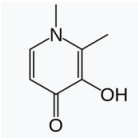
Notes from the 8th International Symposium on NBIA, Lausanne, October 13-15, 2022

Session 1: NBIA general

Session 1: NBIA general

The name NBIA (Neurodegeneration with Brain Iron Accumulation) indicates a central role of increased brain iron levels in this family of neurodegenerative diseases. **Roland Lill**, University of Marburg, addressed this topic in his keynote lecture on **"Molecular mechanisms of iron-sulfur protein maturation and its link to cellular iron regulation"**. Iron-sulfur protein maturation with its more than 30 components is essential for life, because iron-sulfur clusters serve as protein cofactors with importance for, e.g., catalysis, electron transfer, sensing and regulation. Iron-sulfur protein assembly defects can disturb DNA polymerase, DNA primase or DNA helicase functions, und hence lead to impaired genome maintenance. Accordingly, numerous genetic diseases with neurological, metabolic and hematological phenotypes have been described if this complex system gets disturbed. However, Roland Lill made very clear that the process of iron-sulfur protein maturation also plays a decisive role in both the sensing of cellular iron levels and the signaling of the iron status to the iron-regulatory systems. For example, if the iron-regulatory protein 1 (IRP1), a cytosolic iron-sulfur protein, is not matured properly, the apoform of IRP1 can bind to certain mRNAs encoding proteins for cellular iron-uptake and distribution. Hence, defects in iron-sulfur protein maturation lead to iron accumulation which in turn may cause oxidative damage (ROS).
Intense work is ongoing to stage the mitochondrial iron-sulfur protein biosynthetic pathway and to better understand the cytosolic iron-sulfur assembly machinery. Crystal structure analyses have given mechanistic insights into the role of the protein complexes involved and have allowed, for example, the direct visualization of the sulfur transfer to the nascent iron-sulfur cluster. This work will help to clarify how defects in, e.g., Friedreich's ataxia and other iron-sulfur diseases can be explained on a molecular level.

Ferroptosis is the name of a type of an iron dependent programmed cell death which is associated with iron accumulation and formation of lipidic reactive oxygen species. It can play a role in many types of cancer and in neurodegenerative diseases. Iron overload in the substantia nigra is a particular pathological hallmark in Parkinson. This brain region is rich in iron since dopamine is produced in an Fe(II)-dependent pathway. The question was, if the iron overload induced ferroptosis may be treated by removing excess iron by iron chelation. **David Devos**, University of Lille, summarized in his talk **"Iron chelation in neurodegenerative disorders – state of the art and what it means for NBIA"** the results of a phase 2, multicenter, double-blind clinical trial with 373 Parkinson patients *(D. Devos et.al. N Engl J Med 2022; 387:2045-2055)*. The treated group received 15 mg/kg Bid Deferiprone for 36 weeks, without additional dopamine substitution therapy.



Structure of Deferiprone

In In fact, the nigrostriatal iron content could be reduced. However, there was also a clear worsening of the Parkinson symptoms, most probably due to the shutdown of dopamine synthesis by impairing the iron-dependent tyrosine hydroxylase activity with the Deferiprone chelation. Conversely, in PD patients with the association of dopaminergic treatments, no worsening was observed but improvement. Thus low dose of iron chelator and association of dopaminergic drugs are pivotal for the success of this disease modifying strategy. Further studies are ongoing in patients with PD and ALS. Promising results were reported from treatment in a family with neuroferritinopathy, measured by improvement of walking distance without walker.

Session 2: PKAN & CoPAN

The reason for iron accumulation in PKAN (Pantothenate Kinase-Associated Neurodegeneration) patients is unclear. In an PKAN mice model no brain iron accumulation was observed. **Sonja Levi**, University San Raffaele, Milano, tries to make use of patient derived human inducible pluripotent stem cells to develop cell models for the disease and potentially test compounds in search for a therapy. In her talk **"Massive iron accumulation in PKAN-derived neurons and astrocytes: light on the human pathological phenotype"** she presented 2 cellular models. One PKAN patient derived cell line was differentiated into a stage called "medium spiny neurons", a mixed population including gabaergic, dopaminergic and glia cells. These cells showed increased iron load (control adult and neonatal fibroblast). Viability of these cells was low but could be much prolonged by addition of CoA. The other PKAN patient derived cell line led to astrocytes. After 30 days of differentiation transferrin uptake appeared to be increased. After 50-60 days of differentiation also iron load increased. The iron loaded astrocytes enhanced the death of the neurons by excitotoxicity.

A step higher in complexity to study **PKAN** pathology was presented by **Dario Finazzi**, University of Brescia, in his talk **"Zebrafish models with inborn errors of CoA biosynthesis".** Depending on the phenotype, such models could potentially be used for drug screening. PKAN is characterized by loss of function mutations in the pantothenate kinase 2 gene. Dario Finazzi presented a new zebrafish line that carried a biallelic mutation in the pank2 gene, a homozygous deletion of 4 base pairs, which stopped expression of pantothenate kinase 2. The mutant fish developed quite normally (transient impairment of vascular development at embryonic state), had normal CoA levels and normal neuronal development. At one year of age the fish showed testicular atrophy and decreased anxiety levels (novel tank test) but still no obvious neurodegeneration. Further work is needed to better characterize the behavior phenotype and to evaluate, if it may be a basis for an assay to screen molecules.

Mitochondrial quality control is a complex machinery to ensure supply and functioning of these organelles. In case of small damages, mitochondria may divide and fuse again to regain full functioning. Defective mitochondria may get eliminated by a mitophagic pathway. **Zhihao Wu**, Southern Methodist University Dallas, presented models to study mitochondrial quality control in drosophila in his talk **"Mitochondrial and mitophagy in PKAN: insights from a Drosophila model"**. PKAN2 (in form of the drosophila homolog called *fumble*) is present in mitochondria where it regulates the only autophagy receptor in drosophila, called *ref(2)P*, by acetylation which promotes mitophagy. PKAN2 (fumble) mutations lead to phenotypes and functional losses which may be used to define strategies how to restore their function. A similar approach has been followed for Parkinson using PINK1 mutations.

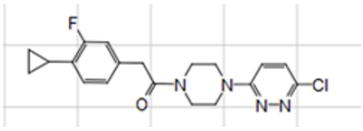
Ivano Di Meo, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, **"Modeling CoPAN in mice"**, described attempts to establish a mouse model for CoPAN (COASY Protein-Associated Neurodegeneration; COASY: Coenzyme A Synthase) suitable for testing potential therapeutic approaches. He showed:
- Constitutive ablation of COASY is incompatible with life (Coasy-null model).
- Neuronal specific knock out of COASY (Syn-Coasy model) shows an early onset severe phenotype (iron dyshomeostasis, reduced mitochondrial respiration, ferritin accumulation, but with no alteration in CoA and acetyl-CoA levels in the brain, and no signs of neurodegeneration). Due to the short lifespan of the animals, this model is not suitable for testing therapeutic approaches.
- A COASY pan-cerebral knock-out mouse is technically challenging due to the lack of a mouse line with pan-cerebral Cre recombinase expression. However, this result has been achieved by injecting a Cre-expressing adeno-associated virus (AAV) directly into the brain of newborn mice. This model (AAV-Coasy), in which COASY is deleted in many neuronal and glial cells, showed a phenotype that resembles the human disease, with additional neuroinflammation, reactive astrogliosis and microglia activation, but again without alteration in brain CoA levels.
- Neuronal specific-inducible COASY knock-out allows to shut down COASY in the adult mouse (induced by tamoxifen at 1 month after birth). The phenotype resembles the human disease (dystonia, neurological impairment) and also shows ferritin accumulation, neurodegeneration, reactive astrogliosis and microglial activation. Life span was about 3 months after induction (SLICK-Coasy model). This model will be used to investigate the disease's pathomechanisms and test experimental therapeutic approaches.

How **"Combination of a specific diet and the microbiome compensates lethality of a PKAN animal model"** showed **Hein Schepers**, University of Groningen. Biosynthesis of Coenzyme A is assumed to exclusively follow the intracellular classical five-step sequence starting from pantothenate (https://www.youtube.com/watch?v=KQSbyhxtW-o). But evidence for alternative routes to CoA exist. In a PKAN drosophila model feeding with the pantothenate precursor pantethine allowed survival. A key role for this rescue plays the microbiome, since the rescue could be abolished by administration of antibiotics (tetracyclin, rifampicin, streptomycin). It seems that bacteria in the microbiome with type I PANK can process pantethine to 4'-phosphopantetheine and all the steps to CoA (Lactococcus lactis). For further information see the published article on: *https://www.cell.com/molecular-cell/fulltext/S1097-2765(22)00440-3"*

How about supplying CoA precursors to PKAN patients? **Penelope Hogarth**, Oregon University, Portland gave an **"Update on the CoA-Z clinical trial"**. CoA-Z is a special formulation of the CoA precursor 4´-phosphopantetheine (bypassing the defective PANK2 and only two synthetic steps away from CoA). Hogarth described the design of a phase 2 study (safety and tolerability) in PKAN patients (classic and atypical) conducted in N. America. Sixty-four participants were randomly assigned to receive one of 3 different doses of 4'-phosphopantetheine or placebo for 6 months, double blind, plus up to an additional 18 months open label phase. The recruitment was unexpectedly very rapid, in part due to a novel trial design with broad inclusion criteria, reduced risks and burden for the participants, entirely in patients home community (which was important during the pandemic), reduced

costs, and strong engagement of the patients and their families. The trial concluded late in 2022; preliminary results are expected in 2023.

Suzanne Jackowski, St. Jude Children's Research Hospital, Memphis, and with support from CoA Therapeutics, Inc., gave an update on their advanced medicinal chemistry program to a potential treatment for PKAN, entitled **"Optimization and efficacy of the clinical compound BBP-671 in a PKAN mouse model".** Their therapeutic approach aimes to compensate the dysfunction of PANK2 by activating the other isoforms PANK1 and PANK3 by a small molecule. Activation of an enzymatic activity by a small molecule is a rather delicate task. As presented and published earlier (L.K. Sharma et al, Nature Communications 2018, 9: 4399) this goal was achieved by a series of molecules named pantazines which block the feedback-loop by which acetyl-CoA stops the activity of the PANK enzymes and which keep the enzymes in an catalytically active conformation. Medicinal chemistry optimization of the series resulted in the clinical candidate BBP-671 with the necessary pharmacokinetic and safety profile.



Structure of the clinical compound BBP-671 *(Subramanian C, Frank MW, Tangallapally R, et al. J Inherit Metab Dis. 2022;1-15. doi:10.1002/jimd. 12570).*

BBP-671 relieves the acetyl-CoA feedback inhibition onto PANK1,2,3. In wt mice it is detected in the brain and elevates brain CoA levels. They used a PKAN mouse model and demonstrated CoA elevation after 30 days on BBP-671 administered in the chow. The mice gained better body weight, improved movement and survival.
The first clinical results were presented by **Susanne A. Schneider** and **Thomas Klopstock**, LMU Munich, **"The PanK Activator BBP-671 for PKAN: results from a phase 1 trial in healthy volunteers, and update on pivotal trial plans".** Orally administered were single doses from 3-120 mg, and repeated doses of 30 mg, 60 mg, 70 mg and 100 mg daily for 7 days. The compound was rapidly absorbed with a T_{max} between 1-2 hours. Elimination half-life was 6-8 hours. The compound was present in CSF and Plasma and its concentrations were measured. Acetyl-CoA levels in whole blood were increased and pantothenate levels decreased. These readouts may be used as potential biomarkers in further clinical studies which are strongly supported by the current data. The compound was well tolerated in 60 healthy adults. BBP-671 is being studied also in patients with propionic acidemia and methylmalonic acidemia (phase 1).

Session 3: MPAN
Defects in C19orf12 lead to MPAN (Mitochondria membrane Protein-Associated Neurodegeneration), a relentlessly progressive rare neurodegenerative disorder. C19orf12 encodes a short transmembrane protein mainly expressed in brain cells, blood cells, and adipocytes. It is localized to mitochondria, endoplasmatic reticulum, and their contact sites. Still, the actual function of the protein needs to be better understood. **Arcangela Iuso**, from the Helmholtz Zentrum München, presented results from her study in the talk "Expanding our understanding of C19orf12 function to develop therapeutic approaches for MPAN".
The study shows no alteration in intracellular Ca++, ROS, GSH, ATP, mitochondrial respiration, or morphology in many fibroblasts of MPAN patients carrying different

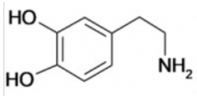
mutations. Instead, a consistent impairment in the initiation of the autophagic process (traced by following LC3 protein, an autophagy marker) was noted. This readout was exploited to screen 14 compounds that modulate autophagy. Paroxetine, carbamazepine, ABT-737, LY294002, and oridonin rescued LC3 levels. Notably, carbamazepine, oridonin, and paroxetine are molecules approved by the Food and Drug Administration, implying a realistic clinical translatability in MPAN.
While further research is needed to identify the primary function of C19orf12, the study moves one step closer to understanding the consequences of C19orf12 dysfunction at the cellular level and establishing a rational therapy for MPAN.
For details see: *Zanuttigh E, et al. Pharmaceutics 2023, 15, 267.* *https://doi.org/10.3390/pharmaceutics15010267*

Loss of C19*orf*12 was studied in drosophila by **Kenta Shiina** et.al., Juntendo University Tokyo, and results were presented in poster 17 entitled **"Loss of MPAN-associated C19orf12 causes alteration of lipid metabolism and autophagosome-lysosome trafficking in Drosophila"**. The group described disturbed lysosome autophagosome trafficking, abnormal autophagosome-like organelles, accumulation of certain autophagy markers and decreased expression of lysosomal channels, transporters and some membrane proteins. They also analyzed changes in lipid metabolism and demonstrated increased phosphatidylethanolamine in total phospholipids, increase of certain triacylglycerols, and decrease of hydroxy-PE-ceramide and hydroxy ceramide. Large lipid droplets in the larval fat body were preserved under starving stress indicating that lipolysis was impaired. It seems that this altered lipid metabolism in combination with impaired autophagic removal could have an effect on α-synuclein accumulation.

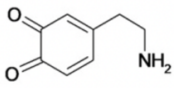
Session 4: BPAN
In BPAN (ß-propeller Protein Associated Neurodegeneration) different mutations spread overall in the WDR45 gene are responsible for the disease with altered ER and mitochondrial morphology, altered iron homeostasis, impaired autophagy and accumulation of protein aggregates that lead to respiratory and ER stress. The corresponding WD-repeat β-propeller protein is important in autophagy, especially in neuronal cells. **Mario Mauthe**, University Groningen, presented in his talk **"Is it all about autophagy? - WDR45's role in autophagy and beyond"**, their lab's fundamental research approach to uncover WDR45 role in autophagy, but also in autophagy independent functions. They primarily focus on full WDR45 knockout cell lines and found a specific mitochondrial phenotype. Currently, they investigate the relationship of the pathways that WDR45 is involved in and how these defects can be restored. For more detailed information about this work, feel free to contact Mario Mauthe (m.mauthe@umcg.nl) directly.

One major hallmark of BPAN is the preferential demise of dopaminergic neurons in the substantia nigra leading to Parkinson-like symptoms. Pathomechanisms such as ROS formation, mitochondrial dysfunction, autophagic defect or reduced lysosomal proteolysis are thought to play a role for this increased neuron susceptibility. However, these pathomechanisms also happen in other cells, not just dopaminergic neurons. **Lena Burbulla**, LMU Munich, addressed this aspect in her talk "Vulnerability of midbrain dopaminergic neurons in BPAN". Midbrain dopaminergic neurons present with certain unique physiological features. On one hand, the morphology of the substantia nigra dopaminergic

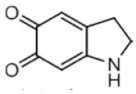
neurons, their large axonal arborization, makes energetic supply and logistics a challenge. On the other hand, handling of dopamine itself is a challenge. High content of iron – present under physiological conditions in the substantia nigra, but even enhanced under pathological conditions in BPAN and Parkinson's disease patient brains – can strongly react with dopamine catalyzing the oxidation of dopamine to dopamine-quinone and the very toxic aminochrome. To a certain extent, neurons can remove such toxic species by formation of neuromelanin. But this rescue pathway may get saturated and problems arise. Burbulla used induced pluripotent stem cells (iPSCs) from BPAN patients which were differentiated in midbrain-specific dopaminergic neurons. Patient neurons showed differential regulation of iron regulatory proteins, and increased neuromelanin formation and accumulation of toxic oxidized dopamine species.



Dopamine



Dopamine quinone.



Aminochrome

org/10.3390/pharmaceutics15010267

BPAN

A novel transgenic mouse model of BPAN adds to the two models already reported in the literature. **Arcangela Iuso** extensively described the new model in her talk "Characterization of a whole-body Wdr45 knock-out, a mouse model for BPAN". Male and female mice survive the whole-body ablation of Wdr45, but Wdr45 knock-out causes neuronal degeneration signs (axonal spheroids) in young mice. Additional phenotypes exhibited by this BPAN model are mitochondrial dysfunction, reduced dopaminergic neurons in substantia nigra, hearing loss, ophthalmic changes, deficits in social interactions, and specific hematological changes. However, no iron accumulation was seen in the brain, even in older mice.
The presence of an early-onset neurological phenotype and neuropathology manifestation qualifies this mouse model to test therapeutic approaches.

C. A. Biagosch et al., Mamm Genome. ***32****, 332–349 (2021).*10.1007/s00335-021-09875-3
H. Wan et al., Autophagy. ***16****, 531–547 (2020).*10.1080/15548627.2019.1630224
Y. G. Zhao et al., Autophagy. ***11****, 881–890 (2015).*10.1080/15548627.2015.1047127

Session 5: PLAN/INAD and FAHN

The term INAD (infantile neuroaxonal dystrophy) and PLAN (phospholipase A2 group 6 = PLA2G6 -associated neurodegeneration) are used as synonyms. Encouraging results on "**Developing gene therapy for PLAN and moving towards clinical trials"** were presented by **Ahad Rahim**, University College London. An adeno-associated viral vector carrying a functioning PLA2G6 gene was administered to genetically modified mice mimicking the INAD-pathology. Intervention at a neonatal stage ameliorated neurodegeneration (reduced the loss of neurons in the brain and spine) and improved behavior (rotarod) and survival. This neonatal proof of concept is promising. But also later intervention at 4 weeks of age led to improved survival and locomotor function.

Author of these highlights: Dr. Roland Jakob-Roetne