NCL Research Newsletter

–DECEMBER 2021–

CONTENT

1 MAIN TOPICS

Change in our Scientific Advisory Board

After having been an active member of our SAB for the last three years, **Prof. Dirk Isbrandt** is retiring from this role serving the NCL Foundation. Dirk is Professor for Experimental Neurophysiology at the German Center for Neurodegenerative Diseases, DZNE-Bonn/University Hospital Cologne. Dirk provided us with valuable access to the DZNE network.

We would like to express our great thanks to Dirk for his honorary work in our Foundation!

A warm Welcome to our new SAB member, **Dr. Rebecca Ahrens-Nicklas, MD, PhD** at the Children's Hospital of Philadephia, USA. Her research aims at understanding why patients with inherited biochemical disorders often suffer from severe, untreatable neurologic and cardiac symptoms. She works towards elucidating links between biochemistry and neural network excitability with the aim to drive and move forward new approaches to therapy. Rebecca has been part of the NCL community for several years and has published groundbreaking papers towards understanding the fundamentals of neuronal network damage in CLN3 disease. In 2018, she and her colleague Dr. Eric Marsh received the NCL Research Award for their work in this area.

Dr. Rebecca Ahrens-Nicklas

We are happy to have Rebecca on board!

Back to the top

The 7th JNCL Young Investigator Symposium

This year`s Young Investigator (YI) Symposium took place December 5 and 6. The YI meeting serves to **exchange state-of-the-art knowledge** in NCL with focus on JNCL i.e. **CLN3 disease**. Altogether, 24 YIs from laboratories across the globe attended. 22 YIs presented their work and 3 that had just started their research activities briefly introduced themselves. The meeting was initially planned as a hybrid event. However, it became clear in the weeks before the meeting that a face-to-face event was no longer an option. The rise in the number of Corona cases and new travel restrictions forced us last minute to switch to an entirely virtual format. Nonetheless, the meeting turned out to

be very successful. **More than 30 participants including young investigators and plenary speakers** active in clinical and/or academic research attended the meeting. Attendants also included members of NCL and Batten disease Foundations as well representatives from the bio-tech industry. Discussed were many different aspects of NCL clinical and basic research as shown in the attached agenda (*see JNCL Young Investigator Symposium*). The two-day meeting was split into four sessions. Each session focused on a different aspect of NCL-research including **cell models**, **clinical aspects**, **mutant transcripts**, and overlaps with **other NCL** and adult-onset neurodegenerative diseases.

7th JNCL Young Investigator Symposium as a virtual meeting

The first three **keynote lectures** gave an overview on the progress and status of CLN3 clinical research (Angela Schulz, UKE, Hamburg), CLN3 cell biology (Susan Cotman, MGH, Boston), and CLN3 biomarker development (An Dang Do, NIH, Bethesda). Mapping out to understand the natural history of CLN3 disease has progressed tremendously. Besides the Unified Batten Disease Rating Scale (UBDRS) numerous clinical assessment methods and parameters such as MRI, OCT, EEG, ECG, event-related potentials e.a. are being used to understand disease progression.

CLN3 biomarker development has reached a pace never seen before. As discussed by An Dang Do, analysis of the thus far largest collection of CLN3 patient CSF samples is starting to bear fruit. Results using proteomics and metabolomics methods point at a number of interesting parameters that are changed in CLN3 disease including some that seem translational from CLN3 mouse and pig models to human. In brief, there is hope on the horizon for a biomarker armamentarium that eventually can support clinical trials in CLN3 disease.

Ever since the *CLN3* gene was mapped and described as the cause of JNCL in 1995, the function of the **CLN3 protein** has remained an enigma. A great number of cell and organismal models have been generated and used by researchers to try to elucidate the function of CLN3. A recent surge in discoveries based in part on applying novel technologies point at CLN3`s complex role in cell biology and lysosomal function. A true breakthrough in understanding CLN3 function is in sight. Perhaps this will offer a deeper understanding of how so many different cellular phenotypes in cells lacking CLN3 might connect. Not only has CLN3 function been a hard nut to crack. The same holds for the structure of CLN3 protein. Many years and frustrating attempts have not yet led to elucidating its protein structure. Recent progress, some of it presented at the YI meeting, raises hope that we might see the end of this tunnel in the not too far future.

Therapeutic approaches that were high-lighted in this YI meeting were mainly the use of exon-skipping ASOs

and targeting lysosomal ion channels. ASOs aim to try to recover protein expression from the exon7/8 deletion allele that is present in over 80% of all JNCL patients. Finding the most promising exon skipping approach to rescue CLN3 function seems key to move to the clinic. As a pharmacological approach, lysosomal ion channel modulation seems to hold promise as a therapeutic avenue to alleviate CLN3 cell pathological changes.

Two additional keynote lectures covered very different topics: Craig Benson (Beyond Batten Disease Foundation, Austin, USA) reviewed the history and achievements of the BBDF in terms of **patient advocacy** and **leading therapeutic development**. The FDA has awarded Orphan Drug and Rare Pediatric Disease designation for BBDF-101 (a drug combination of Trehalose and Miglustat), and Theranexus and BBDF signed a worldwide exclusive license for this drug candidate that now has IND approval and is scheduled to go into clinical trials in 2022. Alessandro Ori (Leibnitz Institute on Aging, Jena) gave a wonderful lecture illustrating how our **brain proteome** is aging and leading to an imbalance in the proteostasis network. This manifests itself for example in a decoupling of protein and transcript levels, an imbalance in the stoichiometry of protein complexes, and alterations in the half-life of short-lived as well as long-lived proteins. A fish model manifesting rapid aging and a short lifespan (*Notobranchius furzeri*) serves as a vertebrate model.

During the general discussion it was raised that **sharing cell lines** and **access to patient samples** (e.g. fibroblasts) remains difficult. Several clinical sites have patient samples and administrative processes to deal with requests. Nonetheless, the road to access remains bumpy. One advice is to make sure you provide a solid scientific rationale when asking for samples. Institutes will carefully assess the value of samples they have. Bio-samples collected from the same patients in longitudinal studies might be of special value for biomarker studies and certainly deserve special care and consideration.

The NCL Foundation remains committed to promote networking and fund research with the aim and **hope to find and help move forward therapeutic options for CLN3 patients.**

All of us share the **ultimate goal** to improve the quality of life of patients and cure as many aspects of this disease as possible.

Back to the top

The 17th International NCL Congress, St. Louis, USA

In brief, it was impressive to see and hear about the incremental increase of basic and clinical research efforts on CLN3 disease.

Biomarker research has received a major boost, also through projects co-funded by the NCL Foundation. For the first time, results point to promising surrogate markers. These may prove useful for diagnostic purposes, to assess clinical stages, disease progression, and therapeutics including pharmacological agents, gene therapy and antisense oligonucleotide-based exon-skipping.

The next international NCL congress in 2023 will be held in Hamburg – organized by Dr. Angela Schulz. We are looking forward to it!

Back to the top

Interview with Ronald Jansen, Chairman of the "Stichting Beat Batten!"

Ronald, you have been Chairman of the Beat Batten Foundation for almost 10 years now – what is your motivation for this task and is there a special highlight for you during this time?

There is of course a very personal motivation as our son Roel was diagnosed with CLN3 in 2011. First, there was a lot of sadness being confronted with blindness first and then the devastating news that it was caused by a deadly metabolic disease with no treatment. But soon after we decided to fight and to do everything we could to find some kind of treatment or medication to stop the progressive degeneration. The best way to do this was to join the Beat Batten Foundation, to raise money for financing CLN3 research. Our lives changed dramatically because of Batten, but at least we have the feeling that we can contribute a bit in finding a solution to save Roel and other kids with this terrible disease.

It is quite difficult talking about highlights, to be honest. In these 10 years I have seen many other kids suffering and sadly, also dying from Batten. And then last summer, the son of the founders of Beat Batten passed away. I knew him from the day I joined Beat Batten. He was a great kid and we always made jokes and had our laughs. When he

passed away I was very sad, felt knocked out, and I wondered why we were not able to find some kind of treatment in all these years. Will it come in time for Roel? But we have to keep pushing. And a highlight was actually the last Young Investigator Symposium of the NCL Foundation. I visited many NCL meetings in the last couple of years, and normally when they end, I am left confused and very worried, as there are more questions than answers. And for years there were hardly any ideas regarding types of treatment. But I feel this is changing now. With new techniques, a better understanding of the NCL fundamentals, I now have the feeling that we are getting closer to possible ways of treatment. So that is of course a true highlight. Having said that, what remains is our lack of time. We are always in a hurry as each day goes by, Roel and all other kids are facing new seizures.

Where do you see the biggest challenge in developing an effective therapy for NCL?

Although I am not an expert, I do understand the complexity of the brain, the enzymes and all the processes that are going on. Unfortunately, because of this complexity, it means that there is not one simple solution that will end the degenerative process. It also means that we face more challenges at the same time. First of all, its time. As I said, as parents, we are always in a hurry as we all want to save our kids. Next to that, its money. For pharma I understand it is difficult to heavily invest in such a rare disease without having the certainty of developing an approved therapy or treatment. It means a huge role for us as foundations in fundraising. But even if you have all the money in the world, we really need to have a good understanding of the fundamentals. Sure, money is helpful but working together, interact with scientists working in e.g. neurology or other related fields, that might speed up things. You could say, that is another challenge; constantly bringing experts together and sharing knowledge. So as a chairman, the biggest challenge is more money to involve more scientists. But as a father, the biggest challenge is time.

Stichting Beat Batten! and the NCL Foundation are working closely together - how does this partnership help in the fight against NCL?

Especially with a rare disease like CLN3, it's absolutely necessary to work closely together. Efficiency is important because of the lack of time and money. We don't have time (and neither the budget) to lose time in doing things double or make the same mistakes. We need multiple studies or even trials, to see what type of therapy will work. By working together, we share experiences, ideas, scientific data which I think is key for efficiency. Another reason for us to work closely with the NCL Foundation is that we live in a small country. For example, there are not many specialists over here so it is for example very difficult to have our own medical advisory board. Therefore I am extremely happy to work with the German foundation. For years now, we work together in projects, financing studies and sometimes we co-organize events. Within the Beat Batten Foundation, the frequent (online) meetings with the NCL Foundation and the results of these talks, led to enthusiasm that leads

to more action by all Dutch board members and volunteers. So we learn from it, we get inspired by it, and it leads to action. To be a little more concrete, I think that both foundations are financing a very promising (CLN3 function/biomarker) study, led by Monther Abu-Remaileh. His results are already very helpful for others in this field, and we are even more enthusiastic about the results to come next year. For me it's a perfect example that this partnership can really help in the fight against NCL.

Ronald Jansen with his son Roel

Back to the top

2 NEW PROJECT GRANTS

We are very pleased to announce the start of **two new CLN3 research projects** in 2021. One focuses on determining robust disease-relevant CLN3 cellular phenotypes to screen for potential drugs on a large scale that can reverse the phenotype.

The project will run for three years at **Cardiff University/Prifysgol Caerdydd, UK** under the leadership of **Dr. Emyr Lloyd-Evans**. Ms. **Llinos Siân Honeybun** has joined Emyr's group as a new PhD student and will carry out the project. We are happy to be able and support another young scientist joining the fight against CLN3 disease. We wish Llinos and Emyr a great deal of success with the project and we are looking forward to news to come.

Dr. Emyr Lloyd-Evans and Llinos Siân Honeybun

We would also like to thank **Contactpunt NCL**, Belgium, **Eurofins Foundation,** and **Reinhard Frank-Stiftung** for their generous support of this project.

The second project is carried out at the **LMU in Munich, Germany** in the group led by **Prof. Christian Grimm.** It is an extension of a PhD student project that we funded during the past three years, and which was focused on generating a set of isogenic iPSC-lines carrying different CLN3 mutations. These lines have been used to generate neurons, study their pathological phenotypes, and test therapeutic principles based on targeting lysosomal ion channels. Phenotypes include mitochondrial defects which are the focus of this extended study.

Prof. Christian Grimm

We thank the **Dr. med. Carl-August Skröder Stiftung**, the **Reinhard Frank-Stiftung,** and the **Werner Reichenberger Stiftung** for their generous support.

Back to the top

3 NEWS AND RECENT PUBLICATIONS

CLN1

Taysha Gene Therapies is working on a CLN1 infantile Batten disease gene therapy program (currently open IND) that was licensed from Abeona Therapeutics and is now designated TSHA-118 (formerly ABO-202).

Sadhukhan et al. report that ablation of miR-155, which upregulation was seen in CLN1 KO mouse brain, does not alter the neuroinflammation trajectory in the brains of these mice.

Augustine et al. reported limited evidence base for treatment and clinical management guidelines for CLN1 disease. A survey and a meeting of experts was conducted to ascertain points of consensus.

CLN2

Schaefers et al. studied cerliponase alfa ERT in two siblings, one symptomatic and one asymptomatic, and propose that ERT is able to delay the onset of symptoms when treatment is started in a presymptomatic stage of CLN2 disease.

Lourenco et al. reported on the clinical phenotype of 30 Latin American patients with atypical CLN2 disease at 6, rather than the common onset between 2-4 years of

age. The authors reinforce an inclusion of CLN2 in differential diagnosis of children presenting with seizures, behavioral disorders and language abnormalities to allow early initiation of therapy.

Domowicz et al. analyzed transcriptional changes in the brain of aging TPP1-deficient mice and report several astrocytic and microglial genes showing increased expression starting after 2 months of age, noted earliest in cerebellum, and associated with disease progression.

Gissen et al. demonstrate how utility studies enable preference-based quantification of a disease`s impact on health-related quality-of-life of CLN2 patients.

Helman et al. present a case study showing that RNA sequencing is a useful complementary tool to DNA sequencing to inform on variants of unknown or uncertain significance, in particular variants caused by aberrant splicing.

Banning and Tikkanen used minigene approaches and patient cells to show that substances like methylxanthine derivatives and luteonin may be able to modulate splicing of one of the most common TPP1 gene variants.

Thompson et al. reported ERG and OCT findings in a cohort of atypical and typical CLN2 patients showing symmetrical cone-rod dysfunction but a broad range of ages when ERG function is preserved.

Gall et al. used next-generation sequencing-based panels including copy-number variant detection in a cohort of 211 patients (24-60 months of age) with a first unprovoked seizure at/after 24 months. In 14% of these patients CLN2 was diagnosed. This is 12-24 months earlier than reported by natural history of disease supporting NGS as a tool to identify patients early.

CLN2 gene therapy: REGENXBIO reported that it is continuing to evaluate the path forward for their RGX-181 (CNS) and RGX-381 (ocular) CLN2 gene therapy programs and plans to provide an update in 2022.

CLN3

Exicure announced at their virtual R&D-day a CLN3 program using its proprietary Spherical Nucleic Acid (SNA) technology to deliver synthetic nucleic acids.

Theranexus & BBDF announced in September receipt of Investigational New Drug approval from the FDA to launch a Phase I/II clinical trial of their drug candidate BBDF-101.

Soldati et al. described increased levels of globotriaosylceramide (Gb3) in cellular and murine models of CLN3 and CLN7 diseases and developed a cell-based high content imaging screening assay for the repurposing of FDA-approved compounds able

to reduce this accumulation within BD cells. Tamoxifen reduced lysosomal Gb3 accumulation in CLN3 and CLN7 cell models through a mechanism that involves activation of TFEB. Tamoxifen reduced Gb3 and SCAMS accumulation in the CLN7$^{\Delta ex2}$ mouse model and improved neuroinflammation and motor coordination.

Morsy et al. provide an overview of available iPSC models for different NCLs and highlight findings in these models that may spur target identification and drug development.

Ostergaard studied gait phenotype in CLN1, CLN2 and CLN3 Batten disease. He reported for CLN3 patients a reduction in walking speed at age 7-8 years, a parkinsonian gait phenotype in the mid-teens, and peripheral nerve involvement, neurogenic musculoskeletal atrophy and loss of tendon reflexes and postural control in the late-teens and early-twenties.

Yasa et al. showed that CLN5 and CLN3 function as a complex to regulate endolysosome function.

Masten et al. created a reliable diagnostic confidence scheme for CLN3 disease and discuss its utility for future clinical research studies.

Kuper et al. showed that in CLN3 disease, as compared to early-onset Stargardt disease, visual acuity loss is more rapid. Also, severe colour vision abnormalities and abnormal dark-adapted ERG responses are main differentiating features of CLN3 disease.

Do et al. showed that neurofilament light chain levels in CSF and serum correlate with clinical measures in CLN3 disease.

Minnis et al. used yeast-based assays to show that the minor 1-kb deletion transcript both loses and retains functions and acquires abnormal characteristics.

Rechtzigel et al. reported substantial overlap in the protein interactomes of CLN3, CLN6, and CLN8 suggesting a shared etiology. The absence of CLN3, CLN6 and CLN8 leads to synaptic depletion of key SNARE proteins and tethers, and aberrant synaptic SNARE dynamics.

CLN5

Neurogene working with Lincoln University researchers announced FDA clearance of IND for NGN-101 gene therapy to treat CLN5.

Basak et al. used CRISPR-based genome editing to generate CLN5-deficient iPSC-derived neurons and describe neutralized lysosomal acidity, reduced lysosomal enzyme activity, and impaired lysosomal movement.

Russell et al. employed ERG and characterized progressive physiological changes in the degenerating retina of CLN5 and CLN6 forms of ovine NCL.

Robinson Kick et al. described visual system pathology and function (ERG and visual evoked potentials) in a canine model that shows pronounced visual impairment by 21-22 months of age. They highlight the utility of the model because of the similarities with changes seen in CLN5 patients.

McLaren et al. describe findings that support a role for CLN5 in autophagy during the life cycle of *Dictyostelium.*

CLN6

Barry et al. generated sheep from ovine wildtype and CLN6 embryo aggregation chimeras and studied degrees of neurodegeneration. These varied from affected, normal-like to recovering-like. In the latter two cases there was a lack of glial activation and storage bodies. The authors propose that intercellular communication affects pathology.

Shiro et al. employed an earlier finding that CLN6 displays anti-aggregate activity of the myopathy-causing R120G alpha-B-crystallin mutant and showed that this activity is compromised and/or nullified depending on which pathogenic or combination of pathogenic CLN6 mutations are present. These findings may bear on patients that are compound heterozygous for different mutant CLN6 alleles.

Nicolaou et al. report clinical and genetic findings in three patients with a juvenile onset of CLN6 disease which remarkably lack vision loss at presentation.

Cherian et al. report on a CLN6 patient with type B Kufs disease who showed a first documented remarkable life-changing response to levodopa.

Koh et al. show that CLN6 interacts with a CRMP2-KLC4 complex to regulate anterograde axonal transport in developing neurons. CRMP2 fails to properly associate with key neuronal protein partners in the absence of CLN6. Some of these deficits can be rescued in cultured neurons and *in vivo* by treatment with the CRMP2 modulator lanthionine ketimine ester.

CLN7

Taysha Gene Therapies has secured an exclusive option from the University of Texas Southwestern to license worldwide rights to a clinical-stage gene therapy program for CLN7 disease that is currently being evaluated in a Phase 1 clinical trial (NCT04737460).

Neurogene Inc. currently sponsers a Natural History Study for both CLN5 and CLN7 to help advance gene therapy for these two diseases.

Wang et al. identified CLN7 as a novel endolysosomal chloride channel that mainly transports chloride from the lysosomal lumen to the cytoplasm. When overexpressed, CLN7 increases chloride currents and enlarges endolysosomes in a Ca^{2+}/calmodulin-dependent way. CLN7 regulates lysosomal chloride conductance, luminal pH, lysosomal membrane potential, and promotes lysosomal Ca^{2+} release through TRPML1. CLN7 KO mice show pathological features similar to those of patients including retinal degeneration and accumulation of lipofuscin storage material. CLN7 pathogenic mutations decrease chloride permeability with more severe mutations showing more serious defects in chloride channel function.

CLN8

Pesaola et al. used CLN8 knockdown to show increased size of the Golgi, lysosomal alkalization, and decreased complexity and size of the somatodendritic compartment in primary rat hippocampal neurons.

Salpeter et al. generated CLN8$^{-/-}$ mice using CRISPR/Cas9 genome editing and provide a detailed clinical characterization of retinopathy in adult mice. The retinal findings are consistent with those seen in CLN8 patients.

CLN11

Logan et al. showed that Grn$^{-/-}$ mice exhibit a global deficiency in bis(monoacylglycero)phosphate (BMP), and an age-dependent, secondary storage of glucocerebrosidase substrate glucosylsphingosine. PGRN protein replacement enhanced CNS biodistribution of PGRN and its delivery rescued various phenotypes in primary murine macrophages and human iPSC-derived microglia. It also rescued BMP levels, and had beneficial effects on glucosylsphingosine levels, microgliosis, lipofuscinosis, and neuronal damage disease pathology in the Grn$^{-/-}$ mouse CNS.

Zin et al. characterized retinal degeneration in CLN11 (progranulin) knockout mice showing that retinal PGRN gene therapy outcome is time-sensitive and depends on route of administration (systemic versus intravitreal).

Takahashi et al. characterized the retinal phenotype (ERG and histology) in mature PGRN knockout (*Grn*$^{-/-}$) mice. Microglial cells accumulated on the retinal pigment epithelium (RPE) apical layer, and in *Grn*$^{+/+}$ mice, strongest PGRN signals were detected in the RPE-choroid. The authors suggest that subretinal translocation of microglia is a characteristic phenotype in the retina of mature PGRN knockout mice.

Boland et al. showed that levels of bis(monoacylglycero)phosphate (BMP), a lysosomal lipid required for ganglioside catabolism, were markedly reduced in PGRN-deficient cells and patient brain tissue. These data indicate that granulins are required to maintain BMP levels which regulate ganglioside catabolism, and that PGRN deficiency in lysosomes leads to gangliosidosis.

Reifschneider et al. generated Grn/TREM2 double knockout mice and used antibody-mediated TREM2 modulation showing that loss of TREM2 reduces hyperactivation of PGRN-deficient microglia but not lysosomal pathology. Accordingly, microglia hyperactivation is not necessarily contributing to neurotoxicity in PGRN-deficiency.

Devireddy and Ferguson identified an interaction between prosaposin and Surf4 and show that Surf4 is critical for the efficient export of progranulin and prosaposin from the ER.

Lan et al. reviewed the role of progranulin in immune-mediated diseases and its potential as a therapeutic target.

CLN12

Mateeva et al. provided a structural and catalytic mechanism model of ATP13A2 (PARK9) from simulations implicating roles of the conserved Arg686 and Lys859 catalytic residues. When missense mutations occur near an active site residue, they can interfere with the barrier height of the reaction, which can halt the normal enzymatic rate of the protein.

Tillinghast et al. provided structural mechanisms for gating and ion selectivity of the human polyamine transporter ATP13A2. These provide a foundation to understand ATP13A2 mutations associated with disease.

Sim et al. generated a high-resolution cryo-EM structure and provide a structural basis of polyamine transport by human ATP13A2. Five distinct conformational intermediates are described which together represent a near-complete polyamine transport cycle of ATP13A2.