara A., Miyajima T., Makita Y., Hamachi A., Yasukochi M., Inoue T., Yasumoto S., Okada M., Kaneko S., Mitsudome A., Hirose S. A de novo KCNQ2 mutation detected in non-familial benign neonatal convulsions. Brain Dev. 2009;31(1):27-33.
27. Wang J. W., Kurahashi H., **Ishii A**., Kojima T., Ohfu M., Inoue T., Ogawa A., Yasumoto S., Oguni H., Kure S., Fujii T., Ito M., Okuno T., Shirasaka Y., Natsume J., Hasegawa A., Konagaya A., Kaneko S., Hirose S. Microchromosomal involving SCN1A and adjacent genes in severe myoclonic epilepsy in infancy. Epilepsia. 2008;49(9):1528-1534.

Session 5-2

Loss of function *ATP1A3* mutations differentiated by pre-steady-state analysis

Steven Petrou, Florey Institute of Neuroscience and Mental Health, Australia

Melody Li[1, 3], Ian C. Forster[1, 3], K Melodi McSweeney[2], Erin L Heinzen[2], David B Goldstein[2], **Steven Petrou**[1]

[1] Florey Institute of Neuroscience and Mental Health, Australia

[2] Institute for Genomic Medicine, Columbia University Medical Center, United States

[3] Equal contribution

Mutations in *ATP1A3* have been increasingly implicated in various neurological disorders. So far, loss of forward cycling function of the Na^+/K^+ ATPase is a common pathological feature of *ATP1A3* mutants. However, there are no clinically available compounds that enhance the function of the Na^+/K^+ ATPase. We aimed to elucidate effects of pathological *ATP1A3* mutations on the structure-function relationships to aid discovery of mechanism based therapeutics. The biophysical properties of two mutants Q140H and E818K were compared to the human wildtype (WT) α3 subunit by expression in *X.laevis* oocytes and recording using two-electrode voltage clamp. The voltage dependence of the forward cycling rate was significantly altered for the E818K mutant. Analysis of the pre-steady-state current relaxations, that reflect charge displacements associated with cation binding/debinding, revealed previously undocumented functional features. We observed a significant hyperpolarizing shift in the voltage dependence of displacement charge for E818K compared with WT. In contrast, whereas the voltage dependence of Q140H was similar to WT, the rate of current relaxation was significantly slower as compared to the WT or E818K. Pre-steady-state relaxation analysis can offer novel insights into how sodium ion translocation may be distinctly altered in mutants associated with different *ATP1A3* disorders. Furthermore, it provides a robust readout that complements the steady-state pump activity and may reveal novel targeted therapeutic mechanisms.

Professor Steven Petrou PhD FAHMS

Academic Qualifications

1984 B.Sc. Hons University of Melbourne

1992 Ph.D. University of Melbourne

Postdoctoral Training

1992-1994 Samuel A. Levine Research Fellow of the American Heart Association. Department of Physiology, University of Massachusetts Medical School, USA

1995 Research Fellow. Worcester Foundation of Experimental Biology, Massachusetts, USA

Academic Positions

1998-1999 National Heart Research Fellow. Department of Physiology, University of Melbourne

2000-2001 Research Fellow. Department of Physiology, University of Melbourne

2001 Research Fellow. Max Planck Institute for Experimental Medicine and Research, Heidelberg, Germany

2001 Senior Research Fellow. Department of Physiology,

2008 Associate Professor, The University of Melbourne

2002-2009 Head of the Laboratory of Ion Channels and Human Disease, HFI

2009-2011 Associate Director, Florey Neuroscience Institute

2011-2015 NHMRC Research Fellow

2013 Appointed to Department of Electrical Engineering, The University of Melbourne

2014 Professor, The University of Melbourne

2015 Professor of Translational Neuroscience (continuing), Department of Medicine, The University of Melbourne

Representative Publications

1. Oliver KL, Franceschetti S, Milligan CJ, Muona M, Mandelstam SA, Canafoglia L, Boguszewska-Chachulska AM, Korczyn AD, Bisulli F, Di Bonaventura C, Ragona F, Michelucci R, Ben-Zeev B, Straussberg R, Panzica F, Massano J, Friedman D, Crespel A, Engelsen BA, Andermann F, Andermann E, Spodar K, Lasek-Bal A, Riguzzi P, Pasini E, Tinuper P, Licchetta L, Gardella E, Lindenau M, Wulf A, Møller RS, Benninger F, Afawi Z, Rubboli G, Reid CA, Maljevic S, Lerche H, Lehesjoki AE, **Petrou S**, Berkovic SF. Myoclonus epilepsy and ataxia due to KCNC1 mutation: Analysis of 20 cases and K(+) channel properties. Ann Neurol. 2017 May;81(5):677-689.

2. Sadovnick AD, Gu BJ, Traboulsee AL, Bernales CQ, Encarnacion M, Yee IM, Criscuoli MG, Huang X, Ou A, Milligan CJ, **Petrou S**, Wiley JS, Vilariño-Güell C. Purinergic receptors P2RX4 and P2RX7 in familial multiple sclerosis. Hum Mutat. 2017 Jun;38(6):736-744.

3. EuroEPINOMICS-RES Consortium. Electronic address: euroepinomics-RES@ua.ac.be; Epilepsy Phenome/Genome Project; Epi4K Consortium; EuroEPINOMICS-RES Consortium. De Novo Mutations in Synaptic Transmission Genes Including DNM1 Cause Epileptic Encephalopathies. Am J Hum Genet. 2017 Jan 5;100(1):179.

4. Mark A Corbett, Susannah T Bellows, Melody Li, Renee Carroll, Silvana Micallef, Gemma L Carvill, Candace T Myers, Katherine B Howell, Snezana Maljevic, Holger Lerche, Elena V Gazina, Heather C Mefford, Melanie Bahlo, Samuel F. Berkovic, **Steven Petrou**, Ingrid E Scheffer, and Jozef Gécz. Dominant KCNA2 mutation causes episodic ataxia and pharmacoresponsive epilepsy. Accepted, Neurology.
5. Petty SJ, Milligan CJ, Todaro M, Richards KL, Kularathna PK, Pagel CN, French CR, Hill-Yardin EL, O'Brien TJ, Wark JD, Mackie EJ, **Petrou S**. The antiepileptic medications carbamazepine and phenytoin inhibit native sodium currents in murine osteoblasts. Epilepsia. 2016 Jul 21.
6. Epi4K Consortium. De Novo Mutations in SLC1A2 and CACNA1A Are Important Causes of Epileptic Encephalopathies. Am J Hum Genet. 2016 Aug 4;99(2):287-98.
7. Howden SE, McColl B, Glaser A, Vadolas J, **Petrou S**, Little MH, Elefanty AG, Stanley EG. A Cas9 Variant for Efficient Generation of Indel-Free Knockin or Gene-Corrected Human Pluripotent Stem Cells. Stem Cell Reports. 2016 Aug 3. pii: S2213-6711(16)30106-0.
8. Petrovski S, Shashi V, **Petrou S**, Schoch K, McSweeney KM, Dhindsa RS, Krueger B, Crimian R, Case LE, Khalid R, El-Dairi MA, Jiang YH, Mikati MA, Goldstein DB. Exome sequencing results in successful riboflavin treatment of a rapidly progressive neurological condition. Cold Spring Harb Mol Case Stud. 2015 Oct;1(1):a000257.
9. Hildebrand MS, Myers CT, Carvill GL, Regan BM, Damiano JA, Mullen SA, Newton MR, Nair U, Gazina EV, Milligan CJ, Reid CA, **Petrou S**, Scheffer IE, Berkovic SF, Mefford HC. A targeted resequencing gene panel for focal epilepsy. Neurology. 2016 Apr 26;86(17):1605-12.
10. Hildebrand MS, Phillips AM, Mullen SA, Adlard PA, Hardies K, Damiano JA, Wimmer V, Bellows ST, McMahon JM, Burgess R, Hendrickx R, Weckhuysen S, Suls A, De Jonghe P, Scheffer IE, **Petrou S**, Berkovic SF, Reid CA. Loss of synaptic Zn2+ transporter function increases risk of febrile seizures. Sci Rep. 2015 Dec 9;5:17816.
11. EpiPM Consortium. A roadmap for precision medicine in the epilepsies. Lancet Neurol. 2015 Dec;14(12):1219-28.
12. Mikati MA, Jiang YH, Carboni M, Shashi V, Petrovski S, Spillmann R, Milligan CJ, Li M, Grefe A, McConkie A, Berkovic S, Scheffer I, Mullen S, Bonner M, **Petrou S**, Goldstein D. Quinidine in the treatment of KCNT1-positive epilepsies. Ann Neurol. 2015 Dec;78(6):995-9.
13. Hildebrand MS, Tankard R, Gazina EV, Damiano JA, Lawrence KM, Dahl HH, Regan BM, Shearer AE, Smith RJ, Marini C, Guerrini R, Labate A, Gambardella A, Tinuper P, Lichetta L, Baldassari S, Bisulli F, Pippucci T, Scheffer IE, Reid CA, **Petrou S**, Bahlo M, Berkovic SF. PRIMA1 mutation: a new cause of nocturnal frontal lobe epilepsy. Ann Clin Transl Neurol. 2015 Aug;2(8):821-30.
14. Gu BJ, Field J, Dutertre S, Ou A, Kilpatrick TJ, Lechner-Scott J, Scott R, Lea R, Taylor BV, Stankovich J, Butzkueven H, Gresle M, Laws SM, **Petrou S**, Hoffjan S, Akkad DA, Graham CA, Hawkins S, Glaser A, Bedri SK, Hillert J, Matute C, Antiguedad A; ANZgene Consortium, Wiley JS. A rare P2X7 variant Arg307Gln with absent pore formation function protects against neuroinflammation in multiple sclerosis. Hum Mol Genet. 2015 Oct 1;24(19):5644-54.
15. Epilepsy Phenome/Genome Project Epi4K Consortium. Copy number variant analysis from exome data in 349 patients with epileptic encephalopathy. Ann Neurol. 2015 Aug;78(2):323-8.

16. Carvill GL, McMahon JM, Schneider A, Zemel M, Myers CT, Saykally J, Nguyen J, Robbiano A, Zara F, Specchio N, Mecarelli O, Smith RL, Leventer RJ, Møller RS, Nikanorova M, Dimova P, Jordanova A, **Petrou S**; EuroEPINOMICS Rare Epilepsy Syndrome Myoclonic-Astatic Epilepsy & Dravet working group, Helbig I, Striano P, Weckhuysen S, Berkovic SF, Scheffer IE, Mefford HC. Mutations in the GABA Transporter SLC6A1 Cause Epilepsy with Myoclonic-Atonic Seizures. Am J Hum Genet. 2015 May 7;96(5):808-15.
17. Li M, Jazayeri D, Corry B, Melodi McSweeney K, Heinzen EL, Goldstein DB, **Petrou S**. A functional correlate of severity in alternating hemiplegia of childhood. Neurobiol Dis. 2015 Feb 12.
18. Gazina EV, Leaw BTW, Richards KL, Wimmer VC, Kim TH, Aumann TD, Featherby TJ, Churilov L, Hammond VE, Reid CA, **Petrou S**. Neonatal NaV 1.2 reduces neuronal excitability and affects seizure susceptibility and behavior. Human Mol Genetics 2015 March; 24:1457-68.
19. De Novo Mutations in Synaptic Transmission Genes Including DNM1 Cause Epileptic Encephalopathies. EuroEPINOMICS-RES Consortium. Am J Hum Genet. 2014 Oct 2;95(4):360-70.
20. Kim TH, Reid CA, **Petrou S**. Oxcarbazepine and its active metabolite, (S)-licarbazepine, exacerbate seizures in a mouse model of genetic generalised epilepsy. Epilepsia 2015 Jan; 56(1): e6-e9.
21. Muona M, Berkovic SF, Dibbens LM, Oliver KL, Maljevic S, Bayly MA, Joensuu T, Canafoglia L, Franceschetti S, Michelucci R, Markkinen S, Heron SE, Hildebrand M, Andermann E, Andermann F, Gambardella A, Tinuper P, Licchetta L, Scheffer IE, Criscuolo C, Filla A, Ferlazzo E, Ahmad J, Ahmad A, Baykan B, Said E, Topcu M, Riguzzi P, King MD, Ozkara C, Andrade DM, Engelsen BA, Crespel A, Lindenau M, Lohmann E, Saletti V, Massano J, Privitera M, Espay AJ, Kauffmann B, Duchowny M, Møller RS, Straussberg R, Afawi Z, Ben- Zeev B, Samocha KE, Daly MJ, **Petrou S**, Lerche H, Palotie A, Lehesjoki AE. A recurrent de novo mutation in KCNC1 is a major cause of progressive myoclonus epilepsy. Nature Genetics 2015 Jan; 47 (1): 39-46.
22. Wimmer VC, Li MYS, Berkovic SF and **Petrou S**. Cortical microarchitecture changes in genetic epilepsy. Neurology, Accepted 2015
23. Milligan CJ, Li MYS, Gazina EV, Heron SE, Nair U, Trager C, Reid CA, Venkat A, Younkin DP, Dlugos DJ, Petrovski S, Goldstein DB, Dibbens LM, Scheffer IE, Berkovic SF, **Petrou S**. KCNT1 Gain of Function in Two Epilepsy Phenotypes Is Reversed by Quinidine. Annals of Neurology 2014 Accepted
24. Reid CA, Leaw B, Richards KL, Richardson R, Wimmer VC, Yu C, Hill-Yardin EL, Lerche H, Scheffer IE, Berkovic SF, **Petrou S**. Reduced dendritic arborisation and hyperexcitability of pyramidal neurons in a Scn1b-based model of Dravet syndrome. Brain 2014 Accepted
25. Carvill GL, Weckhuysen S, McMahon JM, Hartmann C, Møller RS, Hjalgrim H, Cook J, Geraghty E, O'Roak BJ, **Petrou S**, Clarke A, Gill D, Sadleir LG, Muhle H, von Spiczak S, Nikanorova M, Hodgson BL, Gazina EV, Suls A, Shendure J, Dibbens LM, De Jonghe P, Helbig I, Berkovic SF, Scheffer IE, Mefford HC.GABRA1 and STXBP1: Novel genetic causes of Dravet syndrome. Neurology. 2014 Mar 12.
26. Goldstein DB, Allen A, Keebler J, Margulies EH, **Petrou S**, Petrovski S, Sunyaev S. Sequence-wide studies in human genetics: design and interpretation. Nature Reviews Genetics 2013 Jul;14(7):460-70
27. Richards KL, Kurniawan ND, Yang Z, Kim TH, Keller MD, Low JWJ, Ullmann JFP, Cole S, Foong S, Galloway GJ, Reid CA, Paxinos G, Reutens DC, **Petrou S**. Hippocampal volume and cell density changes in a mouse model of human genetic epilepsy. Accepted. Neurology. 2013 Mar 26;80(13):1240-6

28. Reid CA, Kim TH, Phillips AM, Low JWJ, Berkovic SF, Luscher B, **Petrou S**. Multiple molecular mechanisms for a single GABAA mutation in epilepsy. Accepted. Neurology 2013 Mar 12;80(11):1003-8
29. Heron SE, Smith KR, Bahlo M, Nobili L, Kahana E, Licchetta L, Oliver KL, Mazarib A, Afawi A, Korczyn A, Plazzi G, **Petrou S**, Berkovic SF, Scheffer IE, Dibbens LM. Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy. Accepted Nature Genetics. 2012 Nov;44(11):1188-90
30. Oliva M, Berkovic SF, **Petrou S**. Sodium Channels and the Neurobiology of Epilepsy. Epilepsia. 2012 Nov;53(11):1849-59.
31. Arsov T, Mullen SA, Rogers S, Phillips AM, Lawrence KM, Damiano JA, Goldberg-Stern H, Afawi Z, Kivity S, Trager C, **Petrou S**, Berkovic SF, Scheffer IE. GLUT1-deficiency in the idiopathic generalised epilepsies. Annals of Neurology. 2012 Nov;72(5):807-15.
32. Liao Y, Anttonen AK, Liukkonen E, Gaily E, Maljevic S, Schubert S, Bellan-Koch A, **Petrou S**, Ahonen VE, Lerche H, Lehesjoki AE. SCN2A mutation associated with neonatal epilepsy, late-onset episodic ataxia, myoclonus, and pain. Neurology. 2010 Oct 19;75(16):1454-8.
33. Dibbens LM, Reid CA, Hodgson B, Thomas EA, Phillips AM, Gazina E, Cromer BA, Clarke AL, Baram TZ, Scheffer IE, Berkovic SF, **Petrou S**. Augmented currents of an HCN2 variant in patients with febrile seizure syndromes. Ann Neurol. 2010 Apr;67(4):542-6.
34. Liao Y, Deprez L, Maljevic S, Pitsch J, Claes L, Hristova D, Jordanova A, Ala-Mello S, Bellan-Koch A, Blazevic D, Schubert S, Thomas EA, **Petrou S**, Becker AJ, De Jonghe P, Lerche H. Molecular correlates of age-dependent seizures in an inherited neonatal-infantile epilepsy. Brain. 2010 May;133(Pt 5):1403-14.
35. Thomas EA, Hawkins RJ, Richards KL, Xu R, Gazina EV, **Petrou S**. Heat opens axon initial segment sodium channels: a febrile seizure mechanism? Ann Neurol. 2009 Aug;66(2):219-26.
36. Chiu C, Reid CA, Tan HO, Davies PJ, Single FN, Koukoulas I, Berkovic SF, Tan SS, Sprengel R, Jones MV, **Petrou S**. Developmental impact of a familial GABAA receptor epilepsy mutation. Ann Neurol. 2008 Sep;64(3):284-93.
37. Gunnersen JM, Kim MH, Fuller SJ, De Silva M, Britto JM, Hammond VE, Davies PJ, **Petrou S**, Faber ES, Sah P, Tan SS. Sez-6 proteins affect dendritic arborization patterns and excitability of cortical pyramidal neurons. Neuron. 2007 Nov 21;56(4):621-39.
38. Scheffer IE, Harkin LA, Grinton BE, Dibbens LM, Turner SJ, Zielinski MA, Xu R, Jackson G, Adams J, Connellan M, **Petrou S**, Wellard RM, Briellmann RS, Wallace RH, Mulley JC, Berkovic SF. Temporal lobe epilepsy and GEFS+ phenotypes associated with SCN1B mutations. Brain. 2007 Jan;130(Pt 1):100-9.
39. Berkovic SF, Mulley JC, Scheffer IE, **Petrou S**. Human epilepsies: interaction of genetic and acquired factors. Trends Neurosci. 2006 Jul;29(7):391-7.
40. Wallace RH, Marini C, **Petrou S**, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet. 2001 May;28(1):49-52.

Session 5-3

Modeling Alternating Hemiplegia of Childhood using Patient Derived Stem Cells

Kevin C. Ess, MD, PhD
Vanderbilt University Department of Pediatrics, Neurology, and Cell & Developmental Biology

Alternating Hemiplegia of Childhood (AHC) is an episodic neurological disorder that was recently discovered to be caused to mutations in the *ATP1A3* gene. *ATP1A3* encodes the neuronal specific α3 subunit of the sodium-potassium adenosine triphosphatase (Na,K-ATPase) pump. Patients with AHC present in early childhood, often with triggered unilateral episodes of weakness, unilateral nystagmus, seizures, dystonia, developmental delay, and ultimately intellectual disability. Throughout the world, most patients with AHC have heterozygous *ATP1A3* mutations with D801N, E815K, or G947R missense mutations being the most common. While animal models of AHC have been developed, the complexity of human neural development make mechanistic analyses difficult and laborious using rodent models. This is due to the possible involvement of multiple neuronal lineages such as GABAergic, glutamatergic, and dopaminergic neurons as well as overlapping patterns of expression of α1, α2, and α3 subunits and potential compensation in cells with *ATP1A3* mutations.

In collaboration with the laboratories of Drs. Kathy Swoboda and Al George, we have made numerous lines of induced pluripotent stem cells (iPSCs) from patients with AHC including those with known D801N, E815K, or G947R mutations in the *ATP1A3* gene. These lines have been extensively characterized including verification of pluripotency status, karyotype, and confirmation of mutations in the *ATP1A3* gene. We have also used these iPSCs to optimize neuronal differentiation protocols to various neuronal lineages and to also assess the expression of α1, α2, and α3 subunits using immunoblotting. Ongoing experiments using fluorescent dyes will determine relative concentrations of sodium and calcium as well as ion flux in neuronal cultures differentiated from AHC patient iPSCs. Finally, we are actively making isogenic iPSCs using CRISPR/Cas9 technology to either correcting *ATP1A3* mutations in AHC patient-derived iPSC lines or introduce *ATP1A3* mutations to existing control lines. This approach will allow us to control for genetic variations that may introduce experimental variability. However, the same genetic variations may also underlie variability in disease severity seen in individual patients and lead to the identification of modifier genes that can be exploited in the future as potential druggable targets.

This research was generously supported by the Alternating Hemiplegia of Childhood Foundation as well as the Association Française de l'Hémiplégie Alternante.

Ess, Kevin C., M.D., Ph.D

Gerald M. Fenichel Chair in Neurology; Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Cincinnati, Cincinnati, OH	B. M.	06/1989	Music Performance
Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, OH	Ph.D.	12/1996	Developmental Biology
University of Cincinnati, Cincinnati, OH	M.D.	06/1998	Medicine
University of Colorado, Denver, CO	Intern	06/1999	Pediatrics
St. Louis Children's Hospital/Washington University, St Louis, MO	Resident	06/2002	Child Neurology
St. Louis Children's Hospital/Washington University	Fellow	06/2004	Clinical Neurophysiology
Washington University, St Louis, MO	Postdoc	06/2005	Neuroscience Research

A. Personal Statement

I have dedicated my career to understanding the genetic control of brain development and how aberrations in developmental processes lead to epilepsy, and autism. For the past 11 years at Vanderbilt, my research has focused on tuberous sclerosis complex (TSC) as patients with this disease have prominent brain malformations, white matter disease and a very high prevalence of epilepsy and autism. Resulting from mutations in either the *TSC1* or *TSC2* genes, this disorder involves dysregulation of the mTOR kinase with striking signaling abnormalities of both the mTORC1 (increased) as well as the mTORC2 (decreased) signaling pathways. We have also been interested in a recently defined disorder, alternating hemiplegia of childhood (AHC), a devastating neurodevelopmental disorder due to mutations in the *ATP1A3* gene. To study abnormal developmental processes in TSC and AHC, we have utilized diverse model systems including transgenic mouse, zebrafish, and human induced pluripotent stem cells (iPSCs). Principally employing iPSCs, our basic and translational research approaches to TSC and AHC should culminate in advanced knowledge about pediatric neurological disorders and hopefully lead to the development of novel and more effective therapies.

B. Positions and Honors

Positions and Employment

2002 – 2006 Founder and Director, Tuberous Sclerosis Clinic, St. Louis Children's Hospital
2004 – 2006 Instructor, Department of Neurology, Washington University, St. Louis, MO
2006 – 2014 Assistant Professor, Department of Neurology, Vanderbilt University, Nashville, TN

2006 – 2014	Assistant Professor, Department of Pediatrics, Vanderbilt University, Nashville, TN
2006 – present	Investigator, Vanderbilt Kennedy Center for Research on Human Development.
2006 – present	Founder and Director, Tuberous Sclerosis Clinic, Vanderbilt Children's Hospital
2009 – 2014	Assistant Professor, Department of Cell and Developmental Biology, Vanderbilt University
2009 – present	Member, Vanderbilt Center for Stem Cell Biology, Nashville, TN
2011 – 2014	Assistant Professor, Department of Biological Sciences, Vanderbilt University
2014– present	Associate Professor, Pediatrics, Neurology, Cell & Dev. Biology, Biological Sciences
2002 – 2006	Founder and Director, AHC Clinic, Vanderbilt Children's Hospital

C. Contribution to Science

1. Animal Models of Tuberous Sclerosis Complex

My career focus was initiated during a post-doctoral fellowship in the lab of Dr. David Gutmann at Washington University in St. Louis. In my own lab, it has since encompassed the generation of various mouse as well as zebrafish models of TSC. We have tested various hypotheses about the contribution of specific lineages of the brain, that when deficient in either the *Tsc1* or *Tsc2* genes, may underlie neurologic disease.

Relevant peer-reviewed publications:

1. Kim, S. H., Speirs, C. K., Solnica-Krezel, L. and **Ess, KC.** Zebrafish model of tuberous sclerosis complex reveals cell-autonomous and non-cell-autonomous functions of mutant tuberin. *Disease Models Mechanisms* 4: 255-67 (2011). PMID: 20959633 PMCID: PMC3046101
2. Fu C, Cawthon C, Clinkscales W, Bruce A, Winzenburger P and **Ess, KC.** GABAergic Interneuron Development and Function is Modulated by the *Tsc1* Gene. *Cerebral Cortex* 22: 2111-2119 (2011). PMID: 22021912 PMCID: PMC3412444
3. Carson R, Van Nielen D, Winzenburger P and **Ess, KC.** Neuronal and glia abnormalities in *Tsc1*-deficient forebrain and partial rescue by rapamycin. *Neurobiology of Disease* 45: 369-80 (2012). PMID: 21907282 PMCID: PMC3225598
4. Robert Carson, Nathaniel Kelm, Kathryn West, Mark Does, Mark, Cary Fu, Grace Weaver, Eleanor McBrier, Mark Grier, and **Ess KC**, Hypomyelination following deletion of *Tsc2* in oligodendrocyte precursors". *Annals Clinical and Translational Neurology*. 58(3):440-52 (2015). PMID:25818646

2. Models of human diseases using induced pluripotent stem cells (iPSCs)

Over the past several years, the laboratory efforts have included generation and characterization of human iPSCs. These are typically made from patients at Vanderbilt who have neurological disorders with a known genetic etiology such as TSC and other mTOR related disorders.

Relevant peer-reviewed publications:

1. Neely MD, Litt M, Tidball A, Li G, Aboud A, Hopkins C, Chamberlin R, Hong C, **Ess, KC.**, and Bowman A. DMH1, a highly selective small molecule BMP inhibitor promotes neurogenesis of hiPCSs: Comparison of PAX6 and SOX1 Expression during Neural Induction. *ACS Chemical Neuroscience* 3:482-491 (2012) PMID: 22860217 PMCID: PMC3400384
2. Asad A Aboud; Andrew M Tidball; Kevin K Kumar; M D Neely; Bingying Han; **Ess, KC**; Charles C Hong; Keith M Erikson; Peter Hedera. PARK2 patient neuroprogenitors show increased mitochondrial sensitivity to copper. *Neurobiol Dis.* (2014) Oct 12;73C:204-212. doi: 10.1016/j.nbd.2014.10.002. PMID: 25315681
3. Thomas LR, Wang Q, Grieb BC, Phan J, Foshage AM, Sun Q, Olejniczak ET, Clark T, Dey S, Lorey S,

Alicie B, Howard GC, Cawthon B, **Ess KC**, Eischen CM, Zhao Z, Fesik SW, Tansey WP. Interaction with WDR5 Promotes Target Gene Recognition and Tumorigenesis by MYC. *Mol Cell*. (2015) Mar 25. pii: S1097-2765(15)00142-2. PMID:25818646

4. Tidball AM, Bryan MR, Uhouse MA, Kumar KK, Aboud AA, Feist JE, **Ess KC**, Neely MD, Aschner M, Bowman AB. A novel manganese-dependent ATM-p53 signaling pathway is selectively impaired in patient-based neuroprogenitor and murine striatal models of Huntington's disease. *Hum Mol Genet.* (2015) Apr 1;24(7):1929-44. PMID: 25489053

3. Non-TSC Models of human diseases

Our research focus on TSC has led to collaborations with other investigators as TSC/mTOR signaling pathway and the reagents we created (mouse, zebrafish, iPSCs) have broad connections to many fields of research and medicine.

Relevant peer-reviewed publications:

1. Aboud, A, Tidball, A, Kumar, K, Neely, D, **Ess KC**, Erikson, KM and Bowman, AB, "Genetic risk for Parkinson's disease correlates with alterations in neuronal manganese sensitivity between two human subjects" *Neurotoxicology* 33:1443-49 (2012). PMID: 23099318
2. Boglev,Y, Badrock, P, Trotter, A, Du,Q Richardson, E, Parslow, A, Markmiller, S, Hall, N, de Jong-Curtain, T, Ng, A, Verkade, H, Ober, E, Field, H, Shin, D, Shin, C, Hannan, K, Hannan, R, Pearson, R, Kim, S-H, **Ess, KC**, Lieschke, G, Stainier, D and Joan K. Heath, "Autophagy Induction is a Tor and Tp53-independent Cell Survival Response in a Zebrafish Model of Disrupted Ribosome Biogenesis". *PLoS Genetics*. doi: 10.1371/journal.pgen.1003279. (2013). PMID:23408911
3. Kim, SH, Scott, SA, Bennett, MJ, Carson, RP, Brown, HA, and **Ess, KC**. "Multi-organ Abnormalities and mTORC1 Activation in Zebrafish Model of Multiple Acyl-CoA Dehydrogenase Deficiency *PLoS Genetics* (2013 Jun;9(6) PMID: 23785301
4. Chun YW, Balikov DA, Feaster TK, Williams CH, Sheng CC, Lee JB, Boire TC, Neely MD, Bellan LM, **Ess KC**, Bowman AB, Sung HJ, Hong CC. Combinatorial polymer matrices enhance in vitro maturation of human induced pluripotent stem cell-derived cardiomyocytes. *Biomaterials*. 2015 Oct;67:52-64. PMID: 26204225.

Complete list of published work is in:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41636033/?sort=date&direction=ascending

Session 5-4

Modeling Alternating Hemiplegia of Childhood in Induced Pluripotent Stem Cells

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Alternating hemiplegia of childhood (AHC) is one notable phenotype among a heterogeneous spectrum of disorders associated with mutations in *ATP1A3*. We have investigated the electrophysiological properties of human neurons generated from AHC patient-specific induced pluripotent stem cells (iPSCs) to ascertain functional disturbances underlying this neurological disease. We will present data collected on neurons differentiated from two subjects with the common mutation G947R in which we succeeded in measuring ouabain-sensitive electrogenic outward current ('pump current'), resting membrane potential and evoked action potential firing behavior. We will also present data demonstrating the consequences of a splice site mutation (c.2542T>C) associated with AHC. Our findings are consistent with impaired ion transport function of neuronal Na^+/K^+-ATPase but highlight uncertainties regarding the importance of haploinsufficiency versus dominant-negative mechanisms. The iPSC-neuron model offers opportunities to investigate the pathogenesis of *ATP1A3*-associated disorders and serve as a cellular platform for testing therapeutic strategies.

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- **Present Position**

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1995-98 Associate Professor of Medicine and Pharmacology, Vanderbilt University
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- **Representative Publications**

George, A.L., Jr., Crackower, M.A., Abdalla, J.A., Hudson, A.J., and Ebers, G.C. Molecular basis of Thomsen's disease (autosomal dominant myotonia congenita). *Nature Genetics* 1993;3:305-310.

Bennett, P.B., Jr., Yazawa, K., Makita, N., and **George, A.L., Jr.** Molecular mechanism of an inherited cardiac arrhythmia. *Nature*, 1995;367:683-685.

Lossin, C., Wang, D.W., Rhodes, T.H., Vanoye, C.G., and **George, A.L., Jr.** Molecular basis for an inherited epilepsy. *Neuron*, 2002;34:877-884.

Crotti, L., Johnson, C.N., Graf, E., De Ferrari, G.M., Cuneo, B.F., Ovadia, M., Papagiannis, J., Feldkamp, M.D., Rathi, S.G., Kunic, J.D., Pedrazzini, M., Wieland, T., Lichtner, P., Beckmann, B., Clark, T., Shaffer, C., Benson, D.W., Kääb, S., Meitinger, T., Strom, T.M., Chazin, W.J., Schwartz, P.J., and **George, A.L., Jr.** Calmodulin mutations associated with recurrent cardiac arrest in infants. *Circulation*, 2013;127:1009-17.

Rosewich, H., Sweney, M., DeBrosse, S., Ess, K., Ozelius, L., Andermann, E., Andermann, F., Andrasco, G., Belgrade, A., Brashear, A., Ciccodicola, S., Egan, L., **George, A.L., Jr.**, Lewelt, A., Magelby, J., Merida, M.R., Newcomb, T., Platt, V., Poncelin, D., Reyna, S.P., Sasaki, M., Sotero de Menezes, M.A., Sweadner, K., Viollett, L., Zupanc, M.L., Silver, K., and Swoboda, K.J. Report from the 2014 International AHCF Workshop and Task Force: Consensus on a standardized approach to ATP1A3-related neurologic disorders. *Neurol Genet*, 2017;3:e139.

Huang, J., Vanoye, C.G., Cutts, A., Goldberg, P., Dib-Hajj, S.D., Cohen, C.J., Waxman, S.G. and **George, A.L., Jr.** Cellular mechanism underlying insensitivity to pain associated with Nav1.9 mutations. *J Clin Invest*, in press.

Poster Presentations

No.	1st Author	Title
P- 1	Y Nakamura	**A de novo p.Arg756Cys mutation in *ATP1A3* causes a distinctive phenotype with prolonged paralysis and involuntary movements triggered by fever**
P- 2	L Kremer	***De novo* mutations in *ATP1A3* as differential diagnosis for suspected mitochondrial disease patients**
P- 3	M Kubota	**A 20-year-old male with the right-dominant dystonia and bulbar palsy associated with *ATP1A3* gene mutation**
P- 4	M Soleimani	**De novo missense variants in *ATP1A3* with Alternating Hemiplegia of Childhood associated with childhood-onset schizophrenia: Report of 2 cases**
P- 5	F Gurrieri	**Unusual clinical presentation of familial hemiplegic attacks in two first cousins: possible oligogenic inheritance of AHC-related phenotypes?**
P- 6	F Nery	**Pathologic Findings in Alternating Hemiplegia of Childhood (AHC) Patients**
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P- 8	A Vilamala	**EEG Biofeedback for Relaxation using Deep Neural Networks**
P- 9	N Fedosova	**Application of EPR for description of the ouabain-binding site of Na,K-ATPase**
P-10	R Ragno	**Towards the construction of a Structure-based protocol to select potential ATP1A3 ligands through virtual screening procedures**
P-11	K Ikeda	**Perinatal seizure and defective respiratory rhythm generation in *Atp1a3* knockout homozygous knockout mice**
P-12	FD Tiziano	**Characterization of a human neuroblastoma model of AHC: progress report**
P-13	C Simmons	***ATP1A3* Splice Site Mutation Produces Haploinsufficiency in Alternating Hemiplegia of Childhood**
P-14	J Snow	**An iPSC-Derived Neuronal Model for Determining the Pathophysiology of *ATP1A3* Mutations in Alternating Hemiplegia of Childhood**
P-15	E Akkuratov	**Mice model of rapid-onset dystonia-parkinsonism**

P-1

A de novo p.Arg756Cys mutation in *ATP1A3* causes a distinctive phenotype with prolonged paralysis and involuntary movements triggered by fever

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Recently, p.Arg756Cys mutation in *ATP1A3* has been proven to cause a unique form of *ATP1A3*-related disorders, characterized by prolonged paralysis and involuntary movements triggered by febrile illness. Dard R, et al. expressed this phenotype using a term relapsing encephalopathy with cerebellar ataxia (RECA). We herein report an additional patient with a p.Arg756Cys mutation as the fourth case.

The patient is a 4-year-old boy, who has shown from 9 months of age recurrent paroxysmal prolonged motor functional deterioration triggered by febrile illness, followed by gradual recovering. He showed developmental delay, being able to control his head at 5 months, and sat unassisted at 10 months. He began to show movement disorders and ataxia at 1 year 3 months. Whole-exome sequencing detected a de novo p.Arg756Cys mutation.

The current patient shared the common core symptoms with the previously reported patients of RECA carrying a p.Arg756Cys mutation, confirming the distinctive clinical entity that results from the specific missense mutation.

P-2

De novo mutations in *ATP1A3* as differential diagnosis for suspected mitochondrial disease patients

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Mitochondrial disorders are the most common group of inborn errors of metabolism affecting 1 in 5,000 live births. They present with a large variety of symptoms and are generally suspected upon a severe disease course, often involving multiple tissues. For childhood-onset mitochondrial disease, most patients present with neurological symptoms. While metabolic markers as elevated lactate and biochemical findings like impairment of respiratory chain complex deficiencies can further strengthen the assumption of a mitochondrial disorder, these indicators are unspecific and differential diagnosis needs to be considered. WES on 540 suspected mitochondrial disease patients identified a *de novo* mutation in *ATP1A3* in two unrelated patients. Both patients presented with a strikingly similar phenotype including childhood-onset ataxia, epilepsy, dysarthria, hearing impairment and encephalopathic crisis upon febrile illness. While for childhood-onset mitochondrial disorders a recessive mode of inheritance is suspected, *de novo* mutations are often not investigated. The examples show that *ATP1A3*-related disease mimics mitochondrial disorders and are therefore likely to be missed in molecular diagnostics. It also shows that a screen for *de novo* mutations should always be considered in suspected mitochondrial disease patients.

P-3

A 20-year-old male with the right-dominant dystonia and bulbar palsy associated with *ATP1A3* gene mutation

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Rapid-onset dystonia-parkinsonism (RDP) is a rare autosomal dominant movement disorder with variable clinical course caused by a mutation of *ATP1A3* (Na+/K+ −ATPase α3 subunit gene). We here report the first Japanese case with RDP.

This right-handed 20-year-old male had noticed writing difficulty and right-sided facial myoclonus at 13 years of age. Later, distonic posture and twisting movement of right-sided extremities progressed gradually, resulted in gait disturbance. Because he received MR vaccination 1 month before the onset, he suspected of immune-mediated encephalitis, but IVIG therapy failed to recover. One and a half years later from onset, swallowing difficulty and dysarthria became apparent. Trihexyphenidyl, L-dopa, clonazepam, carbamazepine, and baclofen were not effective for his movement disorders. He showed rigidity in extremities, but no tremor. There was no spasticity or diurnal fluctuation of his dystonia. There was no abnormal findings in brain MRI and SPECT. There was no abnormality in CSF-HVA, 5-HIAA, biopterin and neopterin. After the introduction of botulinum toxin i.m. to right-sided extremities at 15 years of age, his symptoms were stabilized, although severe dystonic features remained. The next-generation sequencing revealed *de novo ATP1A3* mutation (c.2477C>T, p.Ala826Val).

Although RDP is characterized by sudden onset of asymmetrical dystonia and parkinsonism, our patient's symptoms progressed slowly and later stabilized. Currently he is engaged in light work with job support. As Brashear et al. (1996) already reported, the clinical course of RDP may be quite variable.

P-4

De novo missense variants in *ATP1A3* with Alternating Hemiplegia of Childhood associated with childhood-onset schizophrenia: Report of 2 cases

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Childhood-Onset Schizophrenia (COS) is a rare (prevalence 1/40 000) but major mental disorder characterized by a wide spectrum of symptoms, including delusions, hallucinations, disorganisation of speech, negative symptoms, and cognitive deficits. Several publications have shown that some polymorphisms are associated with COS. Identification of rare variants is just beginning, regarding CNV and de novo mutations.

Here we report on two COS cases associated with Alternating Hemiplegia of childhood (AHC), a rare disease characterised by repeated episodes of hemiplegia that alternately affects one side of the body. These comorbidities have guided the genetic exploration and two de novo deleterious missense variants (c.2438.T>C and c.2443G>A) in ATP1A3 were identified.

Both patients exhibited early (<13 years old) and severe psychotic signs with high scores on the standard clinical scales. AHC diagnosis was made at an early age for both patients (< 20 months old). Treatment response to antipsychotics was poor as it is often the case for COS.

To our knowledge, only one case report in a young boy is available, these two cases perfectly underlines the need to seek for all possible genetic conditions in children and adolescents presenting atypical psychotic symptoms and in a clinical point of view, on the importance of an early identification of psychiatric symptoms and disorders in rare organic disease like AHC.

Keywords: psychosis, genetic, mutation, early-onset schizophrenia, hemiplegia

P-5

Unusual clinical presentation of familial hemiplegic attacks in two first cousins: possible oligogenic inheritance of AHC-related phenotypes?

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We report on two first cousins presenting with different clinical manifestations within the AHC phenotypic spectrum. The first patient is a 30 y/o female who shows typical features of classical AHC, including early onset hemiplegic attacks, intellectual disability, movement disorders, and seizures. *ATP1A3* molecular analysis tested negative, whereas she bears an apparently *de novo* variant in a novel gene which is a strong candidate for AHC. Her first cousin (daughter of a maternal uncle) is a female of similar age who was healthy until 25 years, when she experienced the onset of severe migraine, suddenly followed by a persisting hemiplegic episode, lasting a few months. Brain MRI was normal. After the recovery from the first hemiplegic attack, she remained healthy for some years. She has recently had a similar episode of migraine followed by hemiplegia. *ATP1A3* as well as the hemiplegic migraine genes (*ATP1A2*, *CACNA1A*, *SCN1A* and *SLC2A1*) tested negative.
To understand the unusual presentation of a similar phenotype in the two cousins, for which a potential shared pathogenic variant would segregate through at least three unaffected subjects, we have undertaken the study of their whole exome (currently under bioinformatic analysis). A simple autosomal dominant model seems unlikely, unless we take into account a high lack of penetrance: rather, we hypothesize that the two cousins share a single or a few variants that alone are not sufficient to induce the pathological phenotype. Additional inherited or *de novo* variants could explain the low penetrance and the marked phenotypic discrepancies between the two patients.
An update of the ongoing genetic studies will be presented.

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P-6

Pathologic Findings in Alternating Hemiplegia of Childhood (AHC) Patients

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Alternating hemiplegia of childhood (AHC) is a rare and severe neurodevelopmental syndrome characterized by recurrent hemiplegic episodes and distinct neurological manifestations. The majority of individuals with AHC have a de novo mutation in *ATPase alpha subunit 3* (*ATP1A3)* gene. Controversy remains as to whether patients with AHC develop consistent structural neuropathological changes that may contribute to disease progression or the acquisition of fixed neurologic deficits over time.

In this present study, detailed autopsies were performed, including the brains, on three AHC affected subjects carrying mutations in the *ATP1A3* gene, including Exon 17 c.2401G>A; p. D801N, Exon 18 c.2423C>T; p. P808L, and Exon 18 c.2443G>A; p. E815K. Pulmonary edema or congestion was present in all three, with scattered foci of dystrophic calcification present in the lung and kidneys of the patient with E815K mutation. Significant cardiac abnormalities were present in 2 of the 3 patients including thickened ventricular walls and endomyocardium. Examination of brain tissue from these three subjects revealed consistent cerebellar atrophy with granular and Purkinje cell loss, gliosis, and patchy disorganization of the cortical layer. Some Purkinje cells were found in the deeper white matter in two subjects with the P808L and E815 mutations—suggesting possible abnormal neuronal migration and development. The subject with D801N mutation additionally had diffuse acute and chronic ischemic neuronal injury, with shrunken pyknotic neurons and significant cortical gliosis. The subject with the P808L mutation had mild vacuolization in the cerebral white matter, cortex and lateral thalamus, pigmented macrophages in perivascular spaces and rare binucleated neurons in the cortex and hippocampus suggesting subtle developmental abnormalities. The visual cortex had very large and disorganized neurons in the third layer. The subject with the E815K mutation had moderate gliosis of the dentate nucleus, hippocampal sclerosis and astrogliosis, and microglial nodules throughout the spinal cord.

In aggregate, our findings revealed severe cerebellar atrophy in AHC subjects, predominantly in the superior vermis; mild atrophy of hippocampus, predominantly in the dentate gyrus; and diffuse gliosis throughout the brain. Curiously, individuals affected with Rapid-onset Dystonia-Parkinsonism (RDP) who carry different *ATP1A3* gene mutations also have mild cerebellar and hippocampus atrophy. However, in contrast to RDP, we did not observe atrophy of the amygdala or substantia nigra. More studies are needed to confirm the unique neuropathologic findings related to brain maldevelopment in these well-defined AHC subjects by comparing them to age- and sex-matched controls. Such pathologic data are essential to underscore the clinical studies and further our understanding of the pathogenesis of AHC.

P-7

Alternating Hemiplegia of Childhood: a magnetic resonance spectroscopy, tractography and functional 3T study exploring ictal and interictal disease mechanisms

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Background: Alternating Hemiplegia of Childhood (AHC) is a very severe and intractable disorder, characterized by a combination of paroxysmal movement disorders and permanent neurological features. Despite the recent discovery of *ATP1A3* mutations in 75% of cases, there is little knowledge about the disease pathophysiology and no proven effective therapeutic options. Moreover, although the use of Magnetic Resonance (MR) techniques in neurological diseases has rapidly grown, long-term MR imaging studies and spectroscopy, tractography and functional studies are lacking in this group of patients.
Aim of the study: Aim of the project is to explore brain pathways and metabolic changes involved in AHC and to describe long-term brain morphological alterations in older patients using a 3T MR unit.
Methods: 8 genetically confirmed AHC patients (6 males, 2 females, age range 10 – 25 years) recruited through the Italian Biobank and Clinical Registry for AHC (www.ibahc.org) underwent MRI with spectroscopy, tractography and functional resting state study. Findings have been collected and reviewed by 2 expert neuroradiologists.
Results: Cerebellar atrophy has been shown in 5/8 patients. Spectroscopy showed slightly reduced cerebellar N-acetilaspartate, used as a potential indicator of neuronal injury/loss, in 6 out of 7 patients. Six patients completed the functional MRI resting state study. Comparison of functional MRI data with normal controls is in progress.
In AHC, multiple stress factors may lead to paroxysmal and permanent movement disorders. In particular, it could be hypothesized a relationship between the role of brainstem and cerebellum in sensory motor and cognitive processing and the site distribution of defective Na-K ATPase pump in AHC disease. Functional 3T MRI analysis will better define the involvement of cerebellum and other brain structures in disease pathophysiology and will give the rationale for the use of newer and more efficacious molecules.

P-8

EEG Biofeedback for Relaxation using Deep Neural Networks

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Background: Alternating Hemiplegia of Childhood (AHC) is a neurodevelopmental condition characterised for transient episodes of partial or total body paralysis. The attacks are usually relieved by relaxation or sleep. EEG biofeedback is a non-invasive technique that has been successfully used for driving subjects to relaxation states, by increasing their measured EEG alpha band power.

Methods: We are currently developing a data processing pipeline to analyse EEG spectrograms using successful artificial intelligence techniques for vision, known as deep neural networks. These networks can extract more informative representations of the subject's cognitive processes than simple alpha band power, providing better feedback to the subject. The system relies on commercial EEG caps connected to portable devices, where the system is deployed. This setting allows its use not only in medical environments, but also at patient's home, making it suitable for regular use.

Results: Preliminary results on sleep stage scoring demonstrate the suitability of the proposed method to analyse EEG spectrograms, obtaining better results as compared to those systems using discrete band power.

Conclusions: We think that this tool might help AHC patients to relieve their hemiplegic episodes by teaching them to bring their emotions to relaxation. Study on this subset of the population is the purpose of future research work.

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P-9

Application of EPR for description of the ouabain-binding site of Na,K-ATPase

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In the long run, the project aims at development of new derivatives of cardiotonic steroids (CTS) with increased selectivity towards individual isoforms of the Na,K-ATPase. The Na,K-ATPase is a target for a number of pharmacological agents from the CTS family, but, due to the enzyme's ubiquity, the inhibitors in use have many adverse effects. A diminished systemic toxicity of the compounds may be acquired by an increase in selectivity towards individual enzyme isoforms. Since the isoform-related differences are found within the cavity leading to the CTS-binding site, we shall supplement the existing description of crystallized enzyme-CTS complexes with information valid under physiological conditions. For this purpose, we shall collect Electron Paramagnetic Resonance (EPR) spectra of our newly synthesized spin-labelled CTS with varying spacer arms specifically bound to the enzyme and correlate the mobility of the label to the distance between the label and the steroid core of CTS. The degree of the motional restriction of the spin label reports on the radius of the cavity at the chosen distance from the steroid core. At present, we screen a number of CTS derivatives for their binding properties to Na,K-ATPase in order to find a core molecule with largest specific signal. We also have several reporting nitroxide groups with different sensitivities to conformational re-arrangements within or in the vicinity of the CTS binding site. Mapping of the CTS-binding cavity and marking out its boundaries under physiological conditions will define the spatial position of the sugar units (known to improve affinity and contribute to isoform selectivity). The preliminary results will be presented.

P-10

Towards the construction of a Structure-based protocol to select potential *ATP1A3* ligands through virtual screening procedures

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The maintenance of the proper Na^+ gradient across the plasma membrane is essential for the function and survival of all eukaryotic cells. Ion gradients are maintained by the Na,K-ATPases of which alpha α subunit carries out the ion transport and ATP hydrolysis.
Mutations in ion-transporting proteins severely affect their ability to traffic ions properly, perturbing the delicate ion gradient balance.
ATP1A3 encodes for an alpha subunit that contributes to the maintenance of Na+ and K+ concentrations across neuron membranes. *ATP1A3* mutations have been indicated as responsible for the vast majority of alternating hemiplegia of childhood (AHC) cases and other related conditions, including DYT12, CAOS and CAPOS.
Due to the lack of any structural data, we have applied homology modeling techniques to build three-dimensional structures of wild type (wt) and mutated *ATP1A3* forms. Starting from the primary sequence of the wt *ATP1A3* protein, and those bearing D801Y and E815K variants, we generated several homology models that were structurally optimized trough molecular mechanics.
Molecular dynamics (MD) techniques have been applied to inspect, at the atomic level, how each point mutation could affect the ion flow through *ATP1A3*. Details of the MD runs and structural insights for future drug design protocol will be reported.

This work was supported by AISEA.

P-11

Perinatal seizure and defective respiratory rhythm generation in *Atp1a3* knockout homozygous knockout mice

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A neuron-specific human α3 subunit isoform of the sodium pump, *ATP1A3*, plays an important role in neuronal excitability. Its point and deletion mutations have been recognized in diverse neurological disorders, such as alternating hemiplegia of childhood (AHC), apnea, and severe infantile epileptic encephalopathy often appear shortly after birth. To get insight into the pathophysiology of the disorders and to understand the functional roles of the sodium pump α3 subunit in the brain *in vivo* during this period of development, we examined the phenotype of *Atp1a3* knockout homozygous mouse fetuses (*Atp1a3*$^{-/-}$). Immediately after extraction from the uterus and release from the placenta, about half of *Atp1a3*$^{-/-}$ showed small spontaneous movements such as mouth opening, responded to pinching with slight body movement, and made an effort to breath. Within several seconds to several minutes, they started to develop atypical seizure with extension of both upper limbs anteroinferiorly and with tongue protrusion. Triggers of seizure seemed to be the body movements such as response to pinching stimulation or the breathing effort they made themselves. The epileptic seizures usually continued for several minutes. During and after the seizure attack, the *Atp1a3*$^{-/-}$ did not respond to pinching, show spontaneous body movements, or breathe on their own. The other *Atp1a3*$^{-/-}$ fetuses showed complete absence of spontaneous body movements, no responses to pinching, and no breathing movements from the very beginning at birth. To investigate the locus of hyperexcitability associated with seizure, we examined c-Fos expression in the brains of *Atp1a3*$^{-/-}$ and found a significantly increased number of c-Fos-expressing cells in various regions of the brains, with unique distribution in the cerebellum, when compared with wild-type littermates (*Atp1a3*$^{+/+}$). We also found various abnormal respiratory rhythms produced in the brainstem of *Atp1a3*$^{-/-}$. The data suggest that *Atp1a3* plays a critical role in neural function during development, survival at perinatal period, and its deficiency causes hyperexitability in the fetal brain.

Reference Ikeda, K et al. *Brain Research, in press, 2017*

P-12

Characterization of a human neuroblastoma model of AHC: progress report

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We are currently refining the characterization of a cellular model of AHC, based on SH-SY5Y neuroblastoma. SH-SY5Y have been stably transfected with constructs expressing the wild type form or four different ATP1A3 variants: E815K, D801N, D801Y, G947R. The rationale behind the choice of this model is related to the fact that SH-SY5H cells display a neuronal-like phenotype and express the endogenous *ATP1A3* gene.
ATP1A3 cDNAs have been cloned into the pIRES-eGFP eukaryotic expression vector. After linearization, cell lines have been permanently transfected with the constructs. The electrophysiological characterization has shown in E815K cells an accumulation of Na^+ and Ca^{2+}, as well as the reduction of the membrane resting potential.
The differentiation protocol of naïve SH-SY5Y cells with retinoic acid (RA) followed by neurobasal medium /B27 supplement (NB/B27) led to the development of neuron-like cells showing Ca^{2+} transients, trains of action potentials, and the expression of MAP2 and NeuN neuronal markers. To characterize the neuronal lineage that we are obtaining upon our differentiation protocol we are currently performing the whole transcriptome analysis of undifferentiated and differentiated (with RA only or with RA-NB/B27) cell lines.
Upon differentiation protocol, cell lines bearing *ATP1A3* mutations started dying two days after the medium switch from RA to NB/B27. By immunocytofluorescence, we demonstrated hyperexpression of activated Caspase 2 in AHC-like cells, suggesting the activation of apoptosis. We are currently planning to perform the transcriptome analysis of mutated cells in order to identify deregulated genes due to the presence of mutated *ATP1A3*.
Further, we are starting a medium throughput screening of candidate compounds to the treatment of AHC, identified by virtual screening of molecules binding to the mutated *ATP1A3* alleles. Candidate compounds will be tested for their effect in: 1) promoting Na^+ and Ca^{2+} scavenging and 2) revert the death phenotype.

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ATP1A3 Splice Site Mutation Produces Haploinsufficiency in Alternating Hemiplegia of Childhood

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AHC is most often associated with heterozygous *ATP1A3* missense mutations. There are no reports of overt loss-of-function mutations such as truncating frameshifts or premature stop codons associated with AHC. However, there have been cases associated with intronic splice site variants.

We investigated the effects of a recurrent intronic splice site mutation (c.2542+2T>C), which disrupts the canonical GT dinucleotide at the start of intron 18, on *ATP1A3* mRNA splicing in human neurons generated from patient-specific induced pluripotent stem cells (iPSCs). We differentiated cortical excitatory neurons from iPSCs from a proband with a clinical history of seizures and alternating hemiplegia presenting in early childhood and a healthy sex-matched control subject. We performed reverse transcription-polymerase chain reaction (RT-PCR) on 25 day post differentiation neurons using primers embedded in exons 17 and 20. We observed a single band of expected size in RT-PCR reactions performed on control neurons but two distinct amplicons in reactions from RNA isolated from AHC iPSC-derived neurons. Sequencing demonstrated that the mutation causes mis-splicing of *ATP1A3* mRNA with an in-frame inclusion of 66 bp from the adjacent intron and a predicted 22 amino acid insertion in a transmembrane domain of the protein. This prediction was consistent with immunoblot experiments performed on HEK-293 cells overexpressing recombinant ATP1A3 engineered with the 22 amino acid insertion, which demonstrated a higher molecular mass than WT protein. Structural modeling indicated that the insertion would affect the ion binding site as well as interactions with the lipid bilayer and beta-subunit thus destabilizing the protein. Immunoblotting of differentiated neurons showed lower amounts of the normal mass ATP1A3 protein with no larger products suggesting that the mutant protein is likely degraded in neurons.

Our findings indicate that a splice site mutation promotes mis-splicing and *ATP1A3* haploinsufficiency in neurons. Electrophysiology studies are underway to assess the cellular consequences associated with this mutation.

P-14

An iPSC-Derived Neuronal Model for Determining the Pathophysiology of *ATP1A3* Mutations in Alternating Hemiplegia of Childhood

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Alternating Hemiplegia of Childhood (AHC) is a rare genetic neurodevelopmental disease caused by heterozygous missense mutations in the *ATP1A3* gene. *ATP1A3* encodes the neuronal specific α3 subunit of the sodium-potassium adenosine triphosphatase (Na,K-ATPase) pump. This protein complex is crucial for many cellular functions including setting the resting membrane potential, regulating ion homeostasis, and generating action potentials. 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, developmental delay, and intellectual disability. It is now known that the majority of these individuals possess one of three heterozygous missense mutations in the *ATP1A3* gene, resulting in D801N, E815K, or G947R mutants of the α3 Na,K-ATPase subunit. However, mechanisms underlying symptoms in patients remain poorly understood and there are no empirically proven treatments for AHC.

The intricate relationships of the *ATP1A3* genotype and AHC phenotype make induced pluripotent stem cell (iPSC) technologies ideal for the investigation of disease mechanisms via creation of patient-specific disease models.

For our studies, we have generated iPSCs from patients with the three most common mutations in AHC. These iPSCs are differentiated into cortical neurons to test our hypothesis that mutant α3 decreases Na,K-ATPase function in a dominant-negative manner. We further hypothesize that mutant α3 protein perturbs normal neurodevelopment and results in neuronal depolarization that is exacerbated during cellular stress. Initial studies have focused on characterizing Na,K-ATPase subunit expression in developing cortical neurons. Results indicate that neurons derived from all three major *ATP1A3* mutations have increased levels of α3 protein and decreased levels of the α1 subunit compared to iPSCs. Expression during neuronal differentiation has similar temporal dynamics to control cells. Ongoing studies are exploring the neurodevelopmental consequences of mutant α3 protein by analyzing synaptic formation, neuronal maturation, and lineage specification. Fluorescent indicators of sodium and calcium concentration in patient-specific iPSC-derived cortical neurons are being used to investigate ion homeostasis in the presence of *ATP1A3* mutations under both physiological and stressed conditions.

Future studies will involve single-cell and multi-electrode electrophysiological analyses, along with testing for lineage-specific consequences of mutant α3 expression in GABAergic iPSC-derived neurons.

Finally, we are increasing the potential impact of our findings by using CRISPR/Cas9 techniques to create isogenic controls by correcting *ATP1A3* mutations in AHC patient-derived iPSC lines.

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P-15

Mice model of rapid-onset dystonia-parkinsonism

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Rapid-onset dystonia-parkinsonism (RDP) is the first identified disease manifestation of ATP1A3 mutation. It is characterized by abrupt onset of several symptoms, including generalized dystonia, severe bradykinesia and gait instability. The most common mutation observed in RDP patients, T613M, is located close to the nucleotide-binding domain. We have recently created a knock-in mouse model of T613M ATP1A3 using CRISPR/Cas technology. Homozygous mice are lethal. Heterozygous mice are born normally, but weigh less and are more hyperactive than their wild type (WT) littermates. During elevated plus-maze test heterozygous mice spend twice as much time in open space as WT. Mice memory has been tested with passive avoidance test and yielded similar results in heterozygous and WT mice. We observed seizures during ketamine anesthesia in heterozygous but not in WT mice. Recent studies have indicated that the neuronal sodium pump plays an important role for control of motor activity and gait. We have identified two heterozygous mice, that had obvious problems moving their hind legs and unable to walk normally. Thus we have in these ongoing studies of heterozygous ATP1A3 mice found several of the phenotypic characteristics observed in patients with ATP1A3 mutations.

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An Introduction of the Illustrator



Ms. Miya Tamura.　She was born in Japan in 1969.
She suffered from repeated hemiplegic attacks since she was 8 months of age.
Although she went to several hospitals, she had not been diagnosed for 14 years.
Finally, she was diagnosed as having Alternating Hemiplegia of Childhood in 1984 at National Center of Neurology and Psychiatry (NCNP) by Dr. Norio Sakuragawa.
Her *ATP1A3* mutation was confirmed as p.D801N through gene analysis in 2012.
She has fun in drawing pictures of animals with crayons.






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