NCL Research Newsletter April 2020

Content

NCL-Stiftung
Für eine Zukunft ohne Kinderdemenz

1. Main Topic

1.1 NCL hotspot for innovative new gene therapy trials

In recent years NCL has become a hotspot for moving potentially disease-transforming innovative new therapies to the clinic (for recent review see Johnson et al. 2019). It started with the success story and **first-in-history approval** by the FDA and the EMA, of an **intracerebral enzyme replacement therapy for CLN2 disease**. Amongst the general boost in gene therapy coming of age (see recent article by McKinsey & Company), we are now also seeing a surge of new gene therapy efforts targeting several NCL subtypes (summarized below). In addition, a **patient customized oligonucleotide therapy** recently started in a single CLN7 patient to try and correct a splice defect, and hopefully reconstitute therapeutic levels of the CLN7 protein. For a recent review see also Liu et al. 2020.

- CLN1, CLN3: Abeona[1]
- CLN2: Spark Therapeutics[2], RegenXbio RGX-181[3]
- CLN3, CLN6, CLN8: Nationwide, Gray Foundation, Amicus[4,5,6]
- CLN5: University Texas Southwestern Medical Center[1] & Neurogene[10]
- CLN7: Batten Hope[7], Mila's Miracle Foundation/stopbatten (ASO)[8], Vigene[9], Neurogene[10]

[1] https://www.abeonatherapeutics.com/science#pipeline; [2]https://sparktx.com/press_releases/spark-therapeutics-announces-presentation-of-preclinical-data-in-pompe-disease-and-cln2-disease-at-15th-annual-worldsymposium/; [3] https://regenxbio.com/cln2/; [4] http://ir.amicusrx.com/news-releases/news-release-details/amicus-therapeutics-announces-phase-12-study-gene-therapy-cln3; [5] https://clinicaltrials.gov/ct2/show/NCT02725580; [4,5] https://www.amicusrx.com/programs-pipeline/batten-disease/; [6] http://ir.amicusrx.com/news-releases/news-release-details/amicus-therapeutics-acquires-gene-therapy-portfolio-ten-clinical; [7] https://battenhope.org/building-a-cln7-clinical-trial-the-natural-history-study-is-here/; [8] https://www.stopbatten.org/our-work; [9] https://www.vigenebio.com/Press-Releases/2017/batten-disease/; [10]https://clinicaltrials.gov/ct2/show/NCT03822650

NCL gene therapy attempts are not restricted to NCL genes encoding soluble lysosomal enzymes, which seem to have the better cards to generate beneficial outcomes in the clinic. These expectations are based on earlier experience gained using intracerebral enzyme delivery, and the finding that soluble lysosomal enzymes can show cross-correction. This mechanism entails that viral transduced cells produce enzyme that can correct the cell's own lysosomal enzyme deficit, as well as that of non-transduced cells because the transduced cells secrete enzyme can reach the lysosomal compartment of non-transduced cells via the endocytic uptake route. Cross-correction is unlikely to occur in the case of transmembrane proteins. Nonetheless, **gene therapy clinical trials** are now underway for NCL caused by mutations in the transmembrane protein CLN6 (an ER resident transmembrane protein) and CLN3 (a lysosomal transmembrane protein). Supportive preclinical evidence for efficacy was obtained using AAV-based gene delivery in existing mutant mouse models (CLN3: Bosch et al. 2016; Pratt et al. 2019 SfN Abstract; CLN6: Cain et al. 2019; Kleine Holthaus et al. 2018). The type of AAV vector used had earlier proven to meet development and safety criteria, and received **FDA approval** for gene therapy in SMA (Spinal Muscular Atrophy). Recently, Amicus reported first positive interim clinical data for the gene therapy trial in CLN6 patients. Data have been collected for up to 2 years post treatment. Some evidence of disease stabilization was reported in 7 out of 8 children.

1.2 A patient-customized ASO Therapy for CLN7

Kim et al. 2019 reported in the New England Journal of Medicine the rapid development of an **investigational antisense oligonucleotide** (ASO) **therapy** (drug name Milasen) for a single CLN7 patient, including clinical outcome observations during the first year of this **"N-of-1" single patient-customized trial**. In patient fibroblasts, the ASO was shown to correct miss-splicing of the CLN7 mRNA that is caused by a mutation that so far seems unique to this one patient. The ASO was also shown to improve lysosomal function. Milasen was reported to have an acceptable side-effect profile. It's too early to tell if and how efficacious this therapy will be on the long-term. This study illustrates the amazingly rapid design, approval, and start of treatment of an individualized genomic medicine. This new drug-discovery paradigm also raises many questions that are not simply restricted to safety. Some of these questions include for example, how good functional data should be before moving forward to administering the drug to the patient, and how to evaluate efficacy in an N-of-1 trial. Other including ethical, societal and drug regulation questions were discussed in an accompanying NEJM article by Woodcock and Marks. A recent article in MedpageToday also provides some interesting thoughts and discussion themes around this topic.

Tim, Mila & Julia © Boston Children's Hospital

Open questions

We have entered a very exciting era of genomic medicine and gene therapy clinical trials in the NCL field. At the same time, it is fair to say, that we still have a long way to go. It is useful to think about "what gene therapy can and can't do today", citing the title of a recent blog article. Will todays delivery vectors carrying healthy versions of **transmembrane proteins** transduce enough brain cells and those cell types most critical to a particular disease? Transmembrane proteins will unlikely benefit from biological mechanisms allowing cross-correction. **Nanotubes**, tiny thin contact tubes between cells that allow some exchange of cytoplasmic components between cells, have even been shown to support organelle transfer between cells. Today, however, we know very little about the role and efficacy of such mechanisms in the living brain. The **AAV gene therapy vectors** used today also largely fail to transduce the innate immune cells in the brain (microglia). How important this caveat is in the context of NCL is largely unknown. Microglia cells will likely be subject to cross-correction when delivering genes encoding lysosomal enzymes but not when delivering transmembrane genes. How relevant each NCL gene defect and its reconstitution in microglial cells is in the context of a given NCL form remains unknown. **Microglial cell activation** is commonly seen in NCL brains. Studies in mouse models suggest significant variations in onset and extent of microglial activation, depending on which NCL gene is defective.

1.3 Biomarkers, not quite there yet

So far, clinical studies for testing new therapies in NCL have to rely entirely on clinical outcome measures in patients. Today, **no clinically validated NCL disease-specific biomarkers** have been identified. The lack of a validated biomarker was not a serious hurdle in the intracerebroventricular infusion ERT trial and the FDA and EMA approval process of the drug cerliponase alpha (recombinant TPP1 enzyme) for CLN2 disease. Decisive were **good natural history data** of CLN2 disease, a rapid progression of disease, and a for the most part rapid progression of the disease with small patient-to-patient variability in disease onset, progression, and clinical outcome measures. For CLN3 disease, an excellent natural history of disease database also already exists and continues to grow. However, unlike in CLN2 disease, most clinical outcome measures in CLN3 disease show very significant patient-to-patient variability in onset, severity, and progression. This bandwidth exists despite the fact that a large fraction of patients carry the same exon 7 and 8 deletion in the CLN3 gene. The situation for CLN6 is similar, and a natural history database for CLN6 disease is lagging behind.

Recently, Ru et al. (Ru et al. 2019) showed that the cross-species translational biomarker of neurodegeneration **Neurofilament Light chain (NF-L)** is a treatment-responsive biomarker in CLN2 disease. The authors showed, that pretreatment NF-L levels did not correlate with disease severity or age. However, in CLN2 patients receiving ERT, NF-L levels decreased by 50% each year over more than 3 years suggesting that circulating NF-L levels can serve as a **treatment-responsive biomarker in CLN2 disease**.

The quest for additional NCL disease biomarkers continues. In mouse models the search for **fluid biomarkers** has so far met with limited success (see e.g. the 2018 published CLN3 mouse model study by Timm and colleagues. Perhaps a recently developed **CLN3 pig model** offers new opportunities (Johnson et al. 2019 SfN Abstract). One earlier study profiled lymphocytes from CLN3 patients. Included were patients with different disease courses and the study identified some dysregulated transcripts (Lebrun et al. 2011). So far, no further confirmative studies have been published.

Sleat and colleagues published a study, in which they used unbiased **proteomic analysis of autopsy brain samples and matching CSF samples** from a few CLN1, CLN2 and CLN3 patients (Sleat et al. 2017). Some alterations in protein expression were identified in samples from each NCL subtype. Confounds of potential postmortem changes in the samples require further validation of these findings. In a more recent study, (Sleat et al. 2019) this group reported a proteomics analysis from mouse CSF and brain samples obtained at different stages of disease progression (CLN1, 4 and 26 weeks; CLN2, 4 and 19 weeks; CLN3, 4 and 52 weeks). They also conducted a label-free analyses of brain proteins that contained the mannose 6-phosphate lysosomal targeting modification. Overall, few changes were seen at presymptomatic timepoints. Later in disease, the expression of a number of proteins was significantly altered in both brain and CSF of CLN1 animals, and fewer in CLN2 animals. Most of these protein levels were elevated. In CLN3, only 4 proteins were significantly elevated. These included lysosomal proteins SCARB2, HEXB, and TPP1 while Sphingomyelin phosphodiesterase 1 (SMPD1; note, mutations cause Niemann Pick type A/B) was significantly decreased in both mouse and human brain CLN3 samples. No proteins were significantly altered in both CLN3 brain and CSF. When considering all proteins elevated in NCL, lysosomal proteins were over represented (CLN1, ~17% lysosomal; CLN2, ~31%; CLN3, 75%,). To what extent these changes reflect compensatory cellular responses remains to be determined. At this point in time, it is premature to predict a biomarker utility for some of the changes seen in CSF.

Sindelar and colleagues (Sindelar et al. 2018) published last year the results of a study using **metabolite profiling of CSF** comparing CSF from normal vs. CLN2 deficient individuals. Main goal of this study was to try and identify a distinct CSF metabolome that could be used to develop a biomarker panel for assessing disease severity in patients with **TPP1 disease** mutations. The combination from targeted and untargeted metabolite profiling resulted in 1486 metabolite features, of which 257 showed significant differential expression between control vs. CLN2 group. 158 were at least 1.5-fold increased or decreased. Of these, 26 features were increased by over 16-fold and were assigned to two drugs (Levetiracetam and Clonazepam) that a significant proportion of the patients received. A further prioritization based on correlation with disease severity scores yielded 6 unique metabolites that showed a significant negative correlation with disease severity scores based on MRI features. Except for glycero-3-phosphoinositol, most of these metabolites with reduced abundance in CLN2 patient CSF carry an acetyl function. As neither the substrates of TPP1 are known, nor is it known why its deficiency causes neuronal death, these metabolite changes offer potential insight into CLN2 function, substrates, and disease pathology. Despite not yet knowing the functional role of these downregulated biomarkers, they constitute a fingerprint for CLN2

disease and may provide an effective tool for monitoring disease progression and the response to therapy.

Dr. Rebecca Ahrens-Nicklas © Ingoboelter photodesign

Ahrens-Nicklas (NCL Research Award 2018) and colleagues reported that **progressive hippocampal dysfunction** occurs in two CLN3 disease models prior to notable lysosomal storage and neuronal loss (Ahrens-Nicklas et al. 2019). CLN3 mice also lose hippocampal sharp wave ripples, an electrophysiological marker of new memory encoding. The authors drew analogues to Alzheimer's disease, where a similar phenomenon has been observed, suggesting a shared electrophysiologic signature of dementia in these diseases. These findings clearly plead for **more detailed EEG assessments** in both pre-symptomatic and symptomatic CLN3 patients, and highlight the need for therapeutic interventions that **target early neuronal circuit deficits**. Interestingly, carbenoxolone, a drug that reduces lysosomal storage burden in CLN3 mutant mice, exacerbated disease-induced hippocampal network changes. Perhaps an important lesson learned that correction of storage burden, which is often used as a surrogate disease marker, is insufficient to restore hippocampal network function.

Ideally, biomarkers correlate with disease severity scores, and they can predict treatment responses and slowing of disease progression in individual patients. At best, they also provide readouts well before clinical assessment scale parameters come into play and provide insight into yes/no improvements in the patient. Currently, the number of patients in the CLN3 and CLN6 gene therapy trials is low and patient-to-patient variability in clinical scores is high which implicates, that it likely will take years to tell, with some degree of certainty, how successful treatment will be. As we are dealing with rare diseases, the number of patients will remain limited. More treatment options will likely emerge which raises concerns about recruiting for each trial new and a sufficient number of patients. Care givers, families and patients may also at some point have to make a decision on more than one treatment option. Once in a trial, patients cannot simply shift to new trials offering novel different treatment modes. How can biomarkers help? These generally do not and cannot replace clinical outcome measures. Nonetheless, good surrogate biomarkers could allow for a better stratification of patients entering trials, and monitoring treatment responses early into the trial.

We hope that some of the ongoing groundbreaking work in unraveling and understanding the function of individual NCL genes will increase our chances of finding and developing **clinically useful biomarkers**. Here, some examples of recent discoveries regarding NCL gene function.

1.4 Shedding light on NCL gene function

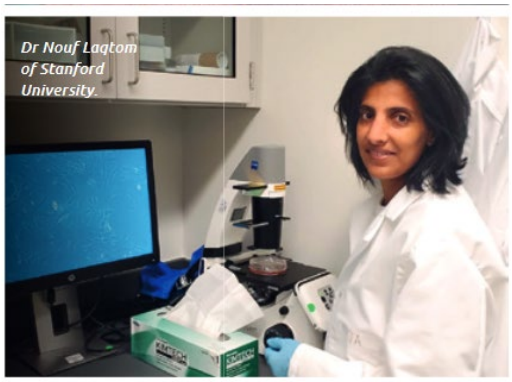
Sardiello (winner of the 5th NCL Research Award) and colleagues provided new insights into the **function of the two ER resident NCL proteins** encoded by the CLN6 gene (Bajaj et al. 2019), and the CLN8 gene (di Ronza et al. 2018). Both CLN6 and CLN8 interact with a large number of lysosomal enzymes and their deficiency results in significant changes in the soluble lysosomal enzyme proteome and its overall degradative capacity. **CLN8 deficiency disrupts ER to Golgi trafficking of lysosomal enzymes**, which results in faster clearance of lysosomal enzymes from the ER via ER-associated protein degradation (ERAD). CLN8 is retrieved from the Golgi to the ER in a COPI interaction-dependent manner, while ER export of CLN8 depends on COPII. Unlike CLN8, CLN6 does not traffic to the Golgi but at the level of the ER, CLN6 and CLN8 proteins form an ER to Golgi relay complex that serves the transport of enzymes of the lysosomal system from the ER to the Golgi. A deficiency in either CLN6 or CLN8 therefore compromises the overall lysosomal content of soluble lysosomal enzymes. What the downstream driving factors inside the lysosome are that might be mainly responsible for their compromised function and cellular toxicity remains to be resolved.

Here, a new **lysosomal immunoprecipitation technology** (LysoIP), developed by Prof. Monther Abu-Remaileh and colleagues in the laboratory of David Sabatini at the Whitehead Institute in Boston, comes to help (Abu-Remaileh et al. 2017). LysoIP is based on the expression of a triple-HA tagged lysosomal transmembrane protein (TMEM192). Its expression in human and mouse cells allows for a very rapid isolation of intact lysosomes, which allows for the first time an in-depth analysis of the **lysosomal metabolome**. Dr. Nouf Laqtom in Prof. Abu-Remaileh's lab was co-funded by the NCL Foundation in Hamburg and the Beat Batten Foundation in the Netherlands, to apply this technology to unravel what goes wrong inside the lysosome of cell lacking CLN3. The results shed an unexpected light on the function of CLN3 and the likely associated toxicity mechanisms. Both investigators have now moved to the ChEM-H institute at Stanford University where they continue their efforts to resolve key CLN3-related questions.

1.5 Interview with Dr. Nouf Laqtom

1. What is your background? And when did you hear about NCL for the first time?

I earned my PhD in Genetics at The University of Edinburgh, UK. The inherited metabolic disorders such as the severe childhood lysosomal storage diseases (LSDs) are commonly encountered in Saudi Arabia (my home country) and other Gulf states due to consanguineous marriage.


Dr Nouf Laqtom of Stanford University.

2. Do the special circumstances, fighting a fatal disease, affect your attitude to your work?


Dr Monther Abu-Remaileh of Stanford University.

In 2016, I joined the lab of Prof. David Sabatini as a visiting postdoctoral researcher. There, I met a senior postdoctoral fellow, Dr. Monther Abu-Remaileh, who was developing a method to isolate high quantities of pure and intact lysosomes from cells and animal tissues. The initial results from his purification method were so far encouraging, providing a better tool to investigate lysosomes in health and diseases. I was so excited to work with him on CLN3-related neuronal ceroid lipofuscinosis (NCL), which is a class of LSDs. CLN3 disease has a devastating impact on children's lives and their health is a major motivation for us.

3. What impact would you like your project to have?

Our current efforts are largely geared towards determining the unknown biochemical function of CLN3. We hope that our work will help to cure Batten disease and slow its progression as well as to contribute to developing novel assays to determine disease severity.

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2. Scientific and Medical Meetings

Due to the Corona crisis many meetings are cancelled or postponed.

NCL2020, the **17th International Congress on the Neuronal Ceroid Lipofuscinoses** (Batten disease) has been postponed. It will take place next year as NCL2021 in October 2021 in St. Louis, MO, USA. Confirmation of the date of NCL 2021 will be announced on the conference website (http://www.ncl2020.org), as soon as this become available.

The NCL Foundation is planning on organizing the national NCL congress as well as the CLN3 young investigator symposium in Dec in Hamburg, Germany. Exact dates will be published soon.

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3. Grants and Awards

Dr. Frank Stehr, Prof. Susan Cotman, Dr. Elisabeth Butz, Dr. Henneke Lütgerath © Ingoboelter photodesign

The **9th NCL Research Award** was awarded to **Prof. Susan Cotman and Dr. Elisabeth Butz** at the Center for Genomic Medicine and Department of Neurology at Massachusetts General Hospital in Boston, USA. The goal of this project is to better understand **microglial physiology in CLN3 pathology** and disease progression. To this end they are using a number of approaches including studying mouse and human CLN3 microglia *ex vivo*, and *in vivo* using a strategy that includes microglia-depletion and reconstitution with microglia-like cells following hematopoietic stem cell transplantation. This project provides excellent synergies with another microglia project that was awarded - our Neurodegeneration Research Award to **Dr. Sabina Tahirovic** at the German Center for Neurodegenerative Diseases (DZNE) in Munich and **Prof. Cotman** at MGH in Boston. This project builds upon complementary expertise in Alzheimer's Disease and Niemann-Pick type C disease in the laboratory of Dr. Tahirovic, and CLN3 disease in Prof. Cotman's laboratory, and aims at uncovering overlapping and distinct features across these different neurodegenerative diseases including biomarkers and therapeutic targets. The 9th NCL Research Award was supported by the **Joachim Herz Stiftung**. We are very grateful for that.

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4. Recent Publications

CLN1

Atiskova et al. performed an in-depth analysis of the retinal phenotype of a CLN1-deficient mouse. Prior to onset of retinal loss, they report reactive retinal astrogliosis and microgliosis, a progressive accumulation of storage material, a dysregulation of lysosomal proteins, and accumulation of p62-positive aggregates in the inner nuclear layer. At advanced stages of the disease, there is a significant loss of ganglion cells, rod and cone photoreceptor cells, and rod and cone bipolar cells.

Eaton et al. generated a novel sheep CLN1 disease model with morphological, anatomical and biochemical disease phenotypes that closely resembles the human condition. Reported phenotypes include an accumulation of autofluorescent storage material, clinical signs including pronounced behavioral deficits, motor deficits, complete loss of vision, reduced lifespan, and profound reduction in brain mass similar to alterations observed in human patients.

CLN2

Gardner et al. provided an update on TPP1 gene variants associated with CLN2 disease comprising 131 unique variants from 389 individuals. This totals 717 alleles including previously unrecorded individuals, two pathogenic variants that collectively occur in 60% of affected individuals in the sample and account for 50% of disease-associated alleles, the

proportion of homozygous allele carriers and alleles private to single families. Atypical disease onset and/or progression alleles represents 13% of individuals recorded with associated phenotype. This information is highly relevant for effective CLN2 disease management that requires early diagnosis.

CLN3

El-Sitt et al. show beneficial effects of long-term exogenous galactosylceramide supplementation on longevity, neurobehavioral parameters, neuronal cell counts, astrogliosis, and diminution in brain and serum ceramide levels in Cln3 $^{\Delta ex7/8}$ knock-in mice. Furthermore, the authors propose the use of serum ceramide as a potential biomarker to track impact of therapies.

Kuper et al. assessed motor function by the 6-Minute Walk Test was in 15 patients with CLN3 disease. 6MWT scores were already impaired from first testing near diagnosis and continuously declined with age and with increasing UBDRS scores, confirming correlation with disease progression.

Adams et al. created a "hub and spoke" model for implementing a 22-week crossover clinical trial of mycophenolate compared with placebo, with two 8-week study arms. This strategy reduced the ability of individuals to participate in this clinical trial.

Kuper et al. described three rare CLN3 patients that are compound heterozygous for the common ~1 kb deletion in combination with a mutation that changes the initiation codon for Methionine into Leucine. These three patients displayed more attenuated phenotypes although their retinal phenotype was similar to that seen in classical cases of CLN3 disease. Whether the mutated mRNA can support low level CLN3 protein production and by what mechanism remains to be investigated.

Wright et al. described that diagnosis and early suspicion of CLN3 disease can be aided by detailed directed history and high-resolution retinal imaging, with subsequent targeted microscopy/genetic testing. Early diagnosis is critical to ensure appropriate management, counseling, support, and social care for children and their families. Also it is likely that successful intervention will be most effective when initiated at the earliest stage of disease.

Gomez-Giro et al. engineered an isogenic pair of CLN3 mutant human hiPSC lines carrying the CLN3 CLN3^{Q352X} pathologic variant. They demonstrate that the CLN3 pathogenic variant gives rise to classical hallmarks of JNCL in vitro, and discovered a splicing alteration caused by this particular mutation. About half of CLN3^{Q352X} cerebral organoids failed to develop normally. Transcriptome analysis of the surviving half demonstrated affected pathways related to development, corticogenesis and synapses. Decreased levels of gamma-amino butyric acid (GABA) also indicate that in this organoid model, the CLN3^{Q352X} mutation causes synapse alterations that precede neuronal damage and storage pathology.

Volz et al. describe that studying retinal phenotypes of Cln3$^{\Delta ex7/8}$ knock-in mice on a C57BL/6 N background is confounded by the rd8 mutation that is present in C57BL/6 N mice. This mouse strain is widely used in ophthalmic research as a background for modeling retinal degeneration. Using electroretinography to investigate the age-dependent functional loss in Cln3$^{+/+\ rd8-/rd8-}$ mice and compare it to C57BL/6 J mice the authors show that the rd8 mutation does affect the retinal function in Cln3$^{+/+\ rd8-/rd8-}$ mice in a variable manner and comparing results obtained in independent studies or on other mouse backgrounds may be misleading.

Rietdorf et al. - the authors review data showing that neuronal ceroid lipofuscinoses (NCLs) have co-morbidities outside the brain. One of these co-morbidities is a decline in cardiac function increasingly recognised in teenagers and adolescents with juvenile CLN3 and some of the other NCLs. The authors summarize current knowledge of the structural and functional changes found in the hearts of animal models and patients diagnosed with NCL, and present evidence of structural changes observed in cardiomyocytes from $CLN3^{\Delta ex7/8}$ mice.

Yasa et al. - using bioluminescence resonance energy transfer (BRET) the authors confirm an interaction between CLN3 and Rab7A in live cells. They show that CLN3 regulates the interaction between Rab7a and retromer, a protein complex required for efficient endosome-Trans-Golgi Network (TGN) trafficking of sorting receptors. In cells lacking CLN3 or expressing disease-causing CLN3 mutations, the lysosomal sorting receptors were degraded and cathepsin D levels were reduced. In line with a role for Rab7A regulating several functions at late endosomes, degradation kinetics of EGFR are delayed. These data provide novel molecular insights in endolysosomal phenotypes and defects observed in CLN3 mutant cells.

Petcherski et al. presented results from a follow-up autophagy modifier screen in neuronal cells that identified several small molecular weight compounds that reduce autophagosome accumulation in CLN3-deficient cells. The results largely confirm earlier results and identify an expanded set of autophagy modifiers that increase or decrease the accumulation of autophagosomes in CLN3 cells. The identified compounds point at several pathways of interest including calcium signaling, microtubule dynamics, and the mevalonate pathway.

Johnson et al. showed that when CLN3 mice received acidified drinking water (pH 2.5-2.9) instead of normal tap water (pH 8.4) for several generations, this normalized motor skills of mutant mice to control levels, indicating a disease-modifying effect of acidified water. The authors also report that the gut microbiota of CLN3 mutant mice was markedly different from control mice, and that acidified water differentially changed the gut microbiota composition in these mice. Relevance of these observations for patients is an open question.

Ahrens-Nicklas et al. focused on the question what mechanisms drive neurologic symptoms in CLN3 disease. Using in vitro voltage-sensitive dye imaging and in vivo electrophysiology the authors report that progressive hippocampal dysfunction occurs before notable lysosomal storage and neuronal loss is detected in two different CLN3 disease mouse models. Pharmacologic reversal of lysosomal storage deposition in young mice does not rescue the circuit dysfunction. Also, CLN3 disease mice lose hippocampal sharp-wave ripples, a marker of new memory encoding. These findings highlight the need for new therapeutic interventions targeting early circuit defects.

Tarczyluk-Wells et al. showed that ibuprofen in combination with the neuroprotective agent lamotrigine improves previously documented beneficial effects of ibuprofen alone. Given the partial efficacy of these treatments, it will be important to test further drugs of this type in order to find more effective combinations.

Mirza et al. compiled all of the available CLN3 gene and protein data from databases, repositories of funded projects, patent and trademark offices, science and technology journals, industrial drug and pipeline reports as well as clinical trial reports and validated the information together with experts in Batten disease and lysosomal storage disease and biology. This compendium provides a roadmap to completed works.

CLN4

Imler et al. expressed CLN4 mutant human CSPα (hCSPα) in Drosphila neurons showing that similar to patients, CLN4 mutations induce excessive oligomerization of hCSPα and premature lethality in a dose-dependent manner. The data suggest that CLN4 alleles have dominant hypermorphic gain of function mutations that drive excessive oligomerization and impair membrane trafficking.

CLN5

Singh et al. investigated the role of CLN5 in the developing brain and showed that loss of CLN5 delays interneuron development during the in utero period. Adult CLN5-deficient mice present deficits in hippocampal parvalbumin-positive interneurons. Furthermore, adult Cln5-/- mice presented deficits in hippocampal parvalbumin-positive interneurons, and age-independent cortical hyper-excitability as measured by EEG. Altogether, this highlights the importance of CLN5 in neurodevelopment.

CLN6

Kleine Holthaus et al. provide further supportive evidence for efficacy of brain directed CLN6 gene therapy by showing that neonatal bilateral intracerebroventricular AAV CLN6 gene therapy increases lifespan by more than 90%, maintains motor parameters, and reduces neuropathological hallmarks of Cln6-deficient mice up to 23 months post vector administration.

CLN7

Connolly et al. show that the CLN7 protein is required for the normal growth of synapses and trans-synaptic communication at the Drosophila larval neuromuscular junction. In a Cln7 mutant, synapses fail to develop fully leading to reduced function and behavioral changes. Cln7 expression is restricted to the post-synaptic cell and the protein localizes to vesicles immediately adjacent to the post-synaptic membrane.

CLN10

Marques et al. show that the administration of recombinant human Cathepsin D (rhCTSD) can improve the biochemical phenotype of CTSD-deficient hippocampal slice cultures in vitro and retinal cells in vivo. Dosing of rhCTSD in the CLN10 mouse model leads to a correction of lysosomal hypertrophy, storage accumulation and impaired autophagic flux in the viscera and central nervous system (CNS). The authors show that direct delivery of the recombinant protease to the CNS is required for improvement of neuropathology and lifespan extension.

CLN11 (Progranulin)

Huin et al. identified homozygous GRN mutations in six new patients which phenotypic spectrum is much broader than previously reported. These include a childhood/juvenile form with an early age at onset as well as distinct delayed phenotypes of frontotemporal

dementia and parkinsonism after 50 years.The authors propose that the hypomorphic effect of some mutations is supported by the presence of residual levels of plasma progranulin and/or low levels of normal transcript. This heterogeneity in phenotypes must be considered in therapeutic trials based on replacement strategies.

Valdez et al. examined the impact of PGRN mutations on the processing of full-length prosaposin to individual saposins, which are critical regulators of lysosomal sphingolipid metabolism. Using Fronto Temporal Dementia (FTD)-PGRN patient-derived cortical neurons differentiated from induced pluripotent stem cells and post-mortem tissue from patients the authors show that PGRN haploinsufficiency results in impaired processing of prosaposin to saposin C, reduced lysosomal Glucocerebrosidase (GCase) activity, lipid accumulation, and increased insoluble α-synuclein. Reduced GCase activity may contribute to FTD-PGRN pathogenesis. Perhaps it is also relevant in the context of CLN11 disease.

CLN12 (also relevant to TRPML1 in last section)

Tsunemi et al. demonstrated a novel role for lysosomal exocytosis in clearing intracellular α-synuclein. The authors show that impairment of this pathway by mutations in the Parkinson Disease-linked gene CLN12 (ATP13A2/PARK9) contributes to α-synuclein accumulation in human dopaminergic neurons. Upregulating lysosomal exocytosis by activation of the lysosomal Ca^{2+} channel transient receptor potential mucolipin 1 (TRPML1) was sufficient to upregulate lysosomal exocytosis, rescue defective α-syn secretion, and prevent α-syn accumulation. These studies identify lysosomal exocytosis as a potential therapeutic target in diseases characterized by the accumulation of α-synuclein and perhaps other lysosomal storage products.

CLN14

Mastrangelo et al. reported two new patients carrying novel pathogenic variants in the CLN14(KCTD7) gene. These two patients presented with a remarkable phenotypic heterogeneity including progressive myoclonus epilepsy without NCL-type lysosomal storages and progressive myoclonus epilepsy with NCL-like lysosomal storage. This is interesting in light of earlier reports (see e.g. Metz et al.) who showed that biallelic KCTD7 mutations define a neurodegenerative disorder with lipofuscin and lipid droplet accumulation but without defining features of NCL or lysosomal storage.

Potential targets - lysosomal ion channels – TPC2, TRPML1

Gerndt et al. show that ion selectivity of the lysosomal ion channel TPC2 depends on the activating ligand. A high throughput screen identified two structurally distinct TPC2 agonists. One of these evoked robust Ca^{2+}-signals and non-selective cation currents, the other weaker Ca^{2+}-signals and Na^{+}-selective currents. These properties were mirrored by the Ca^{2+}-mobilizing messenger NAADP and the phosphoinositide, $PI(3,5)P_2$, respectively. Agonist action was differentially inhibited by mutating a single TPC2 residue. Also, agonist action was coupled to opposing changes in lysosomal pH and exocytosis. Aside from resolving conflicting earlier reports on ion permeability and gating properties of TPC2, these findings may open new avenues for therapeutic applications in NCL as well as in other diseases including coronavirus infections. Ou et al. recently showed that SARS-CoV-2 enters 293/hACE2 cells mainly through endocytosis and that PIKfyve, TPC2, and cathepsin L are critical for entry.

Zhang et al. identified a class of tricyclic anti-depressants (TCAs) as small-molecule agonists of TPC channels. TCAs activate both TPC1 and TPC2 in a voltage-dependent manner. The authors also identified another compound which, like $PI(3,5)P_2$, activates TPC2 independent of voltage, suggesting the existence of agonist-specific gating mechanisms. These findings might explain reported effects of TCAs in the modulation of autophagy and lysosomal functions.

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