Clinical challenges and future therapeutic approaches for the neuronal ceroid lipofuscinoses (Batten disease)

Sara E Mole PhD, Glenn Anderson FIBMS, Heather A Band MSc, Samuel F Berkovic MD, Jonathan D Cooper PhD, Sophia-Martha kleine Holthaus PhD, Tristan R McKay PhD, Diego L Medina PhD, Angela Schulz MD PhD, Alexander J Smith PhD, Ahad A Rahim PhD,

MRC Laboratory for Molecular Cell Biology and UCL GOS Institute of Child Health, University College London, UK (Prof Sara E. Mole, PhD)
Department of Histopathology, Great Ormond Street Hospital, London, UK (Glenn Anderson, FIBMS)
Batten Disease Family Association (BDFA), Farnborough, UK (Heather A. Band, MSc)
Epilepsy Research Centre, University of Melbourne (Austin Health), Australia (Prof Samuel F. Berkovic, MD)
Department of Pediatrics, Washington University School of Medicine, USA (Prof Jonathan D. Cooper, PhD)
UCL Institute of Ophthalmology, University College London, UK (Sophia-Martha kleine Holthaus, PhD, Alexander J. Smith, PhD)
Centre for Bioscience, Manchester Metropolitan University, Manchester, UK (Prof Tristan R. McKay, PhD)
Telethon Institute of Genetics and Medicine, Naples, Italy (Diego L. Medina, PhD)
Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Germany (Angela Schulz, MD PhD)
UCL School of Pharmacy, University College London, UK (Ahad A. Rahim, PhD)
Correspondence to: Prof Sara E. Mole, MRC Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, UK

Prof Sara E. Mole, PhD, MRC Laboratory for Molecular Cell Biology, UCL GOS Institute of Child Health, Department of Genetics, Evolution & Environment, University College London, London WC1E 6BT, UK
Glenn Anderson, FIBMS, Department of Histopathology, Great Ormond Street Hospital, London WC1N 3JH, UK
Heather A. Band, MSc, Batten Disease Family Association (BDFA), The Old Library, 4 Boundary Road, Farnborough, GU14 6SF, UK
Prof Samuel F. Berkovic, MD, Epilepsy Research Centre, University of Melbourne (Austin Health), 245 Burgundy Street, Heidelberg, Victoria 3084, Australia
Prof Jonathan D. Cooper, PhD, Department of Pediatrics, Washington University School of Medicine, 660 S Euclid Ave, St Louis, MO 63110, USA
Sophia-Martha kleine Holthaus, PhD, UCL Institute of Ophthalmology, University College London, London EC1V 9EL, UK
Prof Tristan R. McKay, PhD, Centre for Bioscience, John Dalton Building, Manchester Metropolitan University, Manchester, M1 5GD, UK
Diego L. Medina, PhD, Telethon Institute of Genetics and Medicine, Via Campi Flegrei 34, 80078 (NA), Italy
Angela Schulz, MD PhD, Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg 20246, Germany
Alexander J. Smith, PhD, UCL Institute of Ophthalmology, University College London, London EC1V 9EL, UK
Ahad A. Rahim, PhD, UCL School of Pharmacy, University College London, London, WC1N 1AX, UK

Abstract

Treatment of the neuronal ceroid lipofuscinoses (NCL, Batten disease) is at the start of a new era due to recent therapeutic advances relevant to this group of inherited neurodegenerative and life-limiting disorders that affect children. Diagnostic investigations are changing with the inclusion of comprehensive and simultaneous DNA-based testing for many genes. The identification of disease-causing mutations in 13 genes provides a basis for understanding the molecular mechanisms underlying this group of diseases as well as the development of targeted therapies. These include enzyme replacement therapies, gene therapies targeting the brain and the eye, cell therapies, and the search for pharmacological agents that modulate defective molecular pathways. The first approved treatment is an intracerebroventricularly administered enzyme for CLN2 disease that delays symptom progression. Efforts are underway to make similar progress for other forms of NCL. Such therapeutic developments will change medical practice to enable earlier diagnosis and introduction of innovative therapies.

Introduction

The neuronal ceroid lipofuscinoses (NCL, Batten disease) are a group of monogenic inherited neurodegenerative disorders that mostly present in the first decade (Table 1).[1] They share a broadly similar clinical presentation characterised by seizures, visual failure, and a progressive decline in cognitive and motor abilities due to progressive neuronal death. However, these disorders also show variation, most notably in the age of onset, rate of disease progression and first symptoms.[1] Regardless, all forms of NCL are fatal and no curative treatments are available.

Pathologically, these disorders are profoundly neurodegenerative, and share a common hallmark of accumulation of autofluorescent material in lysosomes, so-called ceroid and lipofuscin, that has a typical ultrastructural appearance under electron microscopy but does not appear to relate directly to neuron loss.[2] Although the precise underlying mechanisms remain elusive, disease-causing mutations have been revealed in 13 different genes.[3;4] The increasing implementation of next generation sequencing panels and exome sequencing as essential diagnostic tools is leading to more diagnoses of patients with NCL especially including those varying from the classically recognised phenotypes due to so-called 'milder' mutations (Table 1). This has necessitated a change in disease nomenclature to one based on the gene defect augmented with age of presentation.[4]

There has been an emphasis on understanding the staging of these disorders and molecular pathways, and advancing experimental therapies such as enzyme replacement and gene therapy in animal models,[5-8] together with the establishment of patient registries[9,10] and disease rating scales.[11,12] These efforts have culminated in the approval (FDA and EMA) of the first treatment – enzyme replacement for CLN2 disease. Much preclinical work followed by clinical trials is required before treatment is available for all forms of NCL. This Review focuses on clinical features, genotype-phenotype correlations, diagnosis and discusses the most promising therapeutic approaches of NCL.

Clinical features

All types, except for a rare congenital form (CLN10 disease), have in common a normal psychomotor development before onset of first symptoms.[1] Age at disease onset ranges from birth to adulthood for most genes, for some even as late as >60 y (Table 1). The main symptoms are the combination of at least two of dementia, epilepsy, motor deterioration, and visual loss.[1] The order in which symptoms occur is variable and depends on the combination of the underlying mutations, which can affect age at onset and the disease phenotype.[1] The number of phenotypes for NCL diseases is growing with widest age of onset for NCLs caused

by lysosomal enzyme deficiencies (Table 1).[3] Increasing knowledge about the natural history of the different forms of NCL has shown that for some genes the phenotype severity can vary substantially even between siblings, as in juvenile CLN3 disease[13]. Symptoms can be also outside the CNS. For example, cardiac involvement has been reported in adolescent and adult patients with CLN3 disease[14,15] and can be treated, eg, fitting a pacemaker has improved psychomotor abilities.[13]

First symptoms in classic infantile (age of onset 6-24 months) and late infantile (age of onset 2-5 years) phenotypes are slowing of psychomotor development, quickly followed by standstill, then progressive loss of acquired psychomotor abilities and onset of epilepsy, followed by vision loss.[1] This regression can be considered a side effect of antiepileptic drugs, delaying diagnosis. In contrast, first symptoms in the juvenile phenotype (age of onset 5-7 years) are usually visual loss, followed by dementia and behaviour changes, then loss of motor skills and epilepsy in the early teenage years.[1] In recessive adult phenotypes (>16 years, Kufs disease), visual loss is usually absent and patients present with progressive myoclonus epilepsy (type A) or dementia with motor decline (type B) typically around age 30 years but this may range from 16 years to the 50s.[16]

Even though all types of NCLs share a similar set of clinical features (eg, dementia, epilepsy, motor deterioration, and visual loss), their clinical severity and presentation often differs even for those caused by mutations in the same gene. Delay in expressive language development is the first sign of regression of psychomotor function in 83% of classic late infantile CLN2 disease and could enable earlier diagnosis.[9] Children with a combination of language acquisition delay and new onset of seizures should be tested for CLN2 disease.[9,17] Epilepsy is therapy-resistant in almost all NCL patients, with especially high seizure frequency and severity in late infantile CLN2 disease up until late disease stages.[17] However, in infantile CLN1 disease, seizure frequency tends to decrease in the later disease stages and in patients with classic juvenile CLN3 disease seizures are infrequent with only mild worsening with increasing age.[18,19] Therapy with more than two anticonvulsants may result in increased side effects rather than reduction of seizures.[1] Some anticonvulsants are particularly recommended - valproate and lamotrigine for CLN2 and CLN3 diseases;[17,18] others (eg, carbamazepine, gabapentin, phenytoin, vigabatrin) may have negative effects, for example exacerbating myoclonic seizures in patients with CLN2 or CLN3 disease.[17,18,20] Also, as disease progresses, anticonvulsive drugs that have been tolerated and effective might cause new side effects, and should be reconsidered critically if symptoms of the disease worsen.[19]

Motor signs range from ataxia (including dysmetria, dysarthria), dysphagia, to myoclonus, chorea, tremor and dystonia, especially in classic infantile and late infantile patients.[1] Others include Parkinsonism, especially in juvenile CLN3 disease, and some stereotypical movements have been reported in various types with late infantile and juvenile age of onset.[1]

Wider NCL disease phenotypes are increasingly being recognised where one of the clinical hallmarks might be more predominant with others missing. For example, in a rare type of CLN2 disease (autosomal recessive spinocerebellar ataxia type 7) ataxia is the primary phenotype with no accompanying epilepsy or vision loss,[21] and in one type of juvenile CLN2 disease associated with a particular mutation, survival is into the fourth decade.[22] Some patients with mutations in *CLN3* have isolated nonsyndromic retinal degeneration only,[23] and others experience visual failure, seizures, and cardiac involvement but no motor deterioration even many decades after disease onset.[24] A pathophysiological link between NCL and an adult degenerative dementia is assumed given that homozygous mutations in *GRN* cause an NCL presenting at around 20 years with visual failure, seizures and ataxia, whereas heterozygous mutations in this gene are a common cause of frontotemporal lobar degeneration with TDP-43 inclusions.[3,25]

Neuroimaging and EEG findings

Brain MRI can be normal in the early stages of the disease or might show some unspecific signs, such as periventricular intensity changes in the early stages of CLN2 disease.[26] Whilst MRI is not sensitive or specific for early diagnosis, it is an excellent tool to objectively monitor the progression of brain changes, particularly with recent advances in resolution and processing, and neuroimaging techniques such as Diffusion Tensor

Imaging to assess disorganisation of white matter tracts as well as atrophy. Two prospective studies in patients with CLN2 disease have shown that the loss of cortical grey matter volume with age may be a sensitive parameter to monitor disease progression.[26,27]

EEG can be helpful in early diagnosis of some NCLs. For example, characteristic posterior spike waves are seen with low frequency photic stimulation early in CLN2 disease (in 13 [93%] of patients in one study).[28] Finding these in a young child with new onset of seizures should trigger testing for CLN2 disease.[28,29] Photosensitivity with low frequency stimulation has also been reported in patients with CLN6 disease, and especially in those with adult CLN6 disease (type A).[30-32] In rapidly progressing NCL forms such as infantile CLN1 disease, early abnormalities disappear as neurons die, leading to a characteristic 'flat EEG' in advanced disease stages.[1] Due to the underlying neurometabolic disease, antiepileptic drugs will not lead to normalisation of the EEG findings.[1] Monitoring by EEG may be useful for detecting signs of encephalitis (which could be a rare consequence of antiepileptic drugs such as valproate in advanced late infantile CLN2 disease[19] or theoretical allergic reaction to new treatments).

Genetic basis and genotype-phenotype correlations

The NCLs are monogenic disorders, so each is in effect a separate disease entity. The genes associated with NCL encode apparently unrelated proteins that include soluble lysosomal enzymes and membrane proteins localised in various organelles, including the lysosome (Table 1).[3,33,34] All types of NCL are autosomal recessive with the exception of one rare adult onset NCL, CLN4 disease.[3] For most NCLs there is a recognizable 'classic' disease phenotype associated with complete loss of gene function, due to intracellular mislocalisation or degradation of the mutant protein.[3] Later onset forms of disease, that may also have a more protracted overall course, also occur and certain anticipated 'classic' phenotypes may be absent as a result of so-called 'milder' mutations that do not completely abolish protein function (Table 1).[16] There are examples of mutations associated with a specific phenotype such as a missense mutation in CLN8 or the 1 kb intragenic deletion that underlies the most common form of NCL, juvenile CLN3 disease.[3] The most prevalent mutations are the 1 kb deletion in *CLN3* and two mutations in *CLN2*.[3] The combination of data collected in disease registries such as DEM-CHILD (www.dem-child.eu/),[9,10] a mutation database (www.ucl.ac.uk/ncl) and application of disease rating scales,[11,12] allows correlations to be drawn between the disease and underlying mutations to provide some guidance to the expected disease course and give an approximation of frequency and occurrence in specific ethnic groups. As the phenotypic spectrum broadens, increasing overlap with other rare and also common diseases, such as retinal dystrophies, through shared disease mechanisms is expected[35] which may lead to better understanding of all these diseases.

Diagnosis

Diagnostic strategies vary according to the age of the patient, and can be guided by diagnostic algorithms.[1] The investigations and order of diagnostic tests are changing as new comprehensive approaches become available. Enzyme testing can rapidly confirm some NCLs (Table 1). The advent of new DNA technologies allows testing for many genes in a single step regardless of how typical the presentation is (eg, a panel can contain NCL genes amongst a larger group of syndromic and nonsyndromic inherited epilepsies). Blood film examination allows identification of vacuolated lymphocytes, a common feature of CLN3 disease.[36] Ultrastructural examination of a skin biopsy or blood sample remains helpful for confirmation of NCL disease for atypical forms that are not enzyme deficiencies or have not yet received a genetic diagnosis (Table 1). Extracerebral storage is readily detected in childhood NCLs but not necessarily in NCL presenting in adulthood.[16]

Prenatal diagnosis is available and can be offered to families with a history of NCL disease. Preimplantation genetic diagnosis[37] or a combination of enzyme assay and mutational analysis, perhaps with ultrastructural examination of chorionic villus samples obtained at 12-15 weeks gestation, can provide a rapid diagnosis.

Pathology

Neuronal loss is profound and widespread in most patients with NCL resulting in cortical grey matter and cerebellar atrophy, and secondary ventricular enlargement. The degree of atrophy and ventricular enlargement varies between forms, but is typically preceded by clinical symptoms.[2,38] Nevertheless, this loss of neurons in the cerebral and cerebellar cortices is selective in the childhood NCLs,[2,38] with the thalamus also severely affected.[2,38] Pronounced microglial and astrocytic activation occurs and precedes neuron loss, accurately predicting its distribution.[39] Indeed, recent evidence suggests glial dysfunction may contribute to this neuron loss.[16,39,40] Brain atrophy of the cerebral cortex and enlargement of the subarachnoid space and ventricles progresses throughout the disease.[2,38] Atrophy of the cerebellum is variable but evident in the latter stages of all NCLs.[26,27,41] Neuronal depletion in the retina commences in the photoreceptor outer segments, proceeding to the inner segments, nerve cell bodies and ganglionic layer, occurring early in CLN3 disease[42,43] and after other symptoms in other types of NCL.[1,38] In adult onset NCL, the loss of neurons may not be as pronounced[2,38] and brain atrophy[2,38] less obvious. In all forms of NCL lipopigment storage material accumulates in macrophages, neurons and some somatic tissues including vascular endothelial and smooth muscle cells.[38] Lipopigments also accumulate in the CNS with age or other conditions, such as mucopolysaccharidoses or GM_1-gangliosidosis,[38] and careful neuropathological assessment is needed to avoid misdiagnosis.[16]

Genetically modified or spontaneous mutant mice exist for all NCL genes[5,44], and their characterisation has provided insights into the staging of neuropathological changes.[6,7] This has revealed differences in the extent, timing and nature of changes (eg, the nature and extent of glial activation)[39,40] leading to a similar pathological end point, despite common pathological themes. The spinal cord has recently been identified as exhibiting significant pathology including loss of neurons, activation of microglia and build-up of storage in Cln1 mice,[45] with impairments of the peripheral nervous system evident resembling paroxysmal sympathetic hyperactivity in juvenile CLN3 disease.[46] Pathology has also been described in somatic tissues including the heart[14] and establishing its relationship to events in the CNS will be important. Knowing where and when pathology occurs is crucial for the effective targeting of experimental therapies. In this respect, larger animal models are proving important for this.[5,8] In addition to their larger and more complex brains for scaling up the delivery of therapies, the extent of neuropathology and its regionalised nature is more pronounced in the brains of sheep and dogs with NCL than in the corresponding mouse model, and appears to more closely resemble that seen in human cases.[6,7] With the ability to engineer genetically modified pigs and sheep, such species may prove invaluable for further understanding the effects of disease for improving therapies.

Technical advances

The development of induced pluripotent stem cells (iPSC) technology is a step change in understanding and treating genetic disease using cell models. Cells obtained from a skin or blood sample can be genetically reprogrammed to a state of pluripotency and these iPSC are capable of differentiating to virtually any cell type including neuronal sub-types.[47] Neural cells differentiated from patient-derived iPSC display autophagic, lysosomal maturation and mitochondrial quality control defects for CLN3 disease and TPP1 enzyme deficiency for CLN2 disease.[48] In 2017, two repositories of NCL iPSC lines became available to academic researchers. The Human Pluripotent Stem Cell Initiative in the UK (http://www.hipsci.org), released 12 lines from patients with CLN1, CLN3, CLN6, CLN7, CLN8 and CLN10 mutations and in the USA the Beyond Batten Disease Foundation, in alliance with the New York Stem Cell Foundation, publicised a resource of iPSC generated from 24 individuals from CLN3 disease families (https://nyscf.org). However, such patient-derived cells are genetically diverse. The use of CRISPR/Cas9 gene editing technology to introduce specific genetic changes into a parental pluripotent line allows the production of isogenic lines representing selected mutations of NCL genes that can more readily be compared.[49] Such repositories, along with further individually generated iPSC lines[50] and engineered stem cells represent the next generation of cellular tools to better understand NCL aetiology. Patient iPSC-derived neural cells have already been used as a therapeutic evaluation platform for the NCLs, including high throughput screening (Appendix).

New experimental therapies

There have been extensive preclinical studies in the path to developing new treatments for the NCLs (Appendix). The current main translational approaches are summarised in the Glossary. Those that have reached clinical trials are described below, beginning with the most advanced and promising approaches, with current clinical trials summarised in Table 2.

Enzyme replacement therapy

Intracerebroventricularly administered enzyme replacement therapy in CLN2 disease is the first FDA and EMA approved treatment for any NCL, with treatment expected to be lifelong.[10,51] The deficient enzyme, tripeptidyl peptidase 1 (TPP1), is administered over 4 h as a recombinant proenzyme via a Rickham or Ommaya reservoir into the lateral cerebral ventricles at a dose of 300 mg protein every two weeks.[10] Safety and efficacy were tested in an open-label, dose-escalation study lasting up to 12 weeks[10] that enrolled 24 patients with CLN2 disease between 3 and 16 years of age, followed by an open-label extension study of at least 48 weeks. Most patients (21, 92%) had lost some language or motor skills according to a long established CLN2 Clinical Rating Scale by trial start. Of the 23 (96%) patients who completed the study,[10] 18 (78%) experienced either a slower than expected or stabilization of the disease measured by rating motor and language function after at least 96 weeks of treatment (median 116, range 96-145 weeks) at the 300 mg dose.[10] Notably, two of those patients who had the highest initial baseline scores maintained these for the duration of the study, indicating that starting treatment early is likely to be most beneficial. Antibodies developed against the drug but were not linked to safety concerns or a poor treatment outcome.[52] The 23 patients continue to be treated and followed to evaluate the long-term efficacy and safety (NCT02485899). A second study has begun to monitor the effects of beginning treatment earlier in the disease course in a separate group of patients (NCT02678689) (Table 2).[10] The cross-corrective approach of enzyme replacement therapy depends on delivered enzyme being recognised by receptors on the surface of cells, taken into the cell and trafficked to the lysosome, and the same enzyme replacement approach might be suitable for other types of NCL caused by mutations in lysosomal enzymes (Table 1).

Gene therapy

Preclinical gene therapy studies for the NCLs have largely focused on targeting the brain for those caused by lysosomal enzyme deficiencies, which should be less challenging than those caused by mutations in integral transmembrane-bound proteins due to advantages provided by cross-correction.[53] Clinical and pre-clinical studies suggest that gene therapy approaches targeting only the brain[10,54] will be unlikely to provide therapeutic benefit for the NCL-related retinal degeneration. A combinatorial gene therapy approach separately treating the brain and the eye is likely to be required for optimal therapy.[55]

The first gene therapy trial was conducted in ten patients with CLN2 disease between 3 to 10 years of age (NCT00151216) essentially to test the safety of introducing a gene therapy vector designed to express TPP1 into the brain. The particular vector was found to be safe but there was no slowing of disease progression.[56] Given the recent experience with ERT[10] a vector and delivery that results in TPP1 reaching more cells is likely to be required to achieve better therapeutic outcome. Orphan drug designation has been granted for AAV9-mediated gene therapy for CLN1 and CLN3 diseases, and clinical trials are anticipated. A phase I/II trial (NCT02725580) in patients with CLN6 disease to assess intrathecal administration of AAV9-mediated gene therapy is ongoing (Table 2). The preceding animal studies in which efficacy of these vectors were tested are not yet published, making critical evaluation of the likelihood of clinical benefit difficult.[57,58]

Pharmacological approaches

As for other lysosomal storage disorders (LSDs), most of the pharmacological treatments for NCLs are palliative, focused on minimising clinical symptoms such as seizures, and not targeting the underlying cause of the disease.[1] Animal models and clinical observations have provided a variety of potential targets to modulate disease (eg, anti-inflammatories, molecular chaperones, enzyme mimics, Bcl-2 upregulators, NMDA and AMPA receptor antagonists, calcium channel blockers, immunosuppressants, antioxidants).[59-64]

However, follow-up studies using these compounds have not shown clinically meaningful benefits for patients,[65,66] including targeting neuroinflammation in the eye for CLN3 disease.[67] Some parents giving the drug flupirtine to their children with juvenile CLN3 disease have anecdotally reported benefit, however quantitative, prospectively obtained data did not show any change in disease progression that could be attributed to this drug.[68] A clinical trial treating 19 children with juvenile CLN3 disease with a non-steroidal immuno-suppressive agent, mycophenolate mofetil, over two 8-week treatment periods with a 4-week intervening washout[63,64] showed this drug was well tolerated, but there was no clinical benefit.[66] A trial in 9 children with CLN1 disease testing the combination of phosphocysteamine and N-acetylcysteine, reported little clinical benefit (Table 2).[65] Thus, to date no efficacy is supported for any of these pharmacological treatments. Rather than targeting secondary downstream effects success is more likely when the underlying disease mechanisms are better understood.

Applying a classical drug discovery approach (disease-target-drug) to the NCLs is challenging as little is known about which intracellular pathways are affected or if they are druggable. In addition, drugs need to cross the blood-brain-barrier to reach the brain. Molecular pathways common to neurodegenerative diseases (neuroinflammation, impairment of autophagy, defects in endocytic trafficking, mitochondrial alterations, or impairment in calcium homeostasis)[69,70] might be promising druggable targets and could be entry points to cell-based phenotypic screening approaches. For example, a screen of bioactive compounds using cells from the Cln3 mouse model that had elevated levels of a marker protein for autophagy identified modulators of autophagy that included some known to target ion channels and especially calcium channels and proteolysis inhibitors.[71,72] Discovery of transcriptional regulation of lysosomal biogenesis and degradative function by the transcription factor EB has opened a new avenue for therapeutic intervention in LSDs.[73-76] For example, stimulating TFEB with trehalose affords benefits in Cln3[75] and mucopolysaccharidosis III[77] disease mice. The FDA-approved lipid lowering drug gemfibrozil also activates the TFEB pathway[78,79] and has moderate beneficial effects in Cln2 mice.[80,81] A clinical trial to test efficacy of these approaches is yet to be launched in any form of NCL.

Cell-based therapy
While the original hope of cell-based therapy lies in its theoretical ability to replace cells lost in advanced disease, the degree of replacement required for lost CNS neurons would need to be substantial and even in mice is very limited.[57] The aim of stem cell trials for the NCLs is to preserve remaining function by providing cells that secrete a missing enzyme.[58] A phase I trial established the safety of neuroprogenitor cell implantation in six patients with advanced CLN1 or CLN2 disease (Table 2).[82] There was little clinical benefit, perhaps because donor engraftment was low and migration limited.[82] There are no results of any other trials of stem cell therapy for any form of NCL. There is an ongoing phase 1 trial (NCT01586455) testing whether transplantation of human placental-derived stem cells benefits patients with a range of diseases that has the option of including the NCLs (Table 2).

Conclusions and future directions

The genetic basis of the NCLs is now well understood with the underlying genes encoding mainly lysosomal enzymes or membrane proteins. Correlation of genotype with clinical phenotype has broadened recognition of an NCL disorder and at the same time provided the gene-based focus and consideration of pathologic targets required for therapeutic advances. This knowledge has prompted therapeutic development beyond current palliative treatments, even without understanding disease mechanisms. Strategies for those NCLs caused by defects in lysosomal enzymes benefit from the phenomenon of 'cross-correction', leading to the first approved treatment for CLN2 disease which delivers recombinant protein directly into the brain at regular intervals. Development of a gene supplement approach is at the preclinical stage for many NCLs, with a clinical trial into the brain underway for CLN6 disease. The results of clinical trials using pharmacological treatments are less convincing, perhaps because primary disease targets or pathways remain to be elucidated. Cell-based therapies have the potential to deliver lysosomal enzymes continually, and may be especially useful for treatment beyond the CNS, but are at a very early stage of development.

To have a long-term clinical benefit it is clear that treatment for the NCLs must begin early, ideally before any symptoms. This will require rapid and earlier diagnosis, which is aided by advances in DNA-based approaches, and highlights the importance of developing appropriate newborn screening.[83,84] It will also be important to consider patients with atypical milder and adult onset forms who may particularly benefit from new treatments.[16] International cooperation is contributing to the ongoing collection of natural history data for all NCL types into the Dementia in Childhood database, which can provide necessary control data for use in future clinical trials as well as data to increase understanding of the spectrum of each genetic type.[10] A natural history database helps to solve the ethical dilemma of treating a proportion of trial patients with placebo so risking rapid progression to death, and speeds up the development of therapy options in rare diseases where availability of suitable patients for a trial can be a limiting factor.

Treatment for the NCLs must reach the most vulnerable cells in the brain and eye, with further study needed to fully understand the burden of disease outside the CNS and the need to target the periphery. Fortunately, new vectors are emerging for gene supplementation therapies that increase the ability to infect cells within the brain or eye.[85] This needs to be balanced against toxicity arising from producing too much recombinant protein within sensitive cells.[86] An ideal least-invasive therapy would be a set of one-off or infrequent gene therapies that together target the whole body supplemented by, probably lifelong, drug-based modulation. Understanding the molecular basis of the NCLs and the means to assess the effectiveness of any new treatment at a molecular level should be a future priority. This is required for a breakthrough in pharmacological treatments that target pathways close to the underlying defect. Such advances will benefit basic research and especially impact medical practice for the NCLs in terms of requiring early diagnosis and providing the first options of disease prevention.

Search strategy and selection criteria:

Articles for this Review were identified by searches of PubMed between 1 Jan 2012 and 30 Sept 2018 using the search terms "ceroid", "Batten", "NCL", "CLN", CLN1, CLN2, CLN3, CLN4, CLN5, CLN6, CLN7, CLN8, CLN10, CLN11, CLN12, CLN13. The final reference list was generated on the basis of relevance to the topics covered in this Review.

References:

1. Schulz A, Kohlschutter A, Mink J, Simonati A, Williams R. NCL diseases - clinical perspectives. *Biochim Biophys Acta* 2013; **1832**: 1801-6.
2. Radke J, Stenzel W, Goebel HH. Human NCL Neuropathology. *Biochim Biophys Acta* 2015; **1852**(10 Pt B): 2262-6.
3. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochim Biophys Acta* 2015; **1852**(10 Pt B): 2237-41.
4. Williams RE, Mole SE. New nomenclature and classification scheme for the neuronal ceroid lipofuscinoses. *Neurology* 2012; **79**(2): 183-91.
5. Faller KM, Gutierrez-Quintana R, Mohammed A, et al. The neuronal ceroid lipofuscinoses: Opportunities from model systems. *Biochim Biophys Acta* 2015; **1852**(10 Pt B): 2267-78.
6. Palmer DN, Barry LA, Tyynela J, Cooper JD. NCL disease mechanisms. *Biochim Biophys Acta* 2013; **1832**(11): 1882-93.
7. Cooper JD, Tarczyluk MA, Nelvagal HR. Towards a new understanding of NCL pathogenesis. *Biochim Biophys Acta* 2015; **1852**(10 Pt B): 2256-61.
8. Weber K, Pearce DA. Large animal models for Batten disease: a review. *J Child Neurol* 2013; **28**(9): 1123-7.
9. Nickel M, Simonati A, Jacoby D, et al. Disease characteristics and progression in patients with late-infantile neuronal ceroid lipofuscinosis type 2 (CLN2) disease: an observational cohort study. *Lancet Child Adolesc Health* 2018; **2**(8): 582-90.
10. Schulz A, Ajayi T, Specchio N, et al. Study of Intraventricular Cerliponase Alfa for CLN2 Disease. *N Engl J Med* 2018; **378**(20): 1898-907.
11. Steinfeld R, Heim P, Von Gregory H, et al. Late infantile neuronal ceroid lipofuscinosis: Quantitative description of the clinical course in patients with CLN2 mutations. *Am J Med Genet* 2002; **112**(4): 347-54.
12. Kwon JM, Adams H, Rothberg PG, et al. Quantifying physical decline in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Neurology* 2011; **77**(20): 1801-7.
13. Lebrun AH, Moll-Khosrawi P, Pohl S, et al. Analysis of potential biomarkers and modifier genes affecting the clinical course of CLN3 disease. *Mol Med* 2011; **17**: 1253-61.
14. Ostergaard JR, Rasmussen TB, Molgaard H. Cardiac involvement in juvenile neuronal ceroid lipofuscinosis (Batten disease). *Neurology* 2011; **76**(14): 1245-51.
15. Dilaveris P, Koutagiar I, Aggeli C, Sideris S, Gatzoulis K, Stefanadis C. Severe sinus node dysfunction in a patient with juvenile neuronal ceroid lipofuscinosis. *Int J Cardiol* 2014; **174**(1): 143-6.
16. Berkovic SF, Staropoli JF, Carpenter S, et al. Diagnosis and misdiagnosis of adult neuronal ceroid lipofuscinosis (Kufs disease). *Neurology* 2016; **87**(6): 579-84.
17. Williams RE, Adams HR, Blohm M, et al. Management Strategies for CLN2 Disease. *Pediatr Neurol* 2017; **69**: 102-12.
18. Augustine EF, Adams HR, Beck CA, et al. Standardized assessment of seizures in patients with juvenile neuronal ceroid lipofuscinosis. *Dev Med Child Neurol* 2015; **57**(4): 366-71.
19. Johannsen J, Nickel M, Schulz A, Denecke J. Considering Valproate as a Risk Factor for Rapid Exacerbation of Complex Movement Disorder in Progressed Stages of Late-Infantile CLN2 Disease. *Neuroped* 2016; **47**(3): 194-6.
20. Mole SE, Williams RE, Goebel HH, editors. The neuronal ceroid lipofusinoses (Batten disease). 2nd ed. Oxford: Oxford University Press; 2011.
21. Sun Y, Almomani R, Breedveld GJ, et al. Autosomal recessive spinocerebellar ataxia 7 (SCAR7) is caused by variants in TPP1, the gene involved in classic late-infantile neuronal ceroid lipofuscinosis 2 disease (CLN2 disease). *Hum Mutat* 2013; **34**(5): 706-13.
22. Kohan R, Carabelos MN, Xin W, et al. Neuronal ceroid lipofuscinosis type CLN2: a new rationale for the construction of phenotypic subgroups based on a survey of 25 cases in South America. *Gene* 2013; **516**(1): 114-21.
23. Ku CA, Hull S, Arno G, et al. Detailed Clinical Phenotype and Molecular Genetic Findings in CLN3-Associated Isolated Retinal Degeneration. *JAMA ophthalmology* 2017; **135**(7): 749-60.
24. Cortese A, Tucci A, Piccolo G, et al. Novel CLN3 mutation causing autophagic vacuolar myopathy. *Neurology* 2014; **82**(23): 2072-6.

25. Smith KR, Damiano J, Franceschetti S, et al. Strikingly different clinicopathological phenotypes determined by progranulin-mutation dosage. *Am J Hum Genet* 2012; **90**(6): 1102-7.

26. Dyke JP, Sondhi D, Voss HU, et al. Brain Region-Specific Degeneration with Disease Progression in Late Infantile Neuronal Ceroid Lipofuscinosis (CLN2 Disease). *Am J Neuroradiol* 2016; **37**(6): 1160-9.

27. Lobel U, Sedlacik J, Nickel M, et al. Volumetric Description of Brain Atrophy in Neuronal Ceroid Lipofuscinosis 2: Supratentorial Gray Matter Shows Uniform Disease Progression. *Am J Neuroradiol* 2016; **37**: 1938-43.

28. Specchio N, Bellusci M, Pietrafusa N, Trivisano M, de Palma L, Vigevano F. Photosensitivity is an early marker of neuronal ceroid lipofuscinosis type 2 disease. *Epilepsia* 2017; **58**(8): 1380-8.

29. Albert DV, Yin H, De Los Reyes EC, Vidaurre J. Unique Characteristics of the Photoparoxysmal Response in Patients With Neuronal Ceroid Lipofuscinosis Type 2: Can EEG Be a Biomarker? *J Child Neurol* 2016; **31**(13): 1475-82.

30. Canafoglia L, Gilioli I, Invernizzi F, et al. Electroclinical spectrum of the neuronal ceroid lipofuscinoses associated with CLN6 mutations. *Neurology* 2015; **85**(4): 316-24.

31. Ozkara C, Gunduz A, Coskun T, et al. Long-term follow-up of two siblings with adult-onset neuronal ceroid lipofuscinosis, Kufs type A. *Epileptic Disord* 2017; **19**(2): 147-51.

32. Lv Y, Zhang N, Liu C, Shi M, Sun L. Occipital epilepsy versus progressive myoclonic epilepsy in a patient with continuous occipital spikes and photosensitivity in electroencephalogram: A case report. *Medicine (Baltimore)* 2018; **97**(15): e0299.

33. Huber RJ, Mathavarajah S. Cln5 is secreted and functions as a glycoside hydrolase in Dictyostelium. *Cell Signal* 2018; **42**: 236-48.

34. Cárcel-Trullols J, Kovács AD, Pearce DA. Cell biology of the NCL proteins: what they do and don't do. *Biochim Biophys Acta* 2015.

35. Cotman SL, Mole SE, Kohan R. Future perspectives: Moving towards NCL treatments. *Biochim Biophys Acta* 2015; **1852**(10 Pt B): 2336-8.

36. Anderson G, Smith VV, Malone M, Sebire NJ. Blood film examination for vacuolated lymphocytes in the diagnosis of metabolic disorders; retrospective experience of more than 2500 cases from a single centre. *J Clin Pathol* 2005; **58**(12): 1305-10.

37. Shen J, Cram DS, Wu W, et al. Successful PGD for late infantile neuronal ceroid lipofuscinosis achieved by combined chromosome and TPP1 gene analysis. *Reprod Biomed Online* 2013; **27**(2): 176-83.

38. Anderson GW, Goebel HH, Simonati A. Human pathology in NCL. *Biochim Biophys Acta* 2013; **1832**(11): 1807-26.

39. Parviainen L, Dihanich S, Anderson GW, et al. Glial cells are functionally impaired in juvenile neuronal ceroid lipofuscinosis and detrimental to neurons. *Acta Neuropathol Commun* 2017; **5**(1): 74.

40. Lange J, Haslett LJ, Lloyd-Evans E, et al. Compromised astrocyte function and survival negatively impact neurons in infantile neuronal ceroid lipofuscinosis. *Acta Neuropathol Commun* 2018; **6**(1): 74.

41. Baker EH, Levin SW, Zhang Z, Mukherjee AB. MRI Brain Volume Measurements in Infantile Neuronal Ceroid Lipofuscinosis. *Am J Neuroradiol* 2017; **38**(2): 376-82.

42. Preising MN, Abura M, Jager M, Wassill KH, Lorenz B. Ocular morphology and function in juvenile neuronal ceroid lipofuscinosis (CLN3) in the first decade of life. *Ophthalmic Genet* 2016: 1-8.

43. Dulz S, Wagenfeld L, Nickel M, et al. Novel morphological macular findings in juvenile CLN3 disease. *Br J Ophthalmol* 2016; **100**(6): 824-8.

44. Bond M, Holthaus SM, Tammen I, Tear G, Russell C. Use of model organisms for the study of neuronal ceroid lipofuscinosis. *Biochim Biophys Acta* 2013; **1832**(11): 1842-65.

45. Shyng C, Nelvagal HR, Dearborn JT, et al. Synergistic effects of treating the spinal cord and brain in CLN1 disease. *Proc Natl Acad Sci U S A* 2017; **114**(29): E5920-E9.

46. Ostergaard JR. Paroxysmal sympathetic hyperactivity in Juvenile neuronal ceroid lipofuscinosis (Batten disease). *Auton Neurosci* 2018.

47. Khurana V, Tardiff DF, Chung CY, Lindquist S. Toward stem cell-based phenotypic screens for neurodegenerative diseases. *Nat Rev Neurol* 2015; **11**(6): 339-50.

48. Lojewski X, Staropoli JF, Biswas-Legrand S, et al. Human iPSC models of neuronal ceroid lipofuscinosis capture distinct effects of TPP1 and CLN3 mutations on the endocytic pathway. *Hum Mol Genet* 2014; **23**(8): 2005-22.

49. Chaterji S, Ahn EH, Kim DH. CRISPR Genome Engineering for Human Pluripotent Stem Cell Research. *Theranostics* 2017; **7**(18): 4445-69.

50. Uusi-Rauva K, Blom T, von Schantz-Fant C, Blom T, Jalanko A, Kyttala A. Induced Pluripotent Stem Cells Derived from a CLN5 Patient Manifest Phenotypic Characteristics of Neuronal Ceroid Lipofuscinoses. *Int J Mol Sci* 2017; **18**(5).

51. Markham A. Cerliponase Alfa: First Global Approval. *Drugs* 2017; **77**(11): 1247-9.

52. Cherukuri A, Cahan H, de Hart G, et al. Immunogenicity to cerliponase alfa intracerebroventricular enzyme replacement therapy for CLN2 disease: Results from a Phase 1/2 study. *Clin Immunol* 2018; **197**: 68-76.

53. Neverman NJ, Best HL, Hofmann SL, Hughes SM. Experimental therapies in the neuronal ceroid lipofuscinoses. *Biochim Biophys Acta* 2015; **1852**(10 Pt B): 2292-300.

54. Whiting RE, Narfstrom K, Yao G, et al. Enzyme replacement therapy delays pupillary light reflex deficits in a canine model of late infantile neuronal ceroid lipofuscinosis. *Exp Eye Res* 2014; **125C**: 164-72.

55. Whiting RE, Pearce JW, Castaner LJ, et al. Multifocal retinopathy in Dachshunds with CLN2 neuronal ceroid lipofuscinosis. *Exp Eye Res* 2015; **134**: 123-32.

56. Worgall S, Sondhi D, Hackett NR, et al. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther* 2008; **19**(5): 463-74.

57. Tamaki SJ, Jacobs Y, Dohse M, et al. Neuroprotection of host cells by human central nervous system stem cells in a mouse model of infantile neuronal ceroid lipofuscinosis. *Cell Stem Cell* 2009; **5**(3): 310-9.

58. Cairo MS, Tarek N, Lee DA, Delaney C. Cellular engineering and therapy in combination with cord blood allografting in pediatric recipients. *Bone Marrow Transplant* 2016; **51**(1): 27-33.

59. Yoon DH, Kwon OY, Mang JY, et al. Protective potential of resveratrol against oxidative stress and apoptosis in Batten disease lymphoblast cells. *Biochem Biophys Res Commun* 2011; **414**(1): 49-52.

60. Saha A, Sarkar C, Singh SP, et al. The blood-brain barrier is disrupted in a mouse model of infantile neuronal ceroid lipofuscinosis: amelioration by resveratrol. *Hum Mol Genet* 2012; **21**(10): 2233-44.

61. Sarkar C, Chandra G, Peng S, Zhang Z, Liu A, Mukherjee AB. Neuroprotection and lifespan extension in Ppt1(-/-) mice by NtBuHA: therapeutic implications for INCL. *Nat Neurosci* 2013; **16**(11): 1608-17.

62. Kinarivala N, Trippier PC. Progress in the Development of Small Molecule Therapeutics for the Treatment of Neuronal Ceroid Lipofuscinoses (NCLs). *J Med Chem* 2016; **59**(10): 4415-27.

63. Hersrud SL, Kovacs AD, Pearce DA. Antigen presenting cell abnormalities in the Cln3(-/-) mouse model of juvenile neuronal ceroid lipofuscinosis. *Biochim Biophys Acta* 2016; **1862**(7): 1324-36.

64. Seehafer SS, Ramirez-Montealegre D, Wong AM, et al. Immunosuppression alters disease severity in juvenile Batten disease mice. *J Neuroimmunol* 2011; **230**(1-2): 169-72.

65. Levin SW, Baker EH, Zein WM, et al. Oral cysteamine bitartrate and N-acetylcysteine for patients with infantile neuronal ceroid lipofuscinosis: a pilot study. *Lancet Neurol* 2014; **13**(8): 777-87.

66. Augustine EF, Beck CA, Adams HR, et al. Short-Term Administration of Mycophenolate Is Well-Tolerated in CLN3 Disease (Juvenile Neuronal Ceroid Lipofuscinosis). JIMD Rep. 2018/06/21 ed. Berlin, Heidelberg: Springer; 2018.

67. Drack AV, Mullins RF, Pfeifer WL, Augustine EF, Stasheff SF, Hong SD. Immunosuppressive Treatment for Retinal Degeneration in Juvenile Neuronal Ceroid Lipofuscinosis (Juvenile Batten Disease). *Ophthalmic Genet* 2015; **36**(4): 359-64.

68. Cialone J, Augustine EF, Newhouse N, et al. Parent-reported benefits of flupirtine in juvenile neuronal ceroid lipofuscinosis (Batten disease; CLN3) are not supported by quantitative data. *J Inherit Metab Dis* 2011; **34**(5): 1075-81.

69. Santiago JA, Bottero V, Potashkin JA. Dissecting the Molecular Mechanisms of Neurodegenerative Diseases through Network Biology. *Front Aging Neurosci* 2017; **9**: 166.

70. Sharma J, di Ronza A, Lotfi P, Sardiello M. Lysosomes and Brain Health. *Annu Rev Neurosci* 2018; **41**: 255-76.

71. Chandrachud U, Walker MW, Simas AM, et al. Unbiased Cell-based Screening in a Neuronal Cell Model of Batten Disease Highlights an Interaction between Ca2+ Homeostasis, Autophagy, and CLN3 Protein Function. *J Biol Chem* 2015; **290**(23): 14361-80.

72. Medina DL, Di Paola S, Peluso I, et al. Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat Cell Biol* 2015; **17**(3): 288-99.

73. Settembre C, Fraldi A, Medina DL, Ballabio A. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. *Nat Rev Mol Cell Biol* 2013; **14**(5): 283-96.
74. Spampanato C, Feeney E, Li L, et al. Transcription factor EB (TFEB) is a new therapeutic target for Pompe disease. *EMBO Mol Med* 2013; **5**(5): 691-706.
75. Palmieri M, Pal R, Nelvagal HR, et al. mTORC1-independent TFEB activation via Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. *Nat Commun* 2017; **8**: 14338.
76. Bajaj L, Lotfi P, Pal R, di Ronza A, Sharma J, Sardiello M. Lysosome biogenesis in health and disease. *J Neurochem* 2018.
77. Lotfi P, Tse DY, Di Ronza A, et al. Trehalose reduces retinal degeneration, neuroinflammation and storage burden caused by a lysosomal hydrolase deficiency. *Autophagy* 2018: 1-16.
78. Ghosh A, Corbett GT, Gonzalez FJ, Pahan K. Gemfibrozil and fenofibrate, Food and Drug Administration-approved lipid-lowering drugs, up-regulate tripeptidyl-peptidase 1 in brain cells via peroxisome proliferator-activated receptor alpha: implications for late infantile Batten disease therapy. *J Biol Chem* 2012; **287**(46): 38922-35.
79. Ghosh A, Jana M, Modi K, et al. Activation of peroxisome proliferator-activated receptor alpha induces lysosomal biogenesis in brain cells: implications for lysosomal storage disorders. *J Biol Chem* 2015; **290**(16): 10309-24.
80. Ghosh A, Rangasamy SB, Modi KK, Pahan K. Gemfibrozil, food and drug administration-approved lipid-lowering drug, increases longevity in mouse model of late infantile neuronal ceroid lipofuscinosis. *J Neurochem* 2017; **141**(3): 423-35.
81. Abtahi-Naeini B, Sadeghiyan H, Adibi N, Shokrollahi MR, Pourazizi M. Bullous dermatosis of childhood induced by gemfibrozil. *J Res Med Sci* 2015; **20**(2): 207-8.
82. Selden NR, Al-Uzri A, Huhn SL, et al. Central nervous system stem cell transplantation for children with neuronal ceroid lipofuscinosis. *J Neurosurg Pediatr* 2013; **11**(6): 643-52.
83. Barcenas M, Xue C, Marushchak-Vlaskin T, Scott CR, Gelb MH, Turecek F. Tandem mass spectrometry assays of palmitoyl protein thioesterase 1 and tripeptidyl peptidase activity in dried blood spots for the detection of neuronal ceroid lipofuscinoses in newborns. *Anal Chem* 2014; **86**(15): 7962-8.
84. Khaledi H, Liu Y, Masi S, Gelb MH. Detection of Infantile Batten Disease by Tandem Mass Spectrometry Assay of PPT1 Enzyme Activity in Dried Blood Spots. *Anal Chem* 2018.
85. Grimm D, Zolotukhin S. E Pluribus Unum: 50 Years of Research, Millions of Viruses, and One Goal--Tailored Acceleration of AAV Evolution. *Mol Ther* 2015; **23**(12): 1819-31.
86. kleine Holthaus S-M, Ribeiro J, Abelleira-Hervas L, et al. Prevention of Photoreceptor Cell Loss in a Cln6nclf Mouse Model of Batten Disease Requires CLN6 Gene Transfer to Bipolar Cells. *Mol Ther* 2018; **26**(5): 1343-53.

Glossary panel

Gene therapy uses viral vectors to introduce a healthy NCL gene, targeting first the brain or eye. The same approach could also introduce a gene whose activity can compensate for a defective NCL gene. There are several vectors that can be used for gene therapy - for NCL the vector AAV is being used most commonly, with the awareness that different serotypes target tissues with varied efficiencies.

Enzyme replacement therapy is appropriate only for NCL disease caused by defective lysosomal enzymes. This approach is already delivering recombinant TPP1 into the brain of children with CLN2 disease in the clinic.[10] The same approach can be used for all types of NCL caused by defects in soluble lysosomal enzymes or proteins that can be taken up by cells (CLN1/PPT1, CLN2/TPP1, CLN5, CLN10/CTSD, CLN13/CTSF). The enzyme may be delivered frequently and periodically as a recombinant product, or alternatively could be produced and released continually within the body following gene therapy or cell-based therapy.

Cell-based therapy for NCL is most likely to be used to provide cells that secrete lysosomal enzyme for uptake by cells in the locality or at a distance, rather than replace lost cells. These cell factories will either need to be located where the enzyme is needed (eg, brain, eye) or the secreted enzyme be transported in the blood and taken up by distant tissues (eg, heart) including crossing the BBB into the brain. Ideally, such cells would be derived from an individual patient, manipulated to produce the required product by an introduced vector or by correcting the cell's own genome, and then reintroduced.

Drug approaches for NCL may modulate disease consequences, compensate for missing functional activities, and slow or prevent cell death. It is likely that pharmacological treatments will be used supplement other treatments and methods such as gene therapy.

Table 1 Correlation between genotype and phenotype in NCL

Gene[x]	Disease name(s)	Protein and location	No. mutations	Genotype-phenotype correlation[y]	Age of onset	Typical clinical feature	Ultrastructural pathological features
CLN1/PPT1	CLN1 disease, Haltia-Santavuori	Lysosome enzyme	71	**Infantile** Late infantile Juvenile Adult	**6-12 mo** 1.5-4 y 5-7 y >16 y	**Clumsiness, loss of developmental gains**	Granular osmiophilic deposits
CLN2/TPP1	CLN2 disease, Jansky-Bielschowsky	Lysosome enzyme	121	**Late infantile** Juvenile Protracted *SCAR7*	**2-4 y** 8 y >11 y	**Motor decline or speech delay, seizures**	Curvilinear profiles
CLN3	CLN3 disease, Spielmeyer-Vogt-Sjögren-Batten disease	Lysosomal membrane protein	78	**Juvenile** Protracted *Autophagic vacuolar myopathy* *Retinitis pigmentosa* *Adult cone-rod dystrophy*	**4-7 y** >20->40 y	**Visual failure**	Fingerprint profiles
CLN4/DNAJC5	CLN4 disease, Parry disease	Cytoplasmic protein that associates with late endosome and lysosome membrane	2	**Adult autosomal dominant** (Parry disease)	**>20 y**	**Seizures ataxia, behavioural changes**	Diverse and often mixed
CLN5	CLN5 disease	Lysosome enzyme	37	**Late infantile** Juvenile Protracted Adult	**4-5 y** 5-7 y >16 y	**Slowing of psychomotor development, visual failure**	Rectilinear profiles & Condensed storage inclusions
CLN6	CLN6 disease, Kufs disease type A	Endoplasmic reticulum membrane protein	70	**Late infantile** Protracted Teenage-Adult Kufs type A *Juvenile cerebellar ataxia*	**18 mo+** >16 y	**Seizures and motor difficulties**	Rectilinear profiles & Condensed storage inclusions
CLN7/MFSD8	CLN7 disease	Lysosomal membrane protein	38	**Late infantile** Juvenile protracted *Adult macular dystrophy* *Adult cone-rod dystrophy*	**1.5-6 y** >7 y >29->65 y >27 y	**Seizures, developmental regression**	Rectilinear profiles & Condensed storage inclusions

				Phenotype[γ]	Onset	Symptoms	Ultrastructure
CLN8	CLN8 disease	Endoplasmic reticulum membrane protein	35	**Late infantile** Protracted *EPMR/Northern epilepsy*	**2-7 y** 5-10 y	**Seizures**	Rectilinear profiles & Condensed storage inclusions
CLN10/CTSD	CLN10 disease, Congenital NCL	Lysosome enzyme	12	**Congenital** Late infantile Juvenile Adult	**Pre/perinatal** 4 y 8-15 y >20 y	**Seizures, spasticity, central sleep apnoea**	Granular osmiophilic deposits
CLN11/GRN	CLN11 disease	Lysosome enzyme chaperone	2[+]	**Adult** *Frontotemporal lobar dementia (when heterozygous)*	**> 20 y**	**Rapidly progressive visual failure, seizures**	Rectilinear profiles
CLN13/CTSF	CLN13 disease Kufs disease type B	Lysosome enzyme	11	**Adult Kufs type B**	**>20 y**	**Tremor, ataxia, seizures**	Fingerprint profiles (FP)

[γ]bold = phenotype caused by complete loss of gene function

italics – non-NCL disease phenotype that in some cases may be more typically associated with this gene.

[+]only the mutations that cause NCL when present on both disease alleles is enumerated; these mutation, and other mutations in this gene, cause later onset frontotemporal lobar dementia when present in heterozygous form.[25]Smith et al instead.

[x]Variations in further genes have occasionally been linked with NCL-like phenotypes: *CLN12/ATP13A2*, mutations usually cause Kufor-Rakeb syndrome; *CLN14/KCTD7* in cases with infantile and late infantile onset, all other known mutations cause a progressive myoclonic epilepsy or opsoclonus-myoclonus ataxia-like syndrome; *SGSH* in a case with adult onset, all other known mutations cause MPSIIIA; *CLCN6*, perhaps modifying disease phenotype.[3]

Note CLN9 is not identified.

Abbreviations: PPT1 = Palmitoyl protein thioesterase 1; TPP1 = Tri-peptidyl peptidase 1; DNAJC5 = DnaJ homolog subfamily C member 5 (also known as cysteine string protein alpha or CSPa); MFSD8 = Major facilitator superfamily domain-containing protein 8; CTSD = Cathepsin D; GRN = Granulin; CTSF = Cathepsin F; SCAR7 = Spinocerebellar Ataxia, Autosomal Recessive 7; EPMR = Epilepsy, Progressive, With Mental Retardation.

Table 2 Clinical Trials assessing treatment of neuronal ceroid lipofuscinoses

Disease	Study title and NCT number	Treatment	Phase and status	N	Location countries	Outcome
CLN1	Cystagon to treat infantile neuronal ceroid lipofuscinosis (A Combination Therapy With Cystagon and N-Acetylcysteine for INCL Patients) NCT00028262	Cystagon	4	9	USA	Little or no clinical benefit[65]
CLN1 and CLN2	Study of human central nervous system stem cells (HuCNS-SC) cells in patients with infantile or late infantile neuronal ceroid lipofuscinosis (NCL) NCT00337636	Stem cells	1	6	USA	Little or no clinical benefit[82]
CLN1	Safety and efficacy study of human central nervous system stem cells (HuCNS-SC) in subjects with neuronal ceroid lipofuscinosis NCT01238315	Stem cells	1, withdrawn prior to enrollment	0	USA	-
CLN2	Safety study of a gene transfer vector for children with late infantile neuronal ceroid lipofuscinosis NCT00151216	AAV2CUhCLN2 gene transfer	1	10	USA	Little or no clinical benefit[56]
CLN2	Safety study of a gene transfer vector (rh.10) for children with late infantile neuronal ceroid lipofuscinosis NCT01161576	AAVrh.10CUhCLN2 gene transfer	1, ongoing	25	USA	
CLN2	AAVrh.10 administered to children with late infantile neuronal ceroid lipofuscinosis with uncommon genotypes or moderate/severe impairment NCT01414985	AAVrh.10CUhCLN2 gene transfer	1/2, ongoing	8	USA	
CLN2	Safety and Efficacy Study of BMN190 for the Treatment of CLN2 Patients. A Phase 1/2 Open-Label Dose-Escalation Study to Evaluate Safety, Tolerability, Pharmacokinetics, and Efficacy of Intracerebroventricular BMN 190 in Patients With Late-Infantile Neuronal Ceroid Lipofuscinosis (CLN2) Disease NCT01907087	rhTPP1 BMN190 (Cerliponase alfa) (recombinant human tripeptidyl peptidase-1)	1/2	24	Germany, UK, Italy, USA	Clinical improvement or stabilization[10] FDA and EMA approval
CLN2	A Multicenter, Multinational, Extension Study to Evaluate the Long-Term Efficacy and Safety of BMN 190 in Patients With CLN2 Disease NCT02485899	rhTPP1 BMN190 (Cerliponase alfa) (recombinant human tripeptidyl peptidase-1)	1/2, ongoing	23	Germany, UK, Italy, USA	
CLN2	A Safety, Tolerability, and Efficacy Study of Intracerebroventricular BMN 190 in Patients With CLN2 Disease NCT02678689	rhTPP1 BMN190 (Cerliponase alfa) (recombinant human tripeptidyl peptidase-1)	2, ongoing	15	Germany, Italy, USA	
CLN3	Cellcept for treatment of juvenile neuronal ceroid lipofuscinosis NCT01399047	Mycophenolate mofetil	2	19	USA	Little or no clinical benefit[66]

CLN6	Phase I/IIa Gene Transfer Clinical Trial for Variant Late Infantile Neuronal Ceroid Lipofuscinosis, Delivering the CLN6 Gene by Self-Complementary AAV9 NCT02725580	scAAV9.CB.CLN6 gene transfer	1/2, ongoing	12	USA	
NCL & other diseases	UCB Transplant of Inherited Metabolic Diseases With Administration of Intrathecal UCB Derived Oligodendrocyte-Like Cells (DUOC-01) NCT02254863	Adjunct therapy for UCB-derived oligodendrocyte-like cells (DUOC-01) to HSCT	1, ongoing	12	USA	
NCL & other diseases	Human Placental-Derived Stem Cell Transplantation NCT01586455	Stem cells (administered in conjunction with umbilical cord blood stem cells	1, ongoing	43	USA	

Abbreviations: human CNS stem cells (HuCNS-SC)

Note: There is no published information available for some of these trials. This is pending for those that are active or extension trials.