Useful information about CLN3 disease (Batten disease)

Available research tools

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Topics

• **Batten Disease/Neuronal Ceroid Lipofuscinosis**
  Historical perspective, diseases, genes and classification, shared clinical and histopathological phenotypes

• **CLN3 Disease**
  Prevalence, gene, mutations, diagnosis, clinical course, pathology

• **CLN3 Protein**
  Expression, localization, function

• **CLN3 Models**
  Cell models, organisms, deficiency phenotypes

• **Therapeutic Approaches**
  Gene therapy, cell models

• **Additional Resources** & **Back-up**
Historical perspective

1826 first report of Batten disease

1826: Otto Christian Stengel (Norway)

Four children in one family. Initial development normal up to age 6. Then, sight began to deteriorate leading to blindness. He also described progressive mental decline, loss of speech, epileptic fits and premature death.

- 1896, Sachs first presented concept of Amaurotic Family Idiocieties (AFI)
- 1st report of a lysosomal storage disease
- Early 1960s, Spielmeyer/Sjögren showed intraneuronal storage material in juvenile AFIs differed from infantile AFI (now known as Tay-Sachs Disease)

Brean 2004 Tidsskr Nor Laegeforen 124(7): 970-971
Batten Disease = Neuronal Ceroid Lipofuscinosis

• Most common cause of childhood dementia
• Onset varies: before or at birth (congenital), during 1st year of life (infantile), 2nd-4th year (late-infantile), at 5 years or older (juvenile), adult (18 or older)
• 13 genetically diverse forms
  - All rare, monogenetic, inherited lysosomal storage diseases
  - Autosomal recessive inheritance, except CLN4B (autosomal dominant)
• Share clinical phenotypes and histopathology
  - Loss of vision
  - Loss of motor and cognitive function
  - Epilepsy
  - Early death
  - Lipofuscin deposits in central and peripheral neurons and other cell-types

For clinical classification of NCLs see:
Schulz & Kohlschütter 2013 Iran J Child Neurol 7(1):1-8

Bennett and Rakheja 2013 Dev Disab Res Rev 17: 254-259
NCL genes (CLN1-14) identified since 1995

6 Transmembrane proteins
4 Lysosomal hydrolases
1 Soluble lysosomal protein
1 Chaperone
1 Secreted protein

CLN2 (TPP1)  CLN7 (MFSD8)
CLN3  CLN5  CLN8  CLN6  CLN10 (CTSD)
CLN4B (DNAJC5)
CLN11 (GRN)
CLN12 (ATP13A2)
CLN13 (CTSF)
CLN14 (KCTD7)

For reviews see: Cárcel-Trullols et al. 2015 Biochim Biophys Acta 1852: 2242–2255
Mole and Cotman 2015 BBA 1852: 2237-2241
Schulz and Kohlschütter 2013 Iran J Child Neurol Winter 7:1-8
NCLs are rare diseases

- \(\leq 5\) patients/10,000 (EU); <200,000 (US; 6.8/10,000)
- EU and US have each \(~30\) Mio patients (~1/25)
- 5,000 - 8,000 rare diseases
- 80% genetic cause
- Disproportionate contribution to pediatric mortality
- Usually little hope for treatment
- High prices for rare drugs
- Socio-economic burden is significant

Life-time costs\(^2\) CF patient in Germany (2007) estimated EUR 858,604; in US 1,907,384 USD (2006); Gaucher, ERT 250,000 USD/year/patient.......

\(^1\) Franco 2013 Drug Discovery today 18(3): 163-72
\(^2\) Angelis et al. 2015 BMC Health Services Research 15: 428
NCLs are classified as Lysosomal Storage Disorders

- Group (~70) of rare (collective incidence ~1:5,000 live births), inborn, metabolic disorders caused by deficiencies in lysosomal function\(^1\)

- Characterized by intra-lysosomal accumulation of non-degraded substrates (lack of clearance)

Excellent introduction into lysosomal storage diseases:
Webinar by Prof. Dr. Steve Walkley

\(^1\)for list of LSDs see: http://www.worldsymposia.org/lysosomal-diseases/
Because they result in lysosomal dysfunction

The lysosome has a key role in nutrient-sensing and -recycling

- Highly complex catabolic organelle
- Recycling station
- Integrator of cell metabolic signaling
- Contains at least 60 different hydrolases
- Requires many other proteins involved in post-translational modifications, trafficking, supporting hydrolase activity, maintaining ion homeostasis inside the lysosome, substrate delivery, and returning breakdown products to cytoplasm or extracellular milieu.

NCLs occur worldwide

Subtypes show significant variation in their geographical distribution

- NCL-Cases (Literature und personal communications)
- NCL-Research Centers
- NCL Non-Profit Organisations
## NCL genes, proteins and diseases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Subcellular localization</th>
<th>Genotype-phenotype correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLN1</td>
<td>Palmitoyl protein thioesterase 1 (PPT1) (soluble protein)</td>
<td>Lys matrix, extrasomal vesicles, lipid rafts, ER and presynaptic areas in neurons</td>
<td>Infantile, Late infantile, Juvenile, Adult</td>
</tr>
<tr>
<td>CLN2</td>
<td>CLN2/Tripeptidyl-peptidase 1 (TPP1) (soluble protein)</td>
<td>Lysosomal matrix and ER</td>
<td>Late infantile, Juvenile, Protracted, SCAR7</td>
</tr>
<tr>
<td>CLN3</td>
<td>CLN3 (transmembrane protein)</td>
<td>Late endosomal/lysosomal membrane</td>
<td>Juvenile, Protracted, Autophagic vacuolar myopathy, RP, Cone-rod dystrophy</td>
</tr>
<tr>
<td>CLN4B</td>
<td>Cysteine-string protein alpha (CSPα)/DNAJC5 (soluble protein)</td>
<td>Cytosolic, associated to vesicular membranes, to synaptic versicles in neurons, to secretory granules in endo/exo/neurcrine cells</td>
<td>Adult autosomal dominant (Parry disease)</td>
</tr>
<tr>
<td>CLN5</td>
<td>CLN5 (soluble protein)</td>
<td>Lysosomal matrix</td>
<td>Late infantile, Juvenile, Protracted, Adult</td>
</tr>
<tr>
<td>CLN6</td>
<td>CLN6 (transmembrane protein)</td>
<td>ER membrane</td>
<td>Late infantile, Protracted, Adult Kuf type A, juvenile cerebellar ataxia, Teenage progressive myoclonic epilepsy</td>
</tr>
<tr>
<td>CLN7</td>
<td>CLN7/MFSD8 (transmembrane protein)</td>
<td>Lysosomal membrane</td>
<td>Late infantile, Juvenile protracted, Macular dystrophy, Cone-rod dystrophy</td>
</tr>
<tr>
<td>CLN8</td>
<td>CLN8 (transmembrane protein)</td>
<td>ER/ER-Golgi intermediate compartment membrane</td>
<td>Late infantile, Protracted, EPMR/Northern epilepsy</td>
</tr>
<tr>
<td>CLN9</td>
<td>CLN9 (unknown protein); probably caused by mutation in CLN5 gene</td>
<td>Unknown localization</td>
<td>-</td>
</tr>
<tr>
<td>CLN10</td>
<td>CLN10/Cathepsin D (CTSD) (soluble protein)</td>
<td>Lysosomal matrix and extracellular</td>
<td>Congenital, Late infantile, Juvenile, Adult</td>
</tr>
<tr>
<td>CLN11</td>
<td>CLN11/Progranulin/Proepithelin/Acrogranin (soluble protein)</td>
<td>Extracellular</td>
<td>Adult, Frontotemporal labor dementia (when heterozygous)</td>
</tr>
<tr>
<td>CLN13</td>
<td>CLN13/Cathepsin F (soluble protein)</td>
<td>Lysosomal matrix</td>
<td>Adult Kuf type B</td>
</tr>
<tr>
<td>CLN14</td>
<td>CLN14/Potassium channel tetramerization domain-containing protein 7 (KCTD7) (soluble protein)</td>
<td>Cytosolic and also partially associated to plasma membrane</td>
<td>Infantile, Progressice myoclonic epilepsy-3, Opsoclonus-myoclonus ataxia-like syndrome</td>
</tr>
</tbody>
</table>

**Bold:** phenotype caused by complete loss of gene  
**Red:** non-NCL disease phenotypes  

Modified from: Cárcel-Trullols et al. 2015 Biochim Biophys Acta 1852: 2242–2255;  
Mole and Cotman 2015 BBA 1852: 2237-2241
NCLs share clinical phenotypes

- Loss of vision, retinal degeneration
- Decline of speech and cognitive function
- Loss of motor function
- Epilepsy
- Premature death
NCLs share histopathology – lipopigment storage

- Neuronal loss, brain atrophy
- Lipopigment in neurons (brain, retina), peripheral lymphocytes, skin, rectum, skeletal muscle
- In CLN3, lymphocytes vacuolated, Substantia nigra often lacks pigment, Parkinsonism common
- Deposits granular osmiophilic (CLN1 & 10) Curvilinear (CLN2) Fingerprint (CLN3) Mixed (CLN10)

1. Schulz et al. 2013 BBA 1832: 1801-1806
2. Anderson et al. 2013 BBA 1832: 1807-1826
CLN3 disease
prevalence, gene, mutations, diagnosis, clinical course, pathology
Juvenile NCL (CLN3) disease is most prevalent

CLN3 (43%)  
CLN2/TPP1 (15%)  
CLN1/PPT1 (2%)  
Molecularly undefined (8%)  
Single mutation identified (3%)  
DNAJC5 (1%)  
CLN8 (1%)  
CLN7 (2%)  

~350 probands with molecularly confirmed and/or clear clinical and histopathologic evidence of NCL (MGH NCL Registry & DNA diagnostic lab)

NCL Mutation and Patient Database: http://www.ucl.ac.uk/ncl/mutation.shtml  
Cases link: http://www.ucl.ac.uk/ncl/SummaryTableOct2014.htm
The human CLN3 gene and diagnosis

- gene mapped to human Chromosome 16

- most common disease allele (>76% of cases): 966 bp deletion, covers exons 7 & 8, results in severely truncated protein that gets trapped in the ER

- >48 other mutations in the NCL Mutation Database

- CLN3 patients have vacuolated lymphocytes
CLN3 protein & Batten disease-causing mutants

CLN3 protein is highly glycosylated; extreme hydrophobic; low abundance; C-terminal prenylation; two lysosomal targeting motifs

N-glycosylations

1Storch et al. 2004 JBC 279: 53625-53634
Cotman and Staropoli 2012 Clinical Lipidology 7: 79-91
Clinical course of CLN3 juvenile Batten disease

Childhood dementia, starts with loss of vision, ends with premature death

- Loss of eye sight
- Reduction of intellectual abilities
- Changes in child‘s personality
- Degradation of speech
- Epilepsy, cardiac problems
- Reduction of physical abilities
- Need for nursing care
- Bedridden, premature death

- Large patient to patient variability for most of these parameters
- Better and objective clinical readouts needed in trials
- No disease modifying therapy available so far
Online training movies on CLN3 disease

**NCL - medical education movie**

For better overview on the clinical course of juvenile NCL, please watch a medical education movie provided by the NCL Foundation:

https://www.youtube.com/watch?v=M_qYV-MfbtA

**Diagnostics and therapy for juvenile NCL**

This educational online training provided by MedLearning offers CME certified training for physicians.

Two CME points can be acquired (German only).

http://cme.medlearning.de/medlearning/ncl_kinderdemenz/index.htm
cause of blindness in children aged 5–15 years. Children present with rapid progressive visual loss at age 6–7 years, early mental deterioration, and fits about 2–4 years later, and this is the stage at which the diagnosis is usually made. Macular degeneration appears to be a consistent early feature, and peripheral retinal changes become more marked as the disease progresses. Phototoxicity may possibly play a part in the retinal degeneration.
Early striation occurs in human CLN3 retina

- Degenerative process within ganglion cell complex (3 innermost retinal layers)
- Striation of papillar macular bundle (sparing fovea) is specific for CLN3 disease
- Earliest consistent retinal change observed so far across CLN3 patients
- New and non-invasive early diagnostic marker

Dulz et al. 2015 Br J Ophthalmol 0: 1-5
Loss of visual acuity

Tim, age 6, healthy boy, loss of visual acuity, narrowing of visual field progressed rapidly

Quick visual field reduction

Negative ERG

Dulz et al. 2015 Br J Ophthalmol and references therein
Common retinal changes seen in CLN3 patients

**JNCL Patients (age 8-26) examined at annual meeting of BDSRA**

14 years: Orange pigment clumping of macula
16 years: RPE atrophy, fine epiretinal membrane of macula
9 years: Bull’s eye pigment of macula
19 years: RPE atrophy, focal pigment, clumping of macula

- Macular RPE atrophy (63%), Orange pigment clumping (50%), Epiretinal membrane (33%), Bull’s eye pigment changes (25%), Peripheral RPE atrophy (58%), Peripheral bone spicules (46%), Peripheral course pigment stippling (25%)
- **Most consistent changes:**
  - Variable optic disk pallor but present in almost all (22/24) patients
  - Diffuse macular RPE atrophy in 13/14 patients (fluorescein angiograms)
- No retinal abnormalities in heterozygous parents

Hainsworth et al. 2009 Retina 29(5): 657-668
Massive retinal degeneration at end-stage disease

- Loss of photoreceptors
- Reduced lipofuscin in RPE
- Many translocated RPEs
- Hypertrophy of Müller glia

22 year old male

Midperiphery – RPE intact

Macula – RPE and Photoreceptors lost

Normal retina (far peripheral)

RPE lipofuscin

rhodopsin (Rod OS)

JNCL retina

Much less RPE lipofuscin

rhodopsin lost

Bensaoula et al. 2000 Ophthamology 107: 1746-1753
For review see also: Ouseph et al. 2016 Ann N Y Acad Sci
CLN3 mutations causing late-onset retinal disease but not Batten disease

Some CLN3 mutations specifically cause Retinitis pigmentosa or cone-rod dystrophy

Probands

2055 (Y322*/V290L), RP, no Batten, 40 and 45 years

348 (R405W/R405W), RP, no Batten, late 50 and 60s

2044 (c.125+1G>C/R405W), RP, no Batten at age 57

2691 (G189R/G189R), RP, no Batten at age 10

SRF41 (E295K/S131R), CRD, no Batten at age 20
A CLN3 mutation causing Autophagic Vacuolar Myopathy (AVM)

- Glycine165Glu
- Conserved across-species
- Vacuoles in ~50% of fibers
- Vacuoles acid phosphatase\(^+\) (B), some acetylcholinesterase\(^+\) (C), LAMP-2\(^+\) (D),
- Fibers also stained for leukocyte antigen HLA1 (F) and membrane attack complex
- Visual failure, seizures, and prominent cardiac involvement
- Only mild cognitive impairment and no motor deterioration after 40 years of disease

Cortese et al. 2014 Neurology 82: 2072-2076
CLN3 Batten disease patients show a profound, progressive and selective loss of brain neurons

- Staining for aging-related lipofuscin shows severe structural alterations in cortex
- Most compelling, fusion of the two light stripes of layer IV and Vb
- Layer V (main cortical output layer) severely depleted of nerve cells¹
- Small pigment-laden neurons in layer II/III are particularly affected¹


Feldmeyer 2012 Frontiers in Neuroanatomy 6(24): 1-17
Hippocampal cell-loss in CLN3
most profound in CA3 and hilus

- In CLN3, the most profound neuronal loss occurs in hilus and CA3, not in CA1 like in temporal lobe epilepsy\(^1\)
- Big loss in hippocampal volume\(^2\)
- In CLN1, an almost complete loss of DG
- In CLN8, a selective loss of CA2 neurons
- Correlation between microglial activation and neuronal loss not maintained in all subfields like DG (CLN3 and CLN8); astrocyte hypertrophy prominent in CA1
- In CLN3, interneuron loss far less evident than in CLN1 and CLN2

\(^1\)For similarities with hippocampal neuropathology in Gaucher disease see: Wong et al. 2004 Mol Genet Metabol 82:192-207
Neuroinflammation occurs in the CLN3 brain

Activated micro- and astroglia, a double-edged sword?

Defective lysosomes, elevated Ca\(^{2+}\), ER stress, inflammasome activation, neurotransmitter imbalance, blood-Brain barrier defects,…

Contributing T-cell components\(^1\)

1 Groh et al. 2016 Glia 64: 792–809
Hypothetical interfaces of CLN3 disease with late-onset neurodegeneration and other LSDs

Key question is whether these relate to gene product-specific mechanisms or general inefficiency in lysosomal maturation and/or flux?

CLN3

Mitochondrial diseases

LSDs
NPC, GBA, MLIV, NCLs

AD, FTD
GRN, PS1

Retinitis pigmentosa

AMD

Parkinson
LRRK2, GBA

Introduction into lysosomal pathways in adult-onset neurodegeneration:
Webinar by Prof. Dr. Ralf Nixon
CLN3 protein expression, localization, and function
CLN3 encodes an evolutionary conserved protein

From: http://mtc.science/mutation-conservation-in-mendelian-genes
Broad tissue expression of CLN3 gene

CLN3 gene may gave rise to multiple variants of CLN3 protein isoforms

The International Batten Disease Consortium 1995 Cell 82: 949-957
Eliason et al. 2007 J Neurosci 27: 9826-9834
CLN3 expression in retina occurs most likely in different cell types – so far, not well defined

Note: detailed analysis of β-Gal expression was only done in homozygous knock-ins

- CLN3-β-Gal+ neurons in INL of retina are PKCα+ (rod bipolar cells)
- β-Gal in ONL appears cytoplasmic despite nuclear targeting signal present in β-Gal

For expression of CLN3 in adult mouse retinal cell types see:
- Eliason et al. 2007 J Neurosci 27: 9826-9834

Ding et al. 2011 Neurobiol Dis 41: 237-248
Wir gewinnen Menschen
Fortbildungsmaßnahmen bei Medizinern

CLN3 models
gene therapy, cell models
Models used to elucidate CLN3 function

*Mammalian models and human cell models*

**Mouse models**

*Cln3Δex1-6 mice*
Hannah Mitchison (KCL)

*Cln3−/− mice*
Neo insertion replacing exon 7 & 8
Martin Katz (U of Missouri)

*Cln3lacZ knock-in mice*
LacZ insert and deletion of exons 1-8
Beverly Davidson (U of Philadelphia)

*Cln3Δex7/8 mice*
Mimics most common ~1kb deletion
Susan Cotman (MGH)

**Murine cell models**

*Mouse brain endothelial cell lines*
*Cln3lacZ* Mice
B. Davidson (U of Philadelphia)

*Glial cell systems*
*Cln3Δex1-6* and *Cln3Δex7/8* mice
Jon Cooper (KCL)
Tammy Kielian (U of Nebraska)

*Cbln3Δex7/8 cerebellar neuronal progenitor cell lines*
Susan Cotman (MGH)

**Human cell models**

*Lymphoblastoid lines (LCLs)*
MGH-CHGR NCL Biorepository
Coriell Repository
Schulz (U of Hamburg) e.a. labs

*Fibroblast lines*
MGH-CHGR NCL Biorepository
Schulz (U of Hamburg) e.a. labs

*Induced pluripotent stem cells (iPSCs)*
S. Cotman (MGH);
Storch/Hermann/Schöler,
(Dresden/MPI Münster)
New York Stem Cell Foundation

**Mini Pig**
David Pearce (Sanford, in progress)
Lower organism models to study CLN3 function

YEAST
David Pearce (Sandford, USA)
Sara Mole (UCL, UK)
Jeffrey Gerst (Weizmann, Israel)

WORM
Peter Taschner
(LUMC, Leiden, NL)

SOCIAL AMOEBA
Richard Tuxworth, Guy Tear
(KCL, London, UK)
Christopher Korey
(Richmond, VA, USA)

FLY

ZEBAFISH
Claire Russell (Royal Vet.
College, London, UK)
Sanger mutant collection, UK

Social amoeba: Copyright, M.J. Grimson and R.L. Blanton, Biological Sciences
Electron Microscopy Laboratory, Texas Tech University
Reported CLN3 deficiency-associated phenotypes

Endo-lysosome-related
- Vacuolation with/without storage material (human, mouse)
- Defects in acidification (yeast)
- Amino acid transport defects (mouse)
- Early defects in endocytosis (mouse)
- Ion imbalance and osmoregulation defects (yeast, mouse, hiPSC-derived neurons)

Outside endo-lysosomes
- Plasma membrane lipid microdomains and membrane fluidity
- Gap junctions (hemi-channels)
- Endoplasmic reticulum & Golgi morphology
- Post-Golgi trafficking (retrograde and/or anterograde)
- Actin-dependent cytoskeleton
- Mitochondria, ROS, morphological changes, reduced OXPHOS capacity
CLN3 protein-protein interactions

Perturbations may account for (some) phenotypes

→ Interactions need to be better defined, also to understand the role and how distinct functional protein complexes are affected by disease mutations

For review see also:

Uusi-Rauva 2012
Molecular Interactions of Neuronal Ceroid Lipofuscinosi s Protein CLN3 Research 82/2012


Rab7
Interaction involved in late endo-/lysosom al transport

Calsenilin/DREAM/kChIP3
Interaction involved in sensitizing neuronal cells to thapsigargin
Chang et al. 2006 HMG 16: 317-326
In particular, those associated with late endolysosomal compartments where CLN3 localizes.

Note, how different CLN3 disease-causing mutations affect protein levels, trafficking, localization and turnover is not yet understood.

CLN3 roles and CLN3-deficiency associated abnormalities – schematic of experimental findings

ER & Golgi
- Recycling pathways
- Ion and glycolipid exchange
- Retention/accumulation of mutated variants

Mitochondria
- Turnover (mitophagy, MDVs)

Cytoskeleton
- Non-muscle myosin IIb
- Actin

Autophagosomes
- Fusion
- \( \text{Ca}^{2+} \) signaling alterations

Endosomes
- \( \text{Ca}^{2+} \) channels/Na/K transporter
- Fodrin

Plasma membrane
- DREAM/Calsenilin/KCHIP4
- Actin

Adapted from a slide provided by Susan Cotman, MGH, Boston
Early hints for a role of CLN3 in trafficking

**Specific alterations in levels of Man-6-P glycoproteins**

- M6P moiety is rapidly removed in most cell types but not in neurons
- Each NCL brain shows characteristic alterations in M6P glycoprotein levels
- Highly elevated in JNCL (on average 8.7x)
- Band 3 (13x) represents mainly CLN2 (TPP1)
- Phenomenon not seen in JNCL fibroblasts
- Increase not explained by a 2.2-fold increase in M6P⁺/M6P⁻ lys. enzyme activities (16 tested)

At the time not clear whether the phenomenon reflected increased (compensatory ?) synthesis, an accumulation of M6P-labeled enzymes in certain trafficking compartments or simply an addition of more M6P moieties per enzyme molecule.

Sleat et al. 1998 Biochem J 334: 547-551
CLN3 deficiency impairs trafficking of cation-independent M6PR and some lysosomal enzymes.

- TGN exit of GFP-CI-M6PR is impaired in CLN3-deficient (RNAi) HeLa cells\(^1\)
- CI-M6PR-negative fibroblasts missort and secrete a large fraction of CatD (~46%) and β-hexoseaminidase\(^2\)
- CatD processing is affected in CLN3-deficient cells\(^3\)

\(^1\) Metcalf et al. 2008 Traffic 9: 1905-1914
\(^2\) Ludwig et al. 1994 EMBO J 13: 3430-3437
\(^3\) Fossale et al. 2004 BMC Neurosci 5: 57; Golabek et al. 2000 Mol Genet Metab 70: 203-213
Positioning of lysosomes is altered in CLN3 cells

Mostly perinuclear in control vs dispersed throughout cell in CLN3 NPCs

Lysosome number, size and positioning is regulated to meet changing cellular needs

1. Long-range movement of lysosomes requires microtubule-based motors
2. Kinesin and dynein association determines direction of lysosome transport
3. Lysosomal channel TRPML1 is required to promote Ca^{2+}-dependent centripetal transport of lysosomes
4. mTOR directly targets and inactivates TRPML1
5. TRPML1 overexpression can mimic starvation effects
6. Perinuclear accumulation occurs under nutrient deprivation conditions and autophagy induction

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2. Onyenwoke et al. 2015 Biochem J 470: 331-342
3. Wang et al. 2015 PNAS 112: E1373-1381
CLN3 influences membrane-domain coupled transport

- CLN3 has direct influence on membrane domain-coupled transport from TGN to PM\(^1\)
- CLN3 loss alters brain endothelial cell function

Specifics:
- Reduced caveolin-1 transport to PM
- Reduced caveolae and caveolae-dependent endocytosis (caveolae act as portals for transcytosis)
- Co-ordinate miss-localization of caveolin-1 and TGN tSNARE syntaxin-6
- Impaired MDR1-mediated drug efflux
- Abnormal TGN morphology (fractured)

- Decreased fluid-phase endocytosis due to elevated Cdc42-GTP and reduced recruitment of Cdc42 GTPase activating protein (ARHGAP21) (Cdc42 regulates synthesis and break down of actin allowing fluid-phase uptake to occur)\(^2\).

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\(^1\) Tecedor et al. 2013 J Neurosci 33: 18065-18079
CLN3 deficiency increases intra-lysosomal $[\text{Ca}^{2+}]$

*In mouse CbCLN3$^{\Delta \text{ex}7/\Delta \text{ex}8}$ cells and human iPSC-derived NPCs*

**Lysosomal Ca$^{2+}$**

<table>
<thead>
<tr>
<th></th>
<th>GPN (200 $\mu$M) mediated Ca$^{2+}$ release ($\Delta F/F_0$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CbCln3$^{+/+}$</td>
<td>0.000</td>
</tr>
<tr>
<td>CbCln3$^{\Delta \text{ex}7/\Delta \text{ex}8}$</td>
<td>****</td>
</tr>
</tbody>
</table>

SF-22, MK6-83: Chen *et al.* 2014 Nat Comm 5: 4681

Chandrachud *et al.* 2015 JBC 290: 14361-14380
Scheme adapted from: Xu and Ren 2015 Annu Rev Physiol 77: 57-80
Lysosomal Ca\textsuperscript{2+}: central role in health and disease

- Regulates fusion, fission and lysosomal exocytosis
- Perturbed in variety of disease conditions (CLN3, CMT4, MLIV, NPC, LRRK2, heart failure...)
- Therapeutic benefits proposed by modulating e.g. TRPML1-mediated lysosomal Ca\textsuperscript{2+}-release\textsuperscript{1}
- Lysosomal Ca\textsuperscript{2+}-modulation may have broad therapeutic application

\textsuperscript{1}http://www.coipharma.com/portfolio/calporta/
\textsuperscript{2}Zou et al. 2015 J Neurosci 35: 6801-6812
Defects in energy homeostasis, mitochondria, and reactive oxygen species in CLN3-deficient cells

CLN3-deficient mouse cerebellar neurons
- Elongated mitochondria (grp75 labeled)
- ~1.3-fold reduced ATP levels
- ~2-fold more sensitive to oxidative stress ($H_2O_2$)

- Higher ROS levels have been reported in CLN3 patient lymphoblasts and fibroblasts

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1Fossale et al. 2004 BMC Neurosci 5: 57
2Luiro et al. 2006 J Neurosci Res 84: 1124-1138
Reduced levels of high-energy phosphates in CLN3-deficient patient fibroblasts

As compared to fibroblasts from healthy individuals, CLN3 fibroblasts have reduced levels of ATP, ADP and AMP; Creatine Phosphate levels are not affected.
Mitochondrial defects in CLN3-deficient human iPSC-derived neurons

- ~50% of the mitochondria in CLN3 iPSC-derived neurons display reduced cristae

Therapeutic approaches
Several NCLs are targets for enzyme replacement therapy (ERT) and/or gene therapy (GT)

Includes CLN1, CLN2 (Biomarin, BMT190 trial), CLN5, CLN10, CLN13

Principle of cross-correction

- in ER, enzyme glycosylation
- in Golgi, addition of M6P and binding to M6PR
- trafficking to lysosome
- secretion
- binding to M6PR of neighboring cells, endocytosis, and targeting of the enzyme to lysosome

Sands and Davidson 2006 Mol Therapy 13: 839-849
Gene therapy is being pursued for CLN3\(^1\) and CLN6\(^2\) so far, no evidence for a cross-correcting principle

3 endo-lysosomal, 2 located in ER, 1 plasma membrane

Kmoch et al. 2013 BBA 1832: 1831-1841

\(^1\)http://abeonatherapeutics.com/research-and-development/
\(^2\)https://clinicaltrials.gov/ct2/show/NCT02725580
CLN3 Gene therapy

Perinatal injection of mice
Vector: AAVrh.10hCLN3

Human CLN3 gene therapy trial in preparation
Vector: ABO-201 (AAV9 CLN3)
CLN3 under control of the weak MeCP2 promoter

Work based on preclinical data produced by
Dr. Tammy Kielian, University of Nebraska, USA

Partial correction of storage material and gliosis, no correction
of neuronal cell loss

1 http://investors.abeonatherapeutics.com/

2 Sondhi et al. 2014 Hum Gene Ther 25: 223-239
Restoring perturbed lipid microdomains in CLN3 cells

Carbenoxolone reduces Cdc42 activity in CLN3-null mouse brain endothelial cells

Davidson et al. PLoS ONE 9(5): e96647
Tecedor et al. 2013  J Neurosci 33: 18065-18079
Targeting and reducing neuroinflammation

**Immunosuppression**

**JUMP trial**
Mycophenolate mofetil (Cellcept)
University of Rochester
Erika Augustine, Jonathan Mink

**PDE4 inhibitor** (Tammy Kielian)

1https://clinicaltrials.gov/ct2/show/NCT01399047

2Kielian 2014 Abstract O-14
Other therapies under investigation

966 bp deletion

Exon9-skipping: restores CLN3 reading frame that may result in an at least partially functional CLN3 protein

Michelle Haistings, Rosalind Franklin University¹

Enhancing lysosomal clearance
TFEB enhancers

Restoring lysosomal Ca²⁺ homeostasis
engaging other lysosomal channels

Autophagy enhancers
Susan Cotman (Boston, USA)

Improving mitochondrial function

Premature termination codon (PTC) read through²
(rare CLN3 forms e.a. NCLs caused by nonsense mutations)

¹Jodelka et al. 2014 Abstract 0-33
²Geraets et al. Orphanet Journal of Rare Diseases (2016) 11:40
Restoring autophagy defects

GFP-LC3 assay identifies group of Ca$^{2+}$-modulators

- Thapsigargin (SERCA inhibitor)
- ~2,000 compounds
- DMSO baseline
- Hit threshold

Enriched in:
- Ca$^{2+}$ channel blockers
- HMG CoA Reductase inhibitors (statins)
- Estrogen receptor agonists

Chandrachud et al. 2015 JBC 290: 14361-80
Further development of human CLN3 cell models

Disease-relevant cell types and “organoids“

**Brain cell models**
- Susan Cotman (neurons)
- Jens Schwamborn (neurons)
- Jon Cooper (astroglia)
- Andreas Hermann (neurons)
- Thomas Langmann (microglia)

**Retinal models**
- Mike Karl (RPE, retinal organoids)
- Thomas Langmann (microglia)

Overcoming hurdles to effectively translate treatments to the clinic

What more is needed and closing gaps

- Natural history of disease
  - Becoming increasingly well defined
  - Should facilitate id of clinically measurable endpoints for trial
  - DEM-CHILD NCL patient database (UKE Hamburg, Germany)
  - University of Rochester (USA)
  - MGH (Boston, USA)

- New EU Horizon 2020 BATcure program (2016-2019)

- Peripheral biomarkers
  - Lymphocyte vacuolation
  - Lysosomal enzyme activities
  - Metabolomics
Wir gewinnen Menschen
Fortbildungsmaßnahmen bei Medizinern
DEM-CHILD Patient Database Consortium

NCL patient registry, retro- and prospective patient, static and dynamic patient data, virtual biobank

Clinical status

Static:
- genetic diagnosis
- age @ 1st symptoms
- parents
- healthy and sick siblings
- other relatives affected by disease

Dynamic: JNCL, every 6-12:
- neurologic status
- current medication
- ophthalmologic exam
- cardiologic exam
- EEG
- brain MRI/MRS

Rating scales:
- late infantile NCL Scoring (Steinfeld et al. 2002)
- NCL scoring (Kohlschütter et al. 1988)
- GMFCS (Gross Motor Function Classification)
- BFMF (Bimanual Fine Motor Function)
- QoL (Quality of Life) Questionnaires

Angela Schulz, MD, University Medical Center Hamburg-Eppendorf (UKE)
Additional resources
Additional resources

Research laboratories
Interactive map shows NCL research laboratories worldwide:
http://www.ncl-stiftung.de/main/pages/index/p/373

Annual Requests Proposals
Addressing researchers helping to promote therapies for neuronal ceroid lipofuscinoses to apply for funding from available Research grants.

- NCL Foundation
  http://www.ncl-stiftung.de/main/pages/index/p/612

- BDSRA (Batten Disease Support and Research Association)
  http://bdsra.org/current-rfps/

Rolling Submission Investigator-Initiated Proposals
Year-round letter of intent and invitation to submit full proposal process.

- BBDF (Beyond Batten Disease Foundation)
  http://www.beyondbatten.org
Additional resources

NCL-Non-Profit Organizations:
Foundations

- Charlotte + Gwenvyth Gray Foundation
  (TO CURE BATTEN DISEASE)
  Gray Foundation (USA)

- Batten Research Fonds
  (Stop deze fatale stofwisselingsziekte)
  Batten Research Fonds (NL)

- Beat Batten!
  (Give Batten Kids a chance of life!)
  Beat Batten! (NL)

- Beyond Batten Disease Foundation
  Beyond Batten Disease Foundation (USA)
Additional resources

NCL-Non-Profit Organizations:

Family support groups

- BDSRA (USA)
- Contactpunt NCL (Belgium)
- NCL-Gruppe Deutschland e.V. (Germany)
- Vaincre les Maladies Lysosomales (France)
- The Saoirse Foundation Bee for Batten (IRE)
- Norsk Spielmeyer-Vogt Forening (Norway)
- Dansk Spielmeyer-Vogt Forening (DK)
- BDFA (UK)
Additional resources

**Brain banks & pathologists/histologists**
Collection of brains and tissues for neurologists
- [http://brainbank.ucla.edu/](http://brainbank.ucla.edu/) (LA)

**Research Newsletter**
The NCL Research Newsletter offers the latest scientific and medical information regarding NCL. To subscribe, please choose “NCL Research Newsletter“. [http://www.ncl-stiftung.de/main/pages/index/p/499](http://www.ncl-stiftung.de/main/pages/index/p/499)

Additional Questions: send Email to herman.vanderputten@ncl-stiftung.de
Acknowledgements

We thank all authors whose work and journal articles were cited, we are grateful to Dr. Susan Cotman for sharing slides, and we thank Anton Petcherski for sharing the CLN3 topology model.

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Back up information
Protein topology models and mutations
Wir gewinnen Menschen
Fortbildungsmaßnahmen bei Medizinern

CLN3 mutations causing retinal degeneration

**Proband 2691**
- G189R
- G189R

**CRD Proband SRF41**
- E295K
- S131R

**Proband 2044**
- c.125+1G>C
- R405W

**Proband 2055**
- Y322*
- V290L

**Proband 348**
- R405W
- R405W

Modified from: Cotman and Staropoli 2012 Clin Lipidol 7: 79-91
Wang et al. 2014 Hum Genet 133(3): 331-345
Human CLN3 protein – novel topology model with syndromic and non-syndromic mutations

Location of mutations: Ratajczak et al. 2014 PLoS ONE 9(7): e102593
Protter visualize proteoforms: Omasits et al. 2013 Bioinformatics 30(6): 884-886
Retina relevant information
The retina

- common architecture across many species
- macula mainly cones
- cones: daytime vision
- rods: low light sensors
- RPE: provides key support to photoreceptors
- 3 types of interneurons (horizontal cells, bipolar cells, amacrine cells)
- process RP signals to retinal ganglion cells
- RGCs and their axons form optic nerve

Light

R=rod; C=cone; B=bipolar cell; H=horizontal cell; A=amacrine cell; G=ganglion cell; M=Müller cell; Layers: INL=inner nuclear layer; GCL=ganglion cell layer; IPL and OPL=inner and outer plexiform layers

Wallace 2011 Stem Cells 29: 412-417
Macula and fovea

Apart from primates, **macula is absent!**

Fovea contains largest concentration of cones, responsible for central, high resolution daylight vision

Sung and Chuang 2010 J Cell Biol 190: 953-963
Vertebrate retinas: species-specific differences

Columnner unit comprises
- one Müller Glia cell
- few secondary neurons
- at least 1 ganglion cell
- species-specific variable number of rods/cones
Wir gewinnen Menschen
Fortbildungsmaßnamen bei Medizinern

Mitochondria & energy homeostasis in retina

- “Ellipsoid“ contains most of the transporters, glycolytic enzymes, **mitochondria**¹
- consumes nearly all O₂ available to the retina¹
- Retina relies on glucose, an essential substrate for retinal mitochondria¹
- **Mitochondrial respiration in photoreceptor mitochondria operates close to maximal capacity, with limited reserve capacity**²
- Retina also relies on aerobic glycolysis to produce lactate from glucose¹
- ATP consumption increases in darkness when cGMP activates Na⁺ and Ca²⁺ ion flow into outer segments of photoreceptors. ATP powers ion pumps to maintain ψm¹

¹Hurley et al. 2015 J Neurosci Res 93: 1079-1092
²Kooragayala et al. 2015 Invest Ophtalmol Vis Sci 56: 8428-8436
Retinal Ganglion Cell-loss in CLN3^{Δex7/8} mice

A. Cresyl violet staining of retinal ganglion cells in Cln3^{+/+} and Cln3^{-/-} mice.

B. Immunostaining for NeuN (red) and DAPI (blue) in Cln3^{+/+} and Cln3^{-/-} mice.

C. Immunostaining for Brn3a (red) and DAPI (blue) in Cln3^{+/+} and Cln3^{-/-} mice.

**Statistical significance**
Reduction of the inner but not outer retina and thinning of NFL/GCL/IPL and INL

Fingerprint profiles in GCL and INL

Groh et al. 2014 Acta Neuropathol Comm 2: 54
Optic nerve axonal perturbations in CLN3Δex7/8 mice

Axonal spheroids in 18-month-old mice

Other evidence supporting that loss of retinal function in CLN3 may, in part, be due to loss of retinal ganglion cells

- Optic disk pallor observed in most JNCL patients is suggestive of optic nerve degeneration
- CLN3 KO mice show pronounced loss of number of axons in the optic nerve

1 Hainsworth et al. 2009 Retina 29: 657-668
Groh et al. 2014 Acta Neuropathol Comm 2: 54
Retinal degeneration in $\text{CLN3}^{\Delta \text{ex7/8}}$ (C57Bl/6N) mice

dark-adapted (scotopic) ERG  
b-/a-wave amplitude ratio

light-adapted (photopic) ERG

Photopic ERG seems affected earlier;  
• consistent with Staropoli et al. 2012 PLoS ONE 7(6): e38310  
and Katz et al. 2008

CLN3 mutants causing RP or Cone-Rod Dystrophy could suggest primary defect in photoreceptors

Probands

2055 (Y322*/V290L), RP, no Batten at 40 and 45 years

348 (R405W/R405W) RP, no Batten in late 50s & 60s

2044 (c.125+1G>C/R405W) RP, no Batten at age 57

2691 (G189R/G189R) RP, no Batten at age 10

SRF41 (E295K/S131R) CRD, no Batten at age 20

Wang et al. 2014 Hum Genet 133(3): 331-45
Retina primary site of insult to visual system?

- \(\text{CLN3}^{\Delta\text{ex7/8}}\) mice\(^1\) and \(\text{Cln3}^{\Delta\text{ex1-6}}\) mice\(^2\): late and limited retinal degeneration
- \(\text{Cln3}^{\Delta\text{ex1-6}}\) mice: less neurons in retinal projection nucleus (LGN) in thalamus\(^3\)
- impaired axonal transport of amino acids from retina\(^3\)
- decreased optic nerve axonal density\(^4\)
- loss of thalamocortical neurons\(^5\)
- atrophy of retinal vasculature and optic nerve head in patients\(^6\)
- note: thalamic nuclei seem selectively vulnerable in a variety of LSDs\(^7\)

\(^1\) Staropoli et al. 2021 PLoS One 7(6): e38310
\(^2\) Seigel et al. 2002 Mol Cell Neurosci 19: 515–527
\(^3\) Weimer et al. 2006 Neurobiol Dis 22: 284-293
\(^5\) Pontikes et al. 2005 Neurobiol Dis 20: 823-826
\(^7\) Autti et al. 2007 Eur J Neurol 14: 447-450; Neuroradiology 49: 571-578; Sargeant 2016 Frontiers in Aging Neurosci 8:11

Scheme from: Feldheim and O’Leary 2010 Cold Spring Harbor Perspect Biol 2: a001768
RPE an important contributor to retinal damage?

**Phagosome degradation is deficient in CLN3Δex1-6 mice RPE and fibroblasts**

- SubC accumulation and fingerprint profiles in RPE lysosomes
- Abnormal accumulation of basally localized phagosomes in the RPE
- Defect in a late stage of fusion between phagosomes and lysosomes in RPE
- Phagosome processing defects in null-fibroblasts

Wavre-Shapton *et al.* 2015 HMG 24: 7060-7074
Open questions regarding retinal degeneration in CLN3 disease

Cell type(s) involved
- RPE ?
- Rod and Cones ?
- Horizontal cells ?
- Bipolar cells ?
- Amacrine cells ?
- Ganglion cells ?
- Müller cells ?
- LGN thalamic neurons ?

Mechanism driving cell-loss
- Energy homeostasis ?
- Defects in visual cycle ?
- Autoimmunity ?
- Intracellular transport ?
- Protein clearance ?
- Protein mistargeting ?
- Retrograde axonal ?

Storage material in NCLs
normal function of SubC and saposins
Saposins A-D

Glycosphingolipid activator proteins in lysosomal degradation pathway

Mainly A and D are part of lipofuscin deposits in various NCLs

Solubilizer (a) and liftase (b) model of SapC activating GCase

Subunit C is the rotor part of the mitochondrial $F_1F_0$ ATPase molecular motor complex

**Assembly of the molecular motor complex**

- Located in inner mitochondrial membrane
- The energy transmembrane proton-motive-force is generated by respiration and coupled mechanically to the synthesis of ATP from ADP and phosphate
- ATP synthesis occurs in membrane-extrinsic catalytic domain by rotating the asymmetrical central stalk in a clockwise direction at about 100 times/sec

Jonckheere *et al.* 2012 J Inherit Metab Dis 35: 211-225
Stewart *et al.* 2013 BioArchitecture 3: 2-12
F$_1$F$_0$ ATPase also part of energy-dissipating Mitochondrial Permeability Transition Pore

Opening of the MPTP results in

- Inner membrane collapse
- Uncoupling of the respiratory chain
- Halt of ATP synthesis
- Mitochondrial swelling
- Cell death

Kwong and Molkentin 2015 Cell Metab 21: 206-214
Bernardi et al. 2015 Physiol Rev 95: 1111-1155
Subunit C has multiple isoforms

- 3 Isoforms (P1-P3) encoded by 3 different nuclear genes located on different chromosomes
- Mature Subunit C has 76 amino acids
- P1-P3 have different N-terminal mitochondrial targeting peptides (P1, 61 AAs; P2, two splice variants with 82 and 123 AAs; P3, 67 AAs)
- All three forms are functional and contribute to the $F_0$ structure
- P1-P3 have variable expression patterns in different tissues
- Silencing of subunit C forms individually impairs ATP synthesis
- Isoforms do not cross-complement and are non-redundant
- Targeting peptides fused to GFP can rescue ATP synthesis in an isoform-specific fashion

$F_{1}F_{0}$ rotary ATPase found also on cell-surface

*Endothelial cells, adipocytes, hepatocytes, tumor cells.......*

**Figure 6.** Model of transendothelial transport of apoA-I and HDL. By lipidating apolipoprotein A-I (apoA-I) ABCA1 generates a particle that is processed by a mechanism which, like the transport of mature HDL, involves ABCG1 and SR-BI. The $F_{0}F_{1}$ ATPase hydrolyzes ATP on binding of apoA-I. The produced ADP binds to P2Y$_{12}$ and stimulates the internalization and transendothelial transport of lipidated apoA-I and HDL via the activation of G proteins.

Cavelier et al. 2013 Arterioscl Throm Vasc Bio 32: 131-139
More questions than answers

- What role does the prosequence play and is one particular SubC isoform giving rise to storage? (prosequence has key role in targeting to mitochondria\(^1\))

- Does ROS damage in mitochondria trigger SubC clearance via a mitophagy pathway (e.g. involving mitochondria derived vesicles/MDVs), and do CLN3-lacking lysosomes fail to degrade SubC?

- Is stored SubC mitochondrial or plasma membrane-derived?

- Is mitophagy/MDV pathway enhanced?

- What role plays aberrant lysine tri-CH\(_3\)-ation* of SubC in NCLs?\(^2\)

\(^*\)K-CH\(_3\)-methylation often changes protein-protein interactions, either directly or by influencing other PTMs (Moore & Gozani 2014 BBA online)

\(^1\)Zhang et al. 2013 FEBS 280: 3425-3435
\(^2\)Katz et al. 1995 Biochem J 310: 887-892
CLN3 brain pathology in context of cortex circuitry
Loss of thalamocortical neurons\(^1\)
- Loss of neurons in VPM thalamic nucleus is reported in a variety of LSDs\(^2\)

\(^1\)Pontikes et al. 2005 Neurobiol Dis 20: 823-826
Functional relevance of Layer Va neurons - Output

Main cortical output
(example from Barrel cortex)
To and from other somatosensory & motor cortices

Contribute and important source of projections to other cortical areas

- Within column vertical signaling to deeper layers V
- Horizontal signaling between columns

Feldmeyer 2012 Frontiers in Neuroanatomy 6(24): 1-17
Miscellaneous
Overlap of CLN3 disease with later-onset neurodegenerations

Are we dealing with gene product-specific mechanisms in diseases or rather a general inefficiency in lysosomal flux that occurs across diseases?

Local impairment of retrograde axonal transport of lysosome precursors blocks their maturation¹

¹Gowrishankar et al. 2015 PNAS 112 (28): E3699-3708
Defects in a host of mechanisms can lead to LSD

Mutations of lysosomal hydrolases, lysosomal membrane proteins or non-lysosomal proteins

Primary storage (for example, GAGs, glycogen) → Secondary storage (for example, lipids) → Lysosomal dysfunction → Autophagy impairment → Tertiary storage (cytosolic autophagic substrates) → Pathogenic cascades

- Ca^{2+} defects
- Impaired signalling
- Lysosomal membrane permeabilization