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8th International Symposium on **NBIA**

PROGRAM BOOKLET

October 13-15, 2022 | **Website:** >> more information << | **Lausanne, Switzerland**
Mövenpick Hotel Lausanne

Welcome to Lausanne for the 8th international symposium on NBIA. While the 7th meeting in 2020 had to be held as a virtual event, we are very happy to meet in person again. We are looking forward to review recent progress in NBIA research, and will put emphasis on the interactive discussion of urgent needs and next steps for the NBIA disorders. We hope you will enjoy the meeting and the beautiful city of Lausanne!

the program committee:
Susan Hayflick, Oregon Health & Science University, Portland (US) | Thomas Klopstock, LMU Klinikum, Munich (DE) | Markus Nielbock, Hoffnungsbaum e.V., Würselen (DE) | Agnès Rötig, Institut Imagine, Paris (FR) | Valeria Tiranti, IRCCS Istituto Neurologico Carlo Besta, Milan (IT) | Patricia Wood, NBIA Disorders Association, El Cajon (US)

the local organizing committee:
Patrick Herren, Fatemeh Mollet, Esther Schärer, Olav Zilian, NBIA Suisse (patient advocacy association), Lausanne (CH)

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8th International Symposium on NBIA

October 13-15, 2022 | Mövenpick Hotel Lausanne, Switzerland

SCHEDULE

THURSDAY, October 13, 2022

13:00	Registration	
14:00	Welcome Address **Fatemeh Mollet,** NBIA Suisse, Lausanne, Switzerland **Patricia Wood,** NBIA Disorders Association, El Cajon, USA **Thomas Klopstock,** LMU Klinikum, Munich, Germany	
SESSION 1	NBIA general	Chair: Thomas Klopstock
14:15	Key note lecture: molecular mechanisms of iron-sulfur protein biogenesis and its link to iron metabolism **Roland Lill**, Marburg, Germany	
15:00	Epidemiology of NBIA disorders **Hana Kolářová**, Prague, Czech Republic	
15:20	Unreported NBIA in known disease genes **Agnès Rötig**, Paris, France	
15:40	Iron chelation in neurodegenerative disorders – state of the art and what it means for NBIA **David Devos,** Lille, France	
16:00	Coffee break	
SESSION 2	PKAN & CoPAN	Chair: Valeria Tiranti
16:30	Massive iron accumulation in PKAN-derived neurons and astrocytes: light on the human pathological phenotype **Sonia Levi,** Milan, Italy	
16:50	Zebrafish models with inborn errors of CoA biosynthesis **Dario Finazzi,** Brescia, Italy	
17:10	Mitochondrial and mitophagy in PKAN: insights from a *Drosophila* model **Zhihao Wu,** Dallas, USA	
17:30	Yeast as a model for functional analysis of mutations associated with PKAN disorder and for the identification of therapeutic molecules **Paola Goffrini,** Parma, Italy	
17:50	Modelling CoPAN in mice **Ivano Di Meo,** Milan, Italy	
18:10	Combination of a specific diet and the microbiome compensates lethality of a PKAN animal model **Hein Schepers,** Groningen, the Netherlands	
18:30	End of the day	
19:30	Dinner	

FRIDAY, October 14, 2022

08:30	**Update on the CoA-Z clinical trial** **Penelope Hogarth,** Portland, USA
08:50	**Optimization and efficacy of the clinical compound BBP-671 in a PKAN mouse model** **Suzanne Jackowski,** Memphis, USA
09:10	**The PanK Activator BBP-671 for PKAN: results from a phase 1 trial in healthy volunteers, and update on pivotal trial plans** **Susanne A. Schneider / Thomas Klopstock**, Munich, Germany
09:30	**Roundtable discussion on urgent needs and next steps in PKAN/CoPAN**
10:30	Coffee break

SESSION 3 MPAN — Chair: Agnès Rötig

11:00	**Expanding our understanding of *C19orf12* function to develop therapeutic approaches for MPAN** **Arcangela Iuso,** Munich, Germany
11:20	**Modelling *c19orf12* deficiency in zebrafish** **Barbara Gnutti,** Brescia, Italy
11:40	**Roundtable discussion on urgent needs and next steps in MPAN**
12:30	Lunch break

SESSION 4 BPAN — Chair: Susan Hayflick

13:30	**Is it all about autophagy? - WDR45's role in autophagy and beyond** **Mario Mauthe,** Groningen, the Netherlands
13:50	**Towards an understanding of the molecular function of WDR45/WIPI4 in autophagy and BPAN** **Tassula Proikas-Cezanne,** Tübingen, Germany
14:10	**Vulnerability of midbrain dopaminergic neurons in BPAN** **Lena Burbulla,** Munich, Germany
14:30	**Characterization of a whole-body Wdr45 knock-out, a mouse model for BPAN** **Arcangela Iuso,** Munich, Germany
14:50	**Roundtable discussion on urgent needs and next steps in BPAN**
15:40	Flash talks - selected poster presentations
16:00	Poster session & Coffee break
17:30	End of the day
19:30	Dinner

SATURDAY, October 15, 2022

SESSION 5 PLAN/INAD & FAHN — Chair: Ody Sibon

09:00	**Towards precision therapies for NBIA** **Manju Kurian,** London, UK
09:20	**PLA2G6-associated neurodegeneration – update** **Manju Kurian,** London, UK
09:40	**Developing gene therapy for PLAN and moving towards clinical trials** **Ahad Rahim,** London, UK
10:00	**Moving forward with FAHN research** **Sunita Venkateswaran,** Ottawa, Canada
10:20	**Roundtable discussion on urgent needs and next steps in PLAN/INAD & FAHN**
11:00	Closing remarks **Thomas Klopstock,** LMU Klinikum, Munich, Germany
11:15	End of the conference

Key note lecture: molecular mechanisms of iron-sulfur protein biogenesis and its link to iron metabolism

Roland Lill

Institut für Zytobiologie, Center for Synthetic Microbiology SYNMIKRO,

Philipps-Universität Marburg, Germany

Iron-sulfur (Fe/S) proteins are involved in numerous important cellular processes such as respiration, metabolism, genome maintenance, protein translation and antiviral response. The synthesis of Fe/S clusters and their assembly into apoproteins is a complex process that involves more than 30 proteins located in mitochondria and cytosol. Biogenesis of mitochondrial [2Fe-2S] and [4Fe-4S] proteins is accomplished by the iron-sulfur cluster assembly (ISC) machinery which was inherited from bacteria during evolution [1]. Cytosolic and nuclear Fe/S protein assembly also depends on the function of this machinery, yet additionally requires the mitochondrial ABC exporter ABCB7 and the cytosolic iron-sulfur protein assembly (CIA) machinery [2]. Interestingly, mitochondrial Fe/S protein biogenesis co-evolved with the existence of the entire organelle, defining this process as both the minimal and essential function of mitochondria or related mitosomes and hydrogenosomes [3]. A combination of numerous in vivo and in vitro studies has assigned the different ISC and CIA proteins to defined stages of the overall process. This now allows the elucidation of the molecular mechanisms underlying these individual reaction steps by employing cell biological, biochemical, biophysical, and structural methods. Fe/S protein biogenesis is also indirectly connected to other cellular pathways including cellular iron regulation, nuclear DNA metabolism and the maintenance of genome integrity, all involving crucial Fe/S proteins. Further, mutations in components of FeS protein biogenesis lead to various human diseases ('Fe/S diseases') with diverse hematological, neurological and metabolic phenotypes [1]. Mechanistic insights into the complex mechanisms of Fe/S protein biogenesis will eventually help understanding the connections to other pathways including iron homeostasis, and help elucidating the physiological consequences of "Fe/S diseases".

Recent reviews:

1. Lill, R. and S.A. Freibert, Mechanisms of Mitochondrial Iron-Sulfur Protein Biogenesis. Annu Rev Biochem, 2020. 89: p. 471-499.
2. Lill, R., From the discovery to molecular understanding of cellular iron-sulfur protein biogenesis. Biol Chem, 2020. 401(6-7): p. 855-876.
3. Braymer, J.J., et al., Mechanistic concepts of iron-sulfur protein biogenesis in Biology. Biochim Biophys Acta Mol Cell Res, 2021. 1868(1): p. 118863.

Lill's research is focused on the molecular mechanisms of iron-sulfur (FeS) protein biogenesis in mitochondria, cytosol, and nucleus of eukaryotes. This essential process of life was discovered by the Lill group in 1999. Mutations in mitochondrial biogenesis factors lead to severe diseases. Lill is also interested in processes intimately connected to FeS protein biogenesis such as cellular iron metabolism. For his work, he was awarded several prizes (e.g., Leibniz Prize, Feldberg Prize, and Luigi-Sacconi Medal). Lill is an elected member of Leopoldina and EMBO.

contact:
Lill@staff.uni-marburg.de

Epidemiology of NBIA disorders

Hana Kolářová

Department of Pediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

Friedrich-Baur-Institute, Department of Neurology, University Hospital, Ludwig-Maximilians-University Munich, Germany

Neurodegeneration with brain iron accumulation (NBIA) are a group of clinically and genetically heterogeneous diseases characterized by iron overload in basal ganglia and progressive neurodegeneration. To date, epidemiological data are scarce, but all forms of NBIA are considered to be ultrarare with a combined prevalence of 0.1 – 0.3 per 100,000, estimated based on reported cases. A population-genetic approach, using the allele frequencies of pathogenic *PANK2* variants, showed an estimated lifetime risk of Panthothenate kinase-associated neurodegeneration of approx. 2 per 1,000,000. However, data on lifetime risks of other NBIA types are either limited to isolated populations or completely unknown. We utilized the publicly available Genome Aggregation Database comprising 125,748 exomes and 15,708 genomes, as well as our in-house database of 22,162 exomes in order to calculate the lifetime risk for all 13 autosomal recessive NBIA disorders. The combined lifetime risk estimate is up to 0.92 per 100,000. Moreover, we could rank all 13 disorders and define the disorders with the highest lifetime risk as being caused by mutations in *PLA2G6*, *PANK2*, and *COASY*. This population-genetic estimation on lifetime risks of recessive NBIA disorders exceeds previous population-based epidemiological investigations by almost a magnitude. Importantly, our approach represents lifetime risks from conception, thus including prenatal deaths. Understanding the true lifetime risk of NBIA disorders is important in estimating disease burden, allocating resources and targeting specific interventions.

Dr. Hana Kolarova graduated in General Medicine at Charles University's First Faculty of Medicine in Prague in 2013. Since her graduation she has worked at the Department of Paediatrics and Inherited Metabolic Disorders and at the Laboratory for Study of Mitochondrial Disorders. In 2018, she obtained certification in Pediatrics and defended her PhD thesis in Biochemistry and Pathobiochemistry. She is currently in training to become a certified pediatric neurologist.

Her research activities mainly focus on rare neurometabolic disorders with a particular interest in mitochondrial diseases. She has benefitted from three long-term research stays and practical internships in the Laboratory of Ophthalmic Genetics at the Imagine Institute, Necker University Hospital in Paris, and at the Friedrich-Baur Institute, Ludwig-Maximilians University in Munich.

contact:
hana.kolarova@vfn.cz

Unreported NBIA in known disease genes

Agnès Rötig

Imagine Institute, Laboratory of Genetics of Mitochondrial Disorders,

Université Paris Cité Paris, France

Neurodegeneration with Brain Iron Accumulation (NBIA) is a genetically heterogeneous condition manifesting with dystonia, spasticity, rigidity and choreoathetosis. This group of neurodegeneration is characterized by iron accumulation in basal ganglia, and brain MRI also reveals cerebral and cerebellar atrophy. Several disease genes have been identified such as *PANK2*, *COASY*, *PLA2G6*, *C19orf12*, *FA2H*, *CRAT*, *REPS1* or *WDR45* but the gene mutations are currently unknown for around 40% of NBIA cases.

By whole exome sequencing in patients with NBIA we identified mutations in already known disease genes for which iron deposits have not been previously reported. In three patients from three unrelated families we identified mutations in *AP2M1*, *RAB39B* and *TUBB4A* genes. These three patients have clinical presentations relatively similar to what has been previously reported: intellectual developmental disorder with seizures for *AP2M1*, leukodystrophy for *TUBB4A*, X-linked intellectual developmental disorder for *RAB39B*. We previously showed that iron accumulation is related to accumulation of transferrin receptor (TfR1) at cell membrane and delayed endosome recycling in fibroblasts of patients from several NBIA subtypes [1]. Moreover, we also identified *REPS1*, involved in endocytosis and vesicle transport, as a NBIA gene. *AP2M1* encodes a component of the AP2 coat assembly protein complex of clathrin-coated vesicles. *RAB39B* is involved in the regulation of vesicular trafficking between membrane compartments. *TUBB4A* encodes a beta-tubulin, a component of the microtubules that functions in intracellular transport. In keeping with this mutations in *AP4M1* involved in intracellular transport of proteins and of *VAC14* that regulates the content phosphatidylinositol 3,5-bisphosphate that modulates intracellular transport have been recently reported [2-4]. These data confirm that endocytosis and endosome recycling are part of the mechanisms leading to iron overload in NBIA and make other genes involved in these mechanism as candidate genes for NBIA.

References:

1. Drecourt, A. et al. (2018) Impaired Transferrin Receptor Palmitoylation and Recycling in Neurodegeneration with Brain Iron Accumulation. Am J Hum Genet 102 (2), 266-277.
2. Roubertie, A. et al. (2018) AP4 deficiency: A novel form of neurodegeneration with brain iron accumulation? Neurol Genet 4 (1), e217.
3. Baumann, H. et al. (2020) Altered homodimer formation and increased iron accumulation in VAC14-related disease: Case report and review of the literature. Parkinsonism Relat Disord 80, 41-46
4. Lyon, G.J. et al. (2019) VAC14 syndrome in two siblings with retinitis pigmentosa and neurodegeneration with brain iron accumulation. Cold Spring Harb Mol Case Stud 5 (6).

Agnès Rötig is research director (DR1, INSERM) in Institut Imagine. She has built her research group more than 20 years ago in the field of mitochondrial disorders in very close collaboration with the Genetic Unit of Necker Hospital.

She has a longstanding collaboration with geneticist, pediatric neurology, neuroradiology and metabolic units from Necker hospital with the aim to identify nuclear genes of mitochondrial disorders and to understand their pathophysiology. Her group has set up the biochemical and genetic diagnosis of these diseases and described several novel genes responsible of these disorders to establish genotype-phenotype correlations. Agnès Rötig has developed a patient database that contains more than 1000 patient data and her group associated with the clinical unit has set up a biobank. Recently, her group has been involved in NBIA as NBIA patients present non specific clinical signs reminiscent to those observed in mitochondrial disorders. The mechanism of iron accumulation in these diseases is investigated in collaboration with other groups of the Imagine Institut.

contact:
agnes.rotig@inserm.fr

Iron chelation in neurodegenerative disorders – state of the art and what it means for NBIA

David Devos

Lille Neuroscience & Cognition Center, University of Lille, CHU of Lille, Inserm, France

Focal iron accumulation associated with brain iron dyshomeostasis is a pathological hallmark of various neurodegenerative diseases (NDD). In PD, a progressive degeneration of the substantia nigra pars compacta (SNc) is associated with the appearance of siderotic foci, largely caused by increased labile iron levels resulting from an imbalance between cell iron import, storage and export. At a molecular level, α-synuclein regulates dopamine and iron transport with PD-associated mutations in this protein causing functional disruption to these processes. In ALS, an early iron accumulation is present in neurons of the cortico-spinal motor pathway before neuropathology and secondary iron accumulation in microglia. It has recently been discovered that these alterations can trigger susceptibility to an iron-dependent cell-death pathway with unique lipoperoxidation signatures called ferroptosis. Interestingly, iron accumulation and ferroptosis are highly sensitive to iron chelation. A moderate iron chelation modality that conserves systemic iron offers a novel therapeutic strategy for neuroprotection. Promising preclinical and clinical proof of concept trials has led to several current large randomized clinical trials.
Neuroferritinopathy is a rare inherited neurodegeneration with brain iron accumulation characterized by brain iron overload resulting in progressive movement disorders. No treatment is currently available. We assessed conservative iron chelation with deferiprone at 30 mg/kg/day on the disease progression with controlled periods of discontinuation. 4 patients with confirmed molecular diagnostic of neuroferritinopathy were placed under deferiprone, at different stage of disease progression and with clinical and biological monitoring to control benefit and risk. The 4 patients experienced from slight to high improvement. In one case, we managed to stabilized disease progression for more than 11 years. In another case, we were able to reverse symptoms after a few months of treatment. The earliest the treatment was started, the most efficient it was on disease progression.Conservative iron chelation should be further assessed in neuroferritinopathy.

David Devos, Doctor of Neurology, Doctor of Neuroscience and Professor in Medical Pharmacology CHU, University of Lille, INSERM U1172, France. He develops innovative therapeutic strategies in neurodegenerative diseases (Parkinson's disease and Amyotrophic Lateral Sclerosis) from the concept through preclinical research to large clinical trials (> 225 articles, H-index: 56, 5 patents, cofounder of 3 Start up, 31 clinical studies, 4 H2020 projects; funding > 12M€).

He is coordinating the neurogenetic center of Lille (Neurodegeneration with Brain Iron Accumulation).

contact:
David.DEVOS@chu-lille.fr

Massive iron accumulation in PKAN-derived neurons and astrocytes: light on the human pathological phenotype

Sonia Levi

IRCCS San Raffaele Scientific Institute, San Raffaele University, Milan, Italy

The hallmark of Neurodegeneration associated with defective Pantothenate kinase-2 (PKAN) disease is the huge accumulation of iron in the *globus pallidus* brain region of patients. PKAN is caused by mutations in the PANK2 gene, that encodes the mitochondrial enzyme Pantothenate kinase-2, whose function is to catalyze the first reaction of the CoA biosynthetic pathway. It is still unknown how this alteration can cause the accumulation of iron in the brain. Starting from the previously obtained hiPS-clones, we set up different differentiation protocols that were capable to generate either inhibitory neurons or a pure population of astrocytes. We obtained striatal-like medium spiny neurons composed by about 70-80% of GABAergic neurons and 10-20% of glial cells. Within this mixed population we detected iron deposition in both PKAN cell types, however the viability of PKAN GABAergic neurons resulted strongly affected. CoA treatment was able to reduce cell death and, notably, also iron overload. A further differentiation of hiPS-clones in a pure population of astrocytes showed a particularly evident iron accumulation, with about 50% of cells positive to Perls stain. The analysis of PKAN astrocytes indicated an alteration of iron metabolism, mitochondria morphology, respiratory activity, and oxidative status. Moreover, PKAN astrocytes showed signs of ferroptosis and were prone to develop a stellate phenotype, thus gaining a neurotoxic feature. This feature was confirmed in iPS-derived astrocytes and glutamatergic neurons co-cultures. Furthermore, the investigation of constitutive exo-endocytosis and vesicular dynamics by the activity-enriching biosensor SynaptoZip, proposes a general impairment in constitutive endosomal trafficking in PKAN astrocytes. CoA and 4-Phenylbutyric Acid treatments were found to be effective in partially rescuing the aberrant vesicular behaviour. This astrocyte model results the first in vitro disease model recapitulating the human phenotype and can be further exploited to deeply elucidate the involvement of iron in the pathogenetic mechanisms of the disease.

The financial support from the Telethon-Italia (GGP16234 and GGP20047 to SL) and the Ministry of Health (GR-2018-12365610to IDM) are acknowledged.

Authors: Santambrogio Paolo[1], Cozzi Anna[1], Ripamonti Maddalena[2], Di Meo Ivano[3], Tiranti Valeria[3] and Levi Sonia[1,2].

[1] IRCCS San Raffaele Scientific Institute, Milan, Italy,
[2] Vita-Salute San Raffaele University, Milan, Italy
[3] Medical Genetics and Neurogenetics Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

Professor Sonia Levi is a Full Professor of Applied Biology at the San Raffaele University and Head of Proteomic of Iron Metabolism Research Unit, IRCCS-OSR, Milano. She has a solid track record in iron metabolism and a long experience in the characterization of structure and function of ferritins, both in vitro and in cellular models. Her recent research interest is focused to clarify the relationship between iron and neurodegeneration. With this aim she developed and studied cellular and animal models of neurodegenerative diseases, like neuroferritinophaty, PKAN, CoPAN and Friedreich's ataxia.

contact:
levi.sonia@hsr.it

Zebrafish models with inborn errors of CoA biosynthesis

Dario Finazzi

Department of Molecular and Translational Medicine, University of Brescia, Italy

Coenzyme A (CoA) is an essential cofactor in all living organisms, functioning either as an activator of molecules with carbonyl groups or as a carrier of acyl moieties. In the most recent years, defects in genes involved in CoA biosynthesis have been found in patients affected by rare inherited disorders. While mutations in PPCS, encoding phosphopantothenoylcysteine synthetase, the second step in CoA biosynthesis, are associated with an autosomal-recessive form of dilated cardiomyopathy, sequence variations in pantothenate kinase 2 (PANK2) and coenzyme A synthase (CoASY), the first and last enzyme of the biosynthetic pathway, are found in patient affected by rare forms of neurodegeneration with brain iron accumulation (NBIA), PKAN and CoPAN, respectively. PKAN, is one of the most common forms of NBIA. In most cases, the onset is very early in childhood; the main clinical features are extrapyramidal symptoms, particularly generalized dystonia and dysarthria, and cognition dysfunction. CoPAN is ultra-rare and its clinical phenotype largely overlaps with that of PKAN. Significant research has concentrated on PKAN lately, and the knowledge about the biochemical and molecular features of the pathology has increased a lot. Nonetheless, the main culprit of the pathology is not well defined. In order to contribute to the understanding of these diseases and facilitate the search for therapies, we explored the potential of the zebrafish (*Danio rerio*) animal model and generated lines carrying biallelic mutations in pank2 and coasy genes. The phenotypic characterization of pank2-mutant embryos revealed anomalies in the development of venous vascular structures and of germinal cells. Adult fish showed testicular atrophy and altered behavioural response in an anxiety test, but no evident signs of neurodegeneration. On the contrary, the initial study of coasy-null animals showed larval lethality, with death occurring around 15 dpf. We are investigating the molecular underpinnings of this phenotype.

Authors: Mignani Luca, Deepak Khatri, Gnutti Barbara, Canino Biagio Francesco, Zizioli Daniela, and Finazzi Dario

Dario Finazzi got his MD and specialty school degree in Clinical Chemistry and Biochemistry at the University of Brescia, Italy. He worked as a visiting fellow at the CBMB, NIH, Bethesda, form 1992 until 1995 and became assistant professor at the University of Brescia in 1996. Since 2015 he is associate professor in Molecular Biology. He has been working as a physician at Spedali Civili Hospital, in Brescia, since 1996.

His main research interest is the study of iron metabolism and particularly the role of the metal in neurodegenerative disorders.

contact:
dario.finazzi@unibs.it

Mitochondrial and mitophagy in PKAN: insights from a *Drosophila* model

Zhihao Wu

Department of Biological Science, Southern Methodist University, Dallas, Texas, USA

Human neurodegenerative disorders often exhibit similar pathologies, suggesting a shared aetiology. Key pathological features of Parkinson's disease (PD) are also observed in other neurodegenerative diseases. Pantothenate Kinase-Associated Neurodegeneration (PKAN) is caused by mutations in the human PANK2 gene, which catalyzes the initial step of de novo CoA synthesis. Here, we show that *fumble (fbl)*, the human *PANK2* homolog in *Drosophila*, interacts with PINK1 genetically. *fbl* and *PINK1* mutants display similar mitochondrial abnormalities, and overexpression of mitochondrial Fbl rescues PINK1 loss-of-function (LOF) defects. Dietary vitamin B5 derivatives effectively rescue CoA/acetyl-CoA levels and mitochondrial function, reversing the PINK1 deficiency phenotype. Mechanistically, Fbl regulates Ref(2)P (p62/SQSTM1 homolog) by acetylation to promote mitophagy, whereas PINK1 regulates *fbl* translation by anchoring mRNA molecules to the outer mitochondrial membrane. In conclusion, Fbl (or PANK2) acts downstream of PINK1, regulating CoA/acetyl-CoA metabolism to promote mitophagy, uncovering a potential therapeutic intervention strategy in PD and PKAN treatment. Intriguingly, subsequent biochemical and metabolome analyses revealed severe dyshomeostasis of redox system components in *fbl* LOF flies. Akin to it, molecular hydrogen treatment and overexpression of mitochondrial redox-regulating enzymes, the peroxiredoxins Prx3 and Prx5, rebalances the redox state and rescues the morphological and physiological defects in *Drosophila* PKAN models.

Dr. Wu received his Ph.D. from Tsinghua University and then completed postdoctoral training in the Department of Pathology at Stanford University. He is now an assistant professor in the Department of Biological Sciences at Southern Methodist University.

His laboratory is devoted to studying the molecular mechanisms of human neurodegenerative diseases, especially the pathogenesis associated with mitochondrial dysfunction.

He and his lab have published more than 22 peer-reviewed journal articles on Molecular Cell, Cell Metabolism, Genes and Development, and Nature Communications.

contact:
zhihaowu@mail.smu.edu

Yeast as a model for functional analysis of mutations associated with PKAN disorder and for the identification of therapeutic molecules

Paola Goffrini

Department of Chemistry, Life Sciences and Environmental Sustainability,

University of Parma, Italy

The unicellular eukaryote Saccharomyces cerevisiae is one of the most intensively investigated model organisms in which several basic cellular mechanisms have been elucidated. Moreover, thanks to significant conservation in gene function between yeast and humans, this single cell organism proved to be an important tool to model human diseases. We constructed and characterized a yeast model for Pantothenate Kinase-Associated Neurodegeneration (PKAN), a rare genetic disorder determined by mutations in the PANK2 gene encoding the mitochondrial isoform of pantothenate kinase. The PKAN yeast model is a mutant strain deleted in the unique pantothenate kinase encoding gene, CAB1, and expressing one of the PANK2 mutations found in patients. This model recapitulates the main phenotypes associated to human disease: mitochondrial dysfunction, iron overload, oxidative damage and altered lipid metabolism, thus representing a valuable tool to investigate the mechanism of pathogenesis underlying the disease and to discriminate between pathogenic variants.
To date, more than 150 mutations have been found, many of which are missense and their impact on protein function is not always known. Since S. cerevisiae possess only one gene for pantothenate kinase it represents a low cost, fast and reproducible system for the identification of null or hypomorphic alleles as well as gain-of-function.
Moreover, yeast-based model is a valuable tool also in drug discover, allowing to screen in a short time large libraries of molecules, to identify chemical suppressors of pathological phenotypes. Taking advantage of the respiratory growth defective phenotype of our PKAN yeast model, we used a 'drug repurposing' approach to identify FDA-approved molecules able to rescue the mitochondrial defect associated to PKAN mutations in order to identify potential therapeutic drugs for PKAN treatment.

Paola Goffrini is Associate Professor of Genetics at the Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma.
She has a long and consolidated background in genetics, molecular biology, physiology and biochemistry of yeast.
Based on the knowledge on the genetics of mitochondrial-core systems, she has been committed to the generation of yeast models helpful to investigate the molecular basis underlying the pathogenic mechanism of human diseases, in particular those caused by mutations in genes associated with defects of mitochondrial metabolism.

contact:
paola.goffrini@unipr.it

Modelling CoPAN in mice

Ivano Di Meo

Medical Genetics and Neurogenetics Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

COASY protein-associated neurodegeneration (CoPAN) is a rare but devastating genetic autosomal recessive disorder of inborn error of CoA metabolism, which shares with PKAN similar features, such as dystonia, parkinsonian traits, cognitive impairment and brain iron accumulation. In order to elucidate the mechanisms linking CoA metabolism, iron dyshomeostasis and neurodegeneration, as well as to test potential therapeutic interventions, we generated mouse models lacking Coasy gene, using the Cre-loxP system. While constitutive ablation is not compatible with life, neuronal-specific Coasy knock-out developed a very severe early onset phenotype with sensorimotor defects and dystonia-like movements, altered brain iron homeostasis and mitochondrial dysfunction. However, these mice showed unchanged levels of brain CoA levels, and the premature death to postnatal day 15 prevents the appearance of neurodegenerative events and the possibility to test therapeutic interventions.
In order to slow down and postpone the onset of the symptoms observed in the congenic model, we recently generated an inducible, neuronal-specific Coasy null mouse (SLICK-Coasy), in which Coasy gene removal is induced in adult age, when the brain development is completed. Starting approximatively at 1-month post induction (mpi), mice showed neurological impairment, progressive sensorimotor defects, dystonia-like movements and reduced lifespan to 3 mpi. Again, no alteration of total CoA levels was found in the brain of recombinant animals. Interestingly, besides the alteration of iron homeostasis, we also found a broad neuroinflammation, characterized by microglial activation and astrocytes iper-proliferation, as well as neurodegenerative neuropathology. Furthermore, we observed higher expression of pro-inflammatory cytokines in the brain of ko mice, as well as increased levels of plasmatic GFAP, whose increase was also found in patient with different neurodegenerative conditions. These data suggest that neuroinflammation could plays an important role in the pathogenesis of the disease, representing a potential target for future therapeutic interventions.

Ivano Di Meo has a strong and solid expertise in mitochondrial, metabolic and neurodegenerative disorders. The focus of his research is the generation and characterization of new disease models, the elucidation of molecular and biochemical pathomechanisms and the implementation of experimental pharmacological and molecular therapeutic approaches.

Through a young researcher grant from the Italian Ministry of Health, he is developing his own group and investigating the pathomechanisms underlying NBIA associated to CoA metabolism, as well as testing potential therapeutic interventions.

contact:
Ivano.DiMeo@istituto-besta.it

Combination of a specific diet and the microbiome compensates lethality of a PKAN animal model

Hein Schepers

University Medical Center Groningen, University of Groningen, the Netherland

Coenzyme A (CoA) is essential for metabolism and protein acetylation. Current knowledge holds that cells obtain CoA exclusively through biosynthesis via a canonical five-step pathway, starting with pantothenate uptake. Errors in this CoA biosynthesis process are detrimental, since defects in genes encoding the CoA biosynthesis enzymes cause inherited human diseases, including pantothenate kinase-associated neurodegeneration (PKAN), COASY protein-associated neurodegeneration (CoPAN), and PPCS-deficiency, a cardiac disease. However, several observations indicate that exceptions to the single-known CoA-generating mechanism must exist. For example, several unicellular organisms lack one or more genes encoding CoA biosynthesis enzymes. For multicellular complex organisms as well, evidence indicates that CoA can be obtained via other pathways. dPANK/fbl is the Drosophila (fruit fly) PANK ortholog, and homozygous dPANK/fbl^{null} mutants proceed normally through embryogenesis and early larval development, despite lacking an intact CoA synthesis pathway. Moreover, early human fetal development occurs in PKAN-, CoPAN-, and PPCS-deficient patients, even in individuals who have CoPAN with no functional COASY enzyme activity. Early development of these compromised organisms can only be explained if CoA is obtained from alternative sources.

Here, we uncovered pathways for CoA generation through inter-organismal flow of CoA precursors. Using traceable compounds and dPANK/fbl^{null} mutants fruit flies, we demonstrate that maternal supply of CoA precursors allow survival of early developmental stages in fruit flies with impaired CoA biosynthesis. These precursors are stable, long-lasting, and sufficient to allow normal development of a complex multicellular organism in the absence of endogenous CoA biosynthesis. Later in life, the microbiome can provide essential CoA building blocks to the host, enabling continuation of normal development.

Together, our data demonstrate the presence of alternate mechanisms to maintain CoA homeostasis. These routes enable a flow of CoA precursors from mother to her progeny and from gut microbiome to the host.

Hein Schepers is a senior scientist in the Sibon Group (UMCG, Groningen).
He received his PhD from the University of Groningen in 2007, working in the field of Hematology, dissecting molecular mechanisms underlying the development of human AML. After a postdoc at Oxford University, UK, working with murine stem cells, he returned to Groningen and now works in the Sibon group. Here, he combines his molecular background, primary cell expertise, flexibility-in-model-system-usage and his knowledge in bioinformatics to unravel the mechanisms underlying CoA metabolism and CoA-linked diseases.

contact:
h.schepers@umcg.nl

Update on the CoA-Z clinical trial

Penelope Hogarth

Oregon Health & Science University

We conducted a phase 2 safety and tolerability trial of 4'-phosphopantetheine, a downstream metabolite of pantothenate, in PKAN, using a remote trial design. Our novel approach to study conduct facilitated rapid recruitment into the study, which consisted of a 6-month, dose-ranging, placebo-controlled, double-blind phase, followed by an 18-month open-label phase, allowing subsequent expansion of the primary cohort and addition of a direct-to-open-label cohort. Further, while not planned with a pandemic in mind, the remote design proved resilient in the face of COVID-19 disruptions. Descriptive data including study design elements, population demographics, and trial experience will be presented.

Penelope Hogarth is a Professor of Molecular & Medical Genetics and Neurology at Oregon Health & Science University in Portland, Oregon, USA. Following her training in neurology at the University of Colorado, Dr. Hogarth completed a combined fellowship in movement disorders and experimental therapeutics at the University of Rochester in New York. During her career at OHSU, she has conducted all phases of clinical trials in patients with movement disorders, and she maintains an active program in Parkinson disease clinical research. She joined the OHSU NBIA research and clinical care program more than 20 years ago, shortly after the PKAN gene discovery. Her NBIA research has focused on characterising the phenotypic spectrum and natural history of various forms of NBIA. She is currently principal investigator for a phase 2 clinical trial of a vitamin metabolite in PKAN in the United States and Canada.

contact:
hogarthp@ohsu.edu

Optimization and efficacy of the clinical compound BBP-671 in a PKAN mouse model

Suzanne Jackowski

St. Jude Children's Research Hospital, Memphis, Tennesse, USA

Pantothenate kinase-associated neurodegeneration (PKAN) is a rare neurodegenerative disease caused by mutations in the gene encoding pantothenate kinase 2 (PANK2). PANK2 is one among 4 active PANK isozymes that initiate coenzyme A (CoA) biosynthesis. CoA and CoA-derivatives, e.g., acetyl-CoA, are essential for hundreds of metabolic reactions and processes throughout the body, including energy and neurotransmitter production. Brain CoA deficiency is thought to be the cause of the movement dysfunction, iron accumulation and neurodegeneration in PKAN, but no probes are available to measure CoA in patients. A mouse model of severe brain CoA deficiency with consistent and measurable movement dysfunction was generated to test the efficacy of BBP-671 as an activator of the alternative PANK3 in restoring brain CoA levels. The mouse model had rapidly progressing disease specific to neurons. BBP-671 is a small molecule within a family of high-affinity PANK activators that is optimized for human use, orally bioavailable and efficiently crosses the blood-brain-barrier. Treatment with BBP-671 provided in chow for 3 weeks significantly increased brain CoA and improved the movement dysfunction observed in the mouse model. Specifically, the PKAN mice treated with BBP-671 spent more time moving and travelled a greater distance than untreated animals. BBP-671 also improved the survival of the mice compared to untreated animals and normalized brain glutamate/glutamine levels indicating better neuronal health and function. Iron accumulation, however, was not observed in this mouse model. Altogether these data provide the preclinical foundation for the therapeutic potential of BBP-671 which is now in clinical trials.

Research supported by the American Lebanese Syrian Associated Charities of SJCRH and CoA Therapeutics, Inc.

Authors: Suzanne Jackowski, Charles Rock, Richard Lee, Stephen White and Puneet Bagga

Suzanne Jackowski received the Ph.D. from the Oak Ridge National Laboratory in 1977. Jackowski joined the faculty at St. Jude Children's Research Hospital in 1980 until her retirement in 2021.
She established research programs addressing the regulation of membrane phospholipids and the regulation of coenzyme A biosynthesis, and published >130 original research articles and 36 topical review articles.
Jackowski was a member of the SMAB of the NBIA Disorders Association (2016-20) and is currently on the SAB of CoA Therapeutics, Inc.

contact:
sjack.rock901@gmail.com

The PanK Activator BBP-671 for PKAN: results from a phase 1 trial in healthy volunteers, and update on pivotal trial plans

Susanne A. Schneider, Thomas Klopstock

Department of Neurology, LMU Klinikum, Ludwig-Maximilians-University Munich, Germany

Background:
BBP-671 is an activator of human pantothenate kinases (PanK), key enzymes in the coenzyme A (CoA) biosynthetic pathway. BBP-671 is being developed to treat pantothenate kinase-associated neurodegeneration (PKAN) and organic acidemias, rare diseases associated with CoA deficiency. Safety, PK, and PD of BBP-671 were investigated in healthy subjects in a Phase 1 study (NCT04836494). The interim Phase 1 data supports development of BBP-671 as a potential treatment of PKAN.

Results:
Single doses of 3 to 120 mg BBP-671, and 7-day courses of 30 mg, 60 mg, 70 mg, and 100 mg total daily dosages, were studied in healthy subjects. BBP-671 was readily absorbed after oral dosing with a T_{max} of 1-2 hours; the elimination half-life was of 6-9 hours. Increases in mean BBP-671 exposure were slightly more than dose proportional and there was a 2.5 to 3-fold accumulation at steady state. BBP-671 was detected in human plasma and cerebral spinal fluid (CSF). Increases in whole blood (WB) acetyl-CoA were observed with BBP-671.

Conclusions:
BBP-671 is a novel, potent, oral, and potentially first-in-class PanK activator. BBP-671 was well-tolerated following oral dosing in healthy volunteers. BBP-671 was detected in healthy volunteer plasma and CSF demonstrating that BBP-671 gets into the brain. BBP-671 increased WB acetyl-CoA levels, demonstrating target engagement and proof of mechanism of the drug. Based on these data, BBP-671 may have the potential to be an effective therapy for conditions associated with CoA deficiency. Therefore, these data support initiation of clinical trials of BBP-671 for the treatment of PKAN.

Authors: Susanne A. Schneider[1], Agnieszka Jurecka[2], Thomas Klopstock[1], Daniel Gretler[2], Rajaa Sukhun[2], Satish Rao[2], Uma Sinha[2]

[1] Department of Neurology, LMU University Munich, München, Germany
[2] CoA Therapeutics, a BridgeBio company, San Francisco, CA, USA

Susanne A. Schneider is a consultant neurologist and associate professor at LMU in Munich, Germany, with broad clinical and academic experience and with a great passion for research into the pathophysiology and treatment of movement disorders, incl. rare sporadic and inherited forms such as NBIA. She authored more than 180 papers and 30 chapters and co-edited four books. She serves on several boards for international professional societies.

contact:
susanne.schneider@med.uni-muenchen.de

Thomas Klopstock, Professor of Neurology at LMU Munich. Long-standing clinical and scientific expertise in mitochondrial & neurogenetic disorders. Speaker of German network for mitochondrial disorders (mitoNET) & of international project TIRCON (Treat Iron-Related Childhood-Onset Neurodegeneration). Member of German Center for Neurodegenerative Diseases, Munich Excellence Cluster for Systems Neurology & Board of Directors of Munich Center for Rare Diseases. Author of >300 publications. Ample experience in clinical trials. Main goal: disease-modifying treatments of mitochondrial & other neurogenetic disorders incl. NBIA.

contact:
thomas.klopstock@med.uni-muenchen.de

SESSION 3 MPAN

Expanding our understanding of C19orf12 function to develop therapeutic approaches for MPAN

Arcangela Iuso

Institute of Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany

Pathogenic variants in the gene *C19orf12* (OMIM: 614298) are the genetic cause of Mitochondria membrane Protein-Associated Neurodegeneration (MPAN), a progressive neurodegenerative disorder presenting with dystonia, spasticity, and neuropsychiatric abnormalities. Onset is mostly in childhood to early adulthood with slow progression and survival well into adulthood. Treatment is so far limited to mainly symptomatic measures. Disease-modifying therapies are not available as the function of C19orf12 is largely unknown.
Considering the mitochondrial localization of C19orf12 and previous studies reporting on the involvement of C19orf12 in autophagy upon nutrient starvation, in the current study we investigated the involvement of C19orf12 in mitochondrial bioenergetics, and bulk autophagy in response to mitochondrial stress.
We consistently detected a slight impairment of bulk autophagy in primary fibroblasts from MPAN patients. We made use of this functional readout to perform a screening of molecules modulating autophagy. The screening led to the identification of five compounds ameliorating autophagy in MPAN fibroblasts.
In summary, our study confirmed a role for C19orf12 in autophagy upon mitochondrial stress, showed that autophagy can be used as a readout for the development of treatment strategies in MPAN, and identified compounds potentially beneficial for MPAN.

Arcangela Iuso is a scientist at the Helmholtz Center Munich and Technical University of Munich, where she fosters preclinical studies in the field of Neurodegeneration with Brain Iron Accumulation (NBIA) and metabolically-related disorders (PPCS and SLC25A42 deficiency).
Arcangela has successfully established cellular (fibroblasts, adipocytes, iPSCs, iPSC-derived neuronal cells) and animal models (fruit flies and mice) for these disorders to investigate the effect of pathogenic variants in cellular processes and exploit therapeutic approaches.
She is the scientific coordinator of the NBIA biobank (TUM, Munich).

email:
arcangela.iuso@helmholtz-muenchen.de

Modelling *c19orf12* deficiency in zebrafish

Barbara Gnutti

Department of Molecular and Translational Medicine, University of Brescia, Italy

Mutations in the orphan gene *C19orf12* cause Mitochondrial membrane Protein Associated Neurodegeneration (MPAN), a rare inherited neurologic disorder characterized by progressive spastic para/tetraparesis, dystonia, motor axonal neuropathy, parkinsonisms, psychiatric symptoms, and optic atrophy. *C19orf12* encodes for a small protein found in mitochondria, endoplasmic reticulum, and at mitochondria-associated membranes. The available data suggest an involvement of the protein in lipid metabolism, mitochondrial function, and autophagy.
Zebrafish harbors four co-orthologues of the human *C19orf12* gene, among those *c19orf12a*, located on chromosome 18, is expressed at higher levels during the early stages of development. The other genes, clustered in tandem on chromosome 7, according to WISH assay, showed a similar expression pattern in Central Nervous System and somites to *c19orf12a*, until 24 hours post-fertilization.
A *c19orf12a* zebrafish morphant exhibited unsettled brain morphology, smaller head and eyes, reduced yolk extension, tilted and thinner tail, significant perturbation of musculature formation associated with defective locomotor behaviour, features resembling MPAN. The specificity of the observed phenotype was confirmed by the phenotypic rescue obtained by the co-injection of human *C19orf12* wild-type mRNA, while the microinjection of a mutant mRNA failed to rescue the phenotype. To further investigate the mechanisms underlying neurodegeneration and the late-onset effects in adult animals we are working on the generation of a stable *c19orf12* loss-of-function model using CRISPR/Cas9 technology, to delete all the zebrafish co-orthologues.
To date, we generated two stable *c19orf12a* mutant lines carrying either a 2 bp deletion (Δ2), inducing a premature stop codon, and an in-frame, potentially pathogenic, 3 bp deletion (Δ3). A preliminary characterization of the models did not evidence any significant morphological alteration, even though the Δ3 F4 generation, not maternally provided by wild-type mRNAs, was characterized by significant larval lethality. The embryos harboring those biallelic mutations will be used to establish the complete *c19orf12* knock-out model.

Authors: Barbara Gnutti[1], Paola Agazzi[1], Luca Mignani[1], Biagio Francesco Canino1, Daniela Zizioli[1], Giuseppe Borsani[1], Dario Finazzi[1]

[1] Department of Molecular and Translational Medicine, University of Brescia, Italy

Barbara Gnutti is a Ph.D. student in the Department of Molecular and Translational Medicine at the University of Brescia where she formerly earned her biotechnology degree. She worked at Laboratory of Medical Genetics, at ASST Spedali Civili of Brescia in a project focused on kidney diseases and she spent one year as a postgraduate associate at Yale University, in the Department of Obstetrics, Gynecology and Reproductive Sciences. Currently she is working on an in-vitro and in-vivo model to study the pathogenetic mechanism underlying the Neurodegeneration with Brain Iron Accumulation (NBIA).

contact:
b.gnutti001@unibs.it

SESSION 4 BPAN

Is it all about autophagy? - WDR45's role in autophagy and beyond

Mario Mauthe

Department of Biomedical Sciences of Cells and Systems, University Medical Center Groningen, the Netherlands

The *WDR45* gene is localized on the X-chromosome and different variants in this gene are causative for the neurodegenerative disorder BPAN (ß-propeller protein associated neurodegeneration). WDR45/WIPI4 is a WD-repeat β-propeller protein that belongs to the WIPI (WD repeat domain, phosphoinositide interacting) family. The precise cellular function of WDR45 is still largely unknown, but deletions or variants in *WDR45* can lead to macroautophagy/autophagy defects, malfunctioning mitochondria, endoplasmic reticulum stress and unbalanced iron homeostasis, suggesting that this protein functions in one or more pathways regulating directly or indirectly those processes. Our laboratory is studying the molecular functions of WDR45, particularly in autophagy and mitochondrial homeostasis, using a variety of cellular systems.

Dr. Mario Mauthe is a senior scientist at the Department of Biomedical Sciences of Cells and Systems at the University Medical Center in Groningen, the Netherlands. He obtained his PhD at the University of Tue bingen, Germany on studying the role of WIPI1 and WIPI2 in the regulation of autophagy. Afterwards he joined the laboratory of Prof. Reggiori in the Netherlands. His research focus lies in the mechanism and regulation of autophagy under physiological and pathological conditions. He is specifically interested in the relevance of autophagy in neurodegenerative diseases.

contact:
m.mauthe@umcg.nl

Towards an understanding of the molecular function of WDR45/WIPI4 in autophagy and BPAN

Tassula Proikas-Cezanne

Department of Molecular Biology, Interfaculty Institute for Cell Biology,
University of Tuebingen, Germany

De novo mutations in the WDR45/WIPI4 gene are the cause of the human neurodegenerative disease BPAN (beta-propeller associated neurodegeneration). BPAN patients show impaired autophagy and high iron accumulation in the brain. Normally, intracellular iron levels are regulated by ferritinophagy. As WDR45/WIPI4 plays an important role in autophagic membrane formation, we have started to analyse its function in ferritinophagy in the pathophysiological context of BPAN and here we discuss our first results on this topic.

Prof. Dr. Tassula Proikas-Cezanne is an internationally recognized expert in autophagy research who originally discovered the human WIPI genes, including WDR45/WIPI4, that underlie BPAN. The research in her laboratory has helped advance the mechanistic understanding of autophagy initiation and regulation, including assigning a role for WDR45/WIPI4 as a signaling molecule in the energy control of autophagy, and the interaction of WDR45/WIPI4 with ATG2 as a control module in the autophagosome formation.

contact:
tassula.proikas-cezanne@uni-tuebingen.de

Vulnerability of midbrain dopaminergic neurons in BPAN

Lena Burbulla

Department of Metabolic Biochemistry, Biomedical Center Munich,

Ludwig-Maximilians-University Munich, Germany

Iron accumulation occurs in the human brain during healthy aging, but can also be a sign of underlying pathology. Neurodegeneration with Brain Iron Accumulation (NBIA), a heterogenous group of rare disorders, display aberrant iron build-up in the basal ganglia. The most common subtype, BPAN (Beta-propeller Protein-Associated Neurodegeneration), shows distinct iron overload in the substantia nigra (SN) along with preferential vulnerability and death of nigral dopamine (DA) neurons and early-onset parkinsonism. However, whether iron is a contributor to or consequence of pathogenesis, and how this relates to DA neuron loss in BPAN remains unknown.

Iron mediates the non-enzymatic oxidation of DA into toxic intermediates, precursors of neuromelanin (NM), a polymer forming in DA neurons of the SN. While the build-up of NM is considered neuroprotective, iron overload may saturate this process and promote neurotoxicity. We used induced pluripotent stem cell (iPSC) technology to generate midbrain-specific DA neurons from BPAN patient and healthy controls to investigate pathology at the crossroads of iron dyshomeostasis and DA neuron vulnerability. Our results demonstrate that BPAN patient iPSC-derived dopaminergic neurons display altered protein expression, most notably in regulators of iron and DA metabolism. Further, BPAN patient DAergic neurons show signs of disrupted DA metabolism resulting in accumulation of oxidized DA and enhanced formation of NM-like structures. Altogether, our findings suggest that elevated iron and impaired DA metabolism in BPAN patient neurons leads to robust build-up of toxic intermediates and NM.

Lena Burbulla serves as Heisenberg Professor for Metabolic Biochemistry of Neurodegenerative Diseases at the Biomedical Center, LMU Munich, since July 2021. She spent the past 9 years as postdoctoral research fellow and Research Assistant Professor at Harvard Medical School in Boston, and Northwestern University in Chicago, where she conducted compelling research on pathologic mechanisms in Parkinson's disease and NBIA disorders. Her lab is interested in the interactions between dopamine, iron, and oxidant stress with a focus on dysfunctional dopamine metabolism.

contact:
Lena.Burbulla@mail03.med.uni-muenchen.de

Characterization of a whole-body Wdr45 knock-out, a mouse model for BPAN

Arcangela Iuso

Institute of Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany

Pathogenic variants in the *WDR45* gene mapping on chromosome Xp11 lead to a rare neurodegenerative disease accompanied by iron deposition disorder in the basal ganglia (OMIM: 300526). The disease has been named Beta-propeller Protein-Associated Neurodegeneration (BPAN) after the WDR45 protein, which encodes a beta-propeller scaffold protein involved in autophagy. BPAN is one of the four most common forms of Neurodegeneration with Brain Iron Accumulation (NBIA).
In the current study, we generated and characterized a whole-body *Wdr45* knock-out (KO) mouse model. We used TALENs, to introduce a 20-bp deletion in exon 2 of *Wdr45* that resulted in a frameshift mutation introducing a premature stop codon after 35 amino acids from the initial methionine.
Both homozygous females and hemizygous male mice are viable, proving that systemic depletion of *Wdr45* does not impair viability or male fertility in mice. After four months of age, the KO mice showed the first signs of neuropathology, neurodegeneration that progressed with aging, hearing loss, and specific hematological changes. However, there was no iron accumulation in the brain. There was a significant decrease in CI activity in the brain of *Wdr45* KO mice, suggesting that mitochondrial dysfunction comes with Wdr45 deficiency. Finally, the systemic *Wdr45* KO mice model described here complements two previously described mouse models (PMIDs: 26000824, 31204559) and provides an additional robust model to investigate the pathophysiology and test therapeutic strategies for BPAN.

Arcangela Iuso is a scientist at the Helmholtz Center Munich and Technical University of Munich, where she fosters preclinical studies in the field of Neurodegeneration with Brain Iron Accumulation (NBIA) and metabolically-related disorders (PPCS and SLC25A42 deficiency).
Arcangela has successfully established cellular (fibroblasts, adipocytes, iPSCs, iPSC-derived neuronal cells) and animal models (fruit flies and mice) for these disorders to investigate the effect of pathogenic variants in cellular processes and exploit therapeutic approaches.
She is the scientific coordinator of the NBIA biobank (TUM, Munich).

email:
arcangela.iuso@helmholtz-muenchen.de

Manju Kurian

Great Ormond Street Institute of Child Health, University College London, United Kingdom

Towards precision therapies for NBIA

Neurodegeneration with brain iron accumulation (NBIA) comprise a group of progressive neurological disorders characterised by cognitive and motor deterioration, associated with the accumulation of excess iron in the brain, particularly within the basal ganglia. Although there are currently no disease-modifying therapies for any of the NBIA disorders, this group of diseases is highly amenable to personalised medicine approaches given that they are monogenic, early onset disorders, with a potential therapeutic window in some NBIA subtypes.
In this lecture, I will present an overview of the current pathophysiology underpinning the common NBIA subtypes and how this is driving precision therapy approaches, including a summary of the clinical trials undertaken so far in NBIA. I will also focus on the future of genetic therapies such as viral vector-based DNA therapies, RNA therapies and other gene editing strategies. Recent advances in the field give hope that these novel strategies will, in the future, be scientifically and financially feasible.

PLA2G6-associated neurodegeneration – update

PLA2G6-associated Neurodegeneration (PLAN) is a subtype of Neurodegeneration with brain iron accumulation (NBIA) caused by biallelic loss-of-function mutations in the *PLA2G6* gene. There is a continuum of clinical presentation, from the severe early onset form presenting in infancy or early childhood (infantile neuroaxonal dystrophy, INAD), to the more slowly progressive childhood form (atypical neuroaxonal dystrophy) and adult presentations of dystonia-parkinsonism. The condition is associated with significant morbidity and high risk of premature mortality and currently there are no disease-modifying treatments.

Over the last decade, there have been an number of therapeutic developments including the trial of a modified polyunsaturated fatty acid RT-001 (Retrotope), repurposing of the tricyclic antidepressant Desipramine and preclinical development of a viral vector-mediated gene therapy approach. With these advances, it is becoming increasingly necessary to be 'trial ready' for PLAN given that there are no readily available, robust disease biomarkers: as such I will discuss the development of key outcome measures that will be necessary for clinical trial. These include a (i) large scale natural history study to improve the understanding of the disease course in PLAN (ii) a PLAN disease-specific rating scale for scoring patients according to disease severity (iii) quantification of radiology changes over time and (iv) development of novel biomarkers that will facilitate early diagnosis and differentiate between early and late stages of disease. Such clinical, radiological and proteomic biomarkers will aid our future assessment of the efficacy of these novel therapies that are currently under development.

Professor Manju Kurian is a Professor of Neurogenetics and NIHR Research Professor at UCL-Great Ormond Street Institute of Child Health. She is also a consultant Paediatric Neurologist at Great Ormond Street Hospital. After graduating from Cambridge University, she trained in Paediatrics before subspecialising in Paediatric Neurology. At the end of her clinical training, she undertook a PhD (University of Birmingham) investigating the molecular genetic basis of childhood neurological disorders (2007-2011). She moved to UCL after her PhD, and has since established herself as an independent Principal Investigator at the Institute of Child Health. She was awarded a Wellcome Intermediate Fellowship in 2012, NIHR Professorship in 2017 and holds the The Jules Thorn Award for Biomedical Research (2019-2024).

contact:
manju.kurian@ucl.ac.uk

Developing gene therapy for PLAN and moving towards clinical trials

Ahad Rahim

Department of Pharmacology, University College London / UCL School of Pharmacy, London, United Kingdom

Gene therapy holds significant potential for the treatment of devastating monogenic neurodegenerative diseases. This has been demonstrated by the recent approval of gene therapy for another lethal neurological condition. The aim of this study was to evaluate the potential that gene therapy has for the treatment of INAD.

A knock-in mouse model of INAD was studied to assess its suitability for assessment of gene therapy. The model was shown to have clear hallmarks of pathology, locomotor decline and reduced survival that mimic the human form of the condition. We then constructed an adeno-associated viral (AAV) vector carrying a therapeutic copy of the *PLA2G6* gene. The AAV vector was administered to the mouse model of INAD and significant therapeutic benefits were observed. These included a significant increase in lifespan, locomotor function and neuronal survival in the central nervous system.

The data is supportive of clinical translation of gene therapy for INAD. This has provided the evidence to begin dialogues with clinicians and regulators to move towards clinical trials.

Ahad Rahim is Professor of Translational Neuroscience, the Wellcome Chair and Head of the Pharmacology Research Department at University College London. He completed an undergraduate degree in Genetics from Queen Mary University of London and a PhD in gene therapy form Imperial College London.
After postdoctoral research at the Institute of Cancer Research and UCL on viral vector mediated gene therapy, he was awarded a UCL Excellence Fellowship in 2013 to establish his independent laboratory. He was made lecturer in 2016, Associate Professor in 2018 and Professor in 2020. His laboratory works on the development and pre-clinical testing of various advanced treatments including gene, exosome and peptide therapies.

contact:
a.rahim@ucl.ac.uk

Moving forward with FAHN research

Sunita Venkateswaran

Division of Neurology, Department of Pediatrics, Children's Hospital of Eastern Ontario (CHEO), University of Ottawa, Canada

Fatty acid hydroxylase-associated neurodegeneration (FAHN) is an autosomal recessive progressive childhood onset neurodegenerative condition affecting multiple neurological systems. Children present with gait abnormalities in early childhood with rapid decline in ambulation, along with dysarthria, dysphagia, cognitive decline and visual abnormalities. The underlying function of *FA2H*, the gene responsible for FAHN is still unclear. This presentation will cover the current understanding of FAHN clinical and MRI disease progression. Understanding the stages of disease offers time points for potential intervention. In addition, there will be discussion on the international collaborative efforts being undertaken to better understand the biology of FAHN ranging from the structural biology, lipidomics and animal modeling to the creation of iPSCs and plans for future gene therapy studies.

Sunita Venkateswaran is an Associate Professor in the Division of Neurology, Department of Pediatrics at the Children's Hospital of Eastern Ontario. Her clinical and research interests and expertise are in rare neurological diseases, specifically leukodystrophies and NBIAs. She has developed a collaborative research network to study FAHN, a condition which intersects the fields of leukodystrophies and NBIAs. The team is working towards understanding the clinical progression and the underlying biology of FAHN in order to inform potential treatment options for this condition.

contact:
svenkateswaran@cheo.on.ca

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POSTERSESSION

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3	Özgür Öztop Çakmak	Two siblings with kufor-rakeb: a novel variant in the atp13a2 gen
4	Susan Hayflick / Penelope Hogarth	Abnormal brain iron accumulation is a rare finding in Down Syndrome Regression Disorder
5	Massimo Alessio	Tight-junctions derangement and blood-cerebrospinal fluid barrier alteration in aceruloplasminemia
6	Sunita Venkateswaran	Survey of NBIA Caregiver Perspectives to Identify Relevant QOL Outcomes for Caregivers/Patients
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7	Robert Spaull	Visual electrophysiology testing in PKAN: diagnostic utility and potential biomarker for clinical trials
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9	Puneet Rai	Lessons learned from running a remote clinical trial
10	Suh Young Jeong	A Blood Biomarker for PKAN: Coasy Expression Correlation between Blood and Brain in Pank2-/- Mice
11	Suh Young Jeong	Beyond the Coenzyme A Pathway: Metabolomics Analyses in Pank2-/-Mice Brain
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17	Kenta Shiina	Loss of MPAN-associated C19orf12 causes alteration of lipid metabolism and autophagosome-lysosome trafficking in Drosophila
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19	Rachel Wise	BPAN patient-specific iPSC-derived dopaminergic neurons reveal altered iron metabolism and excessive dopamine oxidation
20	Apostolos Papandreou	The spectrum of neuroradiological findings in early stages of Beta Propeller Protein-Associated Neurodegeneration (BPAN)
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22	Anna Ardissone	A novel WDR45 variant in an encephalopathy mimicking Leigh syndrome
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24	Audrey Ker Shin Soo	Accelerating Clinical Trial Readiness for *PLA2G6*-Associated Neurodegeneration (PLAN)
25	Fatima Efendic	Patient-specific iPSCs for disease modeling in Fatty acid hydroxylase-associated neurodegeneration (FAHN)
26	Sunita Venkateswaran	The natural history of fatty acid hydroxylase-associated neurodegeneration (FAHN)

#1 Program for Neurodegeneration with Brain Iron Accumulation at OHSU

Presenter: Susan Hayflick / Penelope Hogarth

Allison Gregory [a], Puneet Rai [a], Randy Woltjer [b], Jenny L Wilson [c], Alison Freed [a], Dustin Le [a], Kira Anderson [a], Helena Loftus [a], Suh Young Jeong [a], Penelope Hogarth [d], Susan J Hayflick [e]

[a] Department of Molecular and Medical Genetics, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, L103, Portland, OR, 97239, USA, gregorya@ohsu.edu

[b] Pathology, Oregon Health & Science University

[c] Division of Pediatric Neurology, Oregon Health & Science University

[d] Departments of Molecular and Medical Genetics and Neurology, Oregon Health & Science University

[e] Departments of Molecular and Medical Genetics, Pediatrics and Neurology, Oregon Health & Science University

OHSU is home to a robust NBIA program with clinical and research arms spanning 30 years.
Current components include the following:

- Curation of largest and longest-standing international repository of NBIA clinical data and biosamples.
  - The NBIA registry currently contains >700 individuals with confirmed NBIA diagnoses and >200 healthy family members from over 40 countries.
  - The biosample repository includes a brain bank with >30 brains representing 4 NBIA disorders and several idiopathic cases; a primary cell repository with dozens of fibroblast and lymphoblast cell lines that cover 7 NBIA disorders and idiopathic cases; various biological fluid, DNA, and RNA samples from multiple disorders; and various other human tissues.
  - Biosamples linked to key clinical information are available to other investigators worldwide. In the past 3 years, samples and data have been shared 15 times with investigators in the U.S. and 4 European countries.
  - Imaging data with clinical information from >200 individuals suspected to have an NBIA disorder are also available for collaborative research

- OHSU NBIA Center of Excellence with deeply experienced team including a geneticist, movement disorders neurologist, neurodevelopmental pediatrician, and genetic counselors.
  - Provide care onsite at OHSU during 2+ hour clinical evaluations that also involve a research component
  - Remote physician-to-physician consultations, which may involve family members, address dystonic crisis, medication management, new diagnoses, etc. ~20-25 consults are done per year without any fee.
  - Direct support to families including those with a newly diagnosed member and those seeking gui dance on disease management.

- Clinical investigations, including long-term natural history studies of PKAN/BPAN/PLAN with >200 enrollees, a safety and tolerability clinical trial for CoA-Z in PKAN with >50 participants, and development of consensus best practices guidelines for PKAN/BPAN/PLAN.

- Multifaceted support for NBIA community through comprehensive informational website, participation in family meetings/events, and collaboration with NBIA foundations.

contact: Allison Gregory, gregorya@ohsu.edu

#2 A young woman with a peculiar gait

Presenter: Özgür Öztop Çakmak

Özgür Öztop Çakmak[1], Bülent Elibol[2], Sevda Erer Özbek [3], Nazlı Başak [4]

[1] Department of Neurology, School of Medicine, Koç University, Istanbul, Turkey

[2] Department of Neurology, School of Medicine, Hacettepe University, Ankara, Turkey

[3] Department of Neurology, School of Medicine, Uludağ University, Bursa, Turkey

[4] Suna and İnan Kıraç Foundation, Neurodegeneration Research Laboratory, KUTTAM, School of Medicine, Koç University, İstanbul, Turkey.

VAC14 is a scaffold protein encoded by the *VAC14* gene, playing role in intracellular vesicle transport of endo-lysosome pathway. Herein, we report a 25-year-old female patient with a *VAC14* related dystonia with a peculiar gait, who had been born to a consanguineous marriage. Her first symptoms, gait disorder five years earlier, followed by stiffness on her right side and developed difficulty speaking and swallowing over the past year. On her neurologic examination, slow and irregular hand movements on her left side and typical expression on her face with a slightly open jaw and right-sided-dystonia and mild parkinsonism was noted. MRI showed hypointense areas in the globus pallidus and substantia nigra, suggestive of iron accumulation. WES analysis revealed a novel homozygous variant in the *VAC14* gene. Sanger sequencing of her sisters showed the same variant and MRI findings were similar with the index patient. However, the clinical presentation was different in the siblings. The *VAC14* gene should be considered among genes related with neurodegeneration with brain iron accumulation.

contact: ooztop@ku.edu.tr

#3 Two siblings with kufor-rakeb: a novel variant in the atp13a2 gen

Presenter: Özgür Öztop Çakmak

Özgür Öztop Çakmak[1], İbrahim Kamacı [2], Nazlı Başak[3], Zuhal Yapıcı[2]

[1] Department of Neurology, School of Medicine, Koç University, Istanbul, Turkey

[2] Department of Neurology, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey

[3] Suna and İnan Kıraç Foundation, Neurodegeneration Research Laboratory, KUTTAM, School of Medicine, Koç University, İstanbul, Turkey

Kufor-Rakeb, also known as pallido-pyramidal syndrome, is characterised by young-onset dystonia-parkinsonism and caused by ATP13A2 variants. Herein, we report two siblings (23yo male; 13 yo girl) who had been born to a consanguinous marriage and whose symptoms began to surface by early adelosence. In both the siblings, dystonia and parkinsonism were mild at the onset of the disease. Although the syndrome progressed slowly in the elder sibling, the eventual picture was that of near-total disability without much response to various oral medications and deep brain stimulation. MRI scans of both the cases were unremarkable. Whole exome sequencing revealed a novel homozygous variant in the ATP13A2 gene in both.

contact: ooztop@ku.edu.tr

#4 Abnormal brain iron accumulation is a rare finding in Down Syndrome Regression Disorder

Presenter: Susan Hayflick / Penelope Hogarth

Allison Gregory [a], Jenny L Wilson [b], Penelope Hogarth [c], Susan J Hayflick [d]

[a] Department of Molecular and Medical Genetics, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, L103, Portland, OR, 97239, USA, gregorya@ohsu.edu (corresponding author)

[b] Division of Pediatric Neurology, Oregon Health & Science University, Portland, OR, USA

[c] Departments of Molecular and Medical Genetics and Neurology, Oregon Health & Science University, Portland, OR, USA

[d] Departments of Molecular and Medical Genetics, Pediatrics and Neurology, Oregon Health & Science University, Portland, OR, USA

Background: From 2015-2021, our Neurodegeneration with Brain Iron Accumulation (NBIA) Center of Excellence reviewed 3 cases of Down syndrome (DS) with magnetic resonance imaging (MRI) findings suspicious for abnormal brain iron. These patients had brain imaging after onset of new symptoms, such as depression, near mutism, tremor, incontinence, sleep disturbances, and others consistent with Down Syndrome Regression Disorder (DSRD). Characterized by the sudden loss of adaptive function, cognitive-executive function, and expressive language with development of sleep and motor disorders, abnormal brain iron accumulation has not previously been reported in DSRD.

Methods: Clinical, laboratory, MRI and computed tomography (CT) data from 3 individuals with DSRD and brain imaging suspicious for increased basal ganglia iron were reviewed by clinicians with expertise in NBIA.

Results: All 3 individuals had abnormal signal hypointensity on T2-weighted imaging, which appeared more hypointense on susceptibility sequences, indicative of iron accumulation in the globus pallidus (GP) and the substantia nigra. The degree of signal abnormality suggests mildly increased iron for age in all 3 cases. In 2 of the 3, calcification was also detected by CT in the central GP at relatively young ages in a pattern that did not entirely overlap with the region of increased iron. Molecular diagnostic testing for NBIA was negative in the 2 individuals tested.

Discussion: These cases suggest a subset of individuals with DSRD have abnormal brain iron accumulation. Motor control symptoms reported in DSRD, such as stereotypies and parkinsonism, may reflect this basal ganglia involvement. We propose that iron accumulation in DSRD cases may not be indicative of a second diagnosis of NBIA, but instead be a part of their disease process—a process that may share a common end pathway with the NBIA disorders. The presence of abnormal brain iron should not delay or preclude diagnosis and treatment for DSRD.

contact: Allison Gregory, gregorya@ohsu.edu

#5 Tight-junctions derangement and blood-cerebrospinal fluid barrier alteration in aceruloplasminemia

Presenter: Massimo Alessio

Alan Zanardi [a], Antonio Conti [a], Barbara Ferrini [a], Michela Milani [b], Alessio Cantore [b], Cristina Monterisi [c], Angela Coliva [c], Sara Belloli [c,d], Paolo Rainone [c,e], Rosa Maria Moresco [c,d,e], Massimo Alessio [a]

[a] Proteome Biochemistry, Center for Omics Sciences (COSR), IRCCS Ospedale San Raffaele, Milano (Italy)

[b] Liver gene therapy, Telethon Institute for Gene Therapy (TIGET), IRCCS Ospedale San Raffaele, Milano (Italy)

[c] Nuclear Medicine and PET Cyclotron Unit, IRCCS Ospedale San Raffaele, Milano (Italy)

[d] Institute of Molecular Bioimaging and Physiology (IBFM) of CNR, Segrate (MI) (Italy);

[e] Medicine and Surgery Department, University of Milano - Bicocca, Monza (MB) (Italy)

The ferroxidase enzyme ceruloplasmin (Cp) in brain is mainly produced by astrocytes and choroid plexus epithelial cells (CPEpiC). In aceruloplasminemia (Acp), lack of Cp leads to intracellular iron accumulation, causing systemic and neurological symptoms. Previously we reported iron accumulation in CPEpiC of CpKO mice that was reduced by Cp administration, which enters in the brain reducing neurodegeneration1. Since choroid plexus constitutes the blood-cerebrospinal fluid barrier (BCSFB), we investigated the alterations that absence of Cp and iron accumulation produce in CPEpiC barrier properties, which could have consequence on brain physiology.

We generated a CpKO model of CPEpiC line that was loaded with iron mimicking Acp conditions. Barrier properties were analyzed using transwell system, while western blot and fluorescence microscopy were used to visualize the expression and organization of tight- and adherens-junctions' components. Brain barrier permeability was also analyzed in vivo in CpKO and WT mice evaluating by PET the distribution of administered [64]Cu-labelled Cp.

Iron overload promoted larger iron accumulation in CpKO cells compared to WT, altering the expression of iron homeostasis-related proteins. The barrier permeability was increased in CpKO cells upon iron treatment, with reduced expression of ZO1 and E-Cadherin, markers of tight- and adherens-junctions, respectively, and with altered barrier organization as inferred by staining for ZO1 and E-Cadherin molecules. Biodistribution analysis showed higher [64]Cu-Cp signal in the brain of CpKO mice compared to WT, confirming that barrier leakage is present *in vivo*.

These results indicated that iron overload induces BCSFB morphology alteration, which in turn is responsible for increased barrier permeability observed in CpKO cells and mice. This suggests that BCSFB leakage might be considered a new pathological feature in Acp.

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[1] Zanardi, A.; Conti, A.; Cremonesi, M.; D'Adamo, P.; Gilberti, E.; Apostoli, P.; Cannistraci, C. V.; Piperno, A.; David, S.; Alessio, M. *EMBO Mol Med* 2018, 10 (1), 91–106.

contact: alessio.massimo@hsr.it

#6 Survey of NBIA Caregiver Perspectives to Identify Relevant QOL Outcomes for Caregivers/Patients

Presenter: Sunita Venkateswaran

Dr. Brittany Paul [1], Janani Ramamurthy [1], Madhura Thipse [2], Richard Webster [2], Deepti Reddy [2], Dr. Sunita Venkateswaran [1,2,3]

[1] University of Ottawa Medical School, 451 Smyth Rd #2044, Ottawa, ON K1H 8M5

[2] Children's Hospital of Eastern Ontario Research Institute, 401 Smyth Rd, Ottawa, ON K1H 8L1

[3] Children's Hospital of Eastern Ontario Department of Neurology, 401 Smyth Rd, Ottawa, ON K1H 8L1

Neurodegeneration with brain iron accumulation (NBIA) is a rare group of disorders characterized by abnormal accumulation of iron in the basal ganglia manifesting clinically as a progressive neurological disorder. This is the first survey of NBIA caregiver perspectives to identify factors related to their child's health that are impacting caregiver well-being and quality of life. In this survey we assess how caregiver well-being is impacted by the child's NBIA diagnosis, symptoms and their management, treatment decisions, care accessibility, and support and information availability. The digital platform for rare diseases, Rareconnect, will facilitate the distribution of the survey to Canadian NBIA caregivers. The results of the survey will allow the development of NBIA management guidelines for physicians and structure NBIA family conferences. We will present the process of developing this survey for this vulnerable group of caregivers involving the CHEO Research Family Leader Program.

contact: Janani Ramamurthy, jrama023@uottawa.ca

#7 Visual electrophysiology testing in PKAN: diagnostic utility and potential biomarker for clinical trials

Presenter: Robert Spaull

Dr Robert Spaull [1,2], Dr Audrey KS Soo [1,2], Dr Apostolos Papandreou [1,2], Professor Allison Gregory [3], Professor Penelope Hogarth [3], Professor Susan J Hayflick [3], Professor Manju A Kurian [1,2], Dr Dorothy A Thompson [4],

[1] Molecular Neurosciences, Developmental Neurosciences, Zayed Centre for Research into Rare Disease in Children, UCL Great Ormond Street Institute of Child Health, UCL, London, United Kingdom.

[2] Department of Neurology, Great Ormond Street Hospital for Children, London, United Kingdom.

[3] Departments of Molecular & Medical Genetics and Neurology, Oregon Health & Science University, Portland, Oregon, USA.

[4] Tony Kriss Visual Electrophysiology Unit, Clinical and Academic Department of Ophthalmology, Great Ormond Hospital for Children, London, United Kingdom

Research Project Goals

(1) Assess electroretinography (ERG) and visual electrophysiology (VEP) in suspected NBIA.
(2) Assess progressive retinopathy in pantothenate kinase-associated neurodegeneration (PKAN).

Background

Many NBIA disorders are associated with visual dysfunction. PKAN is a form of NBIA associated with pigmentary retinopathy and retinal degeneration, which can be detected early in the disease, causing nyctalopia and eventual blindness. We wished to better define visual electrophysiology abnormalities in a cohort of patients with suspected/confirmed NBIA.

Methods

Clinical records for children presenting at a single tertiary centre were reviewed. Awake ERG and VEP were recorded using established techniques.

Results

41 children (23 female) with suspected/confirmed NBIA had visual electrophysiology testing from 2005-2022. 32/41 were performed at a single institution. Median age at assessment was 7.2 years (range 1-17 years).

ERG was abnormal in 11/34, including all ten patients with PKAN. 23/34 patients with normal ERG had a non-PKAN NBIA disorder [8 PLAN, 2 MPAN, 1 BPAN], or other disorders with radiological suggestion of excess brain iron (alternative genetic diagnosis in 7/23, 5/23 undiagnosed).
Combining our data with published cohorts (by Egan et al., PMID: 16023068, and Jesus-Ribeiro et al., PMID: 28487376), we observe that ERG changes are more severe in classical vs atypical PKAN, and that severity correlates with earlier disease onset (R=-0.54, p=0.0013).

Relevance and anticipated impact

Visual electrophysiology testing confirms the reported clear dichotomy of photoreceptor disease in PKAN compared to retinal ganglion cell or retinal nerve fibre loss in other NBIA disorders. This highly specific investigation may be used to aid diagnosis if the MRI brain is not typical or PANK2 variants are of uncertain significance. In PKAN, ERG changes are measurable and correlate with disease severity, providing an opportunity for use in disease monitoring and as a non-invasive, objective disease biomarker in clinical trials.

Project runtime and funding: Retrospective study of clinical and published data. Future: inclusion as an outcome measure in UK PKAN clinical trial, funded by grants from the NIHR BRC, Great Ormond Street Hospital Children's Charity, and LifeArc.

contact: r.spaull@ucl.ac.uk

#8 Skeletal Injuries in Pantothenate Kinase-Associated Degeneration (PKAN): A Challenging Problem

Presenter: Susan Hayflick / Penelope Hogarth

Laken Berndt [a], Allison Gregory [b], Katrina Wakeman [b], Alison Freed [b], Penelope Hogarth [c], Susan J Hayflick [d], Scott Yang [a]

[a] Department of Orthopedics and Rehabilitation, Doernbecher Children's Hospital and Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, L103, Portland, OR, 97239, USA

[b] Department of Molecular and Medical Genetics, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, L103, Portland, OR, 97239, USA

[c] Departments of Molecular and Medical Genetics and Neurology, Oregon Health & Science University, Portland, OR, USA

[d] Departments of Molecular and Medical Genetics, Pediatrics and Neurology, Oregon Health & Science University, Portland, OR, USA

Background: The most classic symptom of Pantothenate Kinase-Associated Neurodegeneration (PKAN) is progressive dystonia, often presenting in early childhood, with refractory episodes in later disease. This poses an increased risk of atraumatic fracture and dislocations. Though long-bone injury and fracture are known risks of progressive dystonia, there is a paucity of literature on the orthopedic manifestations of PKAN and the effect of progression on surgical outcomes.

Methods: A retrospective case review was conducted on five pediatric and adolescent patients (ages 10-20 years) with classic PKAN, who presented with an orthopedic injury requiring surgical intervention. Data regarding demographics, injury mechanisms, radiographs, surgical treatments, complications and outcomes were collected from the electronic health record.

Results: All patients were non-ambulatory, and all patients had a non-traumatic mechanism of injury, with four of five patients sustaining injury secondary to dystonic activity. Three of these four had dystonia that was reportedly worsened or prolonged from previous episodes. Four of the five patients sustained femoral shaft fractures; one had an additional acetabular fracture. The fifth patient presented with a hip dislocation. Post-operatively, three of the five patients sustained orthopaedic complications requiring surgical revision, two secondary to recurrent dystonia. Overall, four required prolonged hospitalization in the setting of refractory dystonia, with a mean length of stay around 9 weeks. The only patient without refractory dystonia had a total inpatient stay of 4 days.

Discussion: The presentation and complications of atraumatic skeletal injury in PKAN present distinct challenges for successful outcomes. Refractory dystonia during the postoperative period can be more difficult to control and severely complicates care. A multi-disciplinary approach is recommended in caring for skeletal injuries in PKAN, as precise pharmacological management and sedation are critical to controlling post-operative dystonia and preventing subsequent injuries or complications.

contact: Allison Gregory, gregorya@ohsu.edu

#9 Lessons learned from running a remote clinical trial

Presenter: Puneet Rai

Puneet Rai a, Allison Gregory [a], Alison Freed [a], Katrina Wakeman [a], Suh Young Jeong [a], Susan J Hayflick [b], Penelope Hogarth [c]

[a] Department of Molecular and Medical Genetics, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, L103, Portland, OR, 97239, USA, rai@ohsu.edu

[b] Departments of Molecular and Medical Genetics, Pediatrics and Neurology, Oregon Health & Science University

[c] Departments of Molecular and Medical Genetics and Neurology, Oregon Health & Science University

We developed a novel approach to the conduct of an interventional clinical trial in PKAN, designing a protocol that allowed participants to complete the entire study within their home communities rather than travelling to a study site, with the primary goal of reducing risks and burdens of trial participation in a fragile population; however, the design proved exceptionally resilient in the face of a global pandemic, an unprecedented event that resulted in delays and shutdown of many clinical trials. The 2-year study was divided into a 6-month placebo-controlled double-blind phase and an 18-month open-label phase. We screened and consented participants over the phone, obtaining digital signatures on the consent documents during the call. We shipped study product or placebo to participants' homes after they watched a required educational video about study product handling and dosing within the trial database platform. Participants completed phone visits with study staff, and blood draws at local laboratories. They kept dosing diaries, and shipped empty or unfinished product back to our team.

Benefits realized by this novel approach included reduced travel burden and thus risk to participants; lower trial costs; and limited impact of COVID-19 restrictions. In addition, enrollment proceeded much faster than anticipated based on the experience in other rare disease trials, allowing us to add a "direct-to-open-label" cohort after the primary cohort enrollment target was achieved. Challenges included inconsistency between local labs in research biomarker blood draws early in the trial, and compliance issues for some participants with some aspects of the trial. In addition, we had to adapt our approach to accommodate participants without access to modern technology, such as the Amish population. Despite all the challenges, study participants averaged a visit schedule compliance rate of 97.8% over the course of the study, both pre- and post-pandemic.

contact: rai@ohsu.edu

#10 A Blood Biomarker for PKAN: *Coasy* Expression Correlation between Blood and Brain in *Pank2*$^{-/-}$ Mice

Presenter: Suh Young Jeong

Suh Young Jeong, Helena Loftus, Dolly Zhen, Susan J. Hayflick

Department of Molecular & Medical Genetics, Oregon Health & Science University, Portland, OR, USA

PANK2, the disease gene for pantothenate kinase-associated neurodegeneration (PKAN), is known to be involved in coenzyme A (CoA) metabolism. Previously, we have reported that mRNA levels of two enzymes (*PPCS*/*Ppcs* and *COASY*/*Coasy*) in this pathway were significantly decreased under *PANK2*/*Pank2* mutations in both mouse globus pallidus (GP) and in primary PKAN patient cells. We also reported that *COASY*/*Coasy* gene expression and protein levels were corrected by the end of the treatment periods with 4'-phosphopantetheine (4'PPT) both *in vivo* and *in vitro*.

In this study, we took this question further and assessed kinetics of *Coasy* gene expression recovery in *Pank2*$^{-/-}$ mouse blood and GP. Both WT and KO mice were treated with either vehicle or 4'PPT and sacrificed at days 0,1,2,3,4,5,7, and 14 post-treatment. At each time point, both blood and brain were collected and analyzed for *Coasy* expression. Both tissues showed peak *Coasy* expression at days 1-2 post-treatment. However, *Coasy* expression in GP area was normalized and remained at the WT level from day 2 until the end of experiment (day 14). In contrast, blood *Coasy* expression showed greater variation day-to-day but eventually equilibrated at the WT level at day 14.

These data demonstrate that the Coasy expression patterns in blood and brain differ in response to 4'PPT oral treatment. This difference could be due to the relatively rapid turnover rate of blood cells vs. cells in brain. Regardless, it is reassuring that the desired target area for PKAN treatment, GP, showed an unwavering level of *Coasy* expression recovery only two days post-treatment.

This work was supported by R01 awarded to SJH (1R01NS109083-01)

contact: jeonsu@ohsu.edu

#11 Beyond the Coenzyme A Pathway: Metabolomics Analyses in *Pank2*$^{-/-}$ Mice Brain

Presenter: Suh Young Jeong

Suh Young Jeong, Helena Loftus, Susan J. Hayflick

Department of Molecular & Medical Genetics, Oregon Health & Science University, Portland, OR, USA

Pantothenate kinase-associated neurodegeneration (PKAN) is a devastating neurodegenerative disease caused by mutations in *PANK2* gene. PANK2 is the only mitochondrially located pantothenate kinase in humans, and is known to be involved in coenzyme A (CoA) metabolism. CoA is a key cofactor in cell survival, as it is involved in more than 70 pathways. Therefore, a defect in CoA metabolism is likely to cause global metabolic changes in cells. Moreover, among the pantothenate kinase isozymes, *PANK2* is the only one associated with disease. The unique localization of PANK2 and normal CoA levels in mutant tissues raises the question of whether it plays another role, other than simply as a kinase for pantothenate.

To understand metabolic changes caused by loss of *Pank2 in vivo*, we performed two rounds of unlabeled, unbiased metabolomic analyses using CE-FTMS (Capillary Electrophoresis Fourier Transform Mass Spectrometry). The globus pallidus (GP)-enriched mouse brain was dissected and ~2000 metabolites were analyzed. These metabolites were then subjected to pathway analysis to find affected cellular pathways. In the second round, we also included both WT and KO mice treated with 4'-phosphopantetheine (4'PPT) and corresponding controls.

In this study, we found 1) age-related differences in the *Pank2*$^{-/-}$ mouse brain, 2) perturbed neurotransmitter pathways in young and old animals, and 3) normalization of key metabolic changes in the *Pank2*$^{-/-}$ mouse brain by oral treatment with 4'PPT. These data corroborate and extend earlier work showing that 4'PPT directly affects the brain by crossing the blood-brain barrier or indirectly changes brain metabolism by a peripheral action. Insights from neurotransmitter abnormalities in the mouse brain may guide studies of human brain.

contact: jeonsu@ohsu.edu

#12 Endosomal trafficking in PKAN and COPAN hiPS-derived astrocytes

Presenter: Sonia Levi

Maddalena Ripamonti [1,2], Paolo Santambrogio [2], Gabriella Racchetti [3], Anna Cozzi [2] , Ivano Di Meo [4], Valeria Tiranti [4], Sonia Levi [1, 2]

[1] Vita-Salute San Raffaele University, Milan Italy

[2] Proteomics of Iron Metabolism Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

[3] Psychiatry and Clinical Psychobiology, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

[4] Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

GOALS

To define the pathogenic mechanisms triggered by iron/CoA dysregulation in PKAN and CoPAN and provide evidence of the efficacy of specific drugs as therapeutic options. This will clarify pivotal pathways leading to early cellular changes during disease development.

BACKGROUND

PANK2 and COASY-associated neurodegeneration (PKAN and CoPAN) are caused by mutations in genes that codify for key enzymes on Coenzyme A (CoA) biosynthetic chain reactions (first and last two step respectively). Both diseases are characterized by progressive neurodegeneration and excessive iron deposition in the brain. Several models have been developed trying to clarify the molecular events at the basis of these disorders, these models showed various phenotypes common to human but lack iron accumulation in brain. We developed and exploited a cellular model based on human IPS-derived astrocytes from PKAN and CoPAN patients, that showed iron accumulation during in vitro aging and studied their endosomal trafficking. We exploited fluorescent transferrin (Tf) to investigate iron intake via Transferrin Receptor1 (TfR1) mediated endocytosis, and the activity-enriching vesicular biosensor SynaptoZip to estimate the activity and intracellular fate of individual endosomes. Obtained data suggest a general impairment of the constitutive endosomal trafficking and exo-endocytic processes. CoA and 4-PBA (4-phenylbutyric acid) treatments partially rescue aberrant vesicular behaviours and iron uptake. Results are suggestive of aberrant endosomal trafficking in PKAN and CoPAN endosomes, that can be due to intrinsic properties of membranes but also to alterations in their intracellular interactions.

RELEVANCE and IMPACT

CoA deficiency could interfere with pivotal intracellular mechanisms involved in membrane fusions and constitutive vesicular trafficking, leading to an aberrant transferrin receptor-mediated iron uptake and subsequent deadly iron overload. With the use of the astrocyte model, we can dissect intracellular aspects of iron overburden in CoA deficient cells. This will help to disclose pathways that induce iron accumulation and pinpoint potential molecules that inhibit iron accumulation.

FUNDING: Telethon Italy GGP22047.

contact: Maddalena Ripamonti, ripamonti.maddalena@hsr.it

#13 Deep brain stimulation in pediatric patients with Pantothenate Kinase-Associated Neurodegeneration (PKAN) syndrome: case series and perspectives

Presenter: Julie Bonheur

Bonheur Julie [1], Goetz Laurent [1], Doummar Diane [2], Hully Marie [3], Loussouarn Anna [4], Renaldo Florence [2], Altuzzara Cecilia [5], Charollais Aude [6], Darmency Véronique [7], Gignoux Laurence [8], Lagrue Emmanuelle [9], Dorfmüller Georg [1], d'Hardemare Vincent [1], Dorison Nathalie [1,2]

[1] Unité Dyspa, Neurochirurgie Pédiatrique, Hôpital Fondation Rothschild, Paris;
[2] Neuropédiatrie, Hôpital Armand Trousseau, Paris;
[3] Neuropédiatrie, Hôpital Necker, Paris;
[4] Neuropédiatrie, Hôpital Robert Debré, Paris;
[5] Service de Pédiatrie, CHU Besançon;
[6] Service de Pédiatrie, CHU Rouen;
[7] Service de Pédiatrie, CHU Dijon Bourgogne;
[8] Service de Neurologie, CHU Lyon;
[9] Service de Pédiatrie, CHU Tours;

Introduction
Pantothenate-Kinase-2 associated neurodegeneration is a complex genetic disorder with brain iron accumulation leading to progressive and severe generalized dystonia. No curative treatment has been proposed to date. Deep brain stimulation (DBS) of the internal globus pallidus (GPi) can bring a partial improvement of their dystonia. We present our cohort of 7 PKAN pediatric patients who underwent DBS in our center.

Patients
Seven patients (mean age at surgery 7.4 years; Mean disease duration before surgery: 3.1 years). DBS surgery was performed under general anesthesia, with per-operative electrophysiological and imaging (CT-scan) control. Target was the GPI in all patients. In the last 3 patients, additional target was the subthalamic nucleus (STN). Clinical evaluation was performed using the Burke-Fahn-Marsden (BFM), neurobehavioral, cognitive and quality of life (QoL) scales every 3 months.

Results
At 6 months post-op, 5 patients on 7 had a decrease in the BFMRDS-m suggesting a better clinical condition. Two patients worsened in the immediate post-operative period but slowly stabilized with benefit on pain and dystonic storms. At 18 months FU (4 patients), all scores (BFMRDS-m) increased but very slowly in 2 patients. Some functional gains have endured. The benefits on pain and dystonic storm remained at 18 months FU allowing to preserve QoL for months or even a few years. Additional STN stimulation was effective in 1 patient who had a severe deterioration of his clinical condition pre and post-operatively.

Discussion
DBS seems to slow down the clinical progression of the pathology and to prevent (to some extent) motor complications. From our experience, Multi-target electrode implantation (GPi+STN) and a shorter disease duration at surgery seem to be beneficial to control the symptoms (pain, motor function and dystonic storm). Further studies will allow to define the best DBS surgical strategy to improve the clinical condition of the PKAN pediatric patients.

contact: jbonheur@for.paris

#14 Pantothenate kinase-associated neurodegeneration (PKAN): Cross-sectional data analysis of the TIRCON international patient registry

Presenter: Ivan Karin

Karin Ivan [1], Büchner Boriana [1], Iankova Vassilena [1], Klopstock Thomas [1,2,3]

[1] Department of Neurology, Friedrich-Baur-Institute, University of Munich, Ziemssenstr. 1, 80336, Munich, Germany.

[2] Munich Cluster for Systems Neurology (SyNergy), 80336, Munich, Germany.

[3] German Center for Neurodegenerative Diseases (DZNE), 80336, Munich, Germany.

Introduction
NBIA is a group of rare genetic disorders characterized by progressive dystonia and pathologically increased iron levels in the brain. PKAN is one of the most common NBIA subtypes. The TIRCON project has been formed as an international consortium to further investigate pathophysiological mechanisms and therapeutic options in NBIA. The international patient registry was a fundamental part of TIRCON, and is still fully functional for recruitment of patients.

Methods
The TIRCON registry has been designed as a multicenter, prospective, both cross-sectional and longitudinal study. Besides standard demographic characteristics, we also recorded results of clinical examination, MRI, laboratory findings, genetic testing, concurrent medication, non-pharmacological therapies, family history, and others. Several scales have been used to assess motor function, activities of daily living, and quality of life. Data were analyzed using methods of descriptive statistics.

Results
Out of a total of 437 patients, 113 had classic PKAN (cPKAN) onset (< 6 years) and 124 had atypical PKAN (aPKAN) with a later onset. Mean age of onset (and range) in classic PKAN was 2.3 (±1.7) years, and in atypical PKAN 13.4 (±6.5) years. BAD scores (Barry-Albright-Dystonia-Scale) at baseline were 18,9 for cPKAN (with 10,8 years into disease progression at first visit), and 15,6 for aPKAN (with 12,2 years into disease progression at first visit). The yearly progression rate (from baseline) was 2,8 points on the BAD scale for cPKAN, and 1,5 for aPKAN.

Conclusion
Here we present first cross-sectional and longitudinal data for PKAN patients from the TIRCON registry. In the future, the registry will be of great benefit to retrieve quantitative data on the longitudinal course of NBIA disorders, thus helping to better understand disease progression.

Funding: TIRCON has been funded by the European Commission (FP7/2007-2013, HEALTHF2-2011; grant agreement No. 277984, TIRCON) from 2011 to 2015, and sustained through donations from NBIA Alliance (and its members), and from pharmaceutical companies.

contact: Ivan.Karin@med.uni-muenchen.de

#15 COASY protein-associated neurodegeneration: expanding the clinical and molecular phenotype

Presenter: Chiara Cavestro

Chiara Cavestro [1], Francesca Morra [1], Chiara Frascarelli [1], Andrea Legati [1], Eulàlia Turón-Viñas [2], Magalie Barth [3], Arcangela Iuso [4], Susan Hayflick [5], Valeria Tiranti [1], Ivano Di Meo [1]

[1] Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy.

[2] Child Neurology Unit, Pediatrics department, Hospital Sant Pau, Barcelona, Spain.

[3] Department of Genetics and National Reference Center for Mitochondrial Disorders, University Hospital of Angers, Angers, France.

[4] Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany; Institute of Human Genetics, Technical University of Munich, School of Medicine, Munich, Germany.

[5] Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR, USA.

COASY protein-associated neurodegeneration (CoPAN) is a very rare, autosomal recessive form of NBIA caused by mutations in COASY. This gene encodes for the CoA synthase, a bifunctional enzyme that catalyzes the last two steps of cellular coenzyme A (CoA) biosynthesis. So far, only five patients have been identified worldwide, sharing with PKAN similar features, such as early onset dystonia, parkinsonian traits, cognitive impairment, and brain iron accumulation. More recently, mutations of COASY associated with the complete absence of the protein were reported in cases of pontocerebellar hypoplasia, microcephaly, and arthrogryposis with an invariable perinatal lethal phenotype, probably due to the complete absence of CoA. We present five new CoPAN cases carrying 4 novel COASY mutations from four unrelated families. While one of them displays a classical NBIA phenotype, the remaining four patients presented atypical clinical features, such as deafness, language and autism spectrum disorder, multifocal epilepsy, microcephaly, and pontocerebellar hypoplasia. To gain new insights into the pathogenesis of the disease, we conducted unbiased RNA sequencing to profile the transcriptomes of these novel fibroblast cell lines of the two patients described initially with COASY mutations and of three healthy controls. Bioinformatic analyses revealed, among others, significantly upregulated expression of genes encoding for ribosomal components, cytoplasmic protein translation, and protein localization to organelles in CoPAN fibroblasts. Conversely, we observed decreased expression of genes encoding accessory factors and enzymes involved in lysosomal functions, vesicular trafficking, exocytosis, and response to toxic species. Our findings expand the clinical phenotype associated with COASY mutations and suggest cellular pathways and pathway synergies that could have a role in CoPAN pathogenesis.

contact: chiara.cavestro@istituto-besta.it

#16 PPAR gamma agonist leriglitazone recovers alterations due to Pank2-deficiency in iPS-derived astrocytes.

Presenter: Sonia Levi

Santambrogio P., Di Meo I., Cavestro C., Vergara C, Rodríguez-Pascau L, Pizcueta P, Tiranti V. and Levi S.

Goals: To verify the efficacy of leriglitazone in recovering the pathological alterations existing in PKAN iPS-derived astrocytes (d-astrocytes) in comparison with CoA.

Background: Leriglitazone is an agonist of Peroxisome Proliferator-Activated Receptor Gamma (PPARγ), which is permeable through the BBB and previously validated on other neurodegenerative diseases. Leriglitazone is a small molecule which has activity of regulator of mitochondrial function and biogenesis, and anti-oxidation effects. Thus, we hypothesised that it can be useful in ameliorating the mitochondrial defects characterizing the PKAN d-astrocytes. This model was characterized, in addition to mitochondrial dysfunction, also by cytosolic iron deposition, oxidative stress and neurotoxic behaviour. We monitored the effect of leriglitazone in comparison with CoA on d-astrocytes from three healthy subjects and three PKAN patients. iPS-derived astrocytes were differentiated in the presence or not of the optimal concentration of leriglitazone (100nM) or CoA (25μM) for more than 45 days. We noted that the treatment with leriglitazone did not affect the differentiation of neuronal precursor in astrocytes, improved the viability of PKAN cells and oxygen consuming rate, while diminishing the iron accumulation similarly or even better than CoA.

Relevance and Impact: Until now, PKAN patients are treated with drugs that alleviate symptoms but no resolutive cure is available. Thus, it is crucial to find other therapeutic approaches and these data suggest that leriglitazone is well tolerated in neuronal cellular model and thus could be, eventually, be beneficial in the treatment of PKAN.

Funding: Service Publique de Wallonie-SPW. Telethon-Italia GGP 20047.

contact: levi.sonia@hsr.it

#17 Loss of MPAN-associated *C19orf12* causes alteration of lipid metabolism and autophagosome-lysosome trafficking in *Drosophila*

Presenter: Kenta Shiina

Kenta Shiina [1], Akio Mori [1], Yoshimi Miki [2], Makoto Murakami [2], Noriko Ueno [3], Yoshiki Miura [3], Taku Hatano [1], Yuzuru Imai [4], Nobutaka Hattori [1,5]

[1] Department of Neurology, Juntendo University School of Medicine, Japan.

[2] Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Japan.

[3] Laboratory of Proteomics and Biomolecular Science, Biomedical Research Core Facilities, Juntendo University Graduate School of Medicine, Japan.

[4] Department of Research for Parkinson's Disease, Juntendo University Graduate School of Medicine, Japan.

[5] Neurodegenerative Disorders Collaborative Laboratory, RIKEN Center for Brain Science, Japan.

Background: *C19orf12* mutations lead to mitochondria membrane protein-associated neurodegeneration (MPAN). In addition to iron accumulation, MPAN shows Lewy body pathologies in the brain, similar to Parkinson's disease. Although the exact role of C19orf12 is still unclear, our previous *Drosophila* study reported that overexpression of *C19orf12* rescued acyl-chain shortening in phospholipids by loss of another NBIA gene phospholipase A2 group VI (*PLA2G6*), suggesting that *C19orf12* is involved in lipid metabolism. Here, we investigated the changes in lipid metabolism and membrane trafficking by loss of *C19orf12* in *Drosophila*.

Methods: *C19orf12*-deficient fly line was generated by genome editing. Lipids from *C19orf12*-deficient fly heads were extracted by the Bligh and Dyer method, followed by the liquid chromatography-mass spectrometry. Lipid droplets, autophagosomes, and lysosomes in the fat body were observed with a transmission electron microscope or confocal laser scanning microscopy after fasting stress. Autophagy markers, and α-synuclein, were quantified with Western blot analysis. Transcriptomic profiles were also analyzed.

Results: In *C19orf12*-deficient fly brains, contrary to expectations, the phospholipid acyl chain length was not changed. On the other hand, phosphatidylethanolamine (PE) proportion increased in phospholipids. Some molecular species of hydroxy ceramide decreased while some triacylglycerols increased by *C19orf12* loss. Morphological observation revealed that lysosome-recruitment response was impaired, and lipid droplets remained large even after fasting stress. Abnormal autophagosome-like organelle was also observed. Supporting these changes, transcriptome analysis indicated decreased expression of lysosome membrane proteins, and Western blot analysis revealed the accumulation of autophagy markers. α-synuclein tended to accumulate in the brain.

Conclusions and future goals: We revealed that gene ablation of *C19orf12* led to the disruption of lipid metabolism and autophagosome-lysosome trafficking. Alteration of lipid metabolism has been suggested to cause α-synuclein aggregation. Our studies would contribute to understanding risk lipids and pathogenic molecular mechanisms for α-synuclein collection.

contact: k-shiina@juntendo.ac.jp

#18 Natural history study of Mitochondrial membrane protein-associated neurodegeneration (MPAN)

Presenter: Vassilena Iankova

Iankova Vassilena [1], Karin Ivan [1], Büchner Boriana [1], Klopstock Thomas [1,2,3]

[1] Department of Neurology With Friedrich-Baur-Institute, University Hospital of Ludwig-Maximilians-Universität München, Munich, Germany.

[2] German Center for Neurodegenerative Diseases (DZNE), Munich, Germany.

[3] Munich Cluster for Systems Neurology, Munich, Germany.

Background of the project: Mitochondrial membrane protein associated neurodegeneration (MPAN) is an NBIA disorder, caused by mutations in the *C19orf12* gene on Chromosome 19. In the framework of the TIRCON ("Treat Iron-Related Childhood-Onset Neurodegeneration") research consortium we investigated the natural history of the disease in a prospective, longitudinal multicenter study.

Goals of the research project: We aimed to further the understanding of the clinical course of the disease, identify suitable outcome measures and quantify the progression rate in an MPAN cohort thus providing a basis for future therapeutic trials.

Methods: Patients with genetically confirmed, autosomal recessive MPAN from five global cites were seen on a biannual or yearly basis. The primary endpoint of the study was the Unified Parkinson's Disease Rating Scale (UPDRS Parts I, II and III). Secondary endpoints were the Barry-Albright Dystonia (BAD) Scale and the The Schwab and England Activities of Daily Living scale (SEADL). For the analysis of annual disease progression linear mixed effect models with baseline scores used as a fixed main effect were performed.

Findings: Complete UPDRS assessments from at least two timepoints were available from 29 patients. Annual progression rate for the UPDRS total score was estimated to be 5.71 points (SE 1.45) per year since baseline in the overall cohort. Sex, consanguinity or age at onset had no significant effect on the progression rate. The BAD scale showed an annual progression rate of 0.08 points (SE 0.27), the SEADL scale showed annual decrease of 5.73 (SE 2.0).

Relevance and impact: To our knowledge this is the first prospective, longitudinal analysis of the clinical course of MPAN and the first attempt to quantify the progression rate of the disease using established clinical scales. Thus this project provides an important foundation for future therapeutic trials.

Funding: TIRCON has been funded by the European Commission (FP7/2007-2013, HEALTHF2-2011; grant agreement No. 277984, TIRCON) from 2011 to 2015, and sustained through donations from NBIA Alliance (and its members), and from pharmaceutical companies.

contact: Vassilena.Iankova@med.uni-muenchen.de

#19 BPAN patient-specific iPSC-derived dopaminergic neurons reveal altered iron metabolism and excessive dopamine oxidation

Presenter: Rachel Wise

Rachel M. Wise [1], Laura Strohm [2], Christian Behrends [2], Dimitri Krainc [4], Lena F. Burbulla [1,2,3*]

1 Metabolic Biochemistry, Biomedical Center, Ludwig Maximilian University Munich, Munich, Germany

2 Munich Cluster for Systems Neurology (SyNergy), Ludwig Maximilian University Munich, Munich, Germany

3 German Center for Neurodegenerative Diseases (DZNE), Munich, Germany

4 Ken & Ruth Davee Dept. of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Beta-propeller Protein-Associated Neurodegeneration (BPAN), a common subtype of Neurodegeneration with Brain Iron Accumulation (NBIA), displays unique pathological hallmarks including iron build-up in the substantia nigra, prominent degeneration of nigral dopamine neurons, and early-onset parkinsonism. However, the molecular mechanisms driving the selective loss of midbrain dopaminergic neurons, and what role iron dysregulation may play in this, remain significant gaps in knowledge in the field. To address these critical questions, we employed induced pluripotent stem cell (iPSC) technology to model BPAN pathology in patient-specific dopaminergic neurons, with a particular focus on iron homeostasis. Our results demonstrate that BPAN patient iPSC-derived dopaminergic neurons display altered protein expression, most notably in regulators of iron and dopamine metabolism. BPAN patient iPSC-derived dopaminergic neurons show signs of disrupted dopamine metabolism resulting in accumulation of oxidized dopamine. This oxidation is catalysed by iron and culminates in the formation of neuromelanin, a process typically considered neuroprotective due to the sequestration of potentially toxic metals and reactive compounds. However, excess iron and/or reactive intermediates of dopamine oxidation may saturate the neuromelanin system, eventually contributing to neurotoxicity. Altogether, our findings demonstrate disrupted iron homeostasis and dopamine metabolism in BPAN patient iPSC-derived dopaminergic neurons, providing evidence that these interrelated pathways may be associated with the unique vulnerability of this neuronal subset in BPAN pathophysiology. Iron dyshomeostasis and excessive oxidized dopamine has been demonstrated in postmortem tissue and iPSC-derived neurons from diverse Parkinson's disease patients, respectively. Thus, findings from this work may represent shared pathological mechanisms between BPAN and this common neurodegenerative disease. Future explorations into the intersection of these pathways may reveal promising new strategies for targeted protection of the highly vulnerable nigral dopaminergic neurons, potentially impacting patient populations well beyond BPAN and NBIA.

contact: Rachel.Wise@mail03.med.uni-muenchen.de

#20 The spectrum of neuroradiological findings in early stages of Beta Propeller Protein-Associated Neurodegeneration (BPAN)

Presenter: Apostolos Papandreou

Apostolos Papandreou [1,2], Audrey Soo [1,2], Robert Spaull [1,2], Kshitij Mankad [3], Manju A Kurian [1,2*], Sniya Sudhakar [3*]

[1] Molecular Neurosciences, Developmental Neurosciences Programme, Zayed Centre for Research into Rare Disease in Children, University College London Great Ormond Street Institute of Child Health, London, UK

[2] Department of Neurology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

[3] Department of Neuroradiology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

* These authors contributed equally

Background
Beta Propeller Protein-Associated Neurodegeneration (BPAN) is the most common Neurodegeneration with Brain Iron Accumulation disorder. Typical radiological findings are T2 Substantia Nigra and Globus Pallidus hypo-intensity, as well as a T1 halo-like SN hyper-intense signal surrounding a hypo-intense central area. However, these findings are often subtle or absent on initial scans, risking diagnostic delay. Should future disease-specific therapies emerge, the need for a prompt and accurate BPAN diagnosis will become even more pertinent.

Research Project Goals
In this study, we sought to investigate radiological findings that could aid early BPAN diagnosis.

Methods
A retrospective cohort study was performed in a UK national referral centre, including all paediatric patients with confirmed pathogenic *WDR45* mutations and consistent clinical semiology. Magnetic resonance imaging findings were independently reported by two paediatric neuroradiologists.

Results
Fifteen patients were included in the study and 27 scans were available for review. Initial neuroimaging study was undertaken at a mean age of 3.2 years. Iron deposition was uncommon in patients under 4 years of age. Neuroradiological features from very early on included dentate, globus pallidus and substantia nigra swelling, as well as thin corpus callosum and small pontine volume. Optic nerve thinning was also present in all patients.

Relevance and anticipated impact
Our study describes the largest single-centre paediatric neuroimaging study in BPAN. We report a distinct early radiological signature, which only infrequently includes iron deposition in the globus pallidus and substantia nigra under 4 years of age.
Within the appropriate clinical context, the neuroimaging findings described in this study should guide clinicians to actively suspect BPAN, even in the presence of *WDR45* genetic variants of uncertain significance. This, in turn, can facilitate prompt disease identification and implementation of appropriate multidisciplinary management.

Project runtime and funding

Retrospective cohort study, already completed. No particular funding allocated

contact: apostolos.papandreou@ucl.ac.uk

#21 Altered autophagy mechanisms associated with neurodegeneration: study of a cohort of patients with BPAN (Beta-propeller protein-associated neurodegeneration)

Presenter: Alejandra Darling

Leticia Pías Peleteiro, Mar O`Callaghan, Elena Martínez del Val, Otilia Martínez-Mugica Barbosa, Alberto García Oguiza, Marta Amengual Gual, Gema Aznar, Miguel Tomás, Jesica Espósito, Delia Yubero, Judith Armstrong, Alfonso De Oyarzabal Sanz, Angels García-Cazorla, Alejandra Darling

Hospital Sant Joan de Déu, Barcelona, Spain

Introduction:
β-helix protein-associated neurodegeneration (BPAN) is a monogenic defect associated to WDR45 that give us the opportunity to assess autophagy, a fundamental pathway with a critical role in the protein homeostasis maintenance.

Objective:
To describe the clinical, biochemical, radiological, and genetic profile of patients with genetically confirmed BPAN. Demonstrate defects in the autophagy pathway in BPAN patients.

Material and methods:
Observational study in a cohort of BPAN patients. Clinical, biochemical, and radiological data were assessed. Genetic studies of the WDR45 gene. Autophagy markers in fibroblasts were investigated using western blot techniques and immunofluorescence.

Results:
A total of 9 BPAN patients were assessed, 2/9 male, with ages ranging from 2.6-16 years. The main symptom at onset was global neurodevelopmental delay, also associated with seizures in relation to fever (n = 3), language regression (n = 2) and hypotonia (n = 1). The age of onset was less than 15 months in all cases. The course was variable, including different degrees of developmental delay/intellectual disability (9), epilepsy (7), and parkinsonian signs (2). Elevated liver transaminases were present in 6 cases. Brain MRI showed: T2 and SWI hypointense signal symmetrically in globus pallidus (n = 5) and delayed myelination (n=2). The patients presented de novo pathogenic variants in the WDR45 gene, with the exception of one hemizygous case, whose mother showed mosaicism for the variant investigated in oral mucosa DNA. CSF study showed low level of homovanilic acid in the elder patient. Fibroblasts showed alterations in all the autophagy marker that are compatible with a defect in the autophagy flux (LC3BI/II ratio, LAMP1 and p62).

Conclusions:
We describe a cohort of Spanish BPAN patients, including two male patients with a recognizable and particular phenotype. The data set of these patients contribute to explain that the dysfunction of the intracellular autophagy determines the developmental defect and the neuronal degeneration.

contact: alejandra.darling@sjd.es

#22 A novel WDR45 variant in an encephalopathy mimicking Leigh syndrome

Presenter: Anna Ardissone

A. Ardissone [1], E. Lamantea [2], A. Legati [2], C.Panteghini [2], M. Spagnolo [2], B. Garavaglia [2], V. Tiranti [2], G. Zorzi [1], D. Ghezzi [2]

[1] Child Neurology Unit - Department of Pediatric Neuroscience, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20100 Milan, Italy

[2] Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20100 Milan, Italy

BACKGROUND:

Mutations in *WDR45* result in the NBIA disorder known as β-propeller protein-associated neurodegeneration (BPAN). BPAN is characterized by early-onset global developmental delay, epilepsy, and ataxia and evolves to severe intellectual disability and cognitive deterioration, dystonia, and parkinsonism after the second decade of life. The majority of individuals with BPAN are female; however, some males have been reported. Brain MRI diagnostic markers include iron deposition in the substantia nigra and globus pallidus. Leigh syndrome is the most common pediatric mitochondrial disease defined by focal and/or bilateral symmetric lesions in the deep grey matter regions.

CASE REPORT:

We report a 6 year-old boy with early onset of psychomotor delay ataxia and movement disorder . Seizures were not observed. MRI performed at 18 months of age disclosed bilateral and symmetric hyperintensity of dentate nuclei, stable at last follow up (6 years), iron accumulation was not evident. Several diagnostic analyses resulted negative: enzyme activities for gangliosidosis, genetic analyses for PLA2G6 e PANK2, PARK2, array CGH. Mitochondrial disease was suspected: NARP/MILS mutation was negative, biopsy disclosed defects of complex I (residual activity 48% in muscle, 52% in cultured fibroblasts) and complex II (46% in muscle). Screening of nuclear genes related to Mitochondrial disease and mtDNA sequencing resulted negative. Exome sequencing revealed a de novo variant in WDR45 (c.749_751delCCT).

DISCUSSION:

Our case expands the phenotypic spectrum the number of allelic variants associated with *WDR45* defects. This condition should be considered in the differential diagnosis of neurodevelopmental disorders during early childhood in males and in absence of iron accumulation at MRI.

The correlation with biochemical defects disclosed on biopsy is unclear, but it is known that several pathways are involved in NBIA syndromes including mitochondrial dynamics and autophagy.

contact: anna.ardissone@gmail.com

#23 Ferrous iron is up-regulated and starvation-dependent in fibroblasts of patients with Beta Propeller Protein-Associated Neurodegeneration (BPAN)

Presenter: Rosaria Ingrassia

Rosaria Ingrassia [1], Maurizio Memo [1] and Barbara Garavaglia [2]

[1] Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia Brescia, Italy.

[2] Molecular Neurogenetics Unit, Foundation IRCCS Neurological Institute Carlo Besta Milan, Italy

De novo mutations in WDR45 gene, coding for a beta-propeller protein involved in autophagy, have been found in patients affected by Neurodegeneration with Brain Iron Accumulation, NBIA5 (also known as BPAN). BPAN is a movement disorder with Non Transferrin Bound Iron (NTBI) accumulation in the basal ganglia as common hallmark between NBIA classes (Hayflick et al., 2013). WDR45 has been predicted to have a role in autophagy, while the impairment of iron metabolism in the different NBIA subclasses has not currently been clarified. We found the up-regulation of the ferrous iron transporter (-)IRE/Divalent Metal Transporter1 (DMT1) and down-regulation of Transferrin receptor (TfR) in the fibroblasts of two BPAN affected patients with splicing mutations 235C1G>A (BPAN1) and 517_5191Val 173 (BPAN2). The BPAN patients showed a concomitant increase of intracellular ferrous iron after starvation.

Our results highlight an altered pattern of iron transporters, WDR45-dependent, in BPAN human fibroblasts, with the iron overload supporting for a role of DMT1 in NBIA. We here highlight a novel element to the existing knowledge in the field of NBIA about iron accumulation. We focus to the starvation-dependent aspect of iron overload, due to the WDR45-dependent impairment of autophagy, possibly accounting for iron accumulation in the basal ganglia. Further investigation could clarify iron regulation in NBIA/BPAN.

contact: rosaria.ingrassia@unibs.it

#24 Accelerating Clinical Trial Readiness for *PLA2G6*-Associated Neurodegeneration (PLAN)

Presenter: Audrey Ker Shin Soo

Audrey KS Soo [1,2], Robert Spaull [1,2], Kiran Seunarine [1], Katy Barwick [1], Kathleen Gorman [3], Apostolos Papandreou [1,2], Tian Luo [4], Yi Wang [4], Sangeetha Yoganathan [5], Maya Thomas [5], Belen Perez [6], Alejandra Darling [7], Evangeline Wassmer [8], Boriana Büchner [9], Thomas Klopstock [9], Giovanna Zorzi [10], Nardo Nardocci [10], Katrina Wakeman [11], Penny Hogarth [11], Allison Gregory [11], Susan Hayflick [11], Wendy Heywood [1], Chris Clark [1], Kevin Mills [1], Manju A Kurian [1,2] on behalf of the PLAN Research Consortium*

[1] University College London (UCL) Great Ormond Street-Institute of Child Health (GOS-ICH) Zayed Centre for Research into Rare Disease in Children, London, UK
[2] National Institute for Health and Care Research (NIHR) Great Ormond Street Hospital (GOSH) Biomedical Research Centre (BRC), London, UK
[3] Children's Health Ireland at Temple Street, Dublin, Ireland
[4] Children's Hospital of Fudan University, Shanghai, China
[5] Christian Medical College, Vellore, India
[6] Vall d'Hebron Research Institute, Barcelona, Spain
[7] Hospital Sant Joan de Déu, Barcelona, Spain
[8] Birmingham Children's Hospital, Birmingham, UK
[9] Friedrich-Baur-Institute, Dept of Neurology, LMU Hospital, Munich, Germany
[10] IRCCS Foundation Carlo Besta Neurological Institute, Milan, Italy
[11] Oregon Health and Science University, Oregon, USA
* Full list below abstract

Project runtime: 3.5 years ending 1st March 2023

Goals: To accelerate 'clinical trial readiness' through:
1) A large-scale international natural history study
2) Developing a disease-specific rating scale
3) Analysing neuroimaging to delineate the natural time-course of progressive changes
4) Developing robust biomarkers for disease monitoring

Background: There are currently no PLAN-specific biomarkers and limited knowledge on the natural progression of disease. Consequently, although precision medicines (including gene therapy) are in advanced stages of development, there are no robust, objective outcome measures for use in a clinical trial, making it difficult to objectively evaluate the efficacy of any proposed new treatment.

Results: We present the largest PLAN natural history study to date, with a cohort of >300 international cases. There are three distinct subtypes (INAD, aNAD and DP) with statistically significant differences in time to motor regression and Kaplan-Meier survival curves. We identified a pattern for the evolution of disease symptoms and genotype-phenotype correlations. All aNAD and DP patients learnt to walk independently, whereas most INAD patients (66%) did not and if they did, lost ambulation earlier than the aNAD group (median age INAD=2.8years, aNAD=8.7years, $p<0.01$). Qualitative and quantitative analysis of retrospective (n=100) and prospective longitudinal (n=10, 2 time points >12months apart) brain MRI scans allow for detailed description of radiological features and quantification of volumetric differences and iron accumulation over time. Following a Delphi process, a disease-specific rating scale (INAD-DRS) has been developed with good inter-rater reliability. Notably, INAD-DRS scores clearly correlate with age for INAD cases (r=0.76). Diseasespecific biomarkers have also been identified.

Relevance and Anticipated Impact: We present a comprehensive approach to fully characterising and quantifying PLAN disease progression through clinical findings, neuroimaging features and biomarkers. The findings from this project can be used to evaluate treatment efficacy in future precision therapy clinical trials.

Funding: NIHR GOSH BRC

* **PLAN Research Consortium**

- **Natural History Study Group:**
  - Australia: M Farrar, M Cardomone, S Mohamed, N Mahant
  - China: T Luo, Y Wang, Y Wu, TS Wang, X Chang
  - Egypt: H Megahed
  - Germany: T Klopstock, B Büchner, F Hillmer-Rudloff, J Edenfeld
  - India: M Thomas, S Yoganathan, A Hegde, T Nemani, S Kulkarni
  - Italy: G Zorzi, N Nardocci
  - Japan: Y Takiyama, K Hoshino, W Sato, J Takahashi
  - Malaysia: MK Thong, SK Tae, L Li
  - Spain: A Darling, B Perez
  - USA: S Hayflick, A Gregory, P Hogarth, K Wakeman, T Truong, G Evrony
  - UK: E Wassmer, A Parida, F Magrinelli, K Bhatia, S Mordekar, I Hughes, A Maw, K Pysden, G Chow, S Ramdas, M Smith, V Thiyagesh
  - Other: L Bok, T Cai, G Evrony, M Gurevith, A Jansen, M King, K Koop, D Lev, C Stumpel, S Suroviakova
- **Neuroimaging Study Group:** Chris Clark, Kiran Seunarine, Sniya Sudhakar, Ulrike Loebel, Jan Sedlacik, Asthik Biswas, Kshitij Mankad
- **Disease-Specific Rating Scale Study Group:** Belen Perez, Alejandra Darling, Maya Thomas, Roser Pons, Russell Dale, Margaret Kaminska, Dan Lumsden, Giovanna Zorzi, Nardo Nardocci, Penny Hogarth, Susan Hayflick
- **Biomarker Study Group:** Kevin Mills, Wendy Heywood, Alejandra Darling, Rafael Artuch

contact: a.soo@ucl.ac.uk

#25 Patient-specific iPSCs for disease modeling in Fatty acid hydroxylase-associated neurodegeneration (FAHN)

Presenter: Fatima Efendic

Fatima Efendic [a], Christin Völkner [a], Sunita Venkateswaran [b], Steffany L.A. Bennett [c], Andreas Hermann [a,d,e], Moritz J. Frech [a,d]

[a] Translational Neurodegeneration Section „Albrecht Kossel", Department of Neurology, University Medical Center Rostock, 18147 Rostock, Germany

[b] Division of Pediatric Neurology, Department of Pediatrics, Children's Hospital of Eastern Ontario, Ontario, Canada, Ottawa, ON, K1H 8L1

[c] Neural Regeneration Laboratory, Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, ON, Canada, K1H 8M5

[d] Center for Transdisciplinary Neurosciences Rostock (CTNR), University Medical Center Rostock, 18147 Rostock, Germany

[e] Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Rostock/Greifswald, 18147 Rostock, Germany

The Fatty acid hydroxylase-associated neurodegeneration (FAHN) is caused by mutations in the FA2H gene. FA2H catalyzes the hydroxylation of fatty acids, which are precursors for ceramide synthesis, which in turn plays an important role in the synthesis of sphingolipids and the production of myelin. Patients display abnormal myelination, cerebellar atrophy and some have iron deposition in the central nervous system. However, the pathophysiology of FAHN is poorly understood so far. A deeper understanding of the pathophysiological mechanisms would provide both, new clues to elucidate the neurodegeneration in FAHN, and further open up new possibilities for therapeutic approaches. The main objective of this project is to research and understand FAHN disease by studying FAHN in human cell models using induced pluripotent stem cells (iPSCs) derived from fibroblasts of different FAHN patients, as well as iPSCs carrying FAHN mutations inserted by CRISPR/Cas9 technology. Here we describe the generation of a human iPSC line derived from fibroblasts of a female patient carrying the compound heterozygous mutation p.Gly45Arg/c.p56A>G (c.133G>A/p.His319Arg). The generated iPSCs provide the basis to obtain neurons and glia cells, such as astrocytes and especially oligodendrocytes. In a final step we aim to establish an in vitro culture system comprising neurons and myelinating oligodendrocytes. This disease model of FAHN will allow us to systematically characterize the pathophysiology within neurons and oligodendrocytes and their interactions to gain a better understanding of the detailed pathophysiological mechanisms underlying FAHN-related demyelination and neurodegeneration.

The study was funded by the NBIA Disorders association. Fatima Efendic is funded by the Centre for Transdisciplinary Neurosciences Rostock (CTNR). Andreas Hermann is supported by the Hermann und Lilly Schilling-Stiftung für medizinische Forschung im Stifterverband. Christin Völkner was supported by a grant of the Landesgraduiertenförderung Mecklenburg-Vorpommern.
Project was started in January 2021.

contact: Fatima.Efendic@med.uni-rostock.de

#26 The natural history of fatty acid hydroxylase-associated neurodegeneration (FAHN)

Presenter: Sunita Venkateswaran

Sunita Venkateswaran [1], Madhura Thipse [2], Nicholas Barrowman [2], Elise Chan [2], Vid Bijelic [2], Cassandra Kapoor [2], Geneviève Bernard [3], Simon Edvardson [4], Amelia Nadeau [5], Filippo Santorelli [6], Samuel Shribman [7], Michèl Willemsen [8], David Dyment [2]

[1] Children's Hospital of Eastern Ontario (CHEO), Ottawa, Canada.
[2] Children's Hospital of Eastern Ontario Research Institute (CHEO RI), Ottawa, Canada.
[3] Departments of Neurology and Neurosurgery, Pediatrics and Human Genetics, McGill University, Montreal, Canada. Department Specialized Medicine.
[4] Hadassah University Hospital, Mount Scopus; Pediatric Neurology Unit, Hadassah-Hebrew University Medical Center; Monique and Jacques Roboh Department of Genetic Research, Hadassah-Hebrew University Medical Center, Israel
[5] Centre Hospitalier Universitaire de Sherbrooke, Division of Pediatric Neurology, QC, Canada.
[6] Molecular Medicine, IRCCS Fondazione, Stella Maris, Pisa, Italy.
[7] UCL Queen Square Institute of Neurology, London, UK.
[8] Radboud University Medical Center, Amalia Children's Hospital and Donders Institute for Brain, Cognition and Behavior, Department of Pediatric Neurology, Netherlands.

Objective: Fatty Acid Hydroxylase-Associated Neurodegeneration (FAHN) is an autosomal recessive childhood onset complex spastic-ataxic neurodegenerative disorder. A subset of patients demonstrate brain iron accumulation or leukodystrophy on neuroimaging. The detailed natural history of FAHN is unknown.

Methods: Patients with a genetic confirmation of FAHN were recruited via patient-led family organizations, and international collaborators.

When possible, patients were seen in clinic or via video for interview and examination. All other patients were entered into a standardized clinical reporting form by the collaborating neurologist/geneticist.

Results: Our study cohort included new patients and updated information from 36 patients, 6 previously reported but updated via our collaborators. There is no predilection for sex or ethnicity in this condition. All children were developmentally normal initially. Motor regression was the initial symptom in 31 (86%) of patients, affecting lower extremities first, at an average age of 4.7 (SD 3.4). This was followed by dysarthria in 28 (82%), at an average age of 7.3 (SD 3.1), dysphagia in 26 (77%), at an average age of 13.8 (SD 8.4), and cerebellar involvement in 26 (81%), at an average age of 6.9 (NA). Patients were non-ambulatory by the median age of 6.8 (95%CI 4.0 - 8.3) (figure1). Epilepsy was present in 13 (40.6%) patients. No extra-CNS symptoms were present in our patient cohort.

Conclusions: FAHN is complex spastic-ataxic syndrome with relentless motor degeneration with accompanying dysarthria, dysphagia, dystonia and cerebellar involvement. Understanding this natural history is paramount to allow for disease modifying therapies to be accurately and safely introduced. Further research is underway to follow the natural history prospectively and look for potential imaging and biochemical biomarkers.

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contact: MThipse@cheo.on.ca

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Publishing information

Contact:
NBIA Suisse
Fatemeh Mollet
Nbiasuisse@gmail.com

Editor:
Thomas Klopstock
LMU Klinikum München, Germany