Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis

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Treatment of the neuronal ceroid lipofuscinoses, also known as Batten disease, is at the start of a new era because of diagnostic and therapeutic advances relevant to this group of inherited neurodegenerative and life-limiting disorders that affect children. Diagnosis has improved with the use of comprehensive DNA-based tests that simultaneously screen for many genes. The identification of disease-causing mutations in 13 genes provides a basis for understanding the molecular mechanisms underlying neuronal ceroid lipofuscinoses, and for the development of targeted therapies. These targeted therapies include enzyme replacement therapies, gene therapies targeting the brain and the eye, cell therapies, and pharmacological drugs that could modulate defective molecular pathways. Such therapeutic developments have the potential to enable earlier diagnosis and better targeted therapeutic management. The first approved treatment is an intracerebroventricularly administered enzyme for neuronal ceroid lipofuscinosis type 2 disease that delays symptom progression. Efforts are underway to make similar progress for other forms of the disorder.

Lancet Neurol 2018; 18: 107–16

Published Online
November 20, 2018
http://dx.doi.org/10.1016/S1474-4422(18)30368-5

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Introduction

The neuronal ceroid lipofuscinoses (NCLs), also known as Batten disease, are a group of monogenic inherited neurodegenerative disorders that mostly present in the first decade of life.[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 NCLs 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, 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: *PPT1, TPP1, DNAJC5, CLN3, CLN5, CLN6, MFSD8, CLN8, CTSD, GRN, ATP13A2, CTSF*, and *KCDT7*.[3,4] The increasing implementation of next generation sequencing panels and exome sequencing as essential diagnostic tools leads to more diagnoses of patients with NCL, including those varying from the typically recognised phenotypes due to so-called milder mutations. This has necessitated a change in disease nomenclature based on the gene defect augmented with age of presentation.[4]

There has been an emphasis on understanding the staging of these disorders and their 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 by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) of the first treatment for a NCL disorder: enzyme replacement therapy for neuronal ceroid lipofuscinosis type 2 (CLN2) disease.[13,14] Much preclinical work followed by clinical trials is required before treatment is available for all forms of NCLs. This Review provides an update on clinical features, genotype–phenotype correlations, pathology, and discusses the most promising therapeutic approaches of NCLs.

Clinical characteristics

All patients with NCLs, except for those with a rare congenital form (neuronal ceroid lipofuscinosis type 10 [CLN10] disease), have a normal psychomotor development before onset of first symptoms.[1] Age at disease onset is in childhood for most patients, but for some can be as late as 60 years or older (table 1). The main symptoms are a combination of at least two of the following: dementia, epilepsy, motor deterioration, and visual loss.[1] The order in which symptoms occur varies and depends on the combination of the underlying mutations, which can affect age at onset and disease phenotype.[1] The number of phenotypes for NCL diseases is growing and the widest age range of onset is for those NCLs arising from lysosomal enzyme deficiencies (table 1).[3] Increasing knowledge about the natural history of the different forms of NCLs has shown that for some genes the phenotype severity can vary substantially even between siblings, as in juvenile neuronal ceroid lipofuscinosis type 3 (CLN3) disease.[16] Symptoms can also be outside the CNS. For example, cardiac involvement has been reported in adolescent and adult patients with CLN3 disease[17,18] and can be treated (eg, fitting of a pacemaker has improved a patient's psychomotor abilities).[16]

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, and lastly followed by vision loss.[1] This regression is often mistaken for a side-effect of antiepileptic drugs, delaying diagnosis. By contrast, first symptoms in the

	Disease name(s)	Protein and location	Number of mutations	Genotype–phenotype correlation	Age of onset	Typical clinical feature‡	Ultrastructural pathological features
CLN1/*PPT1**†	CLN1 disease, Haltia-Santavuori disease	Lysosome enzyme	71	Infantile‡ Late infantile Juvenile Adult	6–12 months‡ 1·5–4 years 5–7 years >16 years	Clumsiness, loss of developmental gains	Granular osmiophilic deposits
CLN2/*TPP1**†	CLN2 disease, Jansky-Bielschowsky disease	Lysosome enzyme	121	Infantile Late infantile‡ Juvenile, protracted Spinocerebellar ataxia†	<18 months 2–4 years‡ >5 years >11 years	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 years‡ >4 years >7 years >15 years >20 to >40 years	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 years‡	Seizures ataxia, behavioural changes	Diverse and often mixed
*CLN5**†	CLN5 disease	Lysosome enzyme	37	Late infantile‡ Juvenile Adult	4–5 years‡ 5–7 years >16 to >50 years	Slowing of psychomotor development, visual failure	Rectilinear profiles and condensed storage inclusions
*CLN6**†	CLN6 disease, Kufs disease type A	Endoplasmic reticulum membrane protein	70	Late infantile‡ Progressive myoclonic epilepsy Teenage-adult Kufs type A Juvenile cerebellar ataxia†	≥ 18 months‡ >15 years >15 years >7 years	Seizures and motor difficulties	Rectilinear profiles and 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 years‡ >7 years >29 to >65 years >27 years	Seizures, developmental regression	Rectilinear profiles and condensed storage inclusions
*CLN8**†	CLN8 disease	Endoplasmic reticulum membrane protein	35	Late infantile‡ Protracted EPMR/Northern epilepsy†	2–7 years‡ 5–10 years 5–10 years	Seizures	Rectilinear profiles and condensed storage inclusions
CLN10/*CTSD**†	CLN10 disease, Congenital NCLs	Lysosome enzyme	12	Congenital‡ Late infantile Juvenile Adult	Prenatal and perinatal‡ 4 years 8–15 years >20 years	Seizures, spasticity, central sleep apnoea	Granular osmiophilic deposits
CLN11/*GRN**†	CLN11 disease	Lysosome enzyme chaperone	2§	Adult‡ Frontotemporal lobar dementia (when heterozygous)†	>20 years 50–70 years	Rapidly progressive visual failure, seizures	Rectilinear profiles
CLN13/*CTSF**†	CLN13 disease, Kufs disease type B	Lysosome enzyme	11	Adult Kufs type B‡	>20 years	Tremor, ataxia, seizures	Fingerprint profiles

Note *CLN9* is not identified. *CLN*=neuronal ceroid lipofuscinosis type; *PPT1*=palmitoyl protein thioesterase 1; *TPP1*= tripeptidyl 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; NCLs=neuronal ceroid lipofuscinoses; *CTSD*=cathepsin D; NCLs=neuronal ceroid lipofuscinoses; *GRN*=granulin; *CTSF*=cathepsin F; SCAR7= autosomal recessive spinocerebellar ataxia 7; EPMR=Epilepsy, Progressive, With Mental Retardation. *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 mucopolysaccharidosis type IIIA; *CLCN6*, perhaps modifying disease phenotype.[3] † Non-NCL disease phenotype that could be more typically associated with other genes. ‡Phenotype caused by complete loss of gene function. §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.[15]

Table 1: **Correlation between genotype and phenotype in neuronal ceroid lipofuscinoses**

juvenile phenotype (age of onset 5–7 years) are usually visual loss, followed by dementia and behaviour changes, and then loss of motor skills and epilepsy in the early adolescence.[1] In recessive adult phenotypes (more than 16 years of age) also referred to as 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 symptom onset could range from 16 to 50 years of age.[19]

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 patients with classic late-infantile CLN2 disease and could enable early diagnosis.[9] Children with a combination of language acquisition delay and new onset of seizures should be tested for CLN2 disease.[9,20] Epilepsy is therapy-resistant in almost all patients with NCLs, with especially high seizure frequency and severity in late-infantile CLN2 disease up until late stages of disease.[20] However, in infantile neuronal ceroid lipofuscinosis type 1 (CLN1) disease, seizure frequency tends to decrease in the later stages of disease; in patients with classic juvenile CLN3 disease, seizures are infrequent with only mild worsening with later stages of disease.[21,22] Therapy with more than two antiepileptic drugs could result in increased side-effects rather than a reduction of seizures.[1] Some antiepileptic drugs are particularly recommended—valproate and lamotrigine for CLN2 and CLN3 diseases;[21,22] however, others (eg, carbamazepine, gabapentin, phenytoin, or vigabatrin) might have negative effects—they could exacerbate myoclonic seizures in patients with CLN2 or CLN3 disease.[21–23] As disease progresses, anticonvulsive drugs that have been tolerated and effective might cause new side-effects, and should be reconsidered if symptoms of the disease worsen.[22]

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

More NCL disease phenotypes are being recognised in which one of the clinical hallmarks might be more predominant and others absent. 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,[24] and in one type of juvenile CLN2 disease associated with a particular mutation, survival is into the fourth decade of life.[25] Some patients with mutations in *CLN3* have only isolated non-syndromic recessive retinal degeneration,[26] and others experience visual failure, seizures, and cardiac involvement, but no motor deterioration even many decades after disease onset.[27] A pathophysiological link between NCLs and an adult degenerative dementia is assumed given that homozygous mutations in *GRN* cause NCLs presenting at around age 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.[3,15]

Neuroimaging and EEG findings

Brain MRI scans can look normal in the early stages of the disease or might show some unspecific signs, such as periventricular intensity changes in early-stage CLN2 disease.[28] Although MRI is not sensitive or specific for early diagnosis, it is an excellent tool to objectively monitor the progression of brain changes, particularly with advances in resolution and processing, and neuroimaging techniques such as diffusion tensor imaging allow assessment of disorganisation of white matter tracts and atrophy. Two prospective studies[28,29] in patients with CLN2 disease have shown that the loss of cortical grey matter volume with increasing age might be a sensitive biomarker to monitor disease progression.

EEG can be helpful in early diagnosis of some NCLs. For example, characteristic posterior spike waves were reported after low-frequency photic stimulation in 13 (93%) of 14 patients with early stage of CLN2 disease.[30] Finding posterior spike waves after photic stimulation in a child younger than 5 years with a new onset of seizures should trigger testing for CLN2 disease.[30,31] Photosensitivity with low-frequency stimulation has also been reported in patients with neuronal ceroid lipofuscinosis type 6 (CLN6) disease, and especially in those with adult CLN6 disease (type A).[32–34] In NCL forms that rapidly progress, such as infantile CLN1 disease, early abnormalities disappear as neurons die, leading to a characteristic flat EEG at advanced stages of disease.[1] Because of the underlying neurometabolic disease, antiepileptic drugs will not lead to normalisation of the EEG findings,[1] presumably because cells have died or are dying, and this in itself leads to seizures. Monitoring by EEG might 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,[22] or theoretical allergic reaction to new treatments.

Genotype–phenotype correlations

The NCLs are monogenic disorders, so each is a separate disease entity. The genes associated with NCLs encode apparently unrelated proteins that include soluble lysosomal enzymes and membrane proteins localised in various organelles, including the lysosome (table 1).[3,35,36] All types of NCLs are autosomal recessive with the exception of one rare adult onset NCL, neuronal ceroid lipofuscinosis type 4 (CLN4) disease.[3] For most NCLs there is a recognisable 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 might have a more protracted overall course also occur and some anticipated classic phenotypes might be absent as a result of milder mutations that do not completely stop protein function (table 1).[19] 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 NCLs, 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 DEM-CHILD,[9,10] NCL Mutation Database, and application of NCL disease rating scales[11,12] allows correlations to be made between the disease and underlying mutations to provide some guidance to the expected disease course,

For more on **DEM-CHILD** see www.dem-child.eu

For more on the **NCL Mutation Database** see www.ucl.ac.uk/ncl

and to give an approximation of frequency and occurrence in specific ethnic groups. As the phenotypic spectrum broadens, increasing overlap with other rare (eg, inborn errors of metabolism) and common diseases (eg, retinal dystrophies) that share similar disease mechanisms is expected,[37] which could 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] 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 NCLs genes among a larger group of syndromic and non-syndromic inherited epilepsies). Blood film examination allows identification of vacuolated lymphocytes, which is a common feature of CLN3 disease.[38] 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 NCLs presenting in adulthood.[19] Prenatal diagnosis is available and can be offered to families with a history of NCL disease. Preimplantation genetic diagnosis[39] 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 NCLs resulting in cortical grey matter atrophy, cerebellar atrophy, and secondary ventricular enlargement. The degree of atrophy and ventricular enlargement varies between the NCL forms, but is typically preceded by clinical symptoms (eg, epilepsy).[2,40] Nevertheless, this loss of neurons in the cerebral and cerebellar cortices is selective because cell loss is not spread evenly throughout the brain in childhood NCLs, and the thalamus can be severely affected.[2,40] In adult onset NCLs, brain atrophy is less obvious.[2,40] Pronounced microglial and astrocytic activation precedes and perhaps causes neuron loss,[19,41,42] accurately predicting its distribution.[41] Brain atrophy of the cerebral cortex and enlargement of the subarachnoid space and ventricles progresses throughout the disease.[2,40] Atrophy of the cerebellum is variable but evident in the later stages of all NCLs.[28,29,43] Neuronal depletion in the retina commences in the photoreceptor outer segments, proceeding to the inner segments, nerve cell bodies, and ganglionic layer, and occurs early in CLN3 disease,[44,45] while other symptoms (eg, epilepsy) precede visual loss in other types of NCLs.[1,40] In all forms of NCLs lipopigment storage material accumulates in macrophages, neurons, and some somatic tissues, including vascular endothelial and smooth muscle cells.[40] Lipopigments also accumulate in the CNS with increasing age, in healthy people, or neurological conditions such as mucopolysaccharidoses or GM1 gangliosidosis,[40] and careful neuropathological assessment is needed to avoid misdiagnosis (eg, early-onset Alzheimer's disease).[19]

Mice that are genetically modified or have spontaneous mutations exist for all NCL genes,[5,46] 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[41,42] despite leading to a similar pathological endpoint. The spinal cord has been identified as exhibiting important pathology, including loss of neurons, activation of microglia, and build-up of lipopigment storage in *Cln1* mice,[47] with impairments of the peripheral nervous system evident resembling paroxysmal sympathetic hyperactivity in juvenile CLN3 disease.[48] Pathological changes have also been described in somatic tissues, including heart (eg, repolarisation disturbances, ventricular hypertrophy, sinus node dysfunction),[17] 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, studies with animals that have larger brains (eg, sheep and dogs) will be required.[5,8] In addition to their larger and more complex brains for scaling up the delivery of therapies, the extent of pathology and its regionalised nature is more pronounced in the brains of sheep and dogs with NCLs than in corresponding mouse models (possibly because of the size difference), and pathology appears to more closely resemble that of human cases.[6,7] With the ability to engineer genetically modified pigs and sheep, such species might prove invaluable for further understanding the effects of the disease and to improve therapies.

Technical advances

The development of induced pluripotent stem cell (iPSC) technology has been crucial for understanding and treating genetic diseases using cell models. Cells obtained from a skin or blood sample can be genetically reprogrammed to a state of pluripotency and are capable of differentiating into virtually any cell type, including neuronal subtypes.[49] Neural cells differentiated from patient-derived iPSC display autophagic, lysosomal maturation, and mitochondrial quality control defects for CLN3 disease and tripeptidyl peptidase 1 (TPP1) enzyme deficiency for CLN2 disease.[50] In 2017, two repositories of NCLs iPSC lines became available to academic researchers. The Human Pluripotent Stem Cell Initiative in the UK, 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. However, such patient-derived cells are genetically diverse. The use of

For more on the **Human Pluripotent Stem Cell Initiative** see http://www.hipsci.org

For more on the **Beyond Batten Disease Foundation** see https://beyondbatten.org/

Fore more on the **New York Stem Cell Foundation** see https://nyscf.org

clustered regularly spaced short palindromic repeats (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.[51] Such repositories, along with further individually generated iPSC lines[52] and engineered stem cells, represent the next generation of cellular tools to better understand the causes of NCLs. Patient iPSC-derived neural cells have already been used as a therapeutic evaluation platform for NCLs, including high-throughput screening (appendix).

Emerging therapies

There have been extensive studies using animal models in developing new treatments for NCLs (appendix). The main translational approaches are summarised, beginning with the most advanced and promising approaches.

Enzyme replacement therapy

Intracerebroventricularly administered enzyme replacement therapy in CLN2 disease is the first treatment approved by the FDA and EMA, and treatment is expected to be needed throughout the patient's life.[10,53] The deficient enzyme 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 of protein every 2 weeks into the brain of children with CLN2 disease.[10] Safety and efficacy were tested in an open-label, dose-escalation study lasting up to 12 weeks[10] that enrolled 24 patients aged 3–16 years with CLN2 disease, followed by a 48 week period in which they received a stable dose of the protein and then had the option to enroll into a 240 week extension study. Most patients (21 [92%]) had lost some language or motor skills according to the CLN2 Clinical Rating Scale by the trial's start. Of the 23 (96%) patients who completed the study,[10] 18 (78%) had either a slower than expected disease progression or stabilisation of the disease measured by rating the motor and language function of patients after at least 96 weeks of treatment (median 116 weeks, range 96–145) 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.[54] The 23 patients continue to be treated and followed up to evaluate the long-term efficacy and safety (NCT02485899). A second study (NCT02678689) has begun to monitor the effects of beginning treatment earlier in the disease course in a separate group of patients (table 2). 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 NCLs caused by mutations in lysosomal enzymes (CLN1/palmitoyl protein thioesterase [PPT1], CLN2/TPP1, neuronal ceroid lipofuscinosis type 5 [CLN5], CLN10/CTSD, and neuronal ceroid lipofuscinosis type 13 [CLN13]/cathepsin F [CTSF]); (table 1). The therapy could 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.

Gene therapy

Gene therapy studies using viral vectors to introduce a healthy NCL gene into animal models for NCLs have mainly focused on targeting the brain for diseases caused by lysosomal enzyme deficiencies, which should be less challenging to treat than those caused by mutations in integral transmembrane-bound proteins because of advantages provided by cross-correction.[59] Clinical studies and animal models suggest that gene therapy approaches targeting only the brain[10,60] are unlikely to provide therapeutic benefit for the NCL-related retinal degeneration. A combinatorial gene therapy approach that separately treats the brain and the eye is likely to be required for optimal therapy.[61]

See Online for appendix

The first gene therapy phase 1 clinical trial[62] was done in ten patients with CLN2 disease aged 3–10 years to test the safety of introducing a gene therapy vector designed to express TPP1 into the brain. The vector was safe but there was no slowing of disease progression. Given the experience with enzyme replacement therapy,[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 adeno-associated virus-mediated gene therapy for CLN1 and CLN3 diseases, and clinical trials are anticipated. A phase 1/2 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 was tested are not yet published, making evaluation of the likelihood of clinical benefit difficult.[63,64]

Pharmacological approaches

As for most lysosomal storage disorders, many of the pharmacological treatments for NCLs are palliative, focused on minimising clinical symptoms such as seizures, and do not target 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, and antioxidants).[55,65–69] However, follow-up studies using these compounds have not shown clinically meaningful benefits for patients,[57,58] including targeting neuroinflammation in the eye for CLN3 disease.[70] Some parents giving the drug flupirtine to their children with juvenile CLN3 disease have anecdotally reported benefit; however, quantitative,

	Study title and NCT number	Treatment	Phase and status	N	Location	Outcome
CLN1	Cystagon to treat infantile neuronal ceroid lipofuscinosis (a combination therapy with cystagon and N-acetylcysteine for INCL patients), NCT00028262	Mercaptamine	4, published	9	USA	Little or no clinical benefit[55]
CLN1 and CLN2	Study of human central nervous system stem cells (HuCNS-SC) cells in patients with infantile or late infantile neuronal ceroid lipofuscinosis, NCT00337636	Stem cells	1, published	6	USA	Little or no clinical benefit[56]
CLN1	Safety and efficacy study of human central nervous system stem cells in subjects with neuronal ceroid lipofuscinosis, NCT01238315	Stem cells	1, withdrawn prior to enrolment	0	USA	..
CLN2	Safety study of a gene transfer vector for children with late infantile neuronal ceroid lipofuscinosis, NCT00151216	AAV2CUhCLN2 gene transfer	1,, published	10	USA	Little or no clinical benefit[57]
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	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, published	24	Germany, UK, Italy, USA	Clinical improvement or stabilization[28] 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, published	19	USA	Little or no clinical benefit[58]
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	

CLN=neural ceroid lipofuscinosis type; NCL=neuronal ceroid lipofuscinosis; HSCT=haematopoietic-stem-cell transplantation.

Table 2: **Clinical trials assessing treatment of neuronal ceroid lipofuscinoses**

prospectively obtained data did not show any change in disease progression that could be attributed to this drug.[71] A clinical trial treating 19 children with juvenile CLN3 disease with a non-steroidal immunosuppressive drug, mycophenolate mofetil, over two 8-week treatment periods with a 4-week intervening washout[68,69] showed that this drug was well tolerated, but there was no clinical benefit.[58] A trial in nine children with CLN1 disease that tested the combination of phosphocysteamine and N-acetylcysteine, reported no clinical benefit (table 2).[57] Thus, to date no efficacy is supported for any of these pharmacological treatments. Rather than targeting secondary downstream effects (eg, build up of storage material) focus should be directed to understanding the underlying disease mechanisms.

Applying a classic drug discovery approach (from disease mechanism to target to drug) to the NCLs is challenging because little is known about which intracellular pathways are affected or if they are good candidates for drug therapy. 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 homoeostasis)[72,73] might be promising 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.[74,75] Discovery of transcriptional regulation of lysosomal biogenesis and degradative function by the transcription factor EB has opened a new avenue for therapeutic intervention in lysosomal storage disorders.[76–79] For example, stimulation of transcription factor EB with trehalose affords benefits in Cln3[78] and mucopolysaccharidosis type III[80] disease mice. The FDA-approved lipid-lowering drug gemfibrozil also activates the same pathway[81,82] and has moderate beneficial effects in *Cln2* mice.[83,84] A clinical trial to test efficacy of these approaches is yet to be launched in patients with any form of NCLs. Drug approaches for NCLs could compensate for missing functional activities and slow or prevent cell death and these treatments will probably be used to supplement other treatments and methods such as gene therapy.

Cell-based therapy

Although 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. However, this seems unlikely to happen because the therapy is not capable of replacing enough cells in the brain of a mouse (which is smaller than a human brain and its regions are easier to reach) which makes it unlikely that sufficient cell repopulation will happen in a human brain.[63] The aim of stem cell trials for the NCLs is to preserve remaining function by providing cells that secrete a missing lysosomal enzyme.[64] These cell factories will either need to be located where the enzyme is needed (eg, in the brain or eye) or the secreted enzyme will need to be transported in the blood and taken up by distant tissues (eg, heart), and will need to cross the blood–brain barrier to reach 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 being reintroduced. A phase 1 trial established the safety of neuroprogenitor cell implantation in six patients with advanced CLN1 or CLN2 disease (table 2).[56] There was no clinical benefit to this trial, perhaps because donor engraftment was low and migration limited.[56] There is an ongoing phase 1 trial (NCT01586455) testing whether transplantation of human placental-derived stem cells benefits patients with a range of diseases including 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 pathological targets required for therapeutic advances. This knowledge has prompted therapeutic development beyond current palliative treatments, even without understanding disease mechanisms. Strategies for treatment of NCLs caused by defects in lysosomal enzymes benefit from the process of cross-correction, leading to the first approved treatment for CLN2 disease that delivers recombinant protein directly into the brain at regular intervals. Development of a gene supplement approach is at the experimental stage for many NCLs, with the first of an anticipated new wave of clinical trials focusing on 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 might be especially useful for treatment beyond the CNS, but they are in the early stages of development.

To have a long-term clinical benefit, 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.[85,86] It will also be important to consider patients with milder and adult onset NCL forms who might particularly benefit from new treatments because there is a longer time window to diagnose patients before symptoms advance too far and are irreversible.[19] International cooperation is contributing to the ongoing collection of natural history data for all NCL types into the DEM-CHILD database which can provide necessary control data for use in future clinical trials and 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 because these patients' symptoms would advance so quickly that there would be no hope of recovery once actual treatment starts, and speeds up the development of therapy options in rare diseases for which availability of suitable patients for a trial can be a limiting factor.

Treatment for NCLs must reach the most affected cells in the brain and eye, and further studies are 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

Search strategy and selection criteria

We searched PubMed for articles only written in the English language and published between Jan 1, 2012, and Sept 30, 2018, using the search terms "ceroid", "Batten", "NCL", "CLN", "PPT1", "TPP1", "DNAJC5", "MFSD8", "CTSD", "GRN", "CTSF", "CLN1", "CLN2", "CLN3", "CLN4", "CLN5", "CLN6", "CLN7", "CLN8", "CLN10", "CLN11", "CLN12", and "CLN13". The final reference list was generated on the basis of relevance to the topics covered in this Review.

increase the ability to deliver treatments to cells within the brain or eye.[87] This needs to be balanced against toxicity arising from producing too much recombinant protein within sensitive cells.[88] An ideal least-invasive therapy would be a set of one-off or infrequent gene therapies that together target the whole body and are supplemented by probably lifelong drug-based modulation. Understanding of 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 step is required for a breakthrough in pharmacological treatments that target pathways close to the underlying defect. Such advances will be beneficial for basic research, early diagnosis, and disease prevention.

Contributors

SEM devised the structure of the article; performed the search strategy; made the final selection of references; contributed to writing of the summary, introduction, the genetic basis and genotype-phenotype correlations, and tables; and provided oversight, harmonisation, and final editing of all sections. SFB and AS contributed to writing of the clinical features. GA contributed to writing of the diagnosis and pathology. DLM contributed to writing of the pharmacological approaches. TRM contributed to writing of the technical advances and iPSC cells and cell-based therapy. S-MKH, AJS, and AAR contributed to writing of the gene therapy. AS contributed to writing of the enzyme replacement therapy. JDC contributed to writing of the pathology and disease mechanisms and mouse models. SEM and HAB contributed to the writing of the conclusions and future directions.

Declaration of interests

SEM has received grants from the EU Horizon 2020 research and innovation programme under grant agreement No. 666918 (BATCure), the Batten Disease Family Association, and Biomarin; has received personal fees from Biomarin; receives salary support from University College London (UCL); and Medical Research Council (MRC) provides funding to the MRC Laboratory for Molecular Cell Biology University Unit at UCL (award code MC_U12266B) towards lab and office space. SFB has received grants from UCB Pharma, Eisai, and SciGen; has a patent (8288096) for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies, with no financial return; and was a consultant to Bionomics and Athena diagnostics before 2014. JDC has received research support from BioMarin Pharmaceuticals Inc, Abeona Therapeutics Inc, and Cerespir Inc; and also acts as a consultant for Regenexbio Inc. All other authors declare no competing interest.

Acknowledgments

We thank clinicians, scientists, and families who participate in the NCL Disease Registry and NCL Mutation Database, allowing more comprehensive understanding of this disease.

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