NCL Research Newsletter June 2018

Content

NCL-Stiftung
Für eine Zukunft ohne Kinderdemenz

1. Main Topic:

16th National NCL Congress in Hamburg - Microglia & more.

The role of brain resident microglial cells in CLN3 disease will be one of the main topics during this year's annual NCL Congress in Hamburg (December 17, 2018). The role of microglial cells in the context of CLN3 disease remains poorly defined. Recent findings suggest that brain resident microglial cells together with astrocytes do play an active role in neuron loss in the absence of CLN3 (Parviainen et al., 2017). Furthermore, studies by Dr. Katharina Dannhausen, who recently successfully completed her PhD thesis in the lab of Prof. Thomas Langmann in Cologne, provide experimental evidence for an active role of these cells in a CLN3 mouse model of disease. This work was supported by the NCL Foundation.

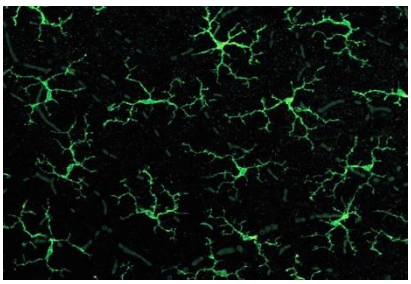
Mechanisms beyond neuron-autonomous processes play an increasing role in many neurodegenerative conditions. Such processes include protein spreading mechanisms in protein misfolding diseases, and contributions to disease processes from non-neuronal cells including astroglia, microglia, brain endothelial cells or infiltrating lymphocytes. In many cases, these mechanisms may not be a primary cause of neurodegeneration in the disease. Nonetheless, non-neuronal cells may have an important role in disease progression.

Microglia are brain-resident innate immune cells that perform vital functions in the brain, both during development and in the adult. Many recent studies point at their important role in neurodegenerative conditions although their precise contribution to maintaining brain homeostasis or exacerbating the progression of neurodegenerative diseases remains poorly understood. Wendeln et al. (2018) showed that peripherally applied inflammatory stimuli can induce innate immune memory in resident brain microglia, and that this can either exacerbate or alleviate neurological disease hallmarks.

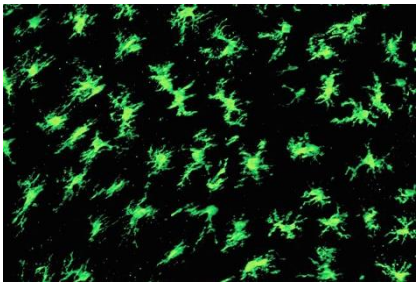
To study microglial biology in disease, scientists until recently had to rely mainly on animal models including freshly isolated brain microglial cells, as well as the use of cell lines. Now several labs have developed protocols to establish human microglia from induced pluripotent stem cells (iPSC), which were derived by reprogramming patient fibroblasts or blood cells (Muffat et al., 2016; Abud et al., 2017; Douvaras et al., 2017; Haenseler et al., 2017; Pandya et al., 2017; Brownjohn et al., 2018). The use of iPSC-derived human microglia paves the road to study human microglial disease biology in the context of specific disease-

associated mutations. Admittedly, there are still gaps in the knowledge on how to best arrive at microglial identities in culture that mimic faithfully these cells features and behavior in the brain (Bohlen et al., 2017; Gosselin et al., 2017). Improvements in culture methodologies (e.g. 2D or 3D microglia/neuron co-cultures) will likely continue to close such gaps.

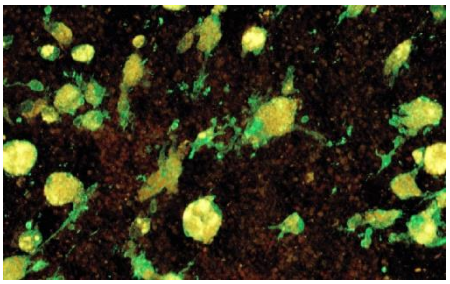
Efforts are on the way to alleviate CLN3 disease using gene therapy. However, none of the currently used gene delivery AAV vectors target microglia.



Healthy retina (Iba1 staining)



Retinitis pigmentosa retina (Iba1 staining)



CLN3$^{\Delta ex7/8}$ retina microglia after light damage (Iba1 staining and lipofuscin autofluorescence)

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2. Scientific and Medical Meetings

4th International JNCL Young Investigator Symposium, London, September 12, 2018

Thanks to the generous financial support from e.g. Contactpunt, Stiftung Kindness for Kids and Beat Batten Stichting! we are able to organize the 4th International JNCL Young Investigator Symposium. It will take place in London on September 12. Eighteen young investigators have been accepted. Each young investigator will be given the chance to give a short oral presentation. The meeting will provide a platform for the young investigators to learn more about daily life with CLN3 disease as the meeting starts with a presentation by Roland Jansen (Beat Batten Stichting!) a father of a child with CLN3 disease. Prof. Beverly Davidson (Children's Hospital of Philadelphia) will give a keynote lecture about CLN3 basic research. We are looking forward to meeting all these young students soon.

Beat Batten!
Stop deze stofwisselingsziekte bij kids

KINDNESS FOR KIDS
Stiftung für Kinder mit Seltenen Erkrankungen

CONTACTPUNT NCL

NCL-Stiftung – DZNE "Adult and Childhood Neurodegenerative Diseases: Common Mechanisms and Markers", Dec 11 - 12, 2017, Bonn, Germany

The NCL Foundation-DZNE joint meeting took place December 11-12, 2017, in the new and recently inaugurated DZNE Building in Bonn, Germany. This meeting provided for the first time a platform to promote scientific exchange and discuss future collaborations between scientists working on adult neurodegenerative diseases (NDs) and scientists working on childhood neurodegenerative diseases. Take home message was that there are several commonalities in molecular and cellular mechanisms underlying adult and childhood onset neurodegenerative diseases. These include in particular the role of the endolysosomal system (including the defects), which is a genetic hot spot for genes causing either childhood and/or adult onset forms of neurodegeneration. It also includes an important role of the innate immune system in the brain as well as neuroinflammatory processes, which play a role in both types of NDs and conditions. During the open plenary session overviews were given on the broad spectrum of adult onset NDs (Thomas Klockether, DZNE Bonn), childhood onset NDs (Jutta Gärtner, DZNE Göttingen), also including vitamin-dependent NDs (Robert Steinfeld, Göttingen), and the role of neuroinflammation as well as the resident brain microglial cell population in ND (Michael Heneka, DZNE Bonn). In both disease fields lessons learned point at "must haves" such as an excellent natural history of disease database, early diagnosis, a patient registry, preclinical models, and biomarkers.

Prof. Nicotera (© DZNE - Christiane Knust)

Susan Cotman (MGH Boston) highlighted histopathological commonalities of two - at first sight - very different neurodegenerative diseases caused by defects in one and the same gene, CLN11. Patients carrying two defective alleles of the CLN11 gene develop neuronal ceroid lipofuscinosis, a lysosomal storage disease, whereas patients with one defective allele develop a form of frontotemporal dementia. Nonetheless, both diseases share a number of very interesting histopathological features including lysosomal deposits and retinal damage. In contrast, heterozygous carriers of CLN3 mutations are healthy and mutations in both alleles are required to develop CLN3 disease. Nonetheless, patients with rare mutations in CLN3 can develop symptoms much later suggesting that also in CLN3 disease, a wider spectrum of clinical phenotypes from juvenile to adult exists. Thomas Wishart (Edinburgh) highlighted age-dependent changes in the synaptic proteome in health and disease, underlying master regulators, and how this technology platform is used to discover novel therapeutic pathway entry points across diseases including CLN3 disease. Neuronal brain iron accumulation (NBIA) is another group of NDs that was discussed (Thomas Klopstock, DZNE Munich). There is hope that iron chelation might give benefit to patients suffering from NBIA caused by mutations in pantothenate kinase 2. Two examples of gene therapy approaches to treat rare mitochondrial diseases were also discussed. In one case, a functional mitochondrial gene is introduced into retinal ganglion cells to prevent optic neuropathy in LHON patients (Rita Horvath, DZNE Munich). To treat Mitochondrial

Neurogastrointestinal Encephalopathy Syndrome (MINGIE), a liver directed gene therapy approach was discussed that should allow clearance of accumulating toxic substances in the body (Thomas Klopstock, Munich). Finally, Volkmar Gieselmann (Bonn) gave an overview on childhood onset lysosomal storage diseases and therapeutics approved. Despite very convincing preclinical data that exist for therapeutics to target some of the diseases that were discussed, one question that often remains is whether rare diseases will escape from being the orphans of medicine. Simply, because funding the late preclinical development phases and clinical translation is not always a given.

"Emerging concepts in mitochondrial biology", February 4 - 8, 2018, Weizmann Institute of Science, Rehovot, Israel

The concept that mitochondria are simply in charge of producing building blocks and ATP (via OXPHOS) has drastically changed over the past years. It has become evident that mitochondria communicate extensively with other cellular compartments. Often, mitochondria are in close proximity and in contact with diverse organelles through specialized contact sites which are likely involved in the exchange of lipids and metabolites. Altogether, the spectrum of roles of mitochondria in health and disease has considerably widened and was well covered by many excellent presentations in this meeting. These covered topics such as the mitochondrial proteome and lipidome, mitophagy, mitochondrial dynamics, channels and carriers, tethering of mitochondria to organelles such as the ER and the lysosome, mechanisms of cross-regulation between mitochondria and lysosomes as well as ER, mitochondrial stress response, mitochondrial metabolism, the role of mitochondria and their metabolites in shaping epigenetic changes, the innate immune driven generation and role of mitochondrial derived vesicles in mitochondrial antigen presentation, and links between impaired mitochondrial function, turnover, and fission/fusion dynamics and neurodegeneration. Mitochondrial defects in CLN3 deficient cells have been described. However, neither the cause nor the consequences for disease pathogenesis and therapy are well understood. Hence, a better understanding of these mitochondrial aspects in CLN3 disease would be beneficial.

"The translational science of rare diseases", April 11 - 13, 2018, Tutzing / Lake Starnberg, Germany

At this international symposium a number of interdisciplinary and international scientific and clinical projects were presented with the goal to elucidate the causes of rare diseases and develop innovative therapies. A wide variety of rare disease topics were discussed such as the NIH undiagnosed disease program, epileptic encephalopathies, imprinting diseases, myelin formation and destruction disorders, fragile X syndrome, chromatin and epigenetically driven

diseases, RASopathies, rare autoinflammatory disorders including idiopathic juvenile arthritis and primary immune deficiency in early IBD, lysosomal storage diseases including MLD and CLN2 (for which intracerebral enzyme replacement therapy was recently approved; Schulz et al., 2018), defects in erythrocyte development, and kidney diseases including Fanconi syndromes.

The meeting also included a lively discussion with panelists on the theme of successful collaborations between academia and industry, and what is fact or fiction. Views presented and topics discussed included, early partnerships to ensure all party needs are met early on including those of patients, smarter use of data, data and placebo group sharing, guiding concepts in companies for developing transformative therapies for rare diseases, sustainability of rare disease therapies, the importance of gathering natural course of disease data at a global level, difficulties to obtain funding to bridge translational gaps including drug repurposing. Genes for about half of the estimated 7.000 rare diseases have been identified today and many more will likely be discovered in the coming years. However, the number of available marketed medicinal products and the pace at which novel treatments are being identified to care for the rare remains low and unsatisfactory. Therefore, the question remains whether rare diseases will escape from being the orphans of medicine.

16th International Conference on Neuronal Ceroid Lipofuscinoses (Batten Disease), Sept 12 - 16, 2018, London, UK

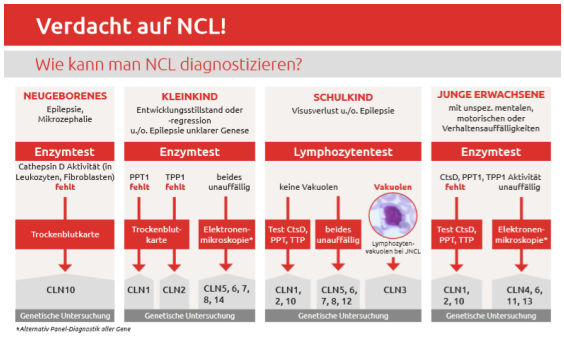
This year the international Conference on NCL will take place in London organized by Dr. Sara Mole. Please, be aware that the early bird registration fee will be until July 2, 2018.

Congress for Paediatricians, Sept 12-15, Leipzig, Germany

Thu, Sept 13, 2018 the NCL Foundation is organizing a symposium, which is included in the annual meeting of the German Society of the Paediatricians. Prof. Das will give an overview about Niemann-Pick Type C and NCL. Dr. Dreha-Kulaczewski will lecture about MRI being used in childhood dementia.

Neurowoche 2018, Oct 10 - Nov 03, Berlin, Germany

During the "Neuroweek" in Berlin, Germany the NCL Foundation will organize a symposium focussing on diagnosis and treatment of different NCL diseases as well as Niemann-Pick Type C. Here you can find the programme.



Information Leaflet for physicians

We have updated our German information leaflet for physicians.

This leaflet was co-authored by Dr. Angela Schulz and Dr. Eva Wibbeler.

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3. Recent Publications

CLN2

Schulz et al. treated CLN2-patients with recombinant human tripeptidyl peptidase 1 (cerliponase alfa) in a multicenter, open-label study. Kids received every 2 weeks an intraventricular infusion with the recombinant lysosomal enzyme. A motor and language Clinical Rating Scale was applied. The mean unadjusted rate of decline in the motor-language score per 48-week period was 0.27±0.35 points in treated patients and 2.12±0.98 points in 42 historical controls.

CLN3

Grünewald et al. showed that the Cln3 knockout ($Cln3^{\Delta ex1\text{-}6}$) mice had an increased anxiety-related behavior and impaired aversive learning as well as markedly affected motor function including disordered coordination. Patch-clamp and loose-patch recordings revealed severely affected inhibitory and excitatory synaptic transmission in the amygdala, hippocampus, and cerebellar networks. Furthermore, loss of specific interneurons in central networks support the hypothesis that degeneration of GABAergic interneurons may be the cause of supraspinal GABAergic disinhibition.

Studniarczyk et al. compared AMPAR properties and synaptic transmission in cerebellar granule cells from wild-type and Cln3 ko mice. Amplitudes of AMPA-evoked whole-cell currents were unchanged. Neither did they detect changes in the amplitude, kinetics, or rectification of synaptic currents evoked by individual quanta, nor in underlying single-channel conductances. Also, no changes were detected expression of GluA2 or GluA4 protein. Quantal events following mossy-fiber stimulation were reduced in number and short-term plasticity was altered in conditions of reduced extracellular Ca^{2+}. Together with reduced mossy fiber vesicle number their results suggest early presynaptic changes but no evidence for altered postsynaptic AMPARs in cerebellum.

Kuper et al. studied the timing of cognitive decline in CLN3 disease. Onset of cognitive decline at a mean age of 6.8 years paralleled onset of visual deterioration at a mean age of 6.4 years. Cognitive dysfunction is universally present around diagnosis in classical CLN3 disease.

Somogyi et al. used cerebellar precursor cell lines generated from wildtype and $Cln3^{\Delta ex7/8}$ mice. Levels of GM1a and GD1a were found to be reduced by both biochemical and cytochemical methods. Quantitative high-performance liquid chromatography analysis revealed an increase in GM3, suggesting a metabolic blockade in the conversion of GM3 to more complex gangliosides. Quantitative real-time PCR analysis revealed reduction in the transcripts of the interconverting enzymes. This data suggest that the complex a-series gangliosides are reduced in $Cln3^{\Delta ex7/8}$ mouse cerebellar precursor cells due to impaired transcription of the genes responsible for their synthesis.

Schultz et al. showed that Cln3 deficient mice have increased astrocyte endfeet area. This phenotype is corrected by treatment with a commonly used GAP junction inhibitor, carbenoxolone (CBX). CBX modifies lipid microdomains and corrects membrane fluidity alterations in Cln3 deficient endothelial cells, which in turn improves defects in endocytosis, caveolin-1 distribution at the plasma membrane, and Cdc42 activity. Cln3 deficient mice

treated orally with CBX exhibited recovery of impaired BBB responses and reduced autofluorescence.

Burkovetskaya et al. followed up on their previous report that Cln3$^{\Delta ex7/8}$ microglia are primed towards a pro-inflammatory phenotype which included an exaggerated caspase-1 inflammasome activation. They now demonstrate heightened caspase activity in the Cln3$^{\Delta ex7/8}$ mouse brain and a reversal of motor behavior deficits and astrocyte activation in Cln3$^{\Delta ex7/8}$ /Casp-1$^{-/-}$ as compared to Cln3$^{\Delta ex7/8}$ mice whereas no significant differences were seen in lysosomal accumulation or microglial activation.

Zhang et al. reported the generation of a new CLN3 mutant human iPSC line derived from a patient with late-onset non-syndromic Retinitis pigmentosa. This patient was compound heterozygous for the common exon$^{7/8}$ deletion and a novel variant (c.175G>A) that causes a missense mutation (p.Ala59Thr) at position 59 in the protein. The authors also generated an isogenic control line by using CRISPR/Cas9 to correct the c.175 G>A mutant allele.

CLN5

Huber & Mathavarajah reported the localization, molecular function, and interactome of Cln5, the CLN5 homolog in the social amoeba *Dictyostelium discoideum*. *Dictyostelium* Cln5 is secreted during growth and starvation. It was revealed that both *Dictyostelium* Cln5 and human CLN5 are glycoside hydrolases. Immunoprecipitation coupled with mass spectrometry identified 61 proteins that interact with Cln5 in *Dictyostelium*. Of the 61 proteins, 67% localize to the extracellular space, 28% to intracellular vesicles, and 20% to lysosomes. The majority of the interacting proteins are involved in metabolism, catabolism, proteolysis, and hydrolysis, and include other NCL-like proteins (e.g., Tpp1/Cln2, cathepsin D/Cln10, cathepsin F/Cln13) as well as proteins linked to Cln3 function in *Dictyostelium* (e.g., AprA, CfaD, CadA).

CLN6

Kleine Holthaus et al. explored the therapeutic potential of an ocular gene therapy to treat sight loss in NCL due to a deficiency in the transmembrane protein CLN6. Supplementation of CLN6 in photoreceptors was not beneficial. In contrast, delivery of CLN6 to bipolar cells using adeno-associated virus (AAV) serotype 7m8 slowed the loss of photoreceptor function and photoreceptor cells.

CLN7

Danyukova et al. performed a SILAC-based quantitative analysis of the lysosomal proteome in mouse embryonic fibroblasts (MEF) derived from a ko mouse. They detected 56 soluble and 29 abundant membrane proteins. The amounts of 12 different soluble lysosomal proteins were significantly reduced in Cln7 ko MEFs. CLN5 was one of the significantly depleted lysosomal proteins, and likely due to its increased proteolytic degradation by cysteine proteases in Cln7 ko lysosomes. The authors also reported defects in the ability of Cln7 ko MEFs to adapt to starvation (impaired reactivation of mTORC1, reduced autolysosome tabulation, increased perinuclear accumulation of autolysosomes).

Progranulin (CLN11)

Valdez et al. used iPSC-derived human cortical neurons from FTD patients harbouring PGRN mutations and showed that both FTD and NCL-like pathology are present in PGRN patient neurons as compared to isogenic controls. Interestingly, PGRN mutant neurons had decreased activity of cathepsin D. PGRN interacts with cathepsin D and a cleavage product of PGRN increases its activity.

Arrant et al. applied an AAV vector (AAV-Grn) to deliver progranulin in $Grn^{-/-}$ mice. Even during the post-symptomatic phase gene replacement was able to reduce lipofuscinosis and microgliosis in several brain regions. Interestingly, AAV-expressed progranulin was only detected in neurons, not in microglia. It corrected abnormal cathepsin D activity.

CLN12

Rayaprolu et al.: Loss-of-function mutations in ATP13A2 are associated with Kufor-Rakeb syndrome (KRS), NCL, and a form of hereditary spastic paraplegia (HSP). They demonstrated that loss of one functional Atp13a2 allele leads to both microgliosis and astrocytosis in multiple brain regions; however, levels of lipofuscin were only modestly elevated in the cortex of heterozygous Atp13a2 knockout mice. This data suggests that partial loss of ATP13A2 causes inflammatory changes within the brain which appear to be independent of robust lipofuscinosis.

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4. Grants, Awards and Open Positions

Prof. Ritva Tikkanen (University of Gießen) receives support to test the potential of substances that can promote full-length protein generation and prevent nonsense mediated decay of mRNAs encoding rare variants of CLN3 carrying nonsense mutations. While the vast majority of CLN3 patients are either homozygous or compound heterozygous for the allele that carries a deletion of exons 7 and 8, a minority of patients are either homozygous or composite heterozygous carriers of nonsense mutations. These nonsense mutations encode a premature termination codon (PTC), causing a stop in protein translation, and often mRNA degradation by nonsense-mediated decay. Prof. Ritva Tikkanen has recently shown that the FDA approved drug amlexanox can induce carrier levels of enzyme activity in cells carrying a nonsense allele for aspartylglucosaminuria (AGU) (Banning et al., 2018).

Prof. Dr. Ritva Tikkanen © Privat

Prof. Henning Tidow © Uni Hamburg

Prof. Henning Tidow (University of Hamburg) receives support to investigate the three dimensional (3D) structure of the CLN3 protein. Prof. Tidow's research is focused on the structural biology of integral membrane proteins. Recent progress in technologies to elucidate the structures of transmembrane proteins including the endolysosomal channel TRPML1 (Schmiege et al., 2017; Li et al., 2017), and TRPML3 (Hirschi et al., 2017) generates hope to cast a glimpse on a 3D model for the CLN3 protein and whether or not this will confirm topologies proposed based on biochemical and bioinformatics approaches (Perland et al., 2017; Ratajczak et al., 2014; Cotman & Staropoli, 2012; Nugent et al., 2008; Mao et al., 2003).

Ultimately, it is expected that a 3D structural model of CLN3 will greatly advance our understanding of CLN3 function and dysfunction in the endo-lysosomal membrane. Our funding partner are:

Furthermore, this year the NCL Foundation will evaluate proposals for its **9th NCL Research Award (50,000 EUR).** The goal of this award is to support an innovative pilot project at the **Postdoctoral fellowship level**. We highly encourage junior scientists, clinical researchers and medical fellows worldwide to submit projects that hold promise to help find and push forward therapies for CLN3 disease. We also highly encourage applicants that work in disease areas outside NCL, provided the proposed research project includes a strong CLN3 focus and is relevant to elucidate its role in disease or finding a cure.

Applicants are requested to follow our 2-stage process. Please, first submit a Letter of Intent. The LOI serves as a basis for discussion with our head of research before submitting a full proposal. Both documents can be requested via email to **Research@ncl-foundation.com**.

Deadline for handing in full proposals: **November 30, 2018.**

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