THE 15TH INTERNATIONAL CONFERENCE ON NEURONAL CEROID LIPOFUSCINOSIS (BATTEN DISEASE)

NCL 2016
BOSTON

October 5-8, 2016

Wyndham Boston Beacon Hill
Boston, Massachusetts, USA

CONTENTS

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October 5-8, 2016

Wyndham Boston Beacon Hill
Boston, Massachusetts, USA

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WELCOME

On behalf of the organizing and planning committees, and with great pleasure, we welcome you to Boston for The 15th International Conference on Neuronal Ceroid Lipofuscinosis (Batten Disease). For the past ~30 years, a conference that is specifically devoted to the neuronal ceroid lipofuscinosis (NCL) disorders, or Batten disease, has been held approximately every two years, typically alternating between the Americas and Europe. This 15th meeting is taking place in Boston, Massachusetts. The Greater Boston region is home to numerous top-ranked hospitals, academic institutions in the life sciences and technology and a rapidly growing number of biotechnology and large pharmaceutical companies, making it an ideal setting for a meeting of the diverse stakeholders working towards improved diagnosis, disease management and treatments for NCL patients. We welcome more than 200 meeting participants, who represent academia, government, research foundations, the biomedical industry, and patient advocacy, from 15 countries, representing the Americas, Asia, Australia, Europe, and Middle East continents.

As in past NCL conferences, the program features numerous opportunities for new investigators (including students, fellows, and recently independent investigators) to gain new knowledge and scientific communication experience by participating in scientific sessions as oral and poster presenters, participating in discussion groups, poster judging and in an active social program that will facilitate informal networking opportunities for all attendees. These new investigators will join a world-class group of established investigators to round out this year's exciting scientific program, which features basic and translational sessions, as well as forums for facilitating research towards improving patient care, disease monitoring and improved patient based research.

We wish to thank all those who have donated their time, funding and encouragement to support NCL 2016. More than 15 organizations have provided generous funding, joining the BDSRA in co-sponsoring this important conference, and supporting the attendance of many of the conference participants.

Thank you for your participation in NCL 2016. We are looking forward to greeting you in Boston!

Susan Cotman, Ph.D.
Chair, NCL 2016 Boston Conference

Margie Frazier, Ph.D., LISW-S
Executive Director of BDSRA

CONFERENCE SUPPORT

NCL 2016 is supported in part by a grant from The National Institutes of Health (R13NS098751).

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CONFERENCE AGENDA

WEDNESDAY, October 5, 2016

5:00 – 6:30pm	**Opening Session**
5:00 – 5:30pm	**Welcome: Susan Cotman, Ph.D.,** Chair, NCL 2016 Organizing Committee **Margie Frazier, Ph.D., LISW-S,** BDSRA Executive Director, NCL 2016 Sponsoring Organization
5:30 – 6:30pm	**Keynote Address: James Gusella, Ph.D.** (MGH/Harvard Medical School) The genetic research cycle in human disease
6:30 – 8:00pm	**Welcome Reception**

THURSDAY, October 6, 2016

7:30am – 8:30am	Breakfast
8:30am – 12:20pm	**Session 1: Genetics and Cell Biology of the NCLs**
8:30 – 8:35am	**Introduction:** Session Chairs: Marco Sardiello, Ph.D., Jill Weimer, Ph.D.
8:35 – 9:00am	**Sara Mole, Ph.D.** (University College London) Perspectives on NCL genetics: genes, mutations and genotype-phenotype correlations (O1)
9:00 – 9:25am	**Monkol Lek, Ph.D.,** (Broad Institute of MIT and Harvard) Using genomic technologies for the diagnosis of rare diseases (O2)
9:25 – 9:40am	**Murat Bastepe, Ph.D.,** (MGH) Identification of large exonic deletions within genes responsible for neuronal ceroid lipofuscinosis (NCL) (O3)
9:40 – 9:55am	**Sreeganga Chandra, Ph.D.,** (Yale University) Identification of PPT1 substrates: Implications for CLN1 (PPT1 and CSPalpha) (O4)
9:55 – 10:20am	**Stephane Lefrancois, Ph.D.,** (Centre INRS-Institut Armand-Frappier) CLN3 and CLN5 regulate endosomal sorting (O5)
10:20 – 10:35am	**Coffee break**
10:35 – 11:00am	**Emyr Lloyd-Evans, Ph.D.,** (Cardiff University) Identifying an ion channel function for CLN3 and a role in regulating lysosomal response to changes in swelling and osmolarity (O6)
11:00 – 11:15am	**Favio Pesaola,** (National University of Cordoba) Implication of CLN8 in the spatial distribution of lysosomes (O7)
11:15 – 11:30am	**Robert Huber, Ph.D.,** (Trent University) Aberrant migration and adhesion in a Dictyostelium model for juvenile neuronal ceroid lipofuscinosis (O8)
11:30 – 11:55am	**Jill Weimer, Ph.D.,** (Sanford Research) CLN6's role in regulation of vesicular trafficking (O9)
11:55am – 12:20pm	**Marco Sardiello, Ph.D.,** (Baylor College of Medicine) TFEB, lysosomal biogenesis, and neuronal ceroid lipofuscinosis (O10)
12:20 – 1:30pm	**Poster Session I** (Lunch will be provided)
1:30 – 4:45pm	**Session 2: Disease models and mechanisms**
1:30 – 1:40pm	**Introduction:** Session Chairs: Sara Mole, Ph.D., Tammy Kielian, Ph.D.
1:40 – 2:05pm	**Michelle Hastings, Ph.D.,** (Rosalind Franklin University of Medicine and Science) Antisense oligonucleotides for the treatment of juvenile neuronal ceroid lipofuscinosis (O11)
2:05 – 2:20pm	**Ryan Geraets,** (Sanford Research) A new cLINCL mouse model for studying various therapeutic approaches (O12)
2:20 – 2:45pm	**Jon Cooper, Ph.D.,** (UCLA) The nature of glial dysfunction and its impact upon neurons varies between forms of NCL (O13)

2:45 – 3:00pm	**Claire Russell, Ph.D.,** (Royal Veterinary College University of London) Valproic acid attenuates seizures and extends lifespan of the zebrafish model of CLN2 disease (O14)
3:00 – 3:15pm	**Coffee break**
3:15 – 3:30pm	**Rosanna Beraldi, Ph.D.,** (Sanford Research) Generation of a Juvenile Batten disease porcine model (O15)
3:30 – 3:45pm	**Carolin Schmidtke, Ph.D.,** (University Medical Center Hamburg-Eppendorf) Transcriptional control of lysosomal components in CLN3 defective cells (O16)
3:45 – 4:00pm	**Megan Bosch,** (University of Nebraska Medical Center) Abnormal astrocyte glutamate regulation and metabolic dysfunction contribute to neuronal pathology in juvenile neuronal ceroid lipofuscinosis (O17)
4:00 – 4:15pm	**Colleen Stein, Ph.D.,** (University of Iowa) Use of a transgenic system to study cell-type specific restoration of CLN3 in a JNCL mouse model (O18)
4:15 – 4:30pm	**Uma Chandrachud, Ph.D.,** (MGH, Harvard Medical School) Targeting Ca2+ homeostasis rescues lysosomal phenotypes in neuronal cell models of juvenile NCL (O19)
4:30 – 4:45pm	**Sophia Kleine-Holthaus, Ph.D.,** (University College London) AAV-mediated transduction of bipolar cells is essential to prolong the photoreceptor survival in Cln6nclf mice. (O20)
5:15pm	**Boarding for Boston Duck Boats Tour and Quincy Market Dinner**

FRIDAY, October 7, 2016

7:30am – 8:30am	Breakfast
8:30am – 11:35pm	**Session 3: Genome-wide and systems level strategies for NCL**
8:30 – 8:40am	**Introduction:** Session Chairs: Beverly Davidson, Ph.D., David Sleat, Ph.D.
8:40 – 9:05am	**Stephen Haggarty, Ph.D.,** (MGH, Broad Institute of MIT and Harvard) Chemical genomics of neurodegeneration: Patient-specific iPSC models (O21)
9:05 – 9:30am	**Carmine Settembre, Ph.D.,** (TIGEM) Physiological role of the lysosome as a signaling organelle (O22)
9:30 – 9:45am	**Elisa Tinelli, Ph.D.,** (University College of London) Screening for lead compounds to treat juvenile CLN3 disease (O23)
9:45 – 10:00am	**Coffee break**
10:00 – 10:25am	**Ethan Perlstein, Ph.D.,** (Perlstein Lab PBC) Using worms and flies to discover drugs for lysosomal storage disorders (O24)
10:25 – 10:50am	**Stephan Storch, Ph.D.,** (University Medical Center Hamburg-Eppendorf) Neurodegeneration and lysosomal pathology in a Cln7 mouse model (O25)
10:50 – 11:05am	**Ursula Heins Marroquin,** (University of Luxembourg) Towards small-molecule therapies for juvenile forms of Batten disease: drug screens in yeast and zebrafish models of JNCL (O26)
11:05 – 11:20am	**Thomas Wishart, Ph.D.,** (University of Edinburgh) Identifying novel therapeutic targets for the NCLs (O27)
11:20 – 11:35am	**Maica Llavero Hurtado,** (University of Edinburgh) Identifying potential peripherally accessible biomarkers in the NCLs (O28)
11:35am – 1:05pm	**Poster Session II** (Lunch will be provided)
1:05 – 5:15pm	**Session 4: Recent advances and challenges in translational research**
1:05 – 1:15pm	**Introduction:** Session Chairs: David Pearce, Ph.D., Sandra Hofmann, M.D.
1:15 – 1:40pm	**Alessandra Biffi, M.D.,** (Dana Farber Cancer Institute, Boston Children's, Harvard Medical School) HSC gene therapy for LSDs: current and new indications
1:40 – 2:05pm	**Mark Sands, Ph.D.,** (Washington University of St. Louis) A new therapeutic target for infantile neuronal ceroid lipofuscinosis (O29)

2:05 – 2:30pm	**David Sleat, Ph.D.,** (Rutgers University) Chronic enzyme replacement therapy to the brain of a mouse model of late infantile neuronal ceroid lipofuscinosis (O30)
2:30 – 2:55pm	**Beverly Davidson, Ph.D.,** (The Children's Hospital of Pennsylvania) Emerging therapies for the NCLs
2:55 – 3:10pm	**Coffee break**
3:10 – 3:35pm	**Tammy Kielian, Ph.D.,** (University of Nebraska Medical Center) Self-complementary AAV9 gene delivery partially corrects neuropathology associated with Juvenile Neuronal Ceroid Lipofuscinosis (CLN3) (O31)
3:35 – 4:00pm	**Ken Hensley, Ph.D.,** (University of Toledo) XN001 promotes autophagy to treat Batten disease (O32)
4:00 – 4:15pm	**Alejandra Rosenberg, Ph.D.,** (University of North Carolina at Chapel Hill) Preclinical Intrathecal Gene Therapy for Infantile Neuronal Ceroid Lipofuscinosis (O33)
4:15 – 4:30pm	**Janos Groh, Ph.D.,** (University Hospital Wuerzburg) Immunomodulatory therapy in mouse models of CLN1 and CLN3 disease (O34)
4:30 – 4:45pm	**Rebecca Whiting, Ph.D.,** (University of Missouri) Retinal function and structure are preserved in a canine model of CLN2 Batten disease after intravitreal implantation of stem cells genetically modified to overproduce TPP1 enzyme (O35)
4:45 – 4:50pm	**Dave Palmer, Ph.D.,** (Lincoln University) An introduction to 'in vivo monitoring of viral gene injection therapy in ovine Batten disease' (P41)
4:50 – 5:15pm	**Nadia Mitchell, Ph.D.,** (Lincoln University) Gene transfer can prevent stereotypical disease development in ovine CLN5 and CLN6 models of NCL (O37)
5:15pm	**Dinner on own**

SATURDAY, October 8, 2016

7:30am – 8:30am	Breakfast
8:00am – 9:15pm	**Late Breaking News and Selected Talks from Posters**
8:00 – 8:10am	**Introduction:** Session Chairs: Jon Cooper, Ph.D., Elisa Tinelli, Ph.D.
8:10 – 8:30am	**Betsy Ferguson, Ph.D.,** (Oregon National Primate Research Center) A non-human primate model of neuronal ceroid lipofuscinosis (O38)
8:30 – 8:45am	Short talk selected from Posters (TBD)
8:45 – 9:00am	Short talk selected from Posters (TBD)
9:00 – 9:15am	Short talk selected from Posters (TBD)
9:15 – 10:15am	**Hot Topic in Clinical Research Workshop: "Neurobehavioral and Psychosocial Function in the NCLs"**
9:15 – 9:20am	**Introduction:** Workshop Chair: Heather Adams, Ph.D. (O39)
9:20 – 9:30am	**Bengt Elmerskog** (Statped midt, Norway) Experiences on education and adaptations for learners with juvenile neuronal ceroid lipofuscinosis (O40)
9:30 – 9:40am	**Anne-Grethe Tøssebro,** (Statped midt, Norway) A study on tactile/body signs or hand signs for students with Batten disease (JNCL) (O41)
9:40 – 9:50am	**Andrea West,** (Batten Disease Family Association, UK) The burden of CLN2 disease on families: home-based surveys with caregivers in Germany and the United Kingdom (O42)
9:50 – 10:15am	Panel discussion
10:15 – 10:45am	**Coffee break**
10:45 – 11:30am	**Margie Frazier, Ph.D., LISW-S** (Batten Disease Support and Research Association), **Erika Augustine, M.D.,** (University of Rochester Medical Center), Engaging families in research workshop: Dialogue with parents and researchers (O43)
11:30am – 1:00pm	**Poster Session III (Lunch will be provided)**

1:00 – 2:10pm	**Session 5: Registries, Biorepositories and Rating Scales**
1:00 – 1:10pm	**Introduction:** Session Chairs: Erika Augustine, M.D., Angela Schulz, M.D.
1:10 – 1:25pm	**Jonathan Mink, M.D., Ph.D.,** (University of Rochester) Rating scales for natural history studies and registries in the NCLs (O44)
1:25 – 1:40pm	**Ines Noher de Halac, Ph.D.,** (National University of Cordoba) Argentina next generation sequencing technology (NGS) in human medicine, and registry of NCL disease in databases focused on genotype-phenotype relationships (O45)
1:40 – 1:55pm	**Angela Schulz, M.D.,** (University Medical Center Hamburg-Eppendorf) The DEM-CHILD Consortium: an international collaboration aimed at improved diagnosis and natural history data for outcomes-oriented research in the NCLs (O46)
1:55 – 2:10pm	**Jan Hochstein, M.D.,** (University Medical Center Hamburg-Eppendorf) Longitudinal 8-years brain volumetric analysis in 35 CLN3 patients: Successful development of a sensitive marker to measure clinical outcome (O47)
2:10 – 2:25pm	**Panel discussion**
2:25 – 2:40pm	**Coffee break**
2:40 – 4:30pm	**Session 6: Progress towards therapies: Clinical trial updates**
2:40 – 2:50pm	**Introduction:** Session Chairs: Ruth Williams, M.D., Jonathan Mink, M.D., Ph.D.
2:50 – 3:15pm	**Ronald Crystal, M.D.,** (Weill Cornell Medical College) Gene therapy for CLN2 disease
3:15 – 3:40pm	**Angela Schulz, M.D.,** (University Medical Center Hamburg-Eppendorf) Intracerebroventricular cerliponase alfa (BMN 190) in children with CLN2 disease: Results from a Phase 1/2, open-label, dose-escalation study (O48)
3:40 – 4:05pm	**Erika Augustine, M.D.,** (University of Rochester) Developing therapies for individuals with CLN3 disease – results from a phase 2, double-blind, crossover study of mycophenolate (O49)
4:05 – 4:30pm	**Emily de los Reyes, M.D.,** (Nationwide Children's Hospital) CLN6: The promise of gene therapy (O50)
4:30 – 4:50pm	**Conference closing and announcement of the 16th International Conference on NCL**
7:30 – 9:30pm	**Boston Batten Brew Bash!**

KEYNOTE

James F. Gusella, PhD

Bullard Professor of Neurogenetics, Harvard Medical School

Director, Center for Human Genetic Research, Massachusetts General Hospital

Dr. James F. Gusella was born and raised in Ottawa, Canada and graduated summa cum laude in 1974 from the University of Ottawa with a B.Sc. in Honours Biology. He continued his education at the University of Toronto, where he earned a M.Sc. degree in Medical Biophysics in 1976 and at the Massachusetts Institute of Technology, where he received his Ph.D. in Biology in 1980. Foregoing the usual period of postdoctoral training, he moved directly to establishing his own independent laboratory at the Massachusetts General Hospital and has risen to the rank of Bullard Professor of Neurogenetics in the Department of Genetics at Harvard Medical School. He pioneered the use of DNA sequence polymorphisms as genetic markers, demonstrating the feasibility of this new approach by mapping the Huntington's disease gene to chromosome 4. This discovery set off a torrent of similar studies aimed at identifying genes by their chromosomal position and provided a major impetus for the development of the Human Genome Project. Dr. Gusella has dedicated his career to the investigation of human neurodegenerative and neurodevelopmental disorders and through this experience, developed a vision of genetic research in human disease as a cycle that begins with patients and their families and uses a series of genetic strategies to understand the mechanism of disease and ultimately to delivering benefit back to patients and families through improved diagnosis and prevention, better disease management and effective, rational therapies. In 2003, he was named Director (a role he fulfilled until earlier this year) of the newly formed Center for Human Genetic Research (CHGR), a multi-disciplinary, cross-departmental, collaborative center aimed at promulgating the "Genetic Research Cycle" in all areas of medicine.

ORAL PRESENTATION ABSTRACTS

Perspectives on NCL genetics: genes, mutations and genotype- phenotype correlations

Sara E Mole

MRC Laboratory for Molecular Cell Biology, UCL GOSH Institute of Child Health, Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK.

The neuronal ceroid lipofuscinoses (NCL, Batten disease) are a group of inherited monogenic neurodegenerative diseases. The age of onset is usually in childhood, but ranges from birth to late in adulthood. Thirteen genes are known to cause NCL, with over 440 mutations listed in the NCL Mutation Database (http://www.ucl.ac.uk/ncl). These genes are named *CLN1/PPT1, CLN2/TPP1, CLN3, CLN4/DNAJC5, CLN5, CLN6, CLN7/MFSD8, CLN8, CLN10/CTSD, CLN13/CTSF*, as well as *CLN11/GRN*, *CLN12/ATP13A2* and *CLN14/KCTD7* described in a few families. There is a characteristic disease phenotype recognised for most NCL genes that is associated with complete loss of gene function, as well as disease with a later age of onset or more protracted that arises when partial gene function remains. Some mutations in NCL genes cause a distinct disease phenotype. The genetic basis of adult NCL is better understood, with some cases carrying mild mutations in genes that usually cause NCL in childhood and others in genes that cause onset only in adulthood. Recent advances in DNA sequencing technologies provide the means to identify the genetic basis of disease in single families. A new nomenclature for NCL has been developed that is gene-based. Study of animals affected with NCL helps us to better understand this disease and its causes.

Using genomic technologies for the diagnosis of rare diseases

Monkol Lek
Massachusetts General Hospital/Broad Institute of Harvard and MIT

An accurate genetic diagnosis is paramount in empowering patients to plan their futures, through family planning, living assistance and therapeutic options. The current cost effective practice involves the use of exome sequencing to identify causal mutations in rare disease patients. However, exome analysis is able to identify causal mutations in only 30-50% of sequenced families, indicating much work remains to be done to discover and interpret the genetic variation that underlies severe disease.

Whole Genome Sequencing (WGS) allows for the unbiased sequencing of both protein-coding and non-coding regions of the genome. Current exome capture technologies provide incomplete coverage of known protein-coding genes, and in any case an estimated 20% of Mendelian mutations lie outside of protein-coding regions and will not be detected by exome sequencing.

RNA sequenicng (RNA-Seq) is a complementary approach to WGS, providing a powerful approach to observe the consequences variants may have on transcript expression and splicing. The availability of accessible tissue from undiagnosed rare disease patients provides a unique opportunity to investigate the effects mutations may have on transcription.

We have established the Exome Aggregation Consortium (ExAC) as a powerful resource, allowing patient variants to be interpreted in the context of over 60,000 healthy individuals. Using the ExAC data set, we are able to assess pathogenic variants in patient exome or genome data and compare against our rare disease cohorts to identify novel disease genes.

The comprehensive identification of genes for each rare disease will provide deeper understanding of common pathways and mechanisms associated with that disease. Treatment known to be effective for particular gene mutations may also be effective for other mutations in common pathways, thus benefiting a larger number of patients. Identifying novel genes and improving the accuracy of patient diagnosis ultimately translates to improvement in patient quality of care; important in anticipation for effective treatments.

Identification of large exonic deletions within genes responsible for neuronal ceroid lipofuscinosis (NCL)

Murat Bastepe, Rosemary Barone, Xin Feng, Katherine Sims, and Winnie Xin

Neurogenetics DNA Diagnostic Lab, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Neuronal ceroid lipofuscinosis (NCL) is a clinically and genetically heterogeneous group of neurological diseases characterized by the intracellular accumulation of autofluorescent lipopigment in brain and other tissues. Patients with NCL display abnormal mental and motor development during early childhood and often present with progressive dementia, seizures, and progressive visual failure. Multiple subtypes of NCL have been described according to the age of onset and clinical features. Most NCL subtypes are inherited in an autosomal recessive manner and thus mutations found in the responsible genes are either homozygous or compound heterozygous. Traditional Sanger sequencing and more recent next-generation sequencing (NGS) techniques have been utilized in NCL mutation detection. To date, various point mutations have been identified in all of the NCL genes. However, neither sequencing method is sensitive for detecting large deletion/duplications that involve an entire exon or multiple exons. Our sequencing analysis of patients with infantile, late infantile, juvenile, or variant-late infantile form of NCL revealed a single heterozygous mutation of the responsible gene in some of the cases. Therefore we suspected that the second mutant allele could be a large deletion that eluded detection by sequencing. To determine whether the other mutant allele in each case carries a deletion, we developed individual MLPA assays by designing synthetic probes for all the exons of *PPT1, TPP1, CLN3,* and *CLN6* genes, which are responsible for infantile, late infantile, juvenile, or variant-late infantile NCL, respectively. In validation experiments, MLPA analysis successfully confirmed a previously known deletion in *CLN6* exon 4 in several cases and the common 1kb deletion comprising *CLN3* exons 7 and 8 (c.461-280_677+382del966bp). These experiments also confirmed a deletion within exon 8 of *TPP1*, which consisted of 8 bp and likely disrupted probe hybridization. We then analyzed 33 NCL cases in whom only a single, non-benign heterozygous mutation was detected in the relevant gene. These MLPA experiments identified a novel deletion comprising *PPT1* exon 2 in a patient carrying a heterozygous nonsense mutation in this gene (c.451C>T, p.Arg151X). Moreover, our analysis revealed, in two unrelated kindreds, a previously undescribed homozygous 913-bp deletion spanning *CLN3* exons 7 and 8 (c.461-354_677+255del913bp). The breakpoints of the novel *CLN3* deletion, similar to those of the common 1kb deletion, are located within AluSx1 repeats. The novel deletion is flanked by two almost identical 22 bp sequences. Because of having different breakpoints, the novel deletion cannot be detected by the published conventional method used to identify the *CLN3* common 1kb deletion. Our MLPA analyses have shown that large deletions do exist in many of the NCL genes and therefore should be considered in NCL diagnosis, especially in patients in whom only a single heterozygous mutation is identified. In addition, the finding of the novel *CLN3* deletion suggests that an alternative deletion detection method, different from the published protocol, should be employed in order to avoid false negative results.

O3

Identification of PPT1 substrates: Implications for CLN1

Vicky Chou[1], Gregory S. Wirak[1], Erica Gorenberg[1], Tukiet Lam[2], Sreeganga S. Chandra[1]

Program in Cellular Neuroscience, Neurodegeneration and Repair (CNNR); Depts. Of Neurology and Neuroscience; W.M. Keck Foundation Biotechnology Resource Laboratory, Yale University, New Haven, CT 06536.

Neuronal Ceroid Lipofuscinoses (NCLs) are a group of inherited neurodegenerative disorders that affect children and adults. The first gene to be causally linked to NCLs was Palmitoyl Protein Thioesterease 1 (PPT1) and over 60 mutations in this gene account for the common CLN1 disease subtype. PPT1 mutations are recessive and associated with a loss of depalmitoylation activity of this enzyme. Hence, there is great interest in identifying PPT1 substrates and understanding how their palmitoylation status impacts neuronal function. In order to identify PPT1 substrates, we purified palmitoylated proteins (palmitome) from wildtype and PPT1 knockout mouse brains, and quantitatively compared them by mass spectrometry. Palmitoylated proteins whose levels were significantly changed in PPT1 knockouts, were considered as putative PPT1 substrates. We then validated their protein level changes and characterized their palmitoylation using chemical biology tools. Newly identified PPT1 substrates could be broadly grouped into synaptic, lysosomal and immune categories. Our results have important implications to the pathophysiology of NCL and will be discussed.

CLN3 and CLN5 regulate endosomal sorting

Stephane Lefrancois

Centre INRS-Institut Armand-Frappier, INRS, Laval, Canada
Department of Anatomy and Cell Biology, McGill University, Montreal, Canada

Mutations in *CLN3* and *CLN5* cause Neuronal Ceroid Lipofuscinosis (NCL), a group of fatal childhood neurodegenerative diseases. Common symptoms include ataxia, vision loss and cognitive regression with death occurring mostly in the second decade of life. The function of these proteins is not well understood, thereby hampering the development of therapies for affected children. To date, the only known function of CLN5 is its role in the activation of the small G protein Rab7 and subsequent recruitment of retromer. Rab7 and retromer are required for the endosome-to-trans Golgi Network trafficking (TGN) of the lysosomal sorting receptors. However, it is unclear how a luminal protein can control the spatiotemporal recruitment of cytosolic proteins such as retromer. CLN3 is a transmembrane domain protein that interacts with Rab7. Using a variety of biophysical and biochemical techniques, we demonstrate that CLN5 regulates the CLN3-Rab7 interaction possibly by modulating the conformation of CLN3. We propose a model whereby CLN5 initiates a signaling cascade, via CLN3, to activate and recruit Rab7. This recruitment step to endosomal membranes could subsequently allow the recruitment of retromer and the efficient endosome-to-TGN trafficking of lysosomal sorting receptors.

Funded by the Canadian Institutes of Health research.

O5

Identifying an ion channel function for CLN3 and a role in regulating lysosomal response to changes in swelling and osmolarity

Emyr Lloyd-Evans, Luke J. Haslett, Morgan Roberts and Mathew W. Walker

Sir Martin Evans Building, School of Biosciences, Cardiff University, Museum Avenue, Cardiff, CF10 3AX

Despite identification of the gene causing CLN3 disease in 1995, the exact function of the lysosomal CLN3 protein has largely remained elusive. In a collaboration with the Cotman lab we recently reported the presence of elevated lysosomal Ca^{2+} in a neuronal cerebellar cell line containing the common 1kb deletion in the *CLN3* gene. We now present evidence that this elevation in lysosomal Ca^{2+}, present also in patient fibroblasts harbouring different mutations in *CLN3*, is a direct consequence of loss of CLN3 function. We have identified key residues in CLN3 that are conserved in ion channels and have functional evidence that the C terminus region of CLN3 can function in ion mobilisation. Furthermore, we have evidence that CLN3 is required for ensuring maintenance of ion balance in response to lysosomal swelling. Ultimately, changes in lysosomal ion homeostasis have knock on detrimental effects on endocytic function, autophagic vacuole clearance and ultimately excitotoxicity of CLN3 disease neurons. These phenotypes can be ameliorated using certain Ca^{2+} antagonists that target lysosomal Ca^{2+} channels.

Implication of CLN8 in the spatial distribution of lysosomes

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BACKGROUND: CLN8p is a putative 286 aa, transmembrane protein encoded by the *CLN8* gene, whose mutations underlay CLN8 disease of NCL. This protein shuttles between Endoplasmic Reticulum (ER) and the ER-Golgi Intermediate Compartment (ERGIC). Its malfunction is associated with the typical NCL aggregates of lipofuscin-like compounds in lysosomes in cells of peripheral tissues. These aggregates are related to neuronal degeneration in the brain. CLN8p role, as well as how mutations trigger lysosomal storage disorder and neurodegeneration is still unknown. Here, we evaluate how *CLN8* expression is involved in the spatial pattern of lysosomes. METHODS: HeLa cells were transfected with one of following constructs: soluble GFP (control), GFP-CLN8wt, shCLN8 or shLuc (control sh). Lysosomes were marked with anti-LAMP1 antibody by immunofluorescence. Images were taken with a Disk Scanning Unit (DSU) microscope and analyzed using ImageJ-Fiji and SpatTrack softwares. RESULTS: Four indexes were calculated to analyze spatial pattern of lysosomes. Nearest Neighborhood (NN) and Clark's Aggregate Index (CAI) refer to distances between particles. Both indexes showed a significant difference ($p < 0.05$) between CLN8wt and shCLN8 or control. Radial Distribution Function (RDF) is calculated by SpatTrack and refers to the number of particles surrounding each particle within a certain radius. This index showed differences ($p < 0.0001$) among all treatments. Finally, we measured distances of each particle to the nucleus that showed difference ($p < 0.01$) between control and shCLN8 but not among the other treatments. DISCUSSION: Different levels of *CLN8* expression cause changes in the spatial distribution of lysosomes in HeLa cells suggesting that lysosomes aggregate more and farther from nucleus at low levels of expression than in the normal condition. Evidences showed altered lysosomal pattern in HeLa cells when the non-lysosomal proteins CLN3 or CLN5 were mutated. A common modified lysosomal distribution pathway may be related with the physiopathology of CLN3-, CLN5- and CLN8 diseases.

O7

Aberrant migration and adhesion in a *Dictyostelium* model for juvenile neuronal ceroid lipofuscinosis

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The social amoeba *Dictyostelium discoideum* has emerged as a significant model system for studying the functions of proteins linked to neuronal ceroid lipofuscinosis (NCL). This model eukaryote contains homologs of 11 of the 13 known NCL genes, and recent research on the *Dictyostelium* homologs of human TPP1/CLN2 and CLN3 suggests that work in this organism may be able to provide novel insight into the precise functions of these and other NCL proteins. In *Dictyostelium* Cln3-deficiency causes aberrant growth and mid-to-late stage multicellular development. During the early stages of development, *cln3*- cells form ~30% more multicellular aggregates that are comparatively smaller than those formed by wild-type cells. Importantly, this phenotype can be rescued by expressing GFP-Cln3 in *cln3*- cells, or treating *cln3*- cells with the calcium chelator EGTA. Loss of Cln3 also reduces cAMP chemotaxis and delays the initiation of the periodic pulsing of cAMP waves. Interestingly, there are no significant differences between wild-type and *cln3*- cells in the expression of genes linked to cAMP signal transduction, and manual pulsing of cells with cAMP fails to correct these defects. However, *cln3*- cells do show reduced cell-substrate and cell-cell adhesion during early development, which correlate with changes in the levels of the calcium-dependent and calcium-independent cell adhesion proteins CadA and CsaA, respectively. Together, these results suggest that the aberrant migration of *cln3*- cells is due to decreased adhesion during the early stages of development. Revealing the molecular basis underlying this phenotype may provide fresh new insight into CLN3 function in humans and an improved understanding of NCL disease pathogenesis.

CLN6's role in regulation of vesicular trafficking

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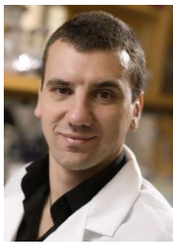
In this study, we focus on regulation of a unique signaling complex composed of CRMP2, CLN6 and kinesin light chain 4 (KLC4), exploring how disruption in this CCK complex in CLN6-Batten disease leads to a loss in many CRMP2-dependent processes. CRMP2 is crucial for axon-dendritic specification and extension and contributes to regeneration/degeneration in the mature brain. CRMP2's ability to specify axon/dendrite fate and regulate cargo transport during axonal growth has been shown to be facilitated and/or antagonized through a complex network of alternative protein-protein interactions, including kinesin-1 light chain, dynein, chimaerin, phospholipase D, calmodulin, L1/numb, neurofibromin 1 (NF1), CaV2.2, and, CLN6. Disruption in CRMP2 impedes axonal formation and promotes degeneration and has been associated with many neurological disorders. We highlight how this novel CCK complex utilizes CLN6 as a "molecular tag" on a pool of ER-vesicles, allowing the complex to segregate cargo to specific locations in neuronal processes. Thus, aberrant modulation of this signaling complex could contribute to the pathogenesis of CLN6-Batten disease through altered axonal/dendritic specification, outgrowth and maintenance.

O9

TFEB, Lysosomal biogenesis and Neuronal Ceroid Lipofuscinoses



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Many neurodegenerative diseases share as a common cellular feature the accumulation of undigested material, which in many instances has been proven to play a causative role in the pathogenesis of the disease. Pathological accumulation of undigested material is often the result of an overwhelmed cellular degradative system, which can be due either to genetic defects that affect the function of lysosomal and autophagy pathways (e.g., lysosomal storage disorders), or to different causes such as the abnormal generation of aggregation-prone proteins (e.g., Alzheimer's, Parkinson's and Huntington's diseases). We and others have provided proof-of-principle evidence that activation of lysosomal pathways may be an effective strategy to ameliorate disease phenotypes in models of these neurodegenerative diseases. Results from our laboratory indicate that the pathways that participate in lysosomal biogenesis are affected in juvenile neuronal ceroid lipofuscinosis (JNCL) and other NCLs. We show that pharmacological activation of the transcription factor EB (TFEB), a master regulator of autophagic-lysosomal pathways, induces lysosomal enhancement in a mouse model of JNCL and reduces neurodegeneration, neuroinflammation and neuronal storage burden, ultimately increasing the life span of the diseased mice. Results from our study might be extended to the whole group of NCLs and lead to future clinical translation.

Antisense Oligonucleotides For The Treatment Of Juvenile Neuronal Ceroid Lipofuscinosis

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Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) is a fatal, pediatric, neurodegenerative, lysosomal storage disease that is caused by the mutation of *CLN3*. Most cases of JNCL are caused by a deletion of *CLN3* exons 7 and 8. There is no effective therapy for JNCL and few options for correcting the defective gene expression associated with this *CLN3*$^{\Delta ex7/8}$ mutation. Here, we describe a new strategy to specifically recover CLN3$^{\Delta ex7/8}$ protein function. The deletion of exons 7 and 8 from *CLN3* results in a shift in the open reading frame of the mRNA, introducing a premature termination codon, which precludes full-length protein production. We *hypothesize* that correcting the reading frame of *CLN3*$^{\Delta ex7/8}$ mRNA will partially restore protein function. Removal of a number of different exons from the mRNA will reframe the transcript and produce a full-length CLN3 protein with an internal deletion. We corrected the *CLN3*$^{\Delta ex7/8}$ reading frame by inducing exon skipping during pre-mRNA splicing using antisense oligonucleotides (ASOs). We find that this frame-correcting approach produces a protein that partially reduces histopathological and phenotypic features of JNCL in Cln3$^{\Delta ex7/8}$ transgenic mice. Transgenic mice with exons 6, 7 and 8 deleted, mimicking the ASO effect, also show evidence of reduced pathology compared to Cln3$^{\Delta ex7/8}$ mice. Together, our results suggest that ASO-mediated reading-frame correction may be a promising therapeutic approach for Batten disease.

O11

A new cLINCL mouse model for studying various therapeutic approaches.

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The Neuronal Ceroid Lipofuscinoses (NCL), also known as Batten disease, result from genetic mutations in over a dozen genes. Depending on the afflicted gene, disease onset can range from infancy to adulthood. Independent of the age of onset, symptomatic hallmarks of the NCLs consist of motor and cognitive decline, seizures, blindness, and ultimately death. Mutations in *CLN1*, *CLN2*, and *CLN3* cause the most common forms of NCL; they are Classic Infantile (cINCL), Classic Late Infantile (cLINCL), and Classic Juvenile (cJNCL), respectively. Based on the NCL Resource Database, up to one third of registered cINCL and cLINCL patients carry at least one allele with a nonsense mutation, with the most common being p.R151X in CLN1 and p.R208X in CLN2. Given the prevalence of nonsense mutations, a cINCL mouse model carrying the p.R151X mutation was generated. Unfortunately, until now, no cLINCL mouse model carrying the p.R208X mutation has existed. Molecular assessment of various *Cln2R207X/R207X* tissues has determined a significant reduction in *Cln2* transcript abundance and TPP1 activity. This reduction leads to the development of behavioral anomalies, neurological impairment, and histological abnormalities. Overall, the various assessments indicate that the *Cln2R207X/R207X* mouse is a valid cLINCL model. Previous cLINCL mouse model genotypes limit which therapeutics can be assessed therefore, using this new cLINCL model we are able to evaluate previously excluded therapies (e.g. read-through compounds).

The Nature Of Glial Dysfunction And Its Impact Upon Neurons Varies Between Forms Of NCL

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In all forms of Neuronal Ceroid Lipofuscinosis (NCLs, or Batten disease), localized glial activation appears to be an early event that accurately predicts where neurons subsequently die. This raises the question whether these events are linked, and what the glial contribution to pathogenesis might be. To begin answering these questions we have grown glial and neuronal monocultures, and a variety of different co-cultures derived from mouse models of the three major forms of NCL (Cln1, Cln2, and Cln3 diseases). This has allowed us to explore whether the biology of glia, which would normally express the deficient proteins, is compromised and if this has any subsequent effects upon neurons.

In the most prevalent form of NCL, Cln3 disease (Juvenile NCL, JNCL), we have found basic defects in both *Cln3* deficient microglia and astrocytes. Mutant microglia failed to transform morphologically and displayed an altered protein secretion profile upon stimulation. These defects were far more pronounced in astrocytes, in which cytoskeletal abnormalities, impaired calcium signalling and reduced glutamate clearance were observed. Furthermore, when grown in a co-culture system, *Cln3* deficient glia were shown to negatively impact the health of both *Cln3* deficient and wildtype neurons, with mutant neurons being the most severely affected.

In Cln1 disease, we have identified a range of different glial phenotypes, with both mutant astrocytes and microglia displaying activated morphologies under basal conditions, but displaying relatively normal responses to stimulation and no cytoskeletal abnormalities. However, unlike *Cln3* deficient cultures, we found a pronounced defect in *Cln1* deficient astrocyte survival, and in the phagocytic ability of *Cln1* microglia. We also found a similar deleterious influence of Cln1 deficient glia upon neuronal morphology and survival, as with *Cln3* deficient glia.

In marked contrast, *Cln2* deficient astrocytes or microglia displayed no obvious phenotypes, when grown under either basal or stimulated conditions. Instead we have found a range of neuronal phenotypes, distinct to those found for either *Cln1* or *Cln2* deficient neurons. Surprisingly, examining sections from Cln2 mouse brains that glial activation occurs much later than in other forms of NCL, accompanying neuron loss instead of preceding it.

Taken together these data reveal that the extent and nature of glial dysfunction appears to vary markedly between the three major forms of NCL. Cln2 disease appears to involve neurons to a greater extent than either Cln1 or Cln3 disease, both of which show more pronounced glial defects which have a negative impact of this upon neuronal health. These data reveal fundamental differences between this group of disorders and raise the possibility that glia should also be considered as therapeutic targets in some forms of NCL.

Valproic Acid Attenuates Seizures And Extends Lifespan Of The Zebrafish Model Of CLN2 Disease

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Objective
CLN2 disease is a subtype of the neuronal ceroid lipofuscinoses (NCLs), a group of lysosomal storage disorders causing progressive, untreatable, neurodegeneration, intractable epilepsy and premature death in children. We have developed a permanent genetic zebrafish model of CLN2 disease due to a mutation in *tpp1* encoding the lysosomal protease Tripeptidyl-peptidase-1 that replicates the neurodegenerative and storage phenotype. We hypothesize that *tpp1-/-* zebrafish display electrical and behavioural evidence of seizure activity that responds to established anti-convulsants.

Results
To validate the presence of seizures we performed single electrode electroencephalography showing *tpp1-/-* zebrafish had increased spiking activity versus wildtype with Fast-Fourier transform showing significantly increased amplitude about 2-4Hz. This was attenuated by valproic acid ($p=0.049$), but not pentobarbitone. We also demonstrate that valproic acid significantly reduces seizure-related movement bouts ($p<0.05$) and total distance moved ($p<0.05$) in 20 minutes, thereby correlating movements and epileptiform activity. Lastly, we show exposure to valproic acid significantly extends the lifespan of our zebrafish model with mortality between 3-6 days post-fertilization 8.33% in treated vs 33.3% in controls ($p=0.01$). A TUNEL assay showed a 50% decrease in the number of apoptotic bodies (10.13 ± 1.3 vs 5.44 ± 0.47; $p<0.001$) and the live Lysotracker assay for lysosomes showed a decrease in fluorescence intensity in valproic acid treated *tpp1-/-* larvae (4.16 ± 0.47 vs 2.81 ± 0.08; $p<0.05$).

Conclusion
These results indicate the mechanisms by which valproic acid can improve not only seizure liability, but also survival. In addition, it provides proof-of-principle that locomotion assays using this seizure model can be used as a surrogate readout for seizures and therefore to screen for novel anti-convulsants.

Generation of Juvenile Batten disease porcine model

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Mutations in the *CLN3* gene cause juvenile CLN3 disease also known as juvenile Batten disease. The most common disease-causing mutation of *CLN3* is a 1.02 kb deletion, $CLN3^{\Delta ex7/8}$, resulting in RNA frameshift, formation of a premature stop codon and ultimately, degradation of the mutant transcript. Data suggests, however, that a part of the mutant *CLN3* mRNA is translated to a truncated protein. Several mouse models of juvenile Batten disease have been developed via targeted disruption of the murine *Cln3* gene, and these models have been valuable for studying some aspects of CLN3 function and the disease mechanisms. To fully replicate the complex clinical symptoms observed in the human disease we have developed a large animal model of JNCL. Pigs may serve as a better model in which to study juvenile Batten disease given that their development, anatomy, and physiology are more closely related to that of humans. The similarities of the porcine visual system offer advantages for modeling the retinal disease phenotype, as well. We bioengineered $CLN3^{\Delta ex7/8}$ miniature pig using homologous recombination in porcine fetal fibroblasts to establish the $CLN3^{\Delta ex7/8}$ heterozygous fetal fibroblast line. The generation of homozygous $CLN3^{\Delta ex7/8}$ cohorts was obtained by somatic cell nuclear transfer. Initial in *vitro* studies in $CLN3^{\Delta ex7/8}$ fibroblasts have found abnormalities in metabolic pathways, and transcriptome analysis has shown novel *CLN3* transcripts.

Transcriptional control of lysosomal components in CLN3 defective cells

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Mutations in the *CLN3* gene, coding for a lysosomal transmembrane protein of unknown function, result in lysosomal dysfunction and accumulation of lysosomal storage material in neuronal cells and non-neuronal tissues. The pathomechanisms underlying neuronal degeneration in CLN3 disease are poorly understood.

To gain insight into CLN3 disease-related alterations in lysosome homeostasis, SILAC-based comparative proteomics of isolated lysosomes from wild-type and $Cln3^{\Delta ex7/8}$ neuronal cerebellar cells was performed. In lysosomes of $Cln3^{\Delta ex7/8}$ cells 37 soluble and 10 membrane proteins were differentially expressed compared to wild-type lysosomes. Among these, 12 enzymes involved in lipid degradation were predominantly decreased $Cln3^{\Delta ex7/8}$ lysosomes, and several were transcriptionally downregulated involving changes in Akt and mTORC1 pathways. LC/MS-based lipidome analysis revealed various alterations in the lipid composition of $Cln3^{\Delta ex7/8}$ membranes. These changes, in particular in sphingolipids, appear to directly affect trafficking of various endocytic clathrin-dependent and clathrin–independent cargo receptors, known to be involved in transport of lysosomal enzymes (Mpr300, LDL receptor, Lrp1 and 2).

In conclusion, the data show that the malfunction of the lysosomal membrane protein CLN3 impairs the biogenesis and composition of lysosomes in neuronal cerebellar cells and moreover, affects trafficking of various endocytic cargo receptors and the delivery of their ligands.

Abnormal Astrocyte Glutamate Regulation and Metabolic Dysfunction Contribute to Neuronal Pathology in Juvenile Neuronal Ceroid Lipofuscinosis

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Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) is a fatal lysosomal storage disease caused by an autosomal recessive mutation in *CLN3*. Children with JNCL experience progressive visual, cognitive, and motor deterioration with a decreased life expectancy (late teens-early 20s). Previous studies have shown that astrocyte activation precedes and predicts regions of neuronal loss in JNCL, suggesting a defect in supportive glial functions. Astrocytes, the major cell type responsible for regulating extracellular glutamate levels in the CNS, provide metabolic and tropic support for neurons and can modulate synaptic activity. Glutamate levels are elevated in the JNCL brain and neuronal loss is thought to occur, in part, via glutamate excitotoxicity. Currently, little is known about aberrant glutamate-glutamine cycling in astrocytes in JNCL. We hypothesized that *CLN3* mutation perturbs glutamate regulatory pathways to induce neuronal dysfunction. To study perturbations in the glutamate cycling pathway, primary astrocytes and neurons were cultured from $CLN3^{\Delta ex7/8}$ and wild type (WT) C57BL/6 mice. Primary $CLN3^{\Delta ex7/8}$ astrocytes displayed decreased glutamate transporter expression and activity when exposed to stimuli present in the JNCL brain. By extension, this disruption in glutamate homeostasis in the context of CLN3 mutation threatens to disrupt neuron-astrocyte signaling pathways, which if chronically perturbed can induce neuron excitotoxicity. Indeed, Ca^{2+} signaling was significantly reduced in $CLN3^{\Delta ex7/8}$ astrocytes, suggesting that disruptions in glutamate cycling interrupt critical homeostatic cell signaling networks. Concurrent with impaired astrocyte glutamate regulation, primary $CLN3^{\Delta ex7/8}$ neurons were hyper-responsive to glutamate stimulation, indicated by heightened and abnormally prolonged Ca^{2+} transients. Glutamate regulation in astrocytes is an energy-demanding process and disruptions in metabolic pathways could further perpetuate disruptions in glutamate cycling leading to neuron excitotoxicity. Accordingly, $CLN3^{\Delta ex7/8}$ astrocytes displayed significantly lower levels of basal mitochondrial respiration, ATP production, and maximal respiration under physiological conditions. These findings support the premise that a neuroinflammatory environment, proposed to be established during JNCL progression, causes perturbations in glutamate regulatory pathways in astrocytes. By extension, Ca^{2+} signaling is elevated in $CLN3^{\Delta ex7/8}$ neurons following glutamate stimulation, bringing the dysregulated glutamate circuit full circle in the context of JNCL pathology. Establishing key regulatory mechanisms of astrocyte-neuron cross-talk in JNCL may unveil novel therapeutic targets to extend the quality-of-life for children suffering from this devastating disease.

Use of a transgenic system to study cell-type specific restoration of CLN3 in a JNCL mouse model

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A normal functioning blood-brain barrier is critical to neuronal health. In previous studies we found that brain microvascular endothelial cells (EC) from CLN3-deficient mice are functionally defective, and we hypothesized that EC impairment is a key contributing factor to JNCL (Juvenile Batten disease) pathogenesis. In the current study, we examined whether selective restoration of CLN3 to endothelial cells, or alternatively to neurons, would alleviate pathology and symptoms in the Δexon7/8 JNCL mouse model. For conditional expression of Cln3, we generated 1665p-FLEX-Cln3R transgenic mice, in which Cln3 expression relies on Cre recombinase-dependent flipping of Cln3R to the forward orientation at the transgene locus. By crossing this mouse to EIIa-cre, Tie2-cre, and Syn1-cre driver strains, we generated Cln3F (restored to all cells), Cln3F-EC (EC restored), and Cln3F-neu (neuron restored) mice, respectively. Control groups included JNCL mice without the transgene or with unaltered transgene (Cln3R), or non-affected C57BL/6 mice (wt). Genomic PCR and transcript analyses showed Cln3 flipping and expression in the expected tissue-specific pattern for all transgenic groups. When assessed for pathology, the Cln3F mice displayed remarkable protection against accumulation of aggregated subunit c of the mitochondrial ATP synthase (SCMAS), as measured by western blot analysis across brain regions and neural retina. Moreover, electroretinogram (ERG) readings indicate significant preservation of retinal function in Cln3F mice relative to JNCL controls. In contrast, assessment of Cln3F-EC or Cln3F-neu mice revealed lack of protective effect as determined by SCMAS accumulation and ERG scores that were not significantly different from JNCL controls. These results indicate that Cln3 expression from the transgenic locus is protective when derived from "all cells" but not when restricted to EC or neurons. We speculate that glial cell expression of CLN3 may be more relevant in this model, and/or that combined restoration to multiple cell types is necessary for substantial therapeutic impact.

Targeting Ca^{2+} homeostasis rescues lysosomal phenotypes in neuronal cell models of juvenile NCL

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Juvenile neuronal ceroid lipofuscinosis (NCL, also referred to as Batten disease) is a lysosomal storage disorder with mostly neurodegenerative symptoms, caused by autosomal recessive mutations in *CLN3*, which encodes a late endosomal/lysosomal transmembrane protein. Previous work established evidence of abnormal Ca^{2+} homeostasis in CLN3 deficiency models including juvenile NCL patient cells, identifying particularly elevated intralysosomal Ca^{2+} levels compared to control cells. We have therefore tested whether treatments expected to reduce the abnormally elevated intralysosomal Ca^{2+} could restore lysosomal system abnormalities and neuronal cell health using genetically accurate mouse and human patient-based cell models. Overexpression of *MCOLN1,* which encodes a cationic efflux channel (TRPML1) of the late endosome/lysosome, in CLN3 mutant mouse neuronal cells normalized lysosomal morphology and reduced the accumulation of lysosomal storage material. Similarly, in a human patient induced pluripotent stem cell (iPSC)-derived neuronal cell system, treatment with the TRPML1 agonist, MLSA1, significantly improved lysosomal morphology and neuronal cell health. CLN3 patient iPSC-derived neuronal cells were more metabolically healthy, and a defect in neuronal differentiation was significantly improved following MLSA1 treatment. These studies emphasize the likely role of CLN3 in lysosomal Ca^{2+} homeostasis and imply that further development of treatments targeting this defect could lead to novel therapies for this devastating and fatal childhood disorder.

AAV-mediated transduction of bipolar cells is essential to prolong the photoreceptor survival in *Cln6*^{nclf} mice.

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A major obstacle to developing gene therapies for the NCLs is the challenge to efficiently deliver agents throughout the brain. Adeno-associated virus (AAV)mediated gene therapies have been used for several monogenic retinal degenerations to restore protein expression and prevent the loss of photoreceptors. As vision loss is one of the first symptoms in NCL, especially in juvenile CLN3 disease, we explored the therapeutic potential of an ocular AAV-based gene therapy with the aim to improve quality of life in patients.

None of the *Cln3*-deficient mouse models present with a severe ocular phenotype. For this reason, we worked with the *Cln6*^{nclf} mouse, an NCL model that is deficient in *Cln6* which, similar to *Cln3,* encodes a transmembrane protein of unknown function. Our work demonstrates that *Cln6*^{nclf} mice present with a predominant loss of photoreceptor function and photoreceptor cells. Aiming to treat the photoreceptor degeneration, we performed subretinal injections of AAV8.CLN6 in mutant animals. Although we achieved a widespread expression of *CLN6* in the treated eyes, no therapeutic effect was observed indicating that *CLN6* supplementation in photoreceptors was not sufficient to prevent the degeneration. Immunostaining on unaffected retinas show that the endogenous expression level of *CLN6* is low in photoreceptors and high in bipolar cells, a cell type of the inner retina that receives input from photoreceptors and transmit signals to retinal ganglion cells. In *Cln6*deficient retinas, however, bipolar cells are not lost at early disease stages. Since conventional AAV serotypes poorly transduce bipolar cells, we investigated the transduction efficiency of bipolar cells following the intravitreal delivery of the AAV variant 7m8. Our data show that 7m8 transduced efficiently cells in all retinal layers including bipolar cells. Based on these findings, we injected *Cln6*^{nclf} mice with 7m8 carrying *CLN6* and a ubiquitous promoter to drive expression in cells of all retinal layers. Treated animals had a significantly thicker photoreceptor layer and increased photoreceptor function as measured by electroretinography compared with untreated mutant eyes. To assess which cell type of the retina is essential for the therapeutic effect, we administered 7m8.CLN6 vector harbouring the bipolar cell type-specific promoter Grm6. Interestingly, the specific expression of *CLN6* in bipolar cells led to increased photoreceptor function and thickness of the photoreceptor layer comparable with the treatment with 7m8.CMV.CLN6.

In summary, these data establish that the transduction of bipolar cells is essential to correct the photoreceptor degeneration in *Cln6*^{nclf} mice. This is the first study indicating that the deficiency of a gene highly expressed in bipolar cells can cause photoreceptors to die through a so far unknown mechanism. We anticipate that this work will aid the development of ocular treatments for CLN3 disease and will complement efforts to develop therapies for the brain in NCL.

Chemical Genomics of Neurodegeneration: Patient-Specific iPSC Models

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The advent of patient-specific, induced pluripotent stem cells (iPSCs) as *ex vivo* models of human disease enables the advancement of a platform for human experimental neurobiology that address the critical goal of identifying new targets and mechanisms for therapeutic intervention. In particular, the ability to differentiate these human iPSCs into neural progenitor cells and neural networks with the capacity to form synapses and regulate genes in an activity-dependent manner provides new avenues for illuminating the genetic and neurobiological mechanisms underlying a range of nervous system disorders. Using examples of neurodegenerative disorders that may share pathophysiological mechanisms in common with NCL/Batten disease, including progranulin (GRN)-deficient frontotemporal dementia, I will summarize strategies being developed for overcoming challenges associated with high-throughput drug screening using patient-derived iPSC models—namely that of scalability and cellular heterogeneity. Examples of using high-content image-based screening and other forms of high-dimensional, molecular signature generation for characterizing known and novel pharmacological probes of pathways implicated in neurodegeneration will be provided. Finally, I will discuss potential future directions for advancing modeling of NCL/Batten disease biology and treatment to help close the loop of the genetic research cycle.

Physiological role of the lysosome as a signaling organelle

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The lysosome plays a key role in cellular homeostasis by controlling both cellular clearance and energy production in response to environmental cues. In recent years, the lysosome has emerged as a key signaling center, which regulates and is in turn regulated by the activity of signaling molecules. However, the physiological relevance of the lysosome as a signaling organelle is still largely unexplored. During my talk I will discuss how the lysosome regulates its own biogenesis and function in response to environmental changes through a lysosome-to-nucleus signaling mechanism mediated by the mTOR kinase and the transcription factor TFEB. In addition, I will present recent data demonstrating how the lysosome regulates anabolic cellular pathways that play fundamental roles during organismal development and growth. Lastly, I will discuss unpublished data showing how an impairment of lysosomal function, such as the one observed in lysosomal storage disorders, affects the capacity of the cells to sense nutrient and how this alteration may in turn contributes to specific disease features observed in lysosomal storage disorder.

Screening for lead compounds to treat juvenile CLN3 disease

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Neurodegenerative diseases are incurable and debilitating conditions, resulting in a progressive degeneration or/and loss of neuron cells. Among these the Neuronal Ceroid Lipofuscinoses (NCLs) have a predominantly paediatric onset and are characterized by progressive loss of cognitive and movement abilities, seizures and early death. Juvenile NCL, or juvenile CLN3 disease, is the most common type of NCL. Development of therapies for CLN3 disease has been complicated by the lack of knowledge of the physiological function of CLN3 and by the need for robust cell phenotypes in mammalian cells compatible with drug screening. However, previous work revealed fission yeast to be a valid model of this disease, with complete loss of function of the orthologous gene, *btn1*, having multiple effects within the cell. Therefore, we used the fission yeast model for CLN3 disease to perform a high throughput image-based screen of FDA approved libraries. This drug screening strategy allowed us to identify many lead compounds that we tested for broader phenotypic rescue on the *btn1* yeast model, three other yeast models of lysosomal storage diseases and on CLN3 patient fibroblasts. In order to prioritize lead compounds, we are currently assessing the effect of these compounds on the metabolism of the yeast disease model through the BATCure Consortium. Our results contribute to identification of putative novel lead compounds for treatment of CLN3 disease. The same drugs may prove to be efficacious in other NCLs and related diseases.

Drug Discovery for Lysosomal Storage Disorders using Model Organisms

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Using CRISPR, we generated null alleles of NPC1 orthologs in *Caenorhabditis elegans* and *Drosophila melanogaster*, resulting in mutant animals that exhibit developmental delay and larval lethality, respectively. We then screened those invertebrate models against a 50,000-compound library to identify small molecules that reverse disease phenotypes in both whole animals and Niemann-Pick C patient fibroblasts. In less than a year, we discovered and validated a pan-mutation pharmacological bypass suppressor called PERL101. PERL101 is a chemically un-optimized primary screening hit from the nematode screen that is orally bioavailable, well tolerated, and CNS penetrant in mice. PERL101 modulates lipophagy, an evolutionarily conserved lipid and sterol mobilization pathway that may be therapeutically relevant for multiple lysosomal storage disorders.

Lysosomal dysfunction and neurodegeneration in the brain and retina of a mouse model for CLN7 disease

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We have disrupted the *Cln7*/*Mfsd8* gene in mice by targeted deletion of exon 2 generating a novel knockout (KO) mouse model for CLN7 disease, which recapitulates key features of human CLN7 disease pathology. *Cln7* KO mice showed increased mortality and a neurological phenotype including hind limb clasping and myoclonus. Lysosomal dysfunction in the brain of mutant mice was shown by the storage of autofluorescent lipofuscin-like lipopigments, subunit c of mitochondrial ATP synthase (SCMAS) and saposin D and increased expression of lysosomal cathepsins B, D and Z. By immunohistochemical co-stainings, increased cathepsin Z expression restricted to Cln7-deficient microglia and neurons was found. Lysosomal storage pathology was observed in the brain, heart, kidney and liver of *Cln7* KO mice. Ultrastructural analyses revealed large storage bodies in Purkinje cells of *Cln7* KO mice containing inclusions composed of irregular, curvilinear and rectilinear profiles as well as fingerprint profiles. MRI analyses revealed neurodegeneration in the olfactory bulb, cerebral cortex and cerebellum of *Cln7* KO mice late in disease. Generalized astrogliosis and microgliosis in the brain preceded neurodegeneration in the brain. Increased levels of LC3-II and the presence of neuronal p62- and ubiquitin-positive protein aggregates suggested that impaired autophagy represents a major pathomechanism in the brain of *Cln7* KO mice. Morphological analyses of the retina of *Cln7* KO mice revealed an early onset and rapidly progressing degeneration of photoreceptor cells in *Cln7* KO mice, resulting in the loss of about 70% photoreceptors by 4 months of age. The combined data identify rod photoreceptor degeneration as a major neurological phenotype of Cln7-deficient mice. The absence of Cln7 in the retina led to increased expression of multiple lysosomal proteins, accumulation of SCMAS and saposin D and reactive astrogliosis and microgliosis. Stable Isotope Labeling by Amino acids in Cell culture (SILAC)-based comparative mass spectrometric analysis of lysosomes from cultured Cln7-deficient mouse embryonic fibroblasts (MEFs) revealed that the amounts of several soluble lysosomal proteins involved in the degradation of different substrates including glycoproteins, glycosphingolipids, sulfatides, gangliosides, glycoproteins, peptides and glycosaminoglycans were decreased in *Cln7* KO MEFs compared with wild type controls. None of the identified lysosomal membrane proteins were significantly altered in *Cln7* KO lysosomes.

The data suggest that loss of the putative lysosomal transporter Cln7 leads to lysosomal dysfunction, impaired constitutive autophagy and neurodegeneration in the brain late in disease and early, rapidly progressing neurodegeneration in the retina. We conclude that the *Cln7* KO mice represent a useful model to elucidate the pathomechanisms ultimately leading to neurodegeneration in CLN7 disease, and to evaluate the efficacy of strategies aimed at developing treatments for this neurodegenerative lysosomal storage disorder.

O25

Towards small-molecule therapies for juvenile forms of Batten disease: drug screens in yeast and zebrafish models of JNCL

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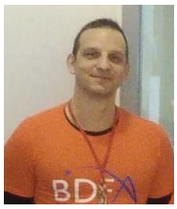
Objective: Our work is focused on *CLN3* and *CLN12*, two genes in which mutations can result in the development of the condition known as Juvenile Neuronal Ceroid Lipofuscinosis (JNCL). Because of their high level of conservation across living species, models of *CLN3* and *CLN12* deficiency have been created in various organisms in order to get a better understanding of the disease mechanism. Despite these efforts, the function of both genes remains unclear and currently there is no therapy to cure this disease. Given that the brain is the main organ affected in Batten disease, screening for small molecule drug candidates that can cross the blood-brain barrier seems like a promising strategy. The aim of our project is to develop disease models for both forms of JNCL in a simple unicellular eukaryote, budding yeast, and in a more complex multicellular organism, zebrafish. The combination of both models should enable a rapid identification of bioactive compounds with therapeutic potential as orphan drug candidates for JNCL. Additionally, we want to exploit these models to study the molecular mechanisms underlying the disease.

Results: We used a high-throughput growth phenotyping method established in-house to screen for conditions leading to slowed growth in strains deleted for the *YHC3* (yeast homolog of *CLN3*) or the *YPK9* (yeast homolog of *CLN12*) genes, as compared to wild-type control strains. In agreement with published results, the *ypk9*□ strains showed impaired growth in the presence of manganese. Building on this phenotype, we have developed a high-throughput drug screening strategy for this disease model and we are currently performing the primary screen of the drug library (Prestwick chemical library containing 1280 FDA- or EMA-approved drugs). So far, although over 400 growth conditions have been tested for the *yhc3*□ strain, we could not find any optimal condition for drug screening in this yeast mutant yet. However, we observed a decreased replicative life span in the *yhc3*□ strain, linking this gene with the aging process. In parallel to the yeast models, we are generating zebrafish models for JNCL based on loss-of-function of *cln3* and *cln12* using morpholino antisense oligonucleotides (PMOs). Two splice-blocking PMOs and one translation-blocking PMO were titrated to achieve maximum knockdown efficiency for each of the *cln3* and *cln12* genes. None of the knockdown embryos revealed morphological defects or early embryonic lethality, suggesting that the phenotypes might appear in a later developmental stage or that those gene knockdowns cause more subtle phenotypes as observed in other NCL zebrafish models. The lack of a gross early phenotype encourages us to generate zebrafish knockout lines in order to study a potential phenotype in juvenile larvaes and exploit it for small compound screening.

Conclusion: We are generating an innovative drug screening pipeline using budding yeast and zebrafish for high-throughput screens in a simple model followed by a more focused screen in a more complex model. By focusing this pilot project on the Prestwick chemical library, any compounds identified and validated through this pipeline will be drugs already marketed for another indication. This will enable accelerated preclinical and clinical development. Additionally, our studies in budding yeast indicate that *YHC3*, and therefore potentially *CLN3*, act as longevity modulators.

Identifying novel therapeutic targets for the NCLs



Maica Llavero Hurtado[1], Sam L. Eaton[1], Heidi R. Fuller[2], Douglas J. Lamont[3], Giusy Pennetta[4], Jon D. Cooper[5], Thomas M. Wishart[1,6]

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Degeneration of synaptic compartments of neurons is an early event contributing to the pathogenesis of many neurodegenerative diseases ranging from conditions such as those associated with advancing age (Alzheimer's & Parkinson's), protein miss folding disorders (Huntington's & Prion) and childhood/early life (SMA & NCLs). Yet, despite the exceptional vulnerability of synapses to such a broad range of neurodegenerative inducing stimuli, the underlying molecular mechanisms governing their exceptional vulnerability remain unclear.

Here, we utilise a "bottom-up" approach combining molecular analysis at the protein level, of differentially vulnerable synaptic subpopulations throughout the time course of disease progression across multiple disease variants (I & J NCL), with *in silico* examination and *in vivo* candidate testing for ability to moderate disease phenotype.

We demonstrate a high degree of molecular overlap between the NCLs and a range of conditions where synapses are an early pathological target. We also identify candidates which not only correlate with the vulnerability status of different neuronal subpopulations, but also show the ability to moderate phenotype *in vivo*. We therefore propose that candidates identified in this manner represent attractive targets for future therapeutic targeting.

O27

Identifying potential peripherally accessible biomarkers in the NCLs

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Main objective of the study: Biomarker is a commonly used term to mean a biologically detectable indicator of disease stage and/or rate of progression. These can be in the form of a behavioural, imaging or molecular readout. In diseases of childhood, where patients are unable to adequately describe the severity of their condition or any potentially mild improvement due to effective therapeutic intervention, an independent reporting mechanism is crucial. In this study we aim to identify peripherally accessible molecular biomarkers for disease progression and/or therapeutic efficacy in CLN1 & CLN3.

Approach: Here we conduct a molecular profiling and tracking study throughout disease progression in order to identify proteins which change in a predictable manner in both CLN1 & CLN3. We use easily accessible samples such as muscle, skin and blood from murine models of theses conditions and validate in human patient samples.

Conclusions from the study: Here we identify multiple biomarkers which may be of use in both I & J NCL forms. Many of which have already been studied as potential biomarkers for cancer. This means that the tools to accurately monitor such candidates may already be available for rapid application to the NCLs.

A New Therapeutic Target for Infantile Neuronal Ceroid Lipofuscinosis

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Infantile Neuronal Ceroid Lipofuscinosis (INCL, Infantile Batten) is an inherited metabolic disorder caused by a deficiency in the lysosomal enzyme palmitoyl-protein thioesterase-1 (PPT1) and is characterized by widespread neuronal loss and cortical atrophy. We recently made the unexpected finding of profound spinal cord pathology that precedes the disease observed in the thalamocortical system and cerebellum. It was not clear how much this spinal cord disease contributes to the clinical deficits observed in INCL, or if it could be effectively treated. Intrathecal administration of an AAV-based gene transfer vector significantly reduced neuropathology in the spinal cord of PPT1-deficient mice and confirmed the clinical importance of this aspect of INCL. Furthermore, the combination of intrathecal and intracranial injections decreased the accumulation of lipofuscin, neuroinflammation and neuronal loss in both the brain and spinal cord to a greater extent than either treatment alone. This also led to dramatic and synergistic improvements in motor function and life span. These data indicate that the spinal cord pathology contributes significantly to the clinical disease and can be an effective therapeutic target.

Chronic enzyme replacement therapy to the brain of a mouse model of late infantile neuronal ceroid lipofuscinosis mouse.

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Objective

Late-infantile neuronal ceroid lipofuscinosis (LINCL) is a fatal inherited neurodegenerative disease caused by loss of the lysosomal protease tripeptidyl peptidase 1 (TPP1). We have investigated the efficacy of long-term treatment using enzyme replacement therapy (ERT) to the brain of an LINCL mouse model.

Results

Chronic intrathecal (IT) administration by lumbar injection ameliorates disease in a dose- and dosing-interval dependent manner, with the most successful regimen preventing a loss of locomotor function and increasing median survival from 126 days to >300 days. However, under some regimens, IT treatment dramatically improves locomotor function without extending lifespan, suggesting that there is correction of anatomically restricted regions of the brain. Morphological studies support this, showing that IT administration effectively delivers TPP1 to ventral brain regions, e.g., the hypothalamus, but not to deeper and dorsal regions. Treatment is most efficacious in presymptomatic (6 weeks) compared to severely affected (15 weeks) LINCL mice. However, a subset of severely affected animals exhibited a dramatic increase in survival with treatment as well as a remarkable improvement in locomotor function after it had declined to a point at which animals normally die. This indicates that some pathology in LINCL is reversible and does not simply reflect neuronal death.

Conclusions

These results provide proof-of-principle for chronic brain ERT in LINCL and have other translational implications. First symptomatic patients may benefit from treatment. Second, measurement of discrete neurological functions, e.g., locomotor, may not necessarily provide an accurate picture of overall disease progression or projected survival.

Self-complementary AAV9 gene delivery partially corrects neuropathology associated with Juvenile Neuronal Ceroid Lipofuscinosis (CLN3)

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Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) is a fatal lysosomal storage disease caused by an autosomal recessive mutation in *CLN3* for which no treatment exists. Symptoms appear between 5-10 years of age, beginning with blindness and seizures, followed by progressive cognitive and motor decline, and premature death (late teens-20s). We explored a gene delivery approach for JNCL by generating two self-complementary AAV9 (scAAV9) constructs to address CLN3 dosage effects using the methyl-CpG-binding protein 2 (MeCP2) and β-actin promoters to drive low vs. high transgene expression, respectively. This was based on the expectation that low CLN3 levels are required for cellular homeostasis due to minimal CLN3 expression postnatally, although this had not yet been demonstrated *in vivo*. One month-old CLN3Δex7/8 mice received one systemic (i.v.) injection of scAAV9/MeCP2-hCLN3 or scAAV9/β-actin-hCLN3, with GFP expressing viruses as controls. A promoter-dosage effect was observed in all brain regions examined, where hCLN3 levels were elevated 3- to 8-fold in CLN3Δex7/8 mice receiving scAAV9/β-actin-hCLN3 vs. scAAV9/MeCP2-hCLN3. However, a disconnect occurred between CLN3 levels and disease improvement, since only the scAAV9 construct driving low CLN3 expression (scAAV9/MeCP2-hCLN3) corrected motor deficits and attenuated microglial and astrocyte activation and lysosomal pathology. This may have resulted from preferential promoter usage, as transgene expression following i.v. scAAV9/MeCP2-GFP injection was primarily detected in NeuN+ neurons, whereas scAAV9/β-actin-GFP drove transgene expression in GFAP+ astrocytes. This is the first demonstration of a systemic delivery route to restore CLN3 *in vivo* using scAAV9 and highlights the importance of promoter selection for disease modification in juvenile animals.

Acknowledgements: This work was supported by the UNMC Dean's Pediatric Research Fund to T.K. and a UNMC Graduate Fellowship to M.E.B. Abeona Therapeutics has provided funding to T.K. for additional preclinical studies of scAAV9/MeCP2-hCLN3.

Disclosure: The intellectual property for the scAAV9/hCLN3 constructs in JNCL by T.K. and K.D.F. (Co-inventors) has been licensed to Abeona Therapeutics.

O31

XN001 promotes autophagy to treat Batten disease

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Objective: Batten disease (neuronal ceroid lipofuscinosis, NCL) is a group of progressive inherited neurodegenerative disorders defined by the accumulation of autofluorescent ceroid lipoprotein deposits in the brain and other tissues. Ceroid accumulation results in part from dysfunction in the fundamental cellular process of autophagy, which is a highly regulated pathway for the controlled digestion of large cellular structures and macromolecular aggregates. XN001 (also called lanthionine ketimine ethyl ester, LKE) is a specific and selective inhibitor of the CDK5 (cyclin dependent kinase-5)/p25 complex. It is an orally bioavailable small molecule that activates beneficial autophagy through a novel mechanism. In the present study we hypothesized that inhibiting CDK5/p25 by XN001, engages autophagy in cell culture systems and in the Cln3□ex7/8 mouse model of juvenile Batten disease to reduce ceroid and improve outcomes.

Results: XN001 inhibits phosphorylation of CRMP2 (collapsin response mediator protein-2) via inhibition of CDK5/p25 activity thus, promoting its activation and leading to autophagy stimulation. Treating human iPSCs derived CLN31kb-del neurons with XN001 inhibited CRMP2 phosphorylation and promoted autophagy flux in a dose response manner. These data suggest a use for XN001 in managing diseases of autophagy impairment. Furthermore, in a cerebellar cell line derived from Cln3□ex7/8 mice, XN001 reduced ceroid deposits. The Cln3□ex7/8 mouse model characterized by developing cerebellar ceroid deposits that increased in size from 3-6 months of age. This process correlated with increased phosphorylation of CRMP2 on CDK5/p25-regulated sites but not on Rho kinase-regulated sites. Increased CDK5/p25 activation was evidenced by disease-associated conversion of the p35, Cdk5 activator protein, to its proteolysis product p25. XN001 *per os* significantly reduced cerebellar ceroid accumulation at 6 months of age. A trend for drug-mediated decrease in neuroinflammatory markers (Iba1+ microglia and GFAP-positive astrocytes) was detected. These outcomes were correlated with reduction in CRMP2 phosphorylation level and restoration of normal p35 levels in cerebellum.

Conclusion: XN001 is an orally available compound that inhibits the CDK5/p25 activity to affect downstream processes stimulating cellular autophagy. Herewith, XN001 is capable of reducing ceroid accumulation rates in cell culture and in a mouse model of juvenile Batten disease.

Disclaimer: KH has financial interests in XoNovo Ltd. and holds intellectual property related to XN001. LBW, IN, and BS are employed by XoNovo Ltd.

Preclinical Intrathecal Gene Therapy for Infantile Neuronal Ceroid Lipofuscinosis

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Infantile Neuronal Ceroid Lipofuscinosis (INCL) is an autosomal recessive neurodegenerative disorder caused by mutations in the *CLN1* gene, which encodes the soluble lysosomal enzyme palmitoyl-protein thioesterase-1 (PPT1). In the absence of PPT1, osmiophilic granules accumulate in cells, leading to cell dysfunction, neuroinflammation and neurodegeneration. The clinical onset of the disease occurs between 6 to 24 months of age, characterized by progressive visual failure, motor and cognitive decline, seizures and premature death. INCL mice (*CLN1*-knockout) recapitulate the major features of the disease, with neurological deficits appearing by 4.5 months of age and premature death around 8 months of age. The objective of the project was to design a global and translationally-relevant gene transfer approach for INCL. A single intrathecal (IT) injection of self-complementary AAV9 (scAAV9) encoding the human *CLN1* gene at a dose of $7x10^{10}$ vector genomes (vg) per mouse was administered via lumbar puncture to INCL mice at 1 week, 1 month (pre-symptomatic), 4.5 months (early-symptomatic) or 6 months of age (symptomatic), with survival as the primary outcome. The treatment time-points were chosen for their relevance in the design of a clinical trial, corresponding to a different stage in the progression of the disease. Treatment at 4.5 months or later did not show any therapeutic benefit at this initial dose. However, mice treated at 1 month survived approximately twice their expected lifespan (16 months). Additionally, mice treated at 1 week (modeling dosing soon after birth in a human) are currently 15 months old in good health. We subsequently tested a 10-fold higher dose ($7x10^{11}$ vg/mouse) at the same ages, monitoring for survival and behavior. Lifespan was significantly extended by 1.5 months in mice treated at the symptomatic stage (6 months). Mice treated at the early-symptomatic stage (4.5 months) had significantly extended lifespan and at 7 months old their performance on the accelerating rotarod and wire-hang test was comparable to the heterozygous controls. The pre-symptomatic cohorts (1 week and 1 month) are in the early phases of behavioral testing. IT-delivered scAAV9 vectors are currently being used in ongoing Phase I clinical trials for Giant Axonal Neuropathy and CLN6. These results clearly demonstrate the importance of early intervention. Our preliminary findings suggest this approach is a feasible and readily translatable treatment for INCL that is likely to provide the most benefit when administered early in the disease development.

Immunomodulatory therapy in mouse models of CLN1 and CLN3 disease

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CLN diseases are characterized by progressive axonal degeneration and neuron loss in the CNS, manifesting in disability and premature death. Visual impairment is often one of the earliest symptoms of CLN disease and pathological alterations in the retinotectal system reflecting disease status as a surrogate tissue have been described in patients and the corresponding mouse models. We have previously demonstrated that secondary neuroinflammation in the CNS contributes to axonal perturbation and neurodegeneration in mouse models of at least two forms of CLN disease. Innate and adaptive immune reactions correlating with disease progression have been detected in the CNS of *Ppt1*$^{-/-}$ and *Cln3*$^{-/-}$ mice, representing models of infantile and juvenile CLN disease, respectively. Using cross-breeding experiments, we could show that CD8+ effector T-lymphocytes and Sialoadhesin-positive, activated microglia/macrophages drive neuroinflammation and promote neuron loss and disease progression in both mouse models.

These findings suggested that immunomodulatory treatment approaches to suppress or attenuate such detrimental immune reactions in the CNS might be suitable to improve disease outcome. Therefore, our current objective was to test the efficacy of distinct clinically approved immunomodulatory drugs in attenuating neuroinflammation, axonal damage and neuron loss in the retinotectal system of *Ppt1*$^{-/-}$ and *Cln3*$^{-/-}$ mice. The respective treatment and control groups were non-invasively monitored for several months by optical coherence tomography and subsequently investigated using flow cytometry, histology, immunohistochemistry and electron microscopy.

Our results demonstrated that not all of the tested approaches were effective in attenuating CNS inflammation and disease progression in *Ppt1*$^{-/-}$ and *Cln3*$^{-/-}$ mice. However, those applied treatment approaches that were able to limit T-cell numbers and/or microglia/macrophage reactions in the CNS also attenuated axonal damage, neuron loss and retinal thinning in both mouse models. The beneficial effects of the treatment approaches were more pronounced when applied early before the onset of pathological alterations. In addition, the applied targeting of specific neuroinflammation-related mechanisms was not accompanied by deleterious side effects.

In conclusion, our data further corroborate the view that immune reactions in the CNS of CLN disease models contribute to axonal damage and neuron loss. Moreover, they show that not all, but some specific clinically approved immunomodulatory drugs can attenuate disease progression in mouse models of both CLN1 and CLN3 disease. These observations suggest that similar immunomodulatory treatment approaches alone or in combination with other therapeutic approaches might also be a promising strategy to attenuate disease progression in CLN1 and CLN3 disease patients.

Retinal function and structure are preserved in a canine model of CLN2 Batten disease after intravitreal implantation of stem cells genetically modified to overproduce TPP1 enzyme

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Objective: While previous treatment studies using a canine model for CLN2 disease have successfully delayed the onset and slowed the progression of neurologic symptoms, these treatments have not mitigated the progressive disease-related vision loss. Dogs with CLN2 disease due to a deficiency in the TPP1 enzyme exhibit clinical signs similar to those in affected children, including progressive retinal degeneration beginning in dogs at 4 to 5 months of age. Retinal degenerative changes are characterized by significantly reduced electroretinogram (ERG) b-wave amplitudes and multifocal retinal detachments. Studies were performed to determine whether supplying the TPP1 enzyme with intravitreal ex vivo gene therapy can inhibit retinal degeneration in CLN2-affected dogs.
Methods: Autologously derived mesenchymal stem cells (MSCs) from CLN2-affected Dachshunds (n=5) were transduced with adeno-associated virus (AAV2)-packaged DNA constructs that direct stable overexpression and secretion of TPP1 or stable expression of green fluorescent protein (GFP). *TPP1*-transduced cells were implanted into the vitreous of one eye and *GFP*-transduced cells into the contralateral eye at 3 months of age. Thereafter, dogs were evaluated monthly with bilateral ERG and *in vivo* retinal imaging until near end-stage neurologic disease was reached around 10 months of age. Following euthanasia, each retina was examined for histopathological abnormalities associated with the CLN2 disease and for possible adverse effects of the implanted cells.
Results: For each dog, the eye treated with TPP1-expressing MSCs had better preserved retinal function and structure compared to the control eye, which exhibited progressive deficits typical of untreated CLN2 disease. Treatment delayed the onset of ERG amplitude decline by 3 months, and ERG amplitudes were significantly improved in the treated eyes ($p<0.001$) compared to untreated eyes throughout the entire disease course. The control retina developed extensive retinal detachment lesions in 4 of 5 dogs, while treatment fully prevented these lesions in 2 of the contralateral TPP1-treated eyes and greatly reduced the prevalence in the remaining treated eyes. The treatment also inhibited the disease-related thinning and disorganization of the retina. No serious complications of the treatment were observed.
Conclusions: We have demonstrated inhibition of retinal degeneration with a continuous supply of therapeutic compound produced by autologous, genetically modified stem cells that have been implanted into the vitreous. This approach to therapeutic drug delivery could offer a long term approach to treatment, without the need for repeated injections, for CLN2 and other forms of NCL and many other chronic diseases affecting not only the retina, but the central nervous system as well.

Gene transfer can prevent stereotypical disease development in ovine CLN5 and CLN6 models of NCL

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Gene therapy represents a promising treatment strategy for the NCLs. Sheep with naturally occurring NCLs are ideal translational subjects for gene therapy studies as they have large complex human-like gyrencephalic brains and recapitulate the key molecular, pathological and clinical features of the human diseases.

Indications of the successful treatment of six CLN5$^{-/-}$ sheep via combinatorial intracerebroventricular and intraparenchymal injections of lentiviral or AAV9 vectors expressing ovine *CLN5* were reported in Cordoba 2014[1]. Both vector platforms afforded sustained protection from stereotypical disease onset and progression. Consequently i*n vivo* monitoring was extended beyond the intended endpoint to 27 months. Cognitive and neurological function was preserved, whilst longitudinal structural neuroimaging by CT and MRI scanning revealed normalisation of intracranial volumes and brain integrity. Quality of life was profoundly improved for the treated sheep and one AAV9 treated sheep is still grazing peacefully at 37 months. The onset of visual deficits was delayed, from 11 months in untreated CLN5$^{-/-}$ sheep to 21-24 months in the treated cohorts. Supporting neuropathological data detailing CLN5 transgene expression and the impact upon the lysosomal storage pathology, glial activation and neuronal loss will be presented.

One of six similarly AAV9-*CLN6* injected CLN6$^{-/-}$ sheep also maintained phenotypic correction. This sheep retained vision and was clinically indistinguishable from age-matched control animals at 26 months of age. *Post mortem* neuropathological studies revealed a significant diminution in disease-associated lysosomal storage, gliosis, atrophy and neurodegeneration after AAV9-mediated *CLN6* transfer.

Second-generation vector studies, using self-complementary AAV9 cassettes and direct intracerebroventricular injections, are underway. Sheep with established disease have been injected and updated *in vivo* monitoring results will be presented. Collectively the *in vivo* assessments and neuropathological results indicate a very good prognoses for translation to human CLN5 and CLN6 gene therapy.

[1]Mitchell NL, Barrell GK, Wellby M, Wicky HE, Palmer DN, Hughes SM. (2014) Gene therapy using adeno-associated virus serotype 9 in the sheep brain. 14th Congreso Internacional de Lipofuscinosis Ceroideas Neuronales (Enfermedad de Batten), Medicina. 74: supl 11 O-48, p 23.

A Non-Human Primate Model of Neuronal Ceroid Lipofuscinosis

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A naturally occurring model of late infantile-onset neuronal ceroid lipofuscinosis (NCL) has been identified in the Japanese macaque breeding colony at the Oregon National Primate Research Center. Five cases presented with neurologic signs of cerebellar dysfunction including incoordination, ataxia, intention tremor and hypermetria. All were female, between 4 and 6 years old, roughly 12-15 human age equivalents. Post-mortem analyses of four animals revealed a dramatic reduction in overall brain size with particularly notable shrinkage of the cerebellum and occipital lobe. Three cases demonstrated a distinct brown discoloration of cerebral and cerebellar gray matter. Microscopic examination demonstrated granular to globular, eosinophilic, yellow-brown material in many neurons, which distended the cytoplasm in the cerebrum, cerebellum, brainstem and spinal cord. Cerebellar atrophy was characterized by loss of Purkinje cells and collapse of the molecular and granular layers. Storage material from affected cases was autofluorescent, and stained positive for periodic acid–Schiff (PAS), sudan black and luxol fast blue. Electron microscopy demonstrated osmophilic intracytoplasmic deposits, some of which had whorling or curvilinear membrane patterns.

Longitudinal retinal imaging was carried out on one currently living subject. Compared to age-matched controls, retinal thickness in the macula measured by OCT was reduced by 12% and 18%, and fundus autofluorescence was 80% and 170% higher at 3 and 4 years old, respectively. Retinal function, measured by multifocal ERG, was markedly reduced throughout the macula with foveal sparing. Retinal sections were hyper-autofluorescent throughout, with the highest levels observed in the photoreceptor inner segments.

Pedigree analysis identified the NCL disorder as being consistent with recessive inheritance. Whole exon sequencing identified a single base deletion in the *CLN7/MFSD8* gene exclusively within obligate carrier and affected individuals. The frame shift mutation predicts a truncation of the CLN7/MSFD8 protein, including loss of transmembrane spanning domains 7-12.

Conclusions: We have identified a spontaneous non-human primate model of NCL in Japanese macaques caused by a point mutation in the *CLN7/MFSD8* gene and characterized by neurological and structural brain abnormalities consistent with human CLN7 disease. This model will be valuable for further characterizing disease-related pathologies, developing biomarkers of disease progression and for evaluating promising therapeutic strategies for this devastating and fatal neurological disease.

Title: Hot Topics Workshop: Neurobehavioral and Psychosocial Function in the NCLs

Heather R. Adams (Workshop leader)

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Each of the Neuronal Ceroid Lipofuscinoses, though genetically distinct, share common clinical features including regression of developmental and cognitive milestones (i.e., dementia) and challenging mood and behaviors that have daily impacts on affected individuals and their families. There are broad differences across daily settings (e.g., school, home) and geographic locations within and across countries, in how these varied symptoms are managed. There are also no established, evidence-based standards to guide symptom management across domains of neurobehavioral and psychosocial function, or to support individual and family quality of life and well-being.

We propose a "Hot Topic Workshop" dedicated to presentation and discussion of new and recent findings related to these issues. We invite submission of abstracts that present results from a variety of methodologic approaches including qualitative and quantitative studies, needs assessment, program outcomes evaluation, survey results, etc. Submissions should address applications (potential or actual) of results to current or future clinical care and education of children with NCLs, and/or support for family, school personnel, or other care providers in managing neurobehavioral/psychosocial symptoms. The latter portion of the workshop will be devoted to an interactive, panel-style discussion (with audience participation as well) of the presentations, with the goal toward identifying common themes in assessment, gaps in our knowledge (and opportunities to address those gaps), and discussion about key areas for potential collaboration on future research initiatives of common interest and need.

Experiences on Education and Adaptations for Learners with Juvenile Neuronal Ceroid Lipofuscinosis (JNCL)

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Background: JNCL is characterized by very early onset dementia. There is little knowledge about educational initiatives provided to learners with JNCL and how to utilize possibilities and compensate for the challenges and vulnerabilities caused by the disease.
Aim: To gain information and a holistic picture about strategies and positive and negative experiences related to educating learners with JNCL. A special aim is to understand the development of dementia in a learning setting.
Method: The study is a part of the ongoing project "Education and Juvenile Neuronal Ceroid Lipofuscinosis" (2014–2017), with participation from Denmark, England, Finland, Germany, Norway, Scotland and USA. The study includes three different questionnaires (parents, bereaved parents and staff) and semi-structured interviews (staff and parents).
NCL associations and clinical institutions distributed information about the study. The study included questions related to the diagnostic process, choice of education setting, educational strategies and experiences, language, school activities, social participation and behavior problems in different age periods. The questionnaires were answered on paper or via secure Internet.
The Regional Committees for Medical and Health Research Ethics in Norway approved the study. Teachers and staff were approached if the parents had agreed to this. The questionnaire included a question whether the parents or staff were willing to take part in an interview. The informants agreed to this by checking the appropriate box.
Results: 190 questionnaires for 140 individuals with JNCL were returned. Seventy parents and staff were interviewed. The answers show large variations in development and loss of skills and abilities and in educational settings and strategies. Participation in social life, well-being and behavior problems, and the impact of different initiatives varied significantly. The analyses reflect possibilities, successes and failures, for instance in reading, writing and communication, social life, behavioral and emotional problems.
Discussion and conclusions: Developmental consequences of dementia versus relevant initiatives are an unknown area in education. The discussion includes implications of findings for adapted education in school and in adult living settings, and suggestions for educational strategies, educational tools, and a working agenda for staff. It appears to be a need to develop properly tailored plans in education and adult living in order to integrate educational and social approaches, early capacity building, pro-active and preventive teaching and "hastened learning" strategies.
Clinical implications: The findings of the study will be used to develop of a JNCL curriculum. I.e. a set of guidelines and strategies to enable staff and families to provide the best possible education, social participation and quality of life for learners with JNCL.

O40

A study on tactile/body signs or hand signs for students with Batten disease (JNCL)

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Background: A small study on learning hand signs for expressive communication for students with JNCL diagnoses was implemented in Norway 2014 – 2015.
Most persons with JNCL experience severe problems with speech fluency and speech production during the course of the disease. The ability to make oneself understood is gradually compromised or lost. Data from the international project *JNCL and Education (2014-2017)* indicate that comprehension of spoken language exceeds the ability to speak in certain stages of the disease. Parents consider this a difficult situation for their child. The project data show that many persons with JNCL have functional use of arms and hands when the ability to speak is lost. However, very few initiatives regarding systematic training in augmentative and alternative expressive communication strategies are offered for students with JNCL according to the *JNCL and Education project.*
Aim of the study: To collect experiences and knowledge about learning of hand signs (signs for deaf or deafblind people) as an augmentative or alternative communication strategy to prepare for future or current speech problems and speech loss. To gain information about the students' learning, the students motivation to learn, and other contextual, social and environmental challenges and barriers of such initiatives.
Method: A small study was conducted with five students in different ages. The study started with a two-day seminar for teachers and parents. The seminar focused on different methods and competence areas of certain importance. The students were subsequently taught signing for a period of 3 months. The project participants were asked to deliver data on prepared registrations forms on a regular basis during the study period. The study also conducted interviews with staff and/or the parents. Four students completed the study according to plan.
Results: The results of the study is presented in a Norwegian report to the funding body "*the Norwegian Directorate of Education and Training"*. All students learned signs. There was a great variation concerning the number of signs learned in the 3-month period. After the conclusion of the study some target persons have stopped using signs and some have continued. New students are today offered training in hand signs because of the study. Denmark has conducted a similar sign project because of the Norwegian study.
Discussion and conclusions: Identification of factors that influence and affect the learning process in the different individuals. Identification of factors not connected to the disease itself or to the specific individuals´ learning abilities, but related to e.g. staff competence, school schedules, attitudes etc. The study experienced several barriers related to services and systems. The long-term effects of the study is the critical point of the study – will the students use hand signs when speech become complicated? These effects will be available some years from now.
Clinical implications: The society should offer signing to students if it turns out to be useful for some children and young people with JNCL. It should be a part of our counselling service. More knowledge and experiences should be collected in the meantime. Barriers not related to the disease itself should in addition be identified and counteracted.

The burden of CLN2 disease on families: home-based surveys with caregivers in Germany and the United Kingdom

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Background and Objectives: CLN2 disease is an ultra-rare rapidly progressing neurodegenerative disorder. Living with and caring for a child with CLN2 disease presents major challenges, impacting on emotional and psychological wellbeing, employment, schooling, finances and health. This study is the first to quantitatively and qualitatively explore this burden on families.

Methods: Mixed-method surveys were developed based on information from a literature review, interviews with clinicians and patient organizations and family focus group. Surveys were administered to caregivers and adult and child siblings in Germany and the United Kingdom. The survey sample consisted of 19 primary caregivers, 10 secondary caregivers, 2 adult siblings and 2 child siblings from 19 different families across different stages of disease.

Results: Families reported that the journey to establishing a correct diagnosis could take as long as two years, resulting in feelings of anger and frustration. Children were prescribed a large number of medications to manage their symptoms, which included seizures, secretions, twitchiness/dystonia, mood changes, difficulty sleeping and problems with/lack of mobility, vision and communication. Most children were attending special needs schools where they received one-to-one support throughout the day. Primary caregivers reported spending 96 hours caring in a usual week and typically slept for 5 hours per night. The financial burden of CLN2 was severe and mainly driven by loss or reduced employment related income as well as the necessity to self-fund healthcare needs of their child, including care equipment and adaptations to home and car.

Conclusions: From pre-diagnosis to death, families caring for a child affected by CLN2 have to cope with many difficult emotional, physical, professional, financial and organizational challenges.

Engaging families in research workshop: Dialogue with parents and researchers

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Background
Patient and family involvement has been integral to the success of rare disease research for decades, though input on the needs, challenges and interest in the phases of research is often not sought. In July, 2016 at the annual BDSRA Family Conference, Drs. Augustine and Frazier gave reviews of the ways in which families' ideas can positively impact the outcome of research and trials from helping to develop research goals to developing trial logistics that make sense.

During the session, audience members answered questions as a group using an audience polling application called Mentimeter. This method allowed for many more ideas to emerge in a short period and encouraged those who usually would not speak in an open forum to express their opinions.

Themes from Audience Poll n= ≥ 75

- The top choice for "expectations for this year's conference" was to learn about new research.
- Nearly 40% of parents felt they were not adequately informed about the latest research.
- 50% had given input into research priorities.
- The majority wanted treatments to slow the disease. Very few interested in developing care guidelines or developing new treatments for specific symptoms as a ***top*** research priority.
- Symptoms that they most wished to have treatments for, in rank order--seizures, vision loss, cognition/dementia, and mobility/motor function.
- What are most important questions for researchers: several themes...
    - finding a cure
    - can symptoms be reversed, if a cure or treatment is discovered that will modify disease
    - What can I do to help?
- Themes related to trial options –
    - want to know more about different types of treatments such as gene therapy, enzyme replacement, stem cell treatment
    - want to know which trial is best to join (if more than one option available); why are there trials for some forms of Batten disease but not others? Why do some kids get into trials but others do not?
- How would you like to be involved in research? Top answers were completing online surveys, participating in clinical trials of experimental treatments, and parent advisory groups.

Conclusion Families and caregivers from the 29th BDSRA Annual Family Conference were engaged in sharing their opinions about research and clinical trials. Particular emphasis was placed on their wish for treatments to slow disease. Individual researchers and patient groups can do more to engage them via online surveys, distance learning and polling opportunities and in-person options.

Rating Scales for Natural History Studies and Registries in the NCLs

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Recent advances in the development and testing of potentially effective disease-altering therapies for specific NCLs has led to substantial excitement about the potential for meaningful therapies for all forms of NCL. However, it has also brought increasing pressure for development and implementation of tools to assess and track disease progression and to gauge meaningful outcomes. These tools must be sensitive to change over both short and long periods of time. They must valid and reliable and must be able to be implemented efficiently in a routine clinical setting. Ideally, individual tools should be relevant to multiple forms of NCL and for the spectrum of disease within a single form of NCL. Most importantly, these tools must be accessible and applicable to centers internationally and there must be consistent application and alignment of data collection methods to allow for multinational collaboration for therapeutic development in these rare diseases.

Rating scales have been used in clinical trial for CLN2 and CLN3 diseases. The differences between scales and between diseases highlight some of the challenges and opportunities for refinement. Data from the "Hamburg" scale for LINCL (CLN2) and the modifications of that scale will be presented and compared to data from the Unified Batten Disease Rating Scale (UBDRS). Data from the UBDRS will be presented from a large natural history study of JNCL (CLN3). Finally, ongoing efforts to align registries for the different NCLs will be discussed.

Registry of NCL Disease In Databases Focused On Genotype-Phenotype Relationships

Inés Noher de Halac

The Ministries of Health and of Science and Technology at National level in Argentina are involved in the adoption and regulations of Next Generation Sequencing (NGS) technologies in human medicine and consequent data recording. The Argentine Human Variome Project node was generated in 2016 and a representative was appointed. Genotypic- phenotypic data registration was activated under a unifying attempt. The Genomic variants recognized in the frame of the NCL-Translational Research Program were recorded for more than 10 years in the disease specific data-base of UCL http://www.ucl.ac.uk/ncl/mutation.shtml. Out of 102 individuals 52 received precision's genotypic characterization and 53 remain unenlightened. These will be analyzed using the facilities of INDEAR- Bioceres Center in Rosario-Argentina operating under Illumina NGS technology (http://www.indear.com/web-services/). Validation of DNA variations as pathological will go on through experimental and bioinformatic tools. Registry options of Argentina go toward participation in databases focusing on the genotype-phenotype relationships like the Leiden Open Variation Databases (LOVD). Of general use are databases of knowledge like the Online Mendelian Inheritance in Man (OMIM) and ORPHANET. The NGS technology and data recording are intended to follow the international recommendations for human medical use. The NEUROREDLAT was created with the aim of increasing knowledge on neuro-genetic disorders in Latin America, expecting to mobilize registry of medical relevant data through graduate's education improvements.
216/250

Current state of the international DEM-CHILD NCL Patient Database: Successful development of tools for the evaluation of therapies in neuronal ceroid lipofuscinoses (NCL)

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Introduction

Neuronal ceroid lipofuscinoses (NCLs) are the most common neurodegenerative diseases in childhood. They are characterized by dementia, visual loss, epilepsy, motor decline, and premature death. Even though no approved curative therapy exists so far for any of these diseases, an increasing number of experimental therapy studies is being developed such as small molecules, gene therapy and enzyme replacement therapy. However, their evaluation is especially difficult as placebo controlled studies in such devastating diseases are considered unethical and as the clinical course of NCLs can be variable. However, in order to closely and prospectively monitor disease progression in patients under experimental therapies, detailed, objective and also organ specific evaluation systems are necessary.

Aim

The international DEM-CHILD database project seeks to develop such outcome measures by (i) unifying and improving existing clinical scoring systems for prospective use in follow-up examinations and (ii) correlating these with organ specific evaluations such as for example MRI-based brain volumetrics, cardiologic and ophthalmologic examinations.

Results

Over the last 3 years, significant progress has been made towards these aims: The DEM-CHILD database consortium has been constantly growing from initially 4 European countries participating to now 23 international partners from 16 countries from all continents actively contributing patient data. Growing financial support has been achieved by successful applications for research grants provided by various national, European, and pharmaceutical industry funding sources. More importantly, generous financial contributions from family associations and other fund raising organisations have allowed us to support various new consortium members.

Data are collected in the international DEM-CHILD NCL patient database which has been established as part of the European FP7-project DEM-CHILD. It currently contains clinical data derived from more than 500 NCL patients with all types of NCLs. A set of these data has already been successfully used as control data in a Phase 1/2 clinical trial on intraventricular enzyme replacement therapy.

Conclusion

The large and growing collection in the DEM-CHILD database of comprehensive sets of data on the natural history of different NCL forms is an indispensable tool for the evaluation of therapies. Its data are already being used for this purpose in current clinical trials.

Longitudinal 8-years brain volumetric analysis in 35 CLN3 patients:Successful development of a sensitive marker to measure clinical outcome

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Introduction
Gray matter atrophy due to neuronal loss is a striking feature of patients with juvenile CLN3 disease. A precise and quantitative description of disease progression is urgently needed in order to establish an evaluation tool for future experimental treatments. This is especially challenging as CLN3 disease may present with variable clinical disease progression despite most patients harboring the same 1 kb deletion in the CLN3 gene. In order to develop a quantitative marker to measure brain volume outcome, we analyzed the longitudinal volumetric development of gray matter (GM), white matter (WM) and lateral ventricles and correlated those with the clinical course.

Methods
One hundred twenty-two MRIs of 35 patients (14 male; 21 female; age 15.3 ± 4.8 years) with genetically confirmed CLN3 disease were performed on 1.5T scanners. A 3D T1-weighted magnetization-prepared rapid gradient-echo (MP-RAGE) sequence was acquired and in 86% of cases the following parameters were used: TR/TE/TI/flip angle= 1900/2.97/1100ms/15°; FOV, 256 mm; matrix, 256×176; slice thickness, 1 mm; 160 slices with whole brain coverage. The other 14% of the image acquisitions were done off-site with different scanners or after a software update of our scanner resulting in slightly different imaging parameters. Volumetric segmentation of the brain was performed with the Freesurfer image analysis suite. The clinical severity was assessed by the Hamburg juvenile NCL score, a disease-specific scoring system.
Results The volumes of cortical GM, cerebellar GM and WM as well as the volume of basal ganglia and thalami ($P=<0.0001$) and hippocampus significantly ($P<0.0001$) decreased with age, while the lateral ventricle volume increased ($P<0.0001$). Supratentorial WM volume correlated much poorer with age then the other observed regions ($R=0.21$, $P= 0.0095$). Decrease of cortical gray matter volume showed the most uniform decrease with strongest correlation with age ($R=0.86$, $P<0.0001$). CLN3 patients lost on average 4.6% (± 0.2 %) of cortical GM per year. In addition, a strong correlation with clinical scoring existed for the cortical GM regions ($R=0.83$, $P= <0.0001$).

Conclusion
Cortical GM volume decline seems to be the most sensitive parameter for assessment of disease progression and represents a potential sensitive outcome measure for evaluation of future therapies.

Intracerebroventricular cerliponase alfa (BMN 190) in children with CLN2 disease: Results from a Phase 1/2, open-label, dose-escalation study

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Background and objectives. CLN2 disease, a rare, inherited, pediatric-onset, neurodegenerative lysosomal storage disorder caused by TPP1 enzyme deficiency, is characterized by seizures, ataxia, rapid loss of language and motor functions, blindness and early death. Cerliponase alfa (BMN 190) is a recombinant human TPP1 enzyme. This phase 1/2, multi-center, open-label, dose-escalation study evaluated the safety, tolerability and efficacy of every other week intracerebroventricular (ICV) infusions of cerliponase alfa in children with CLN2 aged 3 - 16 years.

Design. Following a dose escalation period, all patients received 300mg of cerliponase alfa every two weeks by ICV infusion for 48 weeks. Efficacy was evaluated by monitoring changes in motor and language functions using a CLN2 clinical rating scale.

Results. 24 subjects (9 male, 15 female, mean age 4.3 years [median: 4 years; range: 3-8 years]) enrolled in the study. Almost all subjects (96%) had adverse events assessed as study drug-related, the majority of which were Grade 1-2 and included pyrexia (46%), hypersensitivity (38%), seizure (38%), and epilepsy (17%). Serious adverse events assessed by the investigator as study drug-related were reported in eight (33%) subjects. There were no anaphylaxis/anaphylactoid reactions, study drug discontinuations or deaths due to AEs. The mean (SD)/median rate of decline in CLN2 score for subjects treated 48-91 weeks (n=23) was 0.48 (0.756)/0.00 units/48 weeks, in contrast to 2.09 (0.97)/1.87 units/48 weeks observed in natural history (n=41).

Conclusions. Enzyme replacement therapy with ICV-administered cerliponase alfa is well-tolerated and slows the progression of functional decline in children with CLN2.

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Developing therapies for individuals with *CLN3* disease - results from a phase 2, double-blind, crossover study of mycophenolate

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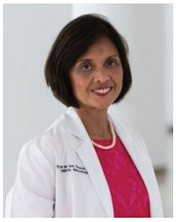
There is pre-clinical evidence of autoimmunity in *CLN3* disease that may contribute to core pathophysiological processes. Knock-down of autoimmunity, genetically or pharmacologically, improves neuropathology and motor function in $CLN3^{-/-}$ mice. On this basis, the safety and tolerability of short-term administration of mycophenolate mofetil was evaluated in a phase 2 study of 19 individuals with genetically confirmed *CLN3* disease. A novel site infrastructure model was successfully employed, and 8-week administration mycophenolate was well-tolerated without significant adverse effects. Study of long-term administration will be needed for evaluation of clinical efficacy.

AAV9 CLN6 gene therapy trial



Emily de los Reyes, M.D.,

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NCL (neuronal ceroid lipofuscinosis) disorders also commonly referred to as Batten's disease are a group of lysosomal storage disorders characterized by progressive degeneration affecting the brain and the retina. They are characterized histologically by the accumulation of abnormal autofluorescent lipopigments in neurons and other cells. Clinically, individuals with mutations in the CLN6 gene present with language delay, cognitive regression, ataxia, pyramidal and extrapyramidal signs. Recently, the enter of gene therapy at Nationwide Children's Hospital has developed a gene therapy approach to deliver a healthy copy of the CLN6 gene to the central nervous system using self-complementary adeno-associated virus (ScAAV) serotype 9. This specific viral vector was chosen based on its proven safety profile in previous pre-clinical studies as well as ongoing clinical trials safety in human subjects.

The CLN6 gene was delivered using self-complementary adeno-associated virus (scAAV) serotype 9 under control of the chicken-β-actin hybrid promoter. This specific viral vector was chosen based on its proven safety in human subjects documented in the currently active Phase I clinical trial for infants with type I spinal muscular atrophy (SMA). Immunohistochemical analysis demonstrated that a single injection of scAAV9.CB.CLN6 resulted in a widespread transduction throughout all the brain regions after a single intracerebroventricular injection (ICV) of scAAV9.CB.CLN6 at post natal day 1. Thus, confirming the utility of CSF delivered AAV9-mediated delivery to target the brain regions critical in the pathogenesis of Batten disease. In addition, administration of scAAV9.CB.CLN6 in CLN6nclf mice by a single ICV injection results in significant reduction of accumulated autofluorescent storage material (Green) and ATP synthase subunit C, one of the proteins that accumulate in the lysosomes of Batten disease patients. Rotarod experiments showed differences in motor function between treated and nontreated CLN6 mice. In conclusion, preliminary data on CLN6 mice demonstrates widespread transduction which laid the ground work on exciting translational studies in humans.

POSTER PRESENTATION ABSTRACTS

Identification of a novel *DNAJC5* mutation missed by Sanger sequencing in a familial case of adult-onset neuronal ceroid lipofuscinosis (ANCL)

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The adult-onset forms of neuronal ceroid lipofuscinosis (ANCL, Kufs disease) are rare inherited neuropsychiatric disorders, which are characterized by intralysosomal accumulation of ceroid lipopigment in tissues. The ceroid accumulation affects primarily brain, leads to loss of neurons and progressive neurodegeneration.

Genetically, homozygous or compound heterozygous mutations in genes *CLN6*, *CTSF*, *GRN*, *CLN1*, *CLN5*, *ATP13A2*, *CLCN6, SGSH* and heterozygous mutations in the *DNAJC5* gene have been described as causative in ANCL cases. The list of the ANCL causal mutations/genes is not complete, as additional families are diagnosed with ANCL based on clinicopathological criteria with an unknown genetic background of the disease.

To support the research on ANCL, **the Adult NCL Gene Discovery Consortium (The Kufs Consortium)** was established involving groups from Australia, UK, Europe, USA and Canada. The suspected ANCL families are evaluated based on clinical and histopathological criteria and further tested for candidate/causal genetic variations by next-generation sequencing based methods including targeted exome sequencing and whole-exome sequencing.

Here we report a discovery of a **30 bp in-frame insertion in the *DNAJC5* gene** in one family with three affected individuals, a mother and two sons, with autosomal dominant (AD) Kufs disease. The 30 bp insertion is predicted to result in a duplication of 10 amino acid residues, primarily cysteine residues, in a highly palmitoylated cysteine string motif and is predicted to affect the hydrophobicity and the palmitoylation of the mutated protein.

This mutation is remarkable in view of the fact that it has been missed by whole exome sequencing with standard approach to data analysis. Moreover, Sanger sequencing of *DNAJC5* in this family using a standard sequencing protocol led to a false-negative result. Considering that *DNAJC5* is the prevalent gene for AD Kufs disease, it cannot be ruled out that this kind of mutation has been missed in other ANCL unsolved cases. This family exemplifies the limitations of next-generation and traditional Sanger sequencing approaches.

P1

Over-expression of wild-type and mutated *CLN1* in SH-SY5Y cells: a transcriptomic, biochemical and morphological study

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Introduction: The Neuronal Ceroid Lipofuscinoses (NCL) are a group of genetically determined, neurodegenerative disorders characterized by endo-lysosomal storage. Classical CLN1 disease is an early childhood onset NCL, which shows rapid progression with loss of neurological functions, blindness and severe epilepsy associated to "vanishing EEG" pattern. CLN1 disease is related to mutations in *CLN1* encoding a hydrolytic enzyme (PPT1), which removes palmitate residues from palmitoylated proteins and localizes to different cellular compartments. *Methods:* SH-SY5Y cells were stably transfected with wild-type *CLN1* cDNA (wtCLN1), cDNAs carrying missense mutations (c.665T>C, c.541G>A), an insertion (c.169-170insA) or an artificially generated deletion (c.124_235del) in *CLN1*. Transfected cells were characterized by RT-PCR, Western Blot (WB), immunofluorescence and PPT1 enzyme assay (EA). Clones were differentiated into a more mature neuronal phenotype (using Retinoic Acid and neurotrophic factors) and assessed using biochemical and morphological methods. RNA sequencing (RNAseq) and bioinformatic analyses were performed on wtCLN1, c.665T>C, c.169-170insA clones. *Results:* Transfected cells exhibited high amounts of *CLN1* RNA. wtCLN1 transfected cells demonstrated a significantly increased signal of PPT1 isoforms on WB, and a 5-fold increase of enzymatic activity. Over-expression of glycosylated PPT1 was observed for c.665T>C/p.L222P and c.541G>A/p.V181M clones. In mock-transfected cells as well as in those carrying a c.169-170insA/p.M57Nfs*45 and an artificially generated p.A43_G145 deletion, a faint 35/37 kDa doublet was detected on WB, corresponding to endogenous PPT1. In all transfected cells carrying PPT1 mutations, enzymatic activity paired the control ones. RNAseq analysis identified 800 differentially expressed genes (DEGs) in wtCLN1 cells and about 200 ones in both mutants, in comparison to the mock expression profile. Bioinformatic predictions indicated down-regulation of genes related to neuritogenesis/axonal elongation processes, according to a gradient of severity from wtCLN1 to missense and nonsense clones. Accordingly, reduced extension of neuronal processes was measured in wtCLN1 transfected cells, and to a lesser extent, for c.665T>C/p.L222P mutant, both under basal conditions and following differentiation. The expression of several axonal cytoskeletal proteins was down-regulated, as assessed by WB. A significant number of DEGs in wtCLN1 transfected cells was assigned to "*Cell-to-Cell Signalling and Interaction*" category, and specifically to *Synaptic Transmission* and *Neurotransmission* annotations, predicting a dysregulation of synaptic functions. About 13.5% of DEGs belonging to this functional category encoded for membrane receptors, subunits of membrane channels and synaptic transporters. Finally, 7% of DEGs identified in wtCLN1 cells encoded for proteins reported to be palmitoylated, the majority of them was further assigned to "*Nervous System Development and Function*" and constituted 12% of the category. Interestingly, two of the identified DEGs encoded for CRMP1, known interacting partner of PPT1, and epilepsy-related GAP43. *Conclusion:* Convergence of transcriptomic data, protein expression, and morphological/morphometric findings outlined a role for PPT1 in axonal growth regulation in differentiated human SH-SY5Y cells, over-expressing wt and mutated *CLN1*. Moreover, the results of bioinformatic predictions pinpointed a dysregulation of genes encoding for ion channels, neuro-transmitter receptors and synaptic proteins. These findings require investigations at cellular levels, before being translated into a pathophysiological model of *CLN1*-related epilepsy disorder.

Whole exome sequencing as a tool for undiagnosed NCL-like cases: outcomes and challenges

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The neuronal ceroid lipofuscinosis (NCL) disorders are a group of 13 distinct neurodegenerative disorders with overlapping neuropathology that is defined by a characteristic accumulation of lysosomal storage material within neurons and non-neuronal cells. Clinical features such as vision loss, seizures, motor and cognitive function deterioration, psychiatric disturbances and premature death are the hallmark of these diseases and are mainly observed in childhood although adult onset cases have also been well characterized. Treatment has long been palliative but gene and enzyme replacement therapy are being clinically developed as potential targeted treatments for several of the NCLs making rapid and accurate genetic diagnosis crucial. Recent genetic advances have considerably broadened the NCL genotypic and phenotypic spectrum but despite testing for all known causative genes, a significant number of NCL-like cases remain molecularly undefined. Here we have tested whole exome sequencing on 35 cases as a tool to improve the molecular diagnosis of NCL. We identified causative mutations in 34% of the cases, possible candidate gene mutations in 25% of the cases, and 41% of the cases remained unsolved. Importantly, in several cases, we identified causative mutations in other known disease genes that had not been raised in a candidate gene approach. This study has highlighted the challenges of this technology as well as its benefit as a diagnostic tool in a group of diseases with increasing genetic heterogeneity and a broadening phenotypic spectrum.

P3

Molecular Genetics Results In A National Ncl Reference Center. A 3 Year Experience

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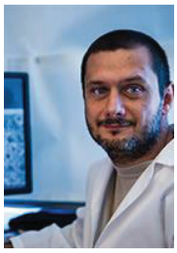
Introduction: The Neuronal Ceroid Lipofuscinoses (NCL) are genetically heterogeneous heritable neurodegenerative disorders with worldwide distribution. They are considered as childhood diseases; however rare adult onset forms are known. Thirteen NCL associated genes have been identified so far, but as yet unidentified NCL genes are foreseen.
Objective To report on the spectrum and relative frequency of NCL form in a national reference molecular genetic laboratory.
Methods: Records of 54 patients in the past 3 years were collected and molecular investigations performed by Sanger sequencing a using a customized NGS gene panel covering all known causes in NCL.
Results: Of 54 patients tested, we detected mutations in 28% with mutations occurring particularly in genes associated with v-LINCL forms. Using NGS rather than traditional Sanger sequencing allowed more rapid but not deeper information on patients' etiologies.
Conclusions: In a reference laboratory, molecular confirmation of NCL is becoming quicker with the adoption of NGS technologies. This, however, did not imply higher relative frequencies of molecularly defined patients, at least in Italy.

Small molecule therapies for JNCL



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Juvenile neuronal ceroid lipofuscinosis (JNCL) is a childhood-onset neurodegenerative disorder caused by mutations in CLN3. Using CLN3 knock-out mice brain endothelial cells (MBEC) as an *in vitro* model, we previously documented membrane instability and brain endothelial cell defects. In current investigations we found that carbenoxolone (CBX) treatment modifies plasma membrane fluidity and rescues caveolin-1 trafficking, fluid-phase endocytosis, and Cdc-42 defects in CLN3null MBEC. Moreover, mice treated orally with CBX exhibited recovery of blood-brain barrier hypotonic stress, improved astrocytic end-feet morphology and reduced autofluorescence in the brain, suggesting that carbenoxolone, or its metabolites may improve JNCL phenotypes. Additional work in our cell-based screen has revealed several other promising small molecules for JNCL therapy. Further studies will examine the effects of these treatments on our recently identified quantifiable seizure phenotypes, an important step to clinical translation.

Natural History of variant Late Infantile Ceroid-lipofuscinosis 5 (CLN5)

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Introduction: CLN5 is a rare form of neuronal ceroid-lipofuscinosis (NCL). Late Infantile onset is most common but juvenile and adult cases have also been described. *CLN5* encodes a soluble lysosomal protein of still undetermined function.

Methods: Records of 15 children from different ethnicities were obtained from the datasets of the DEM-CHILD International NCL Registry and in collaboration with international clinical experts. Scoring of disease evolution was obtained retrospectively by a newly designed scale, which includes 6 domains. Mutation analysis of *CLN5* was performed by Sanger sequencing. Eleven patients underwent EM investigations of either lymphocyte pellets or skin biopsy whereas pCLN5 was characterized by western blotting (WB) in 6 cases using 2 antibodies directed against the N-terminal portion and the core of the protein.

Results: Disease onset was at 2-7 years of age (median age 5); mean age at death was 20 years (5 patients) with mean disease duration of 15 years. The eldest patient is now 26 year old (onset at 5). Behaviour and cognition were the earliest domains to be affected, followed by language decline. Loss of autonomous walking occurred at about 10 years of age (6 years after disease onset); seven children were bedridden at about 14 years of age. Seizures were the initial symptom in one child only; however they were present in all patients from 6 months to 8 years after disease onset. Partial seizures were present first, followed by generalized fits and myoclonus. EEG patterns were characterized by polymorphic slowing of background activities, mutifocal epileptic discharges; photosensitivity gave variable responses. Sleep structure was altered in 7 out 13 patients, 3-10 years after disease onset. Vision impairment occurred relatively late, at about 8 years of age, all patients being affected. Both retinal and visual pathway involvement was detected by ERG and VEP. MRI findings were consistent with progressive cortical atrophy in all examined patients; periventricular white matter hyperintensity were seen in 7/9 patients. Abnormal signal intensity of thalami was reported of 2 patients. Mixed cytosomes were observed by EM: Finger Print Profiles were present in about 70% of the examined tissues, whereas Curvilinear Bodies-like cytosomes were detected in 5 out 11 skin biopsies. Ten mutations were detected of 22 alleles, including 6 mutations predicting a truncated protein. Clinical scoring was overlapping among patients at onset, regardless the mutations. Along time a dramatic decrease was observed, particularly of patients carrying nonsense mutations (either as homozygous or heterozygous mutations), mainly affecting the mid-portion of the protein. WB revealed the mature, cleaved glycosylated form in one patient only, carrying the homozygous p.Tyr258Asp. In three patients the fully expressed, not-cleaved, glycosylated form of pCLN5 was detected. Absence of signal was observed in 2 patients carrying two homozygous mutations (p.Gly177Trpfs*10 and p.Arg199*) predicting a prematurely truncated protein.

Conclusions: School-age onset, followed by rapid decline and protracted course are the clinical features of this cohort of variant late infantile CLN5 patients. Behavioural and cognitive difficulties are early markers of this condition whereas loss of motor and visual skills with severe epilepsy occurs later. Matching the results of the scoring system and patients' genotypes, we observed evidences of more dramatic disease course in patients harboring the most severe mutations.

P6

An electrophysiological approach to analyze endolysosomal membrane proteins

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The membranes of intracellular organelles form more than 95 % of the total cell membrane system. Several types of intracellular ion channels/transporters play a role in the maintenance of ion homeostasis in subcellular organelles including the endoplasmic reticulum, nucleus, lysosome, endosome, and mitochondria. Endolysosomal channels/transporters are implicated in human diseases such as lysosomal storage diseases, metabolic pathologies, mental retardation, non-alcoholic fatty liver disease, hyperlipidemia and infectious disease such as Ebola virus infection. The family of CLN proteins comprises proteins that are found in the matrix of lysosomes, in ER membranes or in endolysosomal membranes. In particular, NCL causing lysosomal membrane proteins including CLN3 are not well characterized since until recently a crucial technique, the endolysosomal patch-clamp technique had not been available to investigate these proteins in more detail. Here we present a novel endolysosomal patch clamp method to analyze intracellular organelle ion channels such as two-pore channels (TPC2) and members of the transient receptor potential family of cation channels (TRPML1-3). With this technique we hope to find out whether CLN3 functions as an ion channel in lysosomes. In parallel we will screen large compound libraries for agonists of CLN3 and other potentially functionally related proteins such as TPCs with the aim to eventually develop a small molecule treatment for NCL caused by mutations in CLN3.

P7

Cell-based screening assay for Autosomal dominant adult onset neuronal ceroid lipofuscinosis

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Background: The pathognomonic hallmark of NCL is the intracellular accumulation of autofluorescent storage material (AFSM). The levels of AFSM are inversely correlated with behavioral changes, response to therapies and severity of clinical and neuropathological presentation of the disease in at least two different models of neuronal ceroid lipofuscinoses (NCL). AFSM is an easily quantifiable phenotype and involves several steps in its formation and degradation, which potentially provides multiple sites for drug action.

Objective: Our aim is to identify compounds that facilitate the disassembly of CSPα aggregates and ASFM in a pathogenic cell-based setting.

Material (Patients) and Methods: Primary fibroblasts from CSPα-p.L115R carriers and healthy control. Primary fibroblasts from both CSPα-deficient and normal mice transduced with CSPα-WT and CSPα-p.L115R.

Results: The dynamics of formation and clearance of AFSM can be recapitulated and quantified *in vitro*. Primary dermal fibroblasts and CSPα-deficient transduced with both CSPα-WT and CSPα-p.L115R recapitulate features of AD-ANCL *in vitro* including CSPα aggregates, a two-fold increase in AFSM and a faster rate of accumulation. AFSM and mutant CSPα aggregates are susceptible to pharmacological intervention *in vitro*. We have identified some compounds that increase AFSM accumulation up to 40% ($p=0.02$) and others that reduce AFSM by 30% ($p=0.03$) in mutation carriers.

Conclusion/Discussion: We have created a human fibroblast system mimicking some features of AD-ANCL *in vitro*. Our results suggest that ASFM can be used as a quantitative fluorometric trait in a cellular model for testing molecular therapy intervention in fluorescence-based screening assays. AFSM can be used as a disease-reporter for testing the efficacy of molecular therapy intervention. This approach could be applied to identify treatments for other NCL.

Revaluation Of Transmission Electron Microscopy (Tem) In The Genomic Era For Accuracy Definition Of Diseases Cln5, Cln6, Cln7 And Cln8

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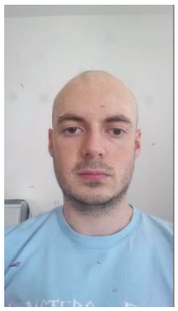
The neuronal ceroid lipofuscinoses (NCLs) are severe inherited neurodegenerative diseases that occur in all ages. They manifest as refractory epileptic syndrome with progressive intractable seizures, visual failure, dementia, movement disorders, and early death. Mutations in 7/13 genes have been found in 9 pediatric patients admitted to the Translational Research Program of NCL in Argentina. Progressive neurodegeneration is a hallmark; it is associated with deposits of ceroid lipofuscin-like materials in the brain, which act by stimulating a cellular apoptotic cascade that leads to neuronal depletion. The correlation of the lipofuscin-like bodies (cytotypes) with the genotype was historically considered pathognomonic for 3 classical NCL diseases: Infantile (CLN1), Granular osmiophylic depots (GROD); late infantile (CLN2), curvilinear bodies (CL); Juvenile (CLN3), fingerprint profiles (FP). Other cytotypes were also described, like rectilinear bodies (RL). Diseases CLN5, CLN6, CLN7 CLN8 lacked definition of cytotype/genotype correlations in Argentinean patients. **Objective:** To correlate the cytotypes/genotypes in CLN5, CLN6, CLN7 and CLN8 diseases in precise genetic defined subjects of Argentina. **Patients and Methods:** In 9 patients suffering from the diseases CLN5, CLN6, CLN7 and CLN8 a study algorithm for NCL was applied including, clinical evaluation with complementary studies, PPT1/CLN1-TPP1/CLN2 enzyme activity tests, transmission electron microscopy (TEM) and mutation screening. **Results:** Enzyme activity tests ruled out diseases CLN1 and CLN2; lacking vacuolated lymphocytes excluded CLN3. TEM lead to the suspicion of other NCL diseases through the observation of lipofuscin-like bodies and oriented the mutation screening. New phenotype/genotype correlations were established. **Conclusion:** The use of TEM as a diagnostic tool is revalued in the context of a NCL Translational study program. TEM analysis of skin biopsies is recommended as a biomarker to guide decision-making for screening of mutations in patients with late infantile, juvenile or congenital phenotype through NCL gene containing-panels, once the enzyme deficiencies and vacuolated lymphocytes were ruled out. This may shorten the diagnostic time and reduce costs with respect to the gene by gene PCR and Sanger sequencing used in the past, and whole exome sequencing (WES) without previous TEM.

P9

Examining the potential function of CLN3 as an ion channel



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Maintenance of lysosomal Ca^{2+} homeostasis is a vital process required to maintain correct function in the endolysosomal system. A number of studies have highlighted changes in lysosomal Ca^{2+} homeostasis as being important in the pathogenesis of lysosomal diseases (e.g. NPC1, ML4, Gaucher). However, to date, little is known about lysosomal Ca^{2+} homeostasis in CLN3 disease.

In a recent publication, we reported elevated lysosomal Ca^{2+} levels in cerebellar cells from a Cln3 mouse model with the most common disease causing mutation. This is the first instance of a human disease with elevated lysosomal Ca^{2+}. Subsequently, we have studied the CLN3 protein and uncovered sequence similarity and functionally important motifs that suggest CLN3 functions as an ion channel. We have identified similarity between the CLN3 c-terminus and certain Ca^{2+} leak channels belonging to the transmembrane bax inhibitor motif (TMBIM) containing family. By creating synthetic peptides, analogous to the CLN3 c-terminus, we are able to release Ca^{2+} from purified lysosomes and have also observed Ca^{2+} flux in single channel recordings utilising these peptides. Furthermore, disease causing mutations in the C terminus of CLN3 render the peptide incapable of transporting Ca^{2+}, and we have observed that lysosomal Ca^{2+} is elevated in fibroblasts from CLN3 patients.

We are currently progressing these studies in order to fully characterise this novel function for the CLN3 protein as a lysosomal ion channel and begin to examine ways in which this function is regulated. In addition we are studying the various ways in which loss of endolysosomal Ca^{2+} homeostasis can lead to endocytic and autophagic dysfunction within cells and how this, along with changes to Ca^{2+} levels throughout the cell, can lead to neuronal dysfunction. In doing so we are identifying therapeutic targets with which we can try and reduce neuronal dysfunction with an ultimate goal of identifying a disease modifying therapy.

Testing safety and efficacy of AAV9-CLN6 gene therapy in a mouse model of CLN6-Batten disease

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Mutations in *CLN6* cause both a late-infantile and adult form of the rare autosomal recessive neurodegenerative disorder, CLN6-Batten disease. In many patients, this condition presents with impaired motor and mental development in early childhood, progressing to severe movement disorders, a decline in intellectual function, recurrent seizures and vision impairment. In the late-infantile form, the disease onset varies between 18 months and 8 years of age, but the outcome is fatal in all cases, leading to death within the first 15 years of life. Importantly, there is no effective cure for CLN6-Batten disease. *CLN6* mutations predominantly lead to the synthesis of highly unstable and/or truncated proteins predicted to be degraded immediately following synthesis and resulting in a complete loss of protein. CLN6 is a transmembrane protein with unknown function located in the endoplasmic reticulum, making it very difficult to develop targeted drug-based therapies for this disorder. Although CLN6 is ubiquitously expressed, it is clear that various neuronal cell-types throughout the central nervous system (CNS) are most sensitive to *CLN6* mutation. In this context, the only practical way to overcome the pathology of CLN6-Batten disease is either to deliver or promote the production of functional CLN6 throughout the CNS.

We have developed an adeno-associated virus serotype 9 (scAAV9) viral vector expressing the human *CLN6* (*hCLN6*) gene under control of the CMV enhancer /chicken-β-actin-hybrid promoter (CB). Gene therapy with this scAAV9.CB.CLN6 offers an efficient way to provide a corrected copy of the faulty gene to the CNS, where the most impaired and involved cells are located, thereby creating the greatest potential for functional benefit. Our pre-clinical data using a single, postnatal ICV injection of scAAV9.CB.CLN6 demonstrates the efficient expression of the hCLN6 protein *in* vivo in both wild type mice and CLN6-Batten disease mouse model. A high level of expression is observed in the most affected areas, including all brain regions, eye and optic nerve. Pathologically, this dosing paradigm results in a reduction in autofluorescent storage material and reactive gliosis by as early as one month of age. Moreover, our extensive toxicology study has also shown that intrathecal administration of scAAV9.CB.CLN6 is safe and well tolerated in wild type mice up to 24 weeks. Based in part on this data, a first-of-its-kind human clinical trial with scAAV9.CB.CLN6 has been initiated.

P11

Expert recommendations for the laboratory diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2 disease): diagnostic algorithm and best practice guidelines for a timely diagnosis

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Background: Neuronal ceroid lipofuscinoses (NCLs), a heterogeneous group of lysosomal storage disorders, include the rare autosomal recessive neurodegenerative disorder CLN2 disease (CLN2). CLN2 is due to mutations in *TPP1/CLN2* gene causing tripeptidyl-peptidase-1 (TPP1) enzyme deficiency. Classic late-infantile CLN2 has pediatric onset with initial symptoms of seizures and language delay followed by progressive dementia, motor and visual deterioration and early death. Variant phenotypes occur more rarely. CLN2 diagnosis is based on laboratory testing following clinical suspicion. Early diagnosis is key to optimizing clinical care and future therapies outcomes, yet delays in diagnosis are common due to low disease awareness, non-specific initial symptoms and limited diagnostic testing access in some regions.

Methods: In May 2015, international experts met to recommend best laboratory practices for early CLN2 diagnosis.

Results: When clinical signs suggest NCLs, TPP1 activity should be the first test performed (along with palmitoyl-protein-thioesterase-1 to exclude CLN1). However, since reaching initial suspicion of CLN2 and NCLs is challenging, where available, use of epilepsy gene panels to investigate unexplained seizures in childhood is endorsed. These panels should include *TPP1/CLN2* besides genes for other NCLs lacking biochemical tests. Diagnostic TPP1 enzyme test in leukocytes is well established and robust and in DBS is considered diagnostic if followed by molecular testing. Future methods to measure TPP1 activity via MS/MS may improve DBS-based TPP1 testing sensitivity allowing also future newborn screening.

Conclusions: To confirm clinical suspicion of CLN2, the recommended gold standard for laboratory diagnosis is demonstrating deficient TPP1 activity and detecting causative mutations in each allele of *TPP1/CLN2* gene

P12

CLN5 Deficiency Results in Alterations in Autophagy Pathway

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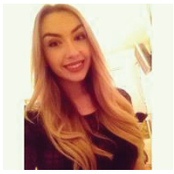
CLN5 encodes a soluble lysosomal glycoprotein. Mutations in CLN5 cause CLN5 disease, a subtype of neuronal ceroid lipofuscinoses. The function of the CLN5 protein is unclear. We previously showed that CLN5 is heavily N-glycosylated, and is proteolytically processed in the lysosomes. Here we show that the autophagy pathway is altered in CLN5 deficient patient fibroblasts.

Results: The basal level of an autophagy marker protein, LC3-II, is elevated in CLN5 deficient cells. By challenging cells with starvation in combination with choloroquine (CQ) treatment to block lysosomal activity, we showed that the autophagy pathway is up-regulated in CLN5 deficient cells. In addition, under the starvation and CQ treatment condition, we observed dramatic lysosomal morphology differences between control and CLN5 deficient cells. Using qRT-PCR, we quantified gene expression at the transcription level of various proteins in the autophagy pathway. The results indicated transcription differences for several genes in the patient compared to the control fibroblasts. A particular gene, SNCA, is highly up-regulated in patient cells. SNCA encodes alpha-synuclein, a protein that is well known to be associated with Parkinson's disease. We further analyzed the alpha-synuclein protein level by Western blotting. We found the protein level of alpha-synuclein is increased in CLN5 deficient patient fibroblasts as well as CLN5 knockdown HeLa cells. By confocal microscopy, we found that alpha-synuclein is localized to the lysosome membrane.

Conclusions: These results suggest a link between alpha-synuclein and autophagy/lysosome pathway dysfunction in CLN5 disease. Further studies will be needed to understand the role of alpha-synuclein in CLN5 deficiency.

P13

Identifying and treating the mechanisms leading to neuronal cell death in neuronal ceroid lipofuscinosis 5 (CLN5).



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The neuronal ceroid lipofuscinoses are a group of autosomal inherited neurodegenerative lysosomal storage diseases characterized by the accumulation of the autofluorescent lipopigment, lipofuscin, throughout all bodily tissues. The Finnish genetic variant of these diseases, *CLN5*, encodes a 46 kDa glycosylated lysosomal protein; the function of which remains elusive. Our project focuses on characterizing the phenotypes of CLN5 disease cells and assessing basic organelle localization, structure and function. Previous studies have found subunit C of the mitochondrial ATP synthase (F1/F0) to be a major storage component in CLN5, indicating that mitochondrial dysfunction may be a major phenotype of this disease. Our data reveal a clear breakdown in the mitochondrial network of CLN5 patient fibroblasts, as well as increased mitochondrial ROS production and higher levels of mitochondrial Ca^{2+}. This coincides with other data we have gathered revealing enlarged lysosomes and ER stress in CLN5 disease cells. This leads us to believe that Ca^{2+} dysregulation and impaired autophagy play a major role in the pathogenesis of this disease. Currently, we are investigating the differences in lipid metabolism and endocytosis in CLN5 disease cells compared with healthy controls. We hope that building on the data we have gathered so far will allow us to identify the earliest pathogenic event in this disease cascade, allowing us to target this therapeutically via high throughput drug screening.

Case Presentation of Neuronal Ceroid Lipofuscinosis 1 and 2

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Objective The neuronal ceroid lipofuscinoses (NCLs) are rare neurodegenerative disorders characterized by accumulation of autofluorescent storage material in neuronal cells. The clinical manifestation shows mental deterioration and visual impairment with onset in childhood or adolescence. More than a dozen responsible genes for NCLs have been identified, though the correlation between genotype and phenotype has not fully understood yet. Herein, we will present the clinical feature and genetic mutations of three Japanese patients with NCL1 and 2.

Case presentations Case1: 8 years old girl with late infantile onset with NCL2. Developmental milestone till 3 years old was normal. She had seizure, ataxia and developmental deterioration from 3 years old. Brain MRI showed both cerebellar atrophy and mild cerebral atrophy, and EEG presents poly sharp and waves mainly in frontal lobe. Ophthalmologic investigation showed optic atrophy. TPP1 activity in DBS and leucocyte was decreased and genetic analysis revealed heterozygote mutations in *TPP1* (R399W/ M244T). Clinical trial of intraventricular enzyme replacement therapy has been performed. Neurological deterioration was diminished after the treatment. Case2: 32 years old female with juvenile onset with NCL-1. She had difficultly leaning and dementia from 10 years old. A retinal pigmentary degeneration was diagnosed at 14 years old. Ataxia was observed since 28 years old. Brain MRI showed diffuse atrophy of both cerebral and cerebellar atrophy. Case3: 27 years old male with juvenile onset with NCL1. He is a younger sibling of Case 2. He had difficulty learning, visual loss at the age of 8 years old and became blind at 12 years old. A retinal pigmentary degeneration was diagnosed at 14 years old. Epileptic seizure and ataxia were not observed. Brain MRI showed mild atrophy. Both Case 2 and 3 showed vacuole of lymphocyte, PTT1 activity in DBS and leucocyte was markedly decreased and genetic analysis revealed heterozygote mutations in *PTT1* (E184K/ K216E). **Conclusion** We experienced late infantile and juvenile onset NCL2 and 1 patients respectively. Intraventricular administration by TTP1-enzyme could treat NCL2 patients. Therefore, early diagnosis and treatment for NCL2 are essential. Furthermore, many of patients with NCL1 also clinically show retinitis pigmentosa with mental retardation. Patients with these unique clinical feature should be screened by enzyme assay with DBS.

P15

Novel mechanisms for distal transport in developing and mature neurons

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The neuronal ceroid lipofuscinoses (NCLs) are a family of devastating neurodegenerative diseases resulting from mutations in as many as 14 different genes. Researchers have long sought a molecular link between various NCLs. Recent studies suggest a common NCL pathway associated specifically with membrane associated protein forms of the disease (CLN3, CLN6, CLN5, CLN8) may be intracellular transport via disrupted interaction with the cytoskeletal network. In support of this concept, we have identified a novel complex containing the ER-associated CLN6, whose mutation results in a variant late infantile NCL (vLINCL), the collapsin response mediator protein 2 (CRMP2), and the kinesin motor protein, KLC4. Acting through a network of protein interactions, CRMP2 regulates axonal/dendritic specification and extension during neurodevelopment and contributes to maintenance/regeneration in the mature brain. We hypothesize that the CRMP2/CLN6/KLC4 (CCK) complex utilizes CLN6 as a "molecular tag" on ER-vesicles for segregation of cargo to distal sites in dendrites and axons. Disruption of this signaling complex could contribute to the pathogenesis of CLN6-Batten disease through altered neuronal process outgrowth and maintenance. The studies we will present focus on 1) determining how the CCK complex regulates ER-vesicle transport development and maintenance of neurons; 2) defining how CCK complex transport is linked to early events in neuronal differentiation; and 3) determining if stabilization of CRMP2-associated complexes, independent of CLN6 rescue, can correct affiliation of CRMP2 with its binding partners. These studies will expands our understanding of CLN6's contribution to crucial cellular processes and start to unravel the biological significance of the CCK complex in developing and mature neurons, as well as its role and the role of intracellular trafficking in neurological disorders such as the NCLs.

Expert opinion on the management of CLN2 disease

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Objectives: CLN2 disease, an inherited, rare, pediatric-onset, rapidly progressive neurodegenerative lysosomal storage disorder caused by TPP1 enzyme deficiency, is characterized by language delay, seizures, movement disorder, motor deterioration, dementia, blindness and early death. No management guidelines exist for this condition. Our aim is to gain insight into current management strategies.

Methods: 24 disease experts (healthcare professionals and patient advocates) completed an online survey with a smaller group participating in a discussion of management practices.

Results: Experts share common goals in the management of patients and their families. Goals and interventions evolve as the disease progresses, with a shift in focus from maintenance of function early in the disease to maintenance of quality of life (QoL). The goal of antiepileptic medication is to achieve sufficient seizure control to support function balancing the side effects. Antiepileptic medications may have unique response in patients with CLN2. Carbamazepine and phenytoin should be used with caution. School and home environments should be adapted to accommodate physical and cognitive/behavioral impairments for ongoing benefit of maintaining social interactions. Physical, occupational and speech therapies should be initiated early and assessed frequently, including use of adaptive devices to support function and independence. Palliative care team engagement is essential soon after diagnosis is made.

Conclusions: CLN2 management practices are consistent among experts worldwide. A multidisciplinary approach is critical for optimizing care and QoL of patients and families throughout the disease course. This effort to identify common management practices represents an initial step towards development of consensus-based management recommendations.

P17

The Danish Batten Disease Team, presentation and parents perspectives

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Background: The Batten Disease Team (BDT) is an interdisciplinary team represented by professionals within education, health/ medicine, social welfare. It is unique that there and two parent representatives in the team, elected by the Family Association. The team was appointed 20 years ago. The BDT is funded by the government of Denmark, and their services are free of charges for families, communities and schools.

The main aims of BDT: The BDT team's main aim is to support and guide families, educational bodies and other public institutions with responsibilities for children, adolescents and young adults with Batten disease. A second aim is to build and disperse competence within education and social services in Denmark.

The BDT sphere of activities: The BDT team is contacted by the local hospital and will join when the diagnose is given. The next meeting between the parents and the BDT is normally organized few days after the primary contact. The BDT team will subsequently offer a life-long support to the target person, his/her family, education and adult institutions in the local community caring for people with Batten disease. The BDT operations includes counselling activities, home based visits, camps, education for teachers, social workers, health workers, helpers etc. Families, teachers, social workers etc. can at any time request for BDT services.

Discussion: The BDT has been operative last 20 years. The BDT service provision is highly appreciated by families, education and social/health sectors in Denmark.

The presentation will in particular be based on parents' views on BDT and how the local community is able to meet special needs of individuals with Batten disease. The introduction will include a short holistic overview on the Danish Batten model. Based on the introduction, the following areas will be elaborated:

- the local impact of BDT activities
- strengths and weaknesses of the Danish model
- how special challenges are met by the Danish model/society such as learning, transitions, speech and behavior

The presentation will conclude with recommendations for the future.

Expert opinion on the management of intracerebroventricular (ICV) drug delivery

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Background: The intracerebroventricular (ICV) route of administration has been used for many decades to treat pediatric and adult patients with a broad range of central nervous system (CNS) disorders. There is no consensus in management of ICV devices and associated rates of reported complications are highly variable. A systematic literature review revealed that noninfectious complication rates per patient range from 1- 33%, while infectious complication rates range from 0- 27%.

Objectives and Methods: 7 healthcare professionals (neurosurgeons, neuro-oncologists, pediatricians, nurse practitioners) with expertise in ICV delivery met to discuss best practices in management of ICV devices and drug administration and to provide guidance on prevention of complications.

Results: Experts share common practices in the management of ICV devices. Most are experienced in delivering drugs through a bolus injection, though one center has had clinical trial experience with an infusion. In either case, extreme care must be taken to follow strict aseptic/sterile techniques. Waiting a minimum of 5 days after device implantation before first use of device is recommended to allow proper wound healing and to reduce risk of backflow of the administered drug through the catheter tract. Experts differ in practice of hair removal over the device, on the type of skin prep solution used, and in use of gown and cap. All experts recommend use of sterile gloves and mask as well as skin disinfection with multiple separate swabs.

Conclusions: ICV drug delivery is an effective way to deliver drugs into the CNS when stringent measures are taken to prevent complications.

P19

Interrogation of mitochondrial function and quality control pathways in iPSC-derived neural cell models of NCL

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A major pathological hallmark of NCL is the autophagolysosomal storage filled over 50% mass with the subunit c of the mitochondrial ATP synthase accumulation. Here, we have initiated studies to test the status of mitochondrial quality control and mitochondrial function in human CLN2 and CLN3 patient iPSC-derived neural cell systems. Immunocytochemistry revealed fragmented, ring-shaped mitochondria localized at the perinuclear region in CLN3 patient-derived neural progenitor cells (NPCs) compared to the stretched, rope-like mitochondria in the cytoplasm of control and CLN2 NPCs. Morphological changes of mitochondria in CLN3 patient-derived NPCs were consistent with further observations of increased levels of three short isoforms (S-OPA1) of OPA1 in CLN3 NPCs. OPA1 is a regulating factor for mitochondria fusion to dilute oxidative stress. Interestingly, we also documented weak MitoSOX signals co-localized with Mitotracker in CLN3 NPCs, suggesting abnormal oxidative respiration process. Indeed, in Seahorse analyses, we have observed diminished basal respiration and maximal respiratory capacity as well as diminished glycolysis in CLN3 patient NPCs, likely leading to reduced cellular ATP levels. In addition, elevated parkin in mitochondrial fractions suggested an impact of *CLN3* mutation on mitophagy. Decreased ATP production and parkin translocation to mitochondrial fraction implicated CLN3 mutation in alteration of mitophagy. These data support further studies of glycolysis and mitophagy efficiency in CLN3 disease.

Considering Valproate as a Risk Factor for Rapid Exacerbation of Complex Movement Disorder in Progressed Stages of Late-Infantile CLN2

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Introduction

Neuronal ceroid lipofuscinosis type 2 (CLN2 disease) is a rare autosomal-recessive lysosomal storage disorder. It is one of the most common neurodegenerative disorders in childhood. Symptoms include epilepsy, rapid motor and language regression, dementia, visual loss and a complex movement disorder in later stages of the disease. The complex movement disorder phenotype represents one of the most challenging symptoms and one of the biggest burden of disease. Improvement of palliative care measures and medication to treat this are urgently needed.

Case Report

We report on two children with genetically confirmed late-infantile CLN2 disease who developed a severe exacerbation of their complex movement disorder leading to hyperthermia, hyper-CK-emia and decreased level of consciousness over several weeks despite different therapeutic approaches. Both patients were on long-term antiepileptic treatment with valproate and only after the withdrawal of valproate, the movement disorder disappeared and level of consciousness improved.

Conclusion

These observations emphasize that valproate has to be considered as a possible risk factor in patients in later stages of late-infantile CLN2 disease who develop a rapidly progressive complex movement disorder.

P21

Quantum dot delivery of Tripeptidylpeptidase to cells

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The inability to deliver missing proteins to the brain is a critical barrier to progress in treating many CNS diseases such as Battens because the brain is such a protected organ. We have tried to overcome this by using 6nm diameter Cd/Se/ZnS Quantum dots (QDs) solubilized with DHLA-chemical ligands and bound through surface Zn to both a unique cell/bbb-penetrating JB577 lipo-peptide (W•G•Dap(N-Palmitoyl)•VKIKK•P9•GG•H6) and tripeptidylpeptidase-1 (TPP1), modified with a C-terminal His6 (to attach to the QD and a c-terminal FLAG, for immunochemical following of the enzyme We can show that His6 enzyme alone is poorly taken up by TPP1-deficient fibroblasts but efficiently taken up when conjugated to QDs. We can deliver H6-tagged proteins to either neurons or glia by modifying the charge on the solubilizing coating of the QD. The TPP1 construct is also taken up by mouse slice cultures mouse brain slice preparations so that eventually we can scale-up to treat whole animals. QDs coupled to JB577 can also reach the brain microvasculature following IV injection and the JB577 structure appears to be working as a cell-penetrating peptide similar to that used by Lobel and associates. We propose that cellular uptake in brain can be improved by treatment with enzymes which degrade extracellular matrix material extracellular matrix (ECM) surrounding neurons (perineural sack) or glia (negative charge) as has been claimed following spinal cord injury. A big stumbling block in following Batten therapy is the absence of an easily quantifiable storage material so we have looked at gangliosides GD3, GM3 and GM2, all markedly elevated in Batten brain, as evidence of progress. TPP1 has been delivered to mouse brain cells by several therapies but restoration of complete brain functions has not been achieved and the mice die prematurely. Correcting the sphingolipid abnormalities in Battens is therefore a prime objective of a successful enzyme replacement therapy program.

Neuronal Ceroid Lipofuscinosis and Sleep Disturbance Treatment

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Purpose:
An aim of this study was to evaluate sleep difficulties in children with neuronal ceroid lipofuscinosis and use this information to develop a guideline for the management of their sleep disturbance.

Method:
We recruited individuals with a confirmed diagnosis of neuronal ceroid lipofuscinosis. We obtained information from the caregiver using the validated Children's Sleep Habits Questionnaire which is a sleep instrument for both behaviorally and medically-based problems. In addition, information was collected including treatment trials and a screen for restless leg syndrome symptoms.

Results:
In our cohort of 54 individuals, 96.3% had questionnaire scores consistent with a sleep disturbance. Sleep subscale analysis provided additional insight into the characteristics of the sleep disturbance. Fifty two of the 54 patients had at least one abnormal sleep subscale.

In addition to strict adherence to sleep hygiene, 88.9% of families had tried additional techniques to treat sleep disturbance. Environmental treatments specifically listed by 48.1% of families included playing music, massaging the child, aromatherapy with nebulizing essential oils, and listening to books on tape. The majority of families, 77.8%, had tried medications with 57.1% having tried at least two medications.

Conclusion:
Patients with neuronal ceroid lipofuscinosis have a high frequency of sleep disturbance, and each child will have a unique pattern. We recommend that the information collected from this study be used to tailor individual treatment based on this collective experience and to develop sleep guidelines.

ClinicalTrials.gov: NCT01966757
IRB 13-00376

P23

Neuronal Ceroid Lipofuscinosis-2 (CLN2) disorder, a type of Batten disease caused by TPP1 enzyme deficiency: Current knowledge of the natural history from international experts

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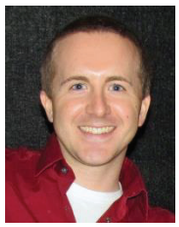
Background and Objectives: The neuronal ceroid lipofuscinoses (NCLs) are the most common group of neurodegenerative disorders in children and adolescents. CLN2, a type of NCL caused by TPP1 enzyme deficiency, is characterized by seizures, rapid deterioration of language, cognition, motor skills and vision, and premature death. Our aim is to describe expert knowledge of CLN2 disease.

Methods: 18 international NCL experts answered a survey on CLN2 natural history.

Results: Clinical suspicion for CLN2 is low due to its rarity and non-specific presenting symptoms. A 1-4 year delay was reported between first onset of symptoms and diagnosis. Speech delay/decline, developmental delay/regression and seizures/epilepsy were identified as initial presenting symptoms. Symptom onset typically occurs between 1.5-5 years of age, but may occur later (9-12 years). Myoclonic epilepsy was the most commonly reported seizure type. Notably, seizures are refractory oftentimes requiring polytherapy. Cardiac rhythm anomalies, not previously associated with CLN2, were also identified.

Conclusions: CLN2 is a severe, progressive, pediatric-onset neurodegenerative disorder. Disease awareness is low, causing delays in diagnosis. Seizures in concert with a regression of language and/or motor milestones should raise suspicion for CLN2. Knowledge of CLN2 is paramount to ensure timely diagnosis and to enable early initiation of future therapies.

Targeting the cannabinoid system as a potential treatment for seizures in infantile Batten disease



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Infantile neuronal ceroid lipofuscinosis (INCL; infantile Batten disease) is an invariably fatal pediatric lysosomal storage disease associated with an inherited mutation in the *Cln1* gene. This gene encodes for palmitoyl-protein thioesterase-1 (PPT1), a lysosomal enzyme responsible for cleaving long-chain fatty acids from proteins. Infants with INCL develop normally until ≈ 6-12 months of age. These children then experience a litany of symptoms beginning with vision loss and progressing to motor function deterioration, cognitive impairment, intractable seizures, and early death. The etiology of seizures in INCL remains unknown. Patients are often treated with conventional anti-epileptic drugs (AEDs) but are subject to issues of tolerance and side effects. First-line AEDs often prove ineffective for controlling INCL seizures or are subject to rapid development of tolerance, and at least two AEDs have been shown to exacerbate the seizures themselves. Cannabidiol (CBD) is one of at least 85 identified cannabinoids found in *cannabis sativa.* Research has shown that CBD is non-psychoactive, and animal studies suggest it is a potent AED. The FDA recently approved a clinical trial examining whether CBD can effectively treat high need pediatric epilepsy cases. Thus far, results from this clinical trial reveal that CBD, when added to an in-progress AED regimen, further reduces the occurrence of seizures by 45%. Our goal is to evaluate the efficacy of CBD in treating seizures caused by INCL disease in the *Cln1*$^{-/-}$ mouse model, with the aim of providing data useful for treating human patients. The *Cln1*$^{-/-}$ mouse is completely deficient in PPT1 activity and exhibits retinal dysfunction, progressive motor/sensorimotor abnormalities, a shorted life span, and spontaneous seizures. To determine whether CBD may be effective for ameliorating INCL seizures, we investigated the distribution of cannabinoid receptor-1 (CNR1) in the *Cln1*$^{-/-}$ mouse brain. Immunohistochemical evaluation reveals similar patterns of CNR1 distribution between wild-type (WT) and *Cln1*$^{-/-}$ mice at 1, 3, and 5 months of age. Abundant CNR1 is seen in the olfactory nucleus, cortex, cerebellum, hippocampus, and substantia nigra. The pattern of CNR1 staining in *Cln1*$^{-/-}$ mice up to 5 months of age is virtually identical to WT mice. Even in the presence of significant neuronal loss and cortical thinning, 7-month-old *Cln1*$^{-/-}$ mice show conspicuous CNR1 staining in these areas. Western blot analysis reveals similar results; CNR1 levels are similar between WT and *Cln1*$^{-/-}$ mice at 1, 3, and 5 months of age. It appears that there may be a decrease in CNR1 levels in 7-month-old *Cln1*$^{-/-}$ mice, though importantly it is clear that the receptor persists. If CBD effectively prevents seizures in our INCL animal model, it may become a standalone treatment, or an important component of a comprehensive treatment strategy, for children suffering from this debilitating disease. Our results reveal that even in the presence of advanced INCL-associated neurodegeneration, CNR1 receptors persist, making them a potential target for alleviating the devastating seizures associated with INCL.

P25

Case Study: Identifying successful educational strategies for twin learners with CLN3 disease and implementing a programme of early intervention for a younger affected sibling

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Background: Children and young people affected by CLN3 disease, Juvenile Batten Disease, experience increasing educational difficulties. Some affected children/young people show early difficulties in short term memory, attention control, behaviour and in acquiring literacy skills before other symptoms including visual loss and the onset of epilepsy are noted. There are few studies on the early impact on cognition and behaviour of CLN3 disease and on strategies and early interventions that maximise learning, enjoyment, social inclusion and quality of life. This case study will consider in detail, the educational journey of three affected children from the same family, identical twin boys and their younger sister.

Aim: To gain information and a holistic picture of the educational journey of the identical twin boys, to identify successes, challenges and vulnerabilities and to use this information to support their on-going education and to validate and facilitate earlier intervention for their younger sister. Successful strategies and resources will also be disseminated more widely for children and young people affected by CLN3 Disease.

Method: This case study is part of the ongoing EU funded Erasmus+ project 'Education and JNCL'. Information on the difficulties experienced by the twin brothers in their mainstream schools has been collected and analysed. Their successful inclusion in a specialist school for the visually impaired has been documented and assessments and test results over time have provided evidence for the success of interventions. The younger sister's increasing cognitive, language and attention control difficulties has been assessed and documented.

Results: The analysis of information, assessments and test results shows clearly that the twin brothers were experiencing very significant learning, behaviour and attention difficulties for many years before their diagnosis. Similar difficulties are evidenced in the younger sister's recent cognitive assessment. Successful strategies have been identified that are enabling the twins to enjoy school and achieve success despite the progression of the disease. Early intervention programmes for the younger sister are being developed, incorporating those strategies.

Discussion and conclusions: The information gained from the detailed analysis of the educational difficulties of the twin brothers will be used to advise those working with the younger sister to maximise her learning and enjoyment. Braille and ICT skills will be taught and the development of functional literacy skills will be prioritised. This study has wider implications for newly diagnosed affected siblings who are not yet showing clinical symptoms. Directed and intensive support will enable children to maximise learning and social interaction opportunities and to acquire adaptive skills before the onset of visual loss and dementia. Successful strategies and resources, evidenced by careful assessment and testing, will be disseminated more widely and contribute to the development of evidence based standards in the education of children and young people with CLN3 disease.

Investigating shared pathologies of Battens disease and Parkinson's disease - a hiPSC and CRISPR/Cas9 based *in vitro* disease model to study the function of ATP13A2

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ATP13A2 belongs to the P-type superfamily of ATPases that uses ATP to transport cations and other substrates across cell membranes. Endogenous ATP13A2 has been shown to localize to multi-vesicular bodies (MVBs), a late endosomal compartment that is part of endosomal and autophagic pathway and has the ability to fuse with the plasma membrane to release extracellular vesicles (exosomes). Depletion of ATP13A2 leads to a dysfunction of the lysosome-autophagy pathway, impaired biogenesis of exosomes, intracellular accumulation of alpha-synuclein and increased sensitivity to zinc. Several loss-of-function mutations in ATP13A2 have been shown to cause Kufor-Rakeb-Syndrom (KRS), a rare autosomal recessive juvenile onset form of Parkinson's disease. Interestingly, the ATP13A2 M810R mutation seems to cause onset of Battens disease. However, the exact molecular mechanism of ATP13A2 function in the context of disease pathogenesis remains largely unknown. Research on neurodegenerative diseases is often restricted by the limited access to patient material. Human induced pluripotent stem cells (hiPSC) can be relatively easy generated from skin fibroblasts of patients and healthy individuals. The CRISPR/Cas9 system has been successfully used to introduce disease-associated mutations into the genome of hiPSC from healthy individuals or to correct mutations in hiPSC derived from patients suffering from familiar forms of different diseases. To understand the impact of disease-associated mutations, it is necessary to generated isogenic cell lines. These are couples of cell lines, which share exactly the same genetic background and differ only in the mutation that is assumed to cause the disease. The combinatorial use of hiPSC and the CRISP/Cas9 system to introduce or correct mutations, provides a valuable tool to generate models to study neurodegenerative diseases *in vitro*. Here, we present a hiPSC and CRISPR/Cas9 based *in vitro* disease model to study the function of ATP13A2 in the context of disease pathogenesis.

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The Role of Reactive Microglial Cells in Retinal Pathogenesis of Juvenile Neuronal Ceroid Lipofuscinosis (jNCL)

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Background: Juvenile neuronal ceroid lipofuscinosis (jNCL) is an inherited autosomal recessive lysosomal storage disorder. Affected children show seizures and mental retardation preceded by early visual impairment between the ages of 2-10 years ultimately followed by vision loss. The course of this NCL form ends lethal in the third decade of life. The causative defect results from mutations in the CLN3 gene which encodes Battenin, a transmembrane protein of unknown function. Because of this early involvement of the eye we aimed to analyzing the pathogenesis of this disease in the retina with a focus on microglia as part of the local innate immune system.

Methods: Reverse transcription PCR was used to show Battenin expression in different microglial cell lines. Primary microglia (pMG) were isolated and cultured from P0 $CLN3^{\Delta ex7/8}$ mouse brains. Samples were treated with Lipopolysaccharide (LPS), Ceramide (C6) and vehicle control. Quantitative real-time PCR was used to analyze changes in the expression of pro-inflammatory marker genes. Griess reagent was used to analyze the amount of NO2- release. Immunofluorescence with anti-IBA1-,TSPO-, NFκB-antibodies and TRITC-labeled Phalloidin were used to quantify amount and localization of these markers and microglial morphology, respectively.

Results: All microglial cell lines (BV2 cells, ESdM, SV40 microglia, pMG) showed prominent Battenin expression. pMG from $CLN3^{\Delta ex7/8}$ mice showed higher basal expression of the pro-inflammatory markers TNFα IL1β, AMWAP, IL6 and CASPASE 11 when treated with the vehicle. A combined stimulation with LPS and C6 lead to an increase of these inflammatory mediators. The same effect was observed for NO2- production. DHE and TSPO staining showed an increase of reactive oxygen species and the pro-inflammatory marker TSPO in LPS stimulated $CLN3^{\Delta ex7/8}$ pMG compared to WT cells. Additionally $CLN3^{\Delta ex7/8}$ pMG show the ability to phagocytose latex beads and apoptotic 661W debris.

Conclusion: Primary brain microglial cells from $CLN3^{\Delta ex7/8}$ mice showed an increased in pro-inflammatory and neurotoxic activity. Further studies are currently under way to verify those findings *in vivo*.

Beyond Batten Disease Foundation

Danielle Kerkovich, Craig Benson, Mary Beth Kiser

Beyond Batten Disease Foundation was established to eradicate juvenile Batten (CLN3) disease. We seek to accomplish our mission in two ways:

1. We helped develop an easy and inexpensive blood test to detect gene mutations for Batten and hundreds of other rare conditions like it that claim the lives of thousands of children each year. Today our focus is on education and genetic testing for orphan diseases.
2. Funding from BBDF has identified a treatment that slows progression of the disease in Batten models. Today we are driving scientific endeavors for the drug development process and continuing to raise awareness and funds to accelerate research to cure the disease.

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Developing a *Cln3*$^{\Delta ex7/8}$ human neural stem cell model using zinc finger endonucleases

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Attempts to design effective therapies for CLN3 disease are hampered by a poor understanding of the consequences of the *Cln3* mutation. We have previously defined defects in both astrocytes and microglia, which have direct consequences for neuron health in CLN3 disease mice. To address whether these same phenotypes are evident in human neural stem cells, we have introduced *Cln3*$^{\Delta ex7/8}$, the most common cause of CLN3 disease, into CTX0E16 cells - a fully characterised human progenitor cell line. Flanking sequences of desired *CLN3* splice sites were first determined and zinc finger endonucleases (ZFNs) were custom designed to cut at sites upstream and downstream of the mutation. A *CLN3* donor construct was then inserted into a pUC57 vector without a selection cassette to facilitate introduction of *Cln3*$^{\Delta ex7/8}$. Plasmids containing the upstream ZFN, downstream ZFN and donor construct were nucleofected into CTX0E16 cells. The SURVEYOR mutation detection assay confirmed the presence of double-stranded DNA breaks and PCR revealed the presence of both wild type and *Cln3*$^{\Delta ex7/8}$. A sub-cloning pipeline was then developed to isolate cells homozygous for the mutation, this involved sib-selection steps and using mouse embryonic fibroblasts to generate single-cell derived colonies of edited and unedited cells. Future steps include differentiating progenitors harbouring *Cln3*$^{\Delta ex7/8}$ into neurons and astrocytes, with the potential to develop drug-screening platforms targeted at clinically relevant CLN3 disease phenotypes.

Impaired lysosome biogenesis due to NCL protein deficiency

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Abstract

NCLs are the most common group of inherited progressive encephalopathies in children, with a frequency in the United States of 1:10000. Inheritance is typically recessive, and patients are characterized by lysosomal accumulation of autofluorescent storage material (ceroid lipopigment), progressive loss of vision, mental and motor deterioration and premature death. To date, 14 different forms of NCLs have been identified, 13 of which have been associated with mutations in different genes. Whereas many NCL proteins are enzymes involved in protein degradation, some others are transmembrane proteins whose function has not been completely characterized. With the use of cell biology, biochemistry, and mass-spectrometry techniques we have examined the role of NCL transmembrane proteins in the biogenesis of the lysosome, and have identified a possible contribution to the maintenance of the steady-state of the lysosomal soluble protein component. In addition, we have identified a molecular connection between CLN3 and the autoimmune response that appears to be specific to this form of NCL. Our data establish a candidate role for NCL membrane proteins and may help explain some of the phenotypes observed in these diseases.

This is our daughter Mari

Trine Paus and Ole Christian Slotten, Oslo, Norway

Parents to a girl with CLN3

Mari was born in 1996. She got her NCL diagnosis in 2004, a few weeks before her eighth birthday. Since then *quality of life* has been the family's guideline for dealing with Batten's disease and our main effort is to give Mari the good life we believe she deserves.

Behind the diagnosis our daughter is still the same girl as she has always been. Mari's personality and identity remains the same even though NCL slowly and constantly degrades her cognitively and physically. Hence, it is crucial that everyone around Mari knows her personality, history, interests and needs. Once, Mari was like every other child and knowledge of her experiences and memories are fundamental for good communication and interaction with Mari after she lost her vision, short-term memory and ability to express herself orally.

Realising the importance of knowing Mari's history we decided to write "The book about Mari" where we gathered all information we thought relevant for friends, family and people working with Mari. An early version of the book was presented to other parents at a workshop during the NCL conference in London 2012.

Two years ago when Mari decided it was time to leave home and start a new life in her own apartment, we felt the need to strenghten the message in the book. The 24/7 staff working with Mari are nice and skilled people but with no prior knowledge of neither NCL nor Mari. Before they were introduced to Mari they were showed a 15 minutes PowerPoint presentation with pictures, Mari's favorite music and text slides emphasizing that Mari's disease has made her totally dependent on assistance. And that a good life for her must be based on elements like predictability, social inclusion, active participation, learning, mastery, physical activity and contact with animals. Mari cannot see – we must be her eyes. Mari doesn't speak very well – we must make sure she is being understood. Mari doesn't always understand what's being said – we must translate for her. Mari doesn't remember too well – we must be her memory stick. Mari's motor skills have deteriorated – we must make up for her loss of skills.

It is a well known fact that appealing to people's emotions might substantiate the communicative effect, something which has also been the purpose with our presentation. We want everyone working with Mari to understand how the disease develops, Mari's history, her needs, and their own crucial role in Mari's life. This is a precondition for them being able to communicate and interact with Mari in a meaningful way. The PowerPoint presentation has proven, together with the book, an important tool for the people around Mari in building the good life she deserves – a life where "time goes so fast because I'm having such a good time", as Mari likes to tell us.

Early spinal cord neuropathology in CLN1 disease.

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Objective: CLN1 disease or Infantile Neuronal Ceroid Lipofuscinosis (INCL) is one of the most rapidly progressing forms of NCL. The *Ppt1*$^{-/-}$ knockout mouse model of CLN1 disease recapitulates most aspects of the human disease, with an average lifespan of 8-8.5 months. Forebrain and cerebellar pathology has been well characterised in *Ppt1*$^{-/-}$ mice as well as in *post-mortem* human samples, however, current understanding of INCL neuropathology cannot entirely explain the sensorimotor deficits seen in this disease and little is known of the progression of pathology in other regions of the central nervous system (CNS). This, along with the limited success of various forebrain-directed experimental therapies for CLN1 disease, have led us to examine the spinal cord, to define the nature and extent of pathology in this region.

Results: Our analysis of *Ppt1*$^{-/-}$ mouse spinal cords revealed unexpectedly early, extensive and progressive pathology, as compared to the brain. This included profound volumetric changes, astrocytosis, microglial activation and the accumulation of autofluorescent storage material as early as 2 months of age in the *Ppt1*$^{-/-}$ mice. Neuron loss was observed as early as 3 months of age, with certain interneuron populations being more susceptible to the disease than motor neurons. There is also evidence for synaptic pathology and lymphocyte infiltration in the *Ppt1*$^{-/-}$ mouse spinal cord, similar to the forebrain. However, we have also shown there to be novel phenotype of white matter pathology, which is more severe than in other CNS regions.

These pathological changes appear to affect all levels of the cord simultaneously, but before pathology occurs within the brain, suggesting that the disease moves rostro-caudally along the sensorimotor pathways and are reflected in changes of the gait of these mice.

Importantly, similar pathology also occurs in human CLN1 disease *post-mortem* spinal cord samples. Furthermore, there is evidence for similar pathological changes in the spinal cord of mouse models of CLN2 and CLN3 disease.

Conclusions: These data reveal the spinal cord to be an important early region of profound pathological change in CLN1 disease, which significantly contributes to the overall progression of the disease and that changes our understanding of its pathophysiology. Thus, targeting the spinal cord in CLN1 disease will be critical for the success of future experimental therapies.

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Novel morphological macular findings in juvenile CLN3 disease

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Introduction
Juvenile CLN3 disease, one of the most common forms of a group of lysosomal storage diseases called neuronal ceroid lipofuscinoses (NCLs), is a progressive neurodegenerative disorder with initial visual deterioration. The objective of this study was to investigate the phenotypic retinal findings in CLN3 disease with the help of recent ophthalmic imaging modalities to distinguish CLN3 disease from other inherited retinal dystrophies.

Methods
11 patients underwent ophthalmic evaluations, including anterior and posterior segment examinations, optical coherence tomography, fundus autofluorescence, near infrared imaging and fundus photography. Patients were also assessed according to the Hamburg JNCL (juvenile NCL) score. Each ophthalmic finding was assessed by three independent examiners and assigned to a clinical severity score.

Results
22 eyes of 11 patients were included. The mean age at examination was 14.4 years (range 11.8–26.4 years) with an average age at initial diagnosis of 8 years (range 4.5–11 years). The mean Hamburg JNCL score was 7.3 (range 0–12 score). All patients showed a specific macular striation pattern on optical coherence tomography that was independent of age and progression of the disease. Previously described retinal features of CLN3 disease, including optic atrophy, bony spicules and orange macular deposits, were classified into 4 severity grades. A lower Hamburg JNCL score was correlated to more severe ocular findings (except for macular orange pigment disposition).

Conclusion
This study represents the first prospective observational case series documenting retinal abnormalities in CLN3 disease with the aid of the spectral domain optical coherence tomography observing a characteristic, striated macular pattern in all patients studied, which was independent of age and disease severity. Particularly in early disease cases, where retinal atrophy is mild, macular striae can potentially help to discriminate CLN3 disease from other inherited forms of retinitis pigmentosa.

Current state of the international DEM-CHILD NCL Patient Database: Successful development of tools for the evaluation of therapies in neuronal ceroid lipofuscinoses (NCL)

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Introduction

Neuronal ceroid lipofuscinoses (NCLs) are the most common neurodegenerative diseases in childhood. They are characterized by dementia, visual loss, epilepsy, motor decline, and premature death. Even though no approved curative therapy exists so far for any of these diseases, an increasing number of experimental therapy studies is being developed such as small molecules, gene therapy and enzyme replacement therapy. However, their evaluation is especially difficult as placebo controlled studies in such devastating diseases are considered unethical and as the clinical course of NCLs can be variable. However, in order to closely and prospectively monitor disease progression in patients under experimental therapies, detailed, objective and also organ specific evaluation systems are necessary.

Aim

The international DEM-CHILD database project seeks to develop such outcome measures by (i) unifying and improving existing clinical scoring systems for prospective use in follow-up examinations and (ii) correlating these with organ specific evaluations such as for example MRI-based brain volumetrics, cardiologic and ophthalmologic examinations.

Results

Over the last 3 years, significant progress has been made towards these aims: The DEM-CHILD database consortium has been constantly growing from initially 4 European countries participating to now 23 international partners from 16 countries from all continents actively contributing patient data. Growing financial support has been achieved by successful applications for research grants provided by various national, European, and pharmaceutical industry funding sources. More importantly, generous financial contributions from family associations and other fund raising organisations have allowed us to support various new consortium members.

Data are collected in the international DEM-CHILD NCL patient database which has been established as part of the European FP7-project DEM-CHILD. It currently contains clinical data derived from more than 500 NCL patients with all types of NCLs. A set of these data has already been successfully used as control data in a Phase 1/2 clinical trial on intraventricular enzyme replacement therapy.

Conclusion

The large and growing collection in the DEM-CHILD database of comprehensive sets of data on the natural history of different NCL forms is an indispensable tool for the evaluation of therapies. Its data are already being used for this purpose in current clinical trials.

Characterisation of key oxidative stress-responsive genes and products in ovine CLN6

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The neuronal ceroid lipofuscinoses, (NCL, Batten disease) are a group of devastating, mainly childhood, neurodegenerative diseases caused by mutations of a number of genes, but the underlying pathogenic mechanisms remain unclear. The major pathological events, including the burden of storage bodies, neuroinflammation and neurodegeneration in NCLs are commonly thought to result from, as well as to cause, oxidative stress. This study investigated the oxidative status of CLN6 affected sheep brains during disease progression using selected oxidative stress markers; mitochondrial manganese superoxide dismutase (MnSOD/SOD2), inducible nitric oxide synthase (iNOS), haem oxygenase -1 (HMOX-1) and the mitochondrial marker, cytochrome c oxidase subunit IV, (COX IV). The expressions and distribution of MnSOD were determined by immunohistochemistry of sagittal CLN6 affected brain sections and matched controls over the span of disease development, 2, 6, 9, 18 and 24 months, three animals at each point. Adjacent sections were immunostained for the mitochondrial marker, COX IV. Quantitative PCR (qPCR) was used to estimate the relative expressions of MnSOD/SOD2 and of iNOS and HMOX-1.

The distribution of MnSOD through the cortical grey matter co-localised to mitochondria and became compressed to the boundary between layers I and II and between layers IV and V in the affected cortices at 18 and 24 months, following severe neurodegeneration. There was no such compression in the non-degenerating cerebellum and brain stem. Quantification of MnSOD and COX1V across the cortical grey matter showed that the expressions were reduced at 18 months and more so at 24 months, indicating that previous reports of enhanced activity are likely to have arisen from unmatched sampling.
iNOS has been described to be induced upon neuroinflammation in murine studies, and HMOX-1 proposed to be responsive to neuroinflammatory stimuli and oxidative stress. However, iNOS was not expressed in ovine brains and HMOX-1 remained unchanged throughout the disease progression. In conclusion oxidative stress is not likely to play a role in pathogenesis and cytoarchitectural changes accompanying neurodegeneration need to be considered when analysing molecular changes to avoid if incorrect inferences.

In vivo drug discovery identifies a compound that reduces seizure-like activity in the zebrafish model of CLN2 disease

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Objective
CLN2 disease is a subtype of the neuronal ceroid lipofuscinoses (NCLs), a group of lysosomal storage disorders causing progressive, untreatable, neurodegeneration, intractable epilepsy and premature death in children. We have developed a permanent genetic zebrafish model of CLN2 disease due to a mutation in *tpp1* encoding the lysosomal protease Tripeptidyl-peptidase-1 that replicates the neurodegenerative and storage phenotype. We performed an *in vivo* screen of an FDA-approved drug library on the zebrafish model of CLN2 disease to identify compounds that might treat one or more aspects of the disease in humans.

Results
One hit was found to have positive effects that continued to be demonstrated in a blind study. This compound reduces seizure-like activity in the zebrafish *tpp1 -/-* mutants, and this is accompanied by a reduction in programmed cell death. The compound has previously been approved for use as a topical agent but it being used in many clinical trials for a variety of neurological disorders, including in young adults.

Conclusion
This study provides proof-of-principle that in vivo drug discovery using the zebrafish *tpp1-/-* mutant can provide positive hits. Next we will test this compound in the mouse model of CLN2 disease and further probe the mechanisms.

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Neurodegeneration in the brain and the retina of a mouse model deficient for the lysosomal membrane protein Cln7

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CLN7 disease is an autosomal recessive, childhood-onset neurodegenerative lysosomal storage disorder caused by the defective lysosomal membrane protein CLN7. We have disrupted the *Cln7*/*Mfsd8* gene in mice by targeted deletion of exon 2 generating a novel knockout (KO) mouse model for CLN7 disease, which recapitulates key features of human CLN7 disease pathology. *Cln7* KO mice showed increased mortality and a neurological phenotype including hind limb clasping and myoclonus. Lysosomal dysfunction in the brain of mutant mice was shown by the storage of autofluorescent lipofuscin-like lipopigments, subunit c of mitochondrial ATP synthase (SCMAS) and saposin D and increased expression of lysosomal cathepsins B, D and Z. By immunohistochemical co-stainings, increased cathepsin Z expression restricted to Cln7-deficient microglia and neurons was found. Ultrastructural analyses revealed large storage bodies in Purkinje cells of *Cln7* KO mice containing inclusions composed of irregular, curvilinear and rectilinear profiles as well as fingerprint profiles. Generalized astrogliosis and microgliosis in the brain preceded neurodegeneration in the olfactory bulb, cerebral cortex and cerebellum in *Cln7* KO mice. Magnetic resonance imaging (MRI) analyses revealed neurodegeneration in the olfactory bulb, cerebral cortex and cerebellum of *Cln7* KO mice late in disease. Increased levels of LC3-II and the presence of neuronal p62- and ubiquitin-positive protein aggregates suggested that impaired autophagy represents a major pathomechanism in the brain of *Cln7* KO mice. Morphological analyses of the retina of *Cln7* KO mice revealed an early onset and rapidly progressing degeneration of photoreceptor cells in *Cln7* KO mice, resulting in the loss of about 70% photoreceptors by 4 months of age. The combined data identify rod photoreceptor degeneration as a major neurological phenotype of Cln7-deficient mice. The absence of Cln7 in the retina led to increased expression of multiple lysosomal proteins, accumulation of SCMAS and saposin D and reactive astrogliosis and microgliosis. The data suggest that loss of the putative lysosomal transporter Cln7 leads to lysosomal dysfunction, impaired constitutive autophagy and neurodegeneration in the brain late in disease and early, rapidly progressing neurodegeneration in the retina. We conclude that the mutant retina presents a useful model to elucidate the pathomechanisms ultimately leading to neurodegeneration in CLN7 disease, and to evaluate the efficacy of strategies aimed at developing treatments for this neurodegenerative lysosomal storage disorder.

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Exploring CRMP2-associated small molecule therapies in a Batten disease model of neurodegeneration

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Collapsin response mediator protein 2 (CRMP2) is an intracellular, cytoskeletal phosphoprotein involved in neurite outgrowth, maintenance, and guidance. These processes are regulated through CRMP2's ability to complex with 20+ neuronal proteins known to be essential for neuronal maturation and maintenance. Thus, small molecules that interact with and regulate CRMP2 offer an interesting opportunity for therapeutic potential in a number of neurodegenerative disorders in which CRMP2 has been linked to the pathophysiology. One such disorder is CLN6-Batten disease, a lysosomal storage disorder resulting from mutations in the ER-membrane associated protein CLN6. Studies from our team have demonstrated that CRMP2, CLN6 and the kinesin motor protein KLC4 complex to move cargo to and from the neuronal cell body (dubbed the CCK complex). When CLN6 is mutated in Batten disease, the CCK complex is disrupted and the dynamic interactions of CRMP2 with its other partners are disrupted. As new therapies are explored for the treatment of CLN6-Batten disease, one avenue of pursuit is compounds that modulate these CRMP2 interactions. LKE (lanthionine ketamine ester) is a cell-permeable derivative of the abundant brain metabolite that binds directly to CRMP2, promoting neurite outgrowth and reducing apoptosis through unknown mechanisms. To elucidate LKE's effects in neurodegeneration, a *Cln6*^{nclf} mouse model of Batten disease was given LKE in chow from weaning until death. LKE treated mice showed improved visual acuity at eight months of age and improved lifespan compared to their untreated counterparts. Further, treated *Cln6*^{nclf} mice showed reduced autofluorescent storage material accumulation, restored cortical thickness deficits, and increased CD68 activation. LKE did not benefit motor decline, astrocyte activation, or the ultimate premature death of *Cln6*^{nclf} mice. Additionally, we explored the therapeutic value of (S)-Lacosamide, a derivative of the clinically available, anti-epileptic Vimpat® that binds directly to CRMP2, modulating its phosphorylation and ability to oligomerize tau. *Cln6*^{nclf} mice treated acutely with daily IP injections of (S)-Lacosamide showed rescued cortical thickness deficits, reduced WBC counts, and reduced RBC deficits, such as lower RDW, MCV, and MCH values compared to untreated counterparts. Autofluorescent storage material and glial activation were not affected by treatment. Behavioral and pathological assessment of mice following long-term, daily IP injections of (S)-Lacosamide is ongoing. In conclusion, these studies offer insight into the therapeutic implications of CRMP2 modulation in disease states, and may allow for the development of combinatorial treatments in the future.

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Epilepsy in patients with neuronal ceroid lipofuscinosis: clinical and electroencephalographic features in cohort of 23 children

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Purpose: Investigation of clinical and EEG features of epilepsy in children with neuronal ceroid lipofuscinosis (NCL) in the West Balkan region.

Method: The study included 23 NCL patients diagnosed by enzyme, genetic, and histopathology analysis in the period 1991-2016. The seizure types according ILAE classification, serial EEG findings during sleeping and awake with prolonged photo-stimulation and hyperventilation, and response to AEDs were evaluated.

Results: In 23 patients (16 males, 7 females) the next NCL types were diagnosed: CLN2 in 15, CLN3 in 2, CLN5 in 2, CLN8 in 2, and in one girl juvenile form of CLN1. Seizures and hyperactivity were the initial manifestations at mean age of 2.8 years in all cases with CLN2, in five cases preceded by atypical febrile seizures. Ataxia and mental regression existed before epilepsy in cases with CLN3, CLN5 and CLN1. All patients suffered different types of seizures: complex and simple focal with/without secondary generalization, atonic attacks, myoclonus; five experienced status epilepticus and three epilepsia partialis continua. Polytherapy was required in all cases, often with side effects to rescue medication. EEG showed occipital discharges during slow--frequency photo-stimulation suggesting diagnosis at early stage in nine CLN2 cases. Slowing and amplitude decreasing of EEG background activity correlated with disease progression and brain atrophy.

Conclusion: CLN2 is the most frequent NCL type in the region. In all cases with late infantile form, epilepsy was initial feature, in a few cases preceded by atypical febrile seizures. The clinical course of epilepsy was complicated by appearance of different seizure types resistant to AEDs. Although inconsistently, EEG discharges provoked by slow-frequency photo-stimulation might be very helpful, easy and fast screening instrument for clinical suspicion to CLN2. Management of seizures and especially of status epilepticus in NCL patients is challengeable due to resistance and side effects of AEDs.

In vivo monitoring of viral gene injection therapy in ovine batten disease

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Recent progress in gene therapy for CLN5 and CLN6 ovine Batten disease (neuronal ceroid-lipofuscinosis, NCL) has highlighted the need for *in vivo* monitoring to provide compelling data for translation to human therapies while surveying the long term impacts of these therapies. Sheep have brains similar to those of non-human primates, have similar cognitive ability and are social domesticated animals. Neurological examinations are carried out monthly. Simple maze tests have proved to be informative and data are now being collected via a precision GPS system. Affected sheep can be distinguished from normal sheep from 6 months of age. Routine computer tomography (CT) scanning is simple and economic. This provides 3-D images of the intracranial space, which is decreased in affected animals by 9 months following cranial thickening into the space left by neurodegeneration. It also allows modelling of the increased volume of the lateral and third ventricles in affected sheep compared to unaffected controls and the identification of cranial markers, ideal for developing co-ordinates for stereotactic injections. CT scans are benchmarked against more sophisticated magnetic resonance structural imaging (MRI) at specific times in disease development. While observations of the sheep in the field and in maze studies indicate a relatively early visual impairment that correlates with the atrophy of the visual cortex, electroretinography (ERG) shows a later onset and slower development of retinal degeneration. Differences are seen in dark-adapted electroretinography between controls and CLN5 affected sheep at 7 months and later in CLN6 affected sheep. All these changes are progressive, differences becoming stark with time. The success of our gene therapies beyond the natural endpoint of disease highlights the desirability of being able to gather *in vivo* data over the long term, to determine the long term efficacy of treatment and to monitor for any other symptoms that may develop, particularly more mild ones that may be amendable to secondary treatments.

Spinal Cord, Heart and Peripheral Nervous System Involvement in CLN3, CLN6 and CLN7 Disease

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Significant progress has been made in testing pre-clinical experimental therapies for enzyme deficient forms of NCL. These depend upon the uptake of exogenously supplied enzymes, but such 'cross correction' is not possible in forms of NCL like CLN3, CLN6 and CLN7 that are due to defects in transmembrane proteins. As such, for these forms of NCL it will be important to gain a better understanding of the pathological processes and where these changes occur.

Relatively little is known about the systemic pathology that occurs outside of the brain in any form of NCL. Recent work in our lab has discovered significant pathology in the spinal cord in CLN1 mice, and suggested involvement of the spinal cord, peripheral nervous system (PNS) and the heart in other forms of NCL. Preliminary data reveals spinal cord pathology in Cln3 knockout mice, including neuron loss and increased microglial activation and astrocytosis. Preliminary data from the spinal cord of $Cln7^{\Delta ex2}$ mice also reveals pronounced microglial activation and astrocytosis. We found that Cln1 and Cln3 mice showed several signs of cardiac abnormalities, including sinoatrial node pathology and heart rate abnormalities.

Based on these findings we are now assessing the onset and progression of neuropathology in the spinal cords of $Cln3^{\Delta ex7/8}$ and $Cln3^{LacZ}$ mice, in addition to $Cln6^{nclf}$ and $Cln7^{\Delta ex2}$ mice. Sciatic nerves from the same mice will be used to assess pathology within the peripheral nervous system. Cardiac involvement will also be appraised via a range of pathological markers, including assessing myocyte size and number, fibrosis ventricular hypertrophy, and the organisation of the sinoatrial node.

A more complete understanding of NCL pathology outside the brain will lead to the development of more effectively targeted therapies, which is especially important for these transmembrane protein-deficient forms of NCL.

Photosensitivity is an early marker of infantile neuronal ceroid lipofuscinosis (NCL-2)

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Introduction: Early diagnosis is essential in neuronal ceroid lipofuscinosis type 2 (NCL-2) because rapid, irreversible clinical deterioration can occur between the onset of the disease and its diagnosis. Early diagnosis of NCL-2 based on EEG findings could provide the key to early treatment, and optimized care and outcomes. The aim of this study was to identify early clinical, MRI and EEG characteristics to enable early diagnosis.
Methods: We retrospectively reviewed clinical charts of a series of patients diagnosed with NCL-2 from 2005 to 2015 in a single centre in Italy. Clinical, MRI and EEG findings were reviewed.
Results: A total of 14 patients were included. For the whole group, median (range) age at disease onset was 3.0 (2.0–3.8) years. Epilepsy was the most common presenting symptom (in 50% of patients), occurring at the age of 3.2 (2.6–3.8) years. All patients walked independently at the age of 12.0 (11.0–18.0) months, but delayed speech or regression of acquired verbal skills was present in 100% of patients at 3 years. First seizure was myoclonic in 36% (5/14), followed by generalized tonic-clonic in 28% (4/14), atonic in 22% (3/14), and focal with motor signs in 14% (2/14). EEGs revealed a photoparoxysmal response (PPR) on intermittent photic stimulation (IPS) in 93% (13/14) of patients. PPR was present from the first EEG in 43% (6/14) of patients, and was documented at low stimulation frequencies in 69% (9/13), in the form of a flash-per-flash response in 69% (9/13). First brain MRI at the age of 3.8 (3.0–5.1) years revealed cerebellar atrophy in all (100%; 14/14) patients, and alteration of the periventricular white matter signal in the posterior hemispheric region in 77%.
Conclusions: The main result of this study is presence of early photosensitivity (typically PPR at low stimulation frequencies) since first EEG performed in patient with NCL-2 disease. This result can improve the chance to reach earlier the diagnosis of NCL-2, above all in infants presenting with different types of seizures, and particularly if accompanied by delayed speech and/or ataxia. MRI revealed cerebellar atrophy in all patients since first evaluation.

New chemical suppressors of nonsense mutations

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Nonsense mutations introduce a premature termination codon (PTC) and underlie ~10% of genetic disease cases. High concentrations of aminoglycosides such as gentamicin can restore gene function by eliciting PTC readthrough but do so with low efficiency and in limited numbers of patient-derived cells. Using a high throughput screen, we identified compounds that potentiated PTC readthrough by aminoglycosides at multiple nonsense alleles in yeast. Chemical optimization generated compound CDX5-1 that was active in human cells. As a single agent, CDX5-1 did not induce PTC readthrough or increase *TP53* mRNA levels in human HDQ-P1 cancer cells with a homozygous *TP53* nonsense mutation. However, in combination with the aminoglycoside G418, it enhanced readthrough up to 180-fold over G418 alone. The combination increased readthrough at all three nonsense codons (TGA, TAG, TAA) and in multiple human cancer cell lines with various *TP53* nonsense mutations. The combination similarly showed superior activity in cells from patients with rare genetic diseases caused by nonsense mutations. For example, it induced full-length TPP1 production and robust enzyme activity in fibroblasts from a late infantile neuronal ceroid lipofuscinosis patient. These findings open up the possibility of treating patients across a spectrum of genetic diseases caused by nonsense mutations.

High Content Screening of Small Molecule Therapies for Cln6-Batten Disease

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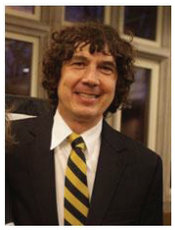
Cln6-Batten Disease is caused by a mutation in the CLN6 gene, a transmembrane protein which localizes to the endoplasmic reticulum and ER-derived vesicles. Cln6Batten Disease is a lysosomal storage disorder that leads to neurodegeneration and loss of neurons. The function of Cln6 remains largely unknown which has slowed progress in the development of targeted therapies and cures. One way to circumvent this problem is to use high throughput techniques to screen many different compounds for potential therapeutic effects. To this end we are combining high content image analysis (HCA) with small molecule drug (SMD, <900 Daltons) screening to search and identify potentially therapeutic compounds. The benefit of using SMD is that these compounds allow for the possibility of rapid diffusion across cell membranes and even the blood-brain barrier. Given the increasing number of SMDs available high throughput screening methods are necessary to identify specific SMDs for further analysis.

Using fibroblasts derived from Cln6-Batten Disease patients we are able to use HCA to rapidly screen SMDs in cell lines with genetic backgrounds from Batten Disease patients. HCA uses automatic image acquisition, analysis, and annotation software to capture multiple data points at the cell level allowing us to follow multiple metrics in thousands of individual cells in real time. These metrics can include the status of lysosomes, autophagosomes, mitochondria, oxidative stress, cell proliferation, and cell death. SMDs identified in these initial analyses will be put forward for additional screening in mouse primary neuronal cultures, neurons derived from patient iPSCs, and in vivo screening in Cln6^{nclf} mice.

P45

Canine models of the neuronal ceroid lipofuscinoses for disease therapy development



Martin L. Katz[1], Dawna L. Voelkl[2], Dietrich H. Volkmann[2], and Gary S. Johnson[2]

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Human neuronal ceroid lipofuscinoses (NCLs) result from mutations in at least 13 different genes. Although all of the NCLs are lysosomal storage diseases characterized by progressive declines in neurological function and neurodegeneration, the underlying disease mechanisms vary depending on the specific causative mutations. Dogs with naturally occurring mutations in the canine orthologs of the NCL genes can serve as models for studying NCL disease mechanisms and for preclinical evaluation of therapeutic interventions. One example is a Dachshund model with a null mutation in *TPP1* that underlies the CLN2 form of NCL. TPP1 enzyme replacement therapy was very effective in retarding disease onset and progression in this dog model. The results of this therapeutic intervention study led to human clinical trials of this treatment that have been successfully completed, making it likely that TPP1 enzyme replacement will become the first effective therapy to receive regulatory approval for treating any of the NCLs. An objective of our research is to identify, preserve, and disseminate canine models for the other forms of NCL so that these models will be available for similar therapeutic intervention studies. To date, 10 different NCL-causing mutations have been identified in the canine orthologs of genes that harbor mutations responsible for the CLN1, CLN2, CLN5, CLN6, CLN7, CLN8, CLN10 and CLN12 forms of NCL. We are continuing our efforts to identify dogs with clinical and histopathological signs of NCL and to identify the mutations responsible for the diseases in these dogs. Our goal is to identify dogs with mutations that cause each of the known forms of NCL and to establish a semen repository with samples from carrier or affected dogs for each of the disease forms. The repository will enable us to generate dogs with these diseases that can be used in therapeutic intervention studies by any investigators with the necessary resources to conduct such studies.

Endogenous Metabolic Profiling As A Fundament In Personalized Theranostics

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Metabolomics has grown into an established tool in research for;
A. Diagnosis, i.e. classification
B. Identification of biomarkers in relation to e.g. diseases.
C. Dynamic studies when identifying effects from, for instance, medical treatment, changes in life style, environmental or genetical changes.

In this presentation the use of metabolomics as a tool in drug discovery and diagnostics will be highlighted. In the first part the differences in biochemical profiles between healthy volunteers and persons with the diagnosis rheumatoid arthritis (RA) are discussed and identification of novel biochemical pathways for understanding the underlying factors of the disease are presented.

In the next part a comparison to different animal models is made, in order to identify the most relevant animal model for describing the disease in humans. The animal models are used for evaluation of novel treatments.

In the last part, an example from the BATCure project will be presented for a CLN3 disease yeast model, comparing the *btn-1* mutant *vs.* wild type. In addition, from the zebrafish (*Danio rerio*) CLN2 disease model, we compared tpp1-/- with the metabolic profile of wild type. Results will be presented and discussed in relation to metabolic profiles and biochemical pathways and how these findings can help us to identify novel methods of treatments.

Gene therapy for the CLN6 Batten disease: in vivo validation and safety study into a non-human primate model.

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CLN6 Batten disease is a devastating neurodegenerative disorder affecting children between 18 months and 8 years of age. The disease is caused by mutations in the CLN6 gene, inherited in an autosomal recessive manner. Symptoms include seizures, progressive dementia, and loss of visual and motor functions. Available treatments are currently only used to ease symptoms, and in all cases the outcome is fatal with death occurring within the first 15 years of life.

CLN6 is a ubiquitously expressed transmembrane protein located in the membrane of the endoplasmic reticulum. The specific function of CLN6 is unknown. However, mutations in the CLN6 gene lead to the synthesis of highly unstable or truncated forms of the protein, thus loss of CLN6 functions are believed to be responsible for the pathology of CLN6 Batten disease. CLN6 patients show the presence of storage material called lipofuscinoses, composed primarily of proteins and lipids, in the brain and in other tissue such as muscles, liver, spleen and skin. It appears that neurons of the central nervous system are particularly sensitive to this the accumulation of lipofuscinoses because CLN6 mutations lead to the death of every neuronal subtype. Therefore, a winning therapeutic approach would aim to restore CLN6 protein function through the expression of the full gene.

We are the first to present a gene therapy approach to treat *CLN6* Batten disease and in the present study we have used an Adeno associated viral vector coding for the human CLN6 protein under control of the chicken β-actin hybrid promoter (scAAV9.CB.CLN6). Following in vitro and in vivo validation, we moved into a non-human primate model and we have shown that the delivery of scAAV9.CB.CLN6 directly into the CNS induced a robust and widespread expression of the transgene. The human CLN6 protein was detected throughout the brain and the spinal cord as early as 4 weeks after the injection and remained sustained for the duration of our study. The animals have never shown any signs of toxicity after the therapy indicating that the treatment is safe and well tolerated. This important milestone was crucial to initiate the first clinical trial for the CLN6 Batten disease.

Modeling CLN6 with patient-derived IPSCs

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Background: Variant Late Infantile neuronal ceroid lipofuscinosis (vLINCL) as a result of mutations in the *CLN6* gene is a rare form of neuronal ceroid lipofuscionosis (NCLs; also known as Batten disease). *CLN6* encodes the CLN6 protein, a transmembrane protein that originates in the endoplasmic reticulum and trafficks through other compartments. Furthermore, the function of CLN6 is unknown. Although the *CLN6* sequence is highly conserved across mammalian species, it has no known homology with other proteins. vLINCL associated with CLN6 is characterized by developmental regression, seizures, loss of vision, and progressive mental and motor deterioration leading to premature death. Similar to other NCLs, the cellular pathology associated with CLN6 dysfunction includes the abnormal accumulation within the lysosome of autofluorescent electron-dense lipopigments and proteins (with subunit C of the mitochondrial ATPase being a predominant component).

Objective: Induced pluripotent stem cells (IPSCs) are renewable cells that can be generated from patient cells by the expression of factors that confer pluripotency. These cells can be differentiated into a wide range of cell types including cells of the CNS. For this reason, IPSCs have become valuable tools in modeling neurodegenerative disorders whose affected tissues are difficult to sample and collect. In order to model CLN6 *in vitro*, IPSCs were produced from affected patients. Subsequent differentiation of these CLN6-IPSCs into neurons and glia were used to determine whether distinctive hallmarks at cellular and molecular level can be recreated and studied in this system. Results: IPSCs derived from the blood (non-T-cell) of normal controls and CLN6-affected subjects were differentiated into neural progenitor cells (NPCs) and cortical neurons in a similar manner. Cellular morphology was undistinguishable from control cells, however, immunostaining for subunit C showed substantial accumulation of this protein within CLN6-derived NPCs and neurons. Of note, this was not seen in CLN6-IPSCs. Immunostaining with markers for vesicular compartments including the endoplasmic reticulum, Golgi apparatus, mitochondria, autophagosome and lysosome are currently being investigated and compared with controls. Conclusions: IPSCs derived from patient cells can be differentiated into neural cells that express markers consistent with the cellular and molecular phenotype previously associated with this disorder. These findings indicate that molecular markers for this disorder can be monitored in human IPSCderived neural cells and used to measure efficacy of therapeutic interventions including small molecule compounds, anti-sense oligonucleotides, and gene therapy.

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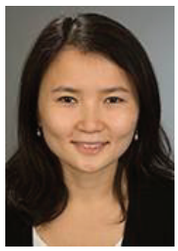
SILAC-based comparative proteomics reveals dysregulation of soluble lysosomal proteins in a cell-based model for CLN7 disease



Khandsuren Ariunbat [1], Melanie Thelen [2], Laura Brandenstein [1], Volkmar Gieselmann [2], Stephan Storch [1]

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By *MFSD8* exon 2 deletion we have generated a *Cln7* knockout (KO) mouse which closely resembles the neuropathology observed in the retina and in the brain of CLN7 patients with variant late-infantile phenotype. In *Cln7* KO brains dysregulated expression of lysosomal proteins, presence of autofluorescent material in neurons, accumulation of subunit c of mitochondrial ATP-synthase and saposin D and storage material containing curvilinear and fingerprint profiles indicate lysosomal dysfunction.

To identify and quantify dysregulated lysosomal proteins we performed a Stable Isotope Labeling by Amino acids in Cell culture (SILAC)-based comparative mass spectrometric analysis of lysosomes from cultured mouse embryonic fibroblasts (MEFs). Wild type and *Cln7* KO MEFs were labeled by SILAC followed by fluid phase endocytosis of magnetite dextran. Magnetite-containing lysosomal fractions of mixed postnuclear supernatants were separated on a magnetic column followed by identification and quantification of proteins by mass spectrometry. From 3422 different proteins identified, we detected all known soluble lysosomal proteins (number: 59) and 30 lysosomal membrane proteins in amounts sufficient for quantification. Mean values of light-to-heavy ratios of more than 1.25-fold and less than 0.75-fold determined in three individual SILAC experiments were considered as significant increase and decrease, respectively. Quantification revealed that the amounts of 24 different soluble lysosomal proteins were decreased in *Cln7* KO MEFs compared with wild type controls. The levels of two soluble lysosomal proteins were increased and interestingly, none of the identified membrane proteins was significantly altered in *Cln7* KO lysosomes. The identified down-regulated soluble lysosomal proteins are involved in the degradation of a number of different substrates including glycoproteins, glycosphingolipids, sulfatides, gangliosides, glycoproteins, peptides and glycosaminoglycans. In addition three NCL-related proteins were down-regulated in *Cln7* KO lysosomes. The data suggest that loss of Cln7 in MEFs leads to lysosomal dysfunction which may be caused by reduced activity of selected soluble lysosomal proteins. The potential role of low abundant lysosomal membrane proteins for lysosomal dysfunction in *Cln7* KO MEFs remains to be investigated.

Using a yeast model of juvenile CLN3 disease to identify novel genetic interactors of CLN3 under stress conditions

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Many disease molecular mechanisms rely on the interplay of a plethora of different proteins. Current drug therapies mostly aim at targeting one specific protein; yet, combination therapies targeting multiple players in a disease pathway are vital in order to overcome acquired resistance mechanisms or to alleviate multiple disease symptoms at the same time. Thus, it is crucial to identify the environment in which a disease-related protein is embedded in order to understand disease pathogenesis, to define novel drug targets and ultimately, to develop novel therapies.

We study novel interactors of *btn1*, the orthologue of human *CLN3* which underlies an early onset neurodegenerative disease (juvenile CLN3 disease, Batten disease), in the fission yeast *Schizosaccharomyces pombe*. *S. pombe* is an excellent model organism for studying CLN3 disease, and its genetic tractability allows for investigating function of genes in a disease-context and on a genome-wide scale. Work previously done in the lab (Bond *et al*., *Microbial Cell* 2015) identified multiple novel interactors of *btn1* using synthetic genetic array analysis (SGA), and showed that the mTOR pathway is a major hub in *btn1* disease-phenotypes. Using SGA again, we will further explore *btn1* and mTOR pathway genome-wide interactions in cells under stress to mimic what may be happening during disease. The data will be analysed to identify genes that show both positive and negative interactions. The most influential novel results will be validated in yeast and mammalian cells before moving onto a higher organism such as a zebrafish model generated using CRISPR.

To complement SGA and further our knowledge of *btn1* regulation and genetic interactions, we will apply a transposon-mediated mutagenesis approach. This allows us to query regulatory, non-translated regions and essential genes by saturating the genome with random insertions. Briefly, we use the *Hermes* transposon library to saturate the genome with insertions of about 1 insertion per 20-50 base pairs. We compare wild-type and *btn1*-deletion yeast transposon libraries and perform next-generation sequencing to assess which genes are synthetic sick/lethal (insertions become under-represented over time) or are suppressor genes/mutations (insertions get enriched over time). By using this novel approach we aim to provide a complete picture of novel genetic interactors of *btn1*, which will help gain insight into juvenile CLN3 disease and can provide novel pathways for future drug development.

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BATCure: Developing new therapies for Batten disease

Sara E Mole, Coordinator of BATCure Consortium

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BATCure is a Consortium of experts from 14 organisations in 7 different European countries (UK, Sweden, Latvia, Italy, Germany, Spain, Denmark) committed to working together towards our common goal. This is to develop effective treatments for patients living with the neuronal ceroid lipofuscinoses (NCL) or Batten disease. We shall provide this real breakthrough in knowledge and innovative therapies to treat three genetically distinct NCL sub-types caused by mutations in intracellular transmembrane proteins (CLN3, CLN6, CLN7 diseases). These affect more than 50% of all children and young adults with NCL living across Europe but are not amenable to the current therapeutic strategies that involve the direct replacement of defective soluble enzymes. They include the most prevalent type of NCL, juvenile CLN3 disease, and about half of all adult-onset cases. We will provide and test novel compounds and gene therapies, and increase understanding of the biochemical pathways and tissues affected to aid the selection and testing of these therapeutic leads. BATCure is funded under the EU Horizon 2020 PHC-14-2015 – new therapies for rare diseases -to provide a concerted, focused and synergistic action over 3 years that comprehensively tackles the challenges and scope of this call. It began in January 2016 to:

1. Create new models, tools and technologies for developing and testing therapies
2. Further delineate disease biology and gene function to identify new therapeutic target pathways
3. Identify biochemical therapeutic target pathways, facilitate effective evaluation of preclinical therapies and improve diagnostics
4. Extend a comprehensive natural history beyond the brain to include cardiology, the spinal cord, peripheral nervous system, psychiatric and metabolic changes
5. Identify new and repurpose existing small molecule therapy
6. Triage new compound treatments in zebrafish, a high-throughput small vertebrate model
7. Deliver and monitor new treatments using mouse models
8. Provide a novel mechanism to involve patients and their families to inform and fully contribute to therapy development and prepare all stakeholders for clinical trials

The project combines the expertise of (i) recognised European research teams, both basic scientists and clinicians, (ii) high-technology SMEs and micro-SMEs, and (iii) an NCL patients' organisation. Some of these partners are participating in NCL-2016. BATCure can be followed at www.batcure.eu, and via Twitter (@BAT_Cure, #battendisease) and Facebook (BATCure).

Altered neuronal activity in the hippocampus and visual cortex during Juvenile Neuronal Ceroid Lipofuscinosis (CLN3)

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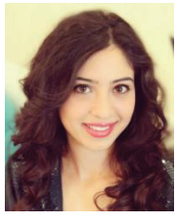
Juvenile Neuronal Ceroid Lipofuscinosis (JNCL) is a fatal lysosomal storage disease caused by an autosomal recessive mutation in *CLN3*. JNCL is typified by progressive neurodegeneration that has been suggested to occur from excessive excitatory and impaired inhibitory synaptic input; however, no studies to date have directly evaluated neuronal activity. To examine temporal changes in neuronal activity with advancing disease pathology, electrophysiological recordings were performed in the CA1 hippocampal area (HPC) and visual cortex (VC) of acute brain slices from $CLN3^{\Delta ex7/8}$ mice at 1, 4, 8, and 12 months of age. Neuronal activity was significantly increased in the HPC of $CLN3^{\Delta ex7/8}$ mice at 1 and 4 months of age as revealed by elevated primary evoked responses of fiber volley (FV), field excitatory post-synaptic potential (fePSP), and population spike (PS) measurements. In older $CLN3^{\Delta ex7/8}$ mice (8 and 12 months), these neuronal responses in the HPC declined to reach levels that were equal or lower than neuronal activity in WT animals. In contrast, paired-pulse facilitation (PPF) of PSs and spike amplitude were significantly decreased in $CLN3^{\Delta ex7/8}$ HPC neurons at all ages. Similar results were observed in the VC. Application of synaptic transmission and sodium channel inhibitors (CNQX, high $[Mg^{2+}]$ and TTX, respectively) revealed that FV was significantly increased in $CLN3^{\Delta ex7/8}$ VC, which can contribute to the non-synaptic component of field potential responses. Additionally, immunostaining of brain tissues from 1 month-old $CLN3^{\Delta ex7/8}$ mice revealed increased expression of the axonal initial segment marker ankyrin G and voltage-dependent sodium channel 1.6 (Nav1.6), both of which are involved in action potential generation, which correlated with increased neuronal activity in 1 month-old $CLN3^{\Delta ex7/8}$ animals. Collectively, these results reveal increased evoked neuronal activity in the HPC and VC of $CLN3^{\Delta ex7/8}$ mice at 1-4 months of age concomitant with reduced short-term plasticity throughout the disease process, which can be associated with modified axonal conductance.

P53

Impact of Galactosylceramide on neurobehavioral parameters and brain ceramide of $Cln3^{\Delta ex7/8}$ knock-in mice.



Sally El-Sitt[1], Jihane Soueid[1], Nadine Makhoul[1], Joelle Makoukji[1], Hayat Harati[1,2], Rose-Mary Boustany[1].

[1]Department of Biochemistry and Molecular Genetics, American University of Beirut Medical Center; [2] Lebanese University/Haigazian College.

Juvenile Neuronal Ceroid Lipofuscinosis (JNCL)/CLN3 disease leads to visual failure followed by seizures, motor decline and death in the second-third decade. It is the most prevalent amongst the Neuronal Ceroid Lipofuscinoses (NCLs). Pathological hallmarks include accelerated apoptosis and accumulation of autofluorescent material, primarily subunit C of mitochondrial ATP synthase in brain, liver, kidneys, lungs, testes, intestines and pancreas. The ~1.02 kb deletion eliminating exons 7/8 of the CLN3 gene resulting in a truncated protein is the commonest human mutation causing JNCL. Wild-type CLN3 protein (CLN3p) is 438 amino acids long. This protein is described as antiapoptotic, and facilitates transport of galactosylceramide (GalCer), from its synthesis in Golgi via early endosomes to lipid rafts (LR) in plasma membrane. Mutated CLN3p results in disrupted anterograde transport of GalCer and retention in Golgi/recycling endosomes without localization to lipid rafts. Ceramide increases, aggravating apoptosis and neuronal loss. GalCer added to CLN3-deficient cells corrects the GalCer deficits in LRs, diminishing apoptosis and ceramide (Rusyn et al, Pediatric Research 2008). Intraperitoneal injection of GalCer into $Cln3^{\Delta ex7/8}$ knock-in mice from 8-17 weeks reduced subunit C accumulation in brain/liver/kidneys and lowered brain ceramide (unpublished data, Harati, Cotman and Boustany). An expanded mouse trial spanning 40 weeks beginning at 4 weeks of age, including 32 $Cln3^{\Delta ex7/8}$ affected mice (Cotman et al., 2002) and 32 wild-type age-matched control mice (GalCer vs. vehicle treated mice) is underway to assess impact of GalCer treatment on neurobiological behavior parameters and mouse brain ceramide. GalCer administration improved grip strength of mouse forelimbs in homozygous $Cln3^{\Delta ex7/8}$ male and female mice in a statistically significant manner (females: $p< 0.05$, males: $p< 0.01$). Slight improvement in motor agility and coordination of $Cln3^{\Delta ex7/8}$ male mice on the pole climbing test was observed (not statistically significant). We determined that wild-type vehicle-treated mice performed better than vehicle-treated affected mice (females:$p<0.01$; males:$p<0.05$). Mouse endurance with the wire hanging test did not differ between vehicle and GalCer-treated mice. GalCer injection improved balance of $Cln3^{\Delta ex7/8}$ female mice in the challenging session of the rotarod test ($p<0.05$). A developmental assessment of ceramide in serum /brain from wild-type mice (ages 0-48 weeks) demonstrated a peak at 3 weeks of age (statistically significant serum: $p<0.05$, brain: $p<0.001$). In brain, and starting at 4 weeks of age, there was a steady increase in ceramide levels that was statistically significant for ages 16, 24 and 48 weeks ($p<0.0001$). A similar developmental assessment of brain ceramide in $Cln3^{\Delta ex7/8}$ mice will be presented, as well as comparison of brain Ceramide in 44 week old GalCer vs. vehicle-treated mice.

Design, Synthesis and Mechanism of Action Studies of Flupirtine Derivatives with Enhanced Neuroprotective Activity

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Introduction: Batten disease is a rare neurodegenerative disorder, affecting around 1 in 12,500 children in the US. It is the most common form of the neuronal ceroid lipofuscinoses (NCLs). Various genes have been identified in which mutations can lead to Batten disease. However, the pathophysiology of the NCLs remains poorly understood. Current treatment is symptomatic and supportive but does not target the underlying disease resulting in a clear urgent and unmet medical need to develop novel therapeutics.

Objective: The non-opioid analgesic flupirtine has been shown to ameliorate apoptosis in NCL patient lymphoblasts as well as a range of other NCL patient cells. No medicinal chemistry structure-activity relationship studies have been conducted to investigate the potential of this compound as a lead for neurodegenerative drug discovery, or for mechanism of action determination. Flupirtine has a range of known mechanisms that may result in neuroprotective activity however, the actual target of action related to Batten disease has never been determined.

Results: We are applying a chemical genetics approach to design and synthesize chemical probe derivatives of flupirtine to identify the mechanism(s) and target of action. A unique library of synthesized derivatives abort etoposide-induced and serum starvation induced-apoptosis in neuron-like PC12 cells with several compounds possessing greater protective effect than the lead. These greater efficacy compounds translate to provide protective effects in phenotypic $CLN3^{-/-}$ knockdown cell lines. Synthesized compounds with greater protective activity function to upregulate Bcl-2 and may have an effect to modulate autophagy. Further chemical synthesis has resulted in an equipotent affinity probe specifically designed for protein target identification studies.

Conclusions: We demonstrate that derivatives with greater neuroprotective ability do not function to ameliorate reactive oxygen species nor do they function as potassium channel agonists (unlike the flupirtine lead compound). We have identified two potential mechanisms of action and are using our developed neuroprotective compounds to further probe and identify the exact target of action.

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Flupirtine Analogues for Treatment of Batten Disease

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Background: Batten disease/the Neuronal Ceroid Lipofuscinoses or NCLs are fatal inherited neurodegenerative diseases with no cure. CLN3 disease is the juvenile and most common. Although rare, the disease often strikes multiple offspring in the same family that carry the defective NCL gene. Current treatment regimens are symptomatic and supportive but do not target the underlying disease. The need for disease-modifying drug candidates is urgent. This work aims to address this requirement by providing and testing lead therapeutic compounds. Previous work had shown that Flupirtine aborts etoposide-induced apoptosis in CLN1/CLN2/CLN3/CLN6-deficient and normal lymphoblasts and prevents death of CLN3 and CLN2-deficient neurons. The end goal is generation of Flupirtine analogues with enhanced anti-apoptotic and neuroprotective properties.

Methods: Optimum drug concentrations of Flupirtine derivatives (Drugs 3, 4, 5, 6, 7, 8 and 9) were tested by establishing cell growth curves under pro-apoptotic conditions following etoposide treatment. Protection was assessed by Trypan Blue and Propidium Iodide assays. Flupirtine derivatives with enhanced activity at optimum concentration were evaluated by Trypan Blue staining of CLN5-deficient patient fibroblasts and PC12 cells following siRNA knockdown of the CLN3 gene. Bcl-2 levels were measured by RT-PCR in treated CLN3 knockdown PC12 cells.

Results: Many of the Flupirtine analogues with specific chemical substitutions around the carbamate moiety proved protective after the application of etoposide to PC12 cells. In fact, with the Trypan Blue assay drugs 3, 6 and Retigabine exerted maximum antiapoptotic activity at 20 μM at 24 hrs, whereas, drug 5 was most protective at 50 μM at 24 hrs. Cells treated with these four drugs were also assessed by propidium iodide under pro-apoptotic conditions (etoposide) at 24 hrs. All four drugs had ~60% less dead cells with respect to vehicle treated control cells, except for drug 3 (~20% less dead cells). Moreover, there was a significant 1.5-fold increase in the percentage of PC12 live cells transfected with siCLN3 and treated with drugs 5 or 6. Also, drugs 3, 5 and 6 significantly prevented cell death of CLN5-deficient patient fibroblasts, with a significant 2-fold increase in the percentage of live cells compared to normal human fibroblasts (Trypan Blue assay). Bcl-2 levels were increased by ~40% in CLN3 knockdown PC12 cells treated with drugs 5 or 6. Ceramide levels in CLN3 knockdown PC12 cells treated with the analogues will be presented. The protective effects against cell death of drugs 3, 5 and 6 was superior to that of Flupirtine.

Conclusion: Analogous compounds to Flupirtine with enhanced activity have been generated and tested for the treatment of Batten disease. These analogues even prove to possess greater protective activity than Flupirtine.

Volumetric Description of Brain Atrophy in CLN2 disease: Supratentorial Gray Matter Shows Uniform Disease Progression

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Introduction
Experimental therapies for CLN2 disease, a genetic disorder of childhood associated with progressive brain atrophy, are currently being developed. Because quantitative descriptions of the natural course of brain volume loss are needed to evaluate novel therapies, we performed MR imaging volumetry of patients with CLN2 to identify a suitable MR imaging marker of disease progression.

Materials and Methods
Thirteen patients (8 females, 5 males) were recruited from prospective natural disease cohort of patients with neuronal ceroid lipofuscinosis. Repeated MR imaging volumetric analysis (29 datasets) was performed by using the FreeSurfer Software Suite. Follow-up time ranged from 8 months to 5.3 years. MR imaging–segmented brain volumes were correlated to patient age and clinical scores.

Results
Segmented brain volumes correlated significantly with patient age (lateral ventricles, *r* 0.606, *P* .001; supratentorial cortical GM, *r* 0.913, *P* .001; supratentorial WM, *r* 0.865, *P* .001; basal ganglia/thalamus, *r* 0.832, *P* .001; cerebellar GM, *r* 0.659, *P* .001; cerebellar WM, *r* 0.830, *P* .001) and clinical scores (lateral ventricles, *r* 0.692, *P* .001; supratentorial cortical GM, *r* 0.862, *P* .001; supratentorial WM, *r* 0.735, *P* .001; basal ganglia/thalamus, *r* 0.758, *P* .001; cerebellar GM, *r* 0.609, *P* .001; cerebellar WM, *r* 0.638, *P* .001). Notably, supratentorial cortical GM showed a uniform decline across the patient cohort. During late stages of the disease when the clinical score was zero, segmented brain volumes still correlated with patient age; this finding suggests that MR imaging volumetry allows quantitative assessment of disease progression at stages when it cannot be detected by clinical assessment alone.

Conclusions
Automated MR imaging volumetry, as a non subjective and highly sensitive tool, is feasible in CLN2 disease and provides a quantitative basis to evaluate novel experimental therapies.

Diagnosis and Misdiagnosis of Adult Neuronal Ceroid Lipofuscinosis (Kufs Disease)

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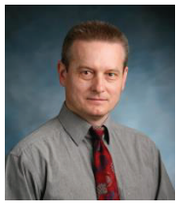
Objective:
To critically re-evaluate cases diagnosed as adult neuronal ceroid lipofuscinosis (ANCL) in order to aid clinicopathological diagnosis as a route to further gene discovery.

Methods:
Through establishment of an international consortium we pooled 47 unsolved cases regarded by referring centers as ANCL. Clinical and neuropathological experts within the Consortium established diagnostic criteria for ANCL based on the literature to assess each case. A panel of three neuropathologists independently reviewed source pathological data. Cases were given a final clinico-pathological classification of 'definite ANCL', 'probable ANCL', 'possible ANCL' or 'not ANCL'.

Results:
Of the 47 cases, only 16 fulfilled the Consortium's criteria of ANCL (5 definite; 2 probable; 9 possible). Definitive alternate diagnoses were made in 10, including Huntington disease, early-onset Alzheimer disease, Niemann-Pick disease, neuroserpinopathy, prion disease and neurodegeneration with brain iron accumulation. Six cases had features suggesting an alternate diagnosis, but no specific condition was identified; in 15 the data were inadequate for classification. Misinterpretation of normal lipofuscin as abnormal storage material was the commonest cause of misdiagnosis.

Conclusions:
Diagnosis of ANCL remains challenging; expert pathological analysis and recent molecular genetic advances revealed misdiagnoses in >1/3 cases. We now have a refined group of cases that will facilitate identification of new causative genes.

Differential effects of acidified drinking water on the behavior and gut microbiome of *Cln3*$^{-/-}$ and wild type mice



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Mutations in the *CLN3* gene cause juvenile CLN3 disease also known as juvenile neuronal ceroid lipofuscinosis (JNCL). The *Cln3*-knockout (*Cln3*$^{-/-}$) mouse model of JNCL displays several characteristic pathological features of the human disease including neurological deficits as measured in the rotarod test and pole climbing test. When *Cln3*$^{-/-}$ mice received acidified drinking water for several generations, their pole climbing ability became similar to wild type (WT) mice. It is known that acidified drinking water can change the microbial flora living in the gut (called microbiome), and this change affects autoimmunity. To test the therapeutic potential of acidified drinking water, a group of *Cln3*$^{-/-}$ mice were switched to acidified drinking water at weaning (postnatal day 21) and their behavior and gut microbiome were compared to *Cln3*$^{-/-}$ and WT mice kept on non-acidified drinking water and to WT mice receiving acidified drinking water from weaning. At 3 and 6 months of age, mice were tested in the pole climbing and rotarod tests, and fecal pellets were collected for gut microbiome analysis. Acidified drinking water in WT mice caused marked alterations in the gut microbiome and significantly changed pole climbing and rotarod performance. In *Cln3*$^{-/-}$ mice, however, acidified drinking water received from weaning did not change the gut microbiome and did not affect the neurological deficits.

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