NEURAL REGENERATION RESEARCH
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Ocular therapies for neuronal ceroid lipofuscinoses: more than meets the eye

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The neuronal ceroid lipofuscinoses (NCLs), also known as Batten disease, are a group of inherited, neurodegenerative, lysosomal storage diseases typically manifesting in childhood. There are currently 13 known forms of NCL resulting from various mutations in the CLN (ceroid lipofuscinoses neuronal) genes (CLN1-8 and CLN10-14). Although varying in onset and severity, the NCLs share several phenotypic features including seizures, motor dysfunction, cognitive decline, and progressive loss of vision (Mole et al., 2011). Current treatment strategies being investigated in pre-clinical studies and early stage clinical trials primarily target the brain and spinal cord. While these potential therapeutics show promise in attenuating neurological disease, protection against retinal dysfunction and degeneration is generally ineffective or not reported.

Vision loss in NCL is believed to be due to early and severe degeneration of the primary visual cortex of the brain, and accompanying dysfunction and death of retinal cells in the eye. There are few comprehensive studies of the visual phenotype in NCL patients, however reduced acuity is reported as the most common initial visual symptom, with the fundus appearing normal in the early stages of disease. Electroretinography (ERG) studies have shown that retinal cell dysfunction occurs early in most forms of the human disease, even in the absence of overt visual symptoms or fundus abnormalities. Both rod and cone driven ERG responses continue to deteriorate until they are completely abolished (Mole et al., 2011). Histopathological evaluation of the retina of NCL patients demonstrates an overall reduction in retinal thickness driven by a loss of cells in the inner and outer nuclear layers, with particularly severe loss of inner and outer segments of photoreceptors at end stage disease (Mole et al., 2011).

Many naturally occurring and genetically engineered animal models of NCL recapitulate both the neurological and visual pathology and symptomology of NCL. The CLN6^{nclf} mouse, a naturally occurring model of CLN6 disease, presents with visual deficits prior to motor dysfunction and neurodegeneration. Studies out of University College London and Sanford Research in 2019 showed that intracerebroventricular delivery of adeno-associated virus serotype 9 (AAV9) carrying the human *CLN6* gene (h*CLN6*) in neonatal CLN6^{nclf} mice protects against motor decline, significantly extends lifespan, and attenuates characteristic NCL neuropathology. Although no retinal outcomes were reported in these initial studies, a subsequent trial of intracerebroventricular delivery of h*CLN6* was able to prevent cell loss in the visual centers of the brain and in the retina of CLN6^{nclf} mice, and preserve visual acuity (White et al., 2021). Yet brain-directed gene therapy was unable to transduce the retina and prevent retinal degeneration in a mouse model of CLN10, despite successfully attenuating pathology in the brain and peripheral organs such as the liver and spleen (Shevtsova et al., 2010). A similar brain-directed gene therapy study in the knock-in mouse model of CLN3 disease (*Cln3*$^{\Delta ex7/8}$) at Weill Medical College also resulted in reduced lysosomal storage in the brain and mild decreases in gliosis and neuronal cell loss, but did not address vision loss or retinal degeneration. However, subsequent trials of intraocular gene therapy in the CLN3 and CLN6 mouse models have shown promise. In both *Cln3*$^{\Delta ex7/8}$ and CLN6^{nclf} mice, targeting of inner retinal cells, particularly bipolar cells, via intravitreal delivery and/or the use of a bipolar cell-specific AAV serotype, was more therapeutically efficacious than targeting photoreceptors via subretinal delivery (Kleine Holthaus et al., 2018, 2020). Expression of CLN3 or CLN6 in the inner retina led to increased survival of bipolar cells or photoreceptors, respectively, and preservation of retinal function. Collectively, aside from the study by White et al. (2021), existing brain-directed studies in NCL mice have either not addressed the retinal disease or shown that the treatment alone is insufficient to correct it. It would be useful to compare retinal efficacy through different brain-directed routes of administration or vector constructs. The success of ocular gene therapies in NCL mice highlight the need for more specific therapeutic targeting of the retina, and even transduction of particular retinal cell types based on the differential expression patterns of the various NCL genes in the retina and selective vulnerability of retinal cells in the different forms of NCL.

NCL also occurs naturally in many breeds of dog, including Border Collies, Dachshunds, English Setters, Australian Shepherds, and American Bulldogs. The most comprehensively studied canine model of NCL is Dachshunds with a null mutation in the tripeptidyl peptidase-1 (*TPP1*) gene, leading to CLN2 disease. *TPP1*-deficient dogs display many of the same symptoms as CLN2 patients including ataxia, tremors, sensory deficits, and vision loss. Trials of brain-directed enzyme replacement therapy and AAV-mediated gene therapy in CLN2 dogs at the University of Missouri demonstrated clinical and neuropathological benefits including increased life span, delay in onset and progression of motor and cognitive symptoms, and reduced brain atrophy. Whilst these brain-directed therapies were able to delay the onset of pupillary light reflex deficits, they did not have any significant impact on other measures of visual function including ERG responses (Katz et al., 2015; Whiting et al., 2014). As with the mouse NCL models, a more direct approach to treating the retinal component of disease in CLN2 dogs was subsequently trialed. Intravitreal delivery of mesenchymal stem cells transduced with human *TPP1* cDNA resulted in dose-dependent reduction in retinal detachment lesions and ERG deficits (Tracy et al., 2016). Similarly, regular intravitreal injections of the human TPP1 enzyme was able to preserve retinal structure and function, however also caused local inflammatory reactions which were more severe the shorter the intervals between doses (Whiting et al., 2020). These trials of enzyme replacement and gene therapies for NCL in a well-studied large animal model again highlight the need for treatment of the eye to be considered and further optimized in pre-clinical therapy trials for NCL, to address the retinal component of disease.

Several breeds of sheep develop naturally occurring forms of NCL, including CLN5 disease in Borderdale sheep and CLN6 disease in South Hampshire and Australian Merino sheep. Affected sheep manifest progressive brain atrophy, motor and cognitive decline, and loss of vision. A single intracerebroventricular delivery of AAV9 expressing ovine *CLN5* (AAV9.o*CLN5*) to both pre- and post-symptomatic CLN5 affected sheep was able to extend their lifespan, maintain pre-treatment brain volumes and significantly slow neurological decline (Mitchell et al., 2018). Interestingly, although treated sheep did still lose their vision, the onset of visual deficits was considerably delayed compared to untreated affected sheep. This suggests that brain-directed treatment can protect against degeneration of the visual cortex, but did not afford long-term protection to the retina. In a

consequent trial, intravitreal administration of the same vector (AAV9.o*CLN5*) in CLN5 affected sheep protected against visual decline and retinal degeneration in the treated eye (Murray et al., 2021). In contrast, intravitreal administration of gene therapy for CLN6 disease (AAV9.o*CLN6*) in affected South Hampshire sheep provided minor attenuation of retinal atrophy but no prevention of visual decline (Murray et al., 2021).

The success of brain-directed treatments in NCL animal models has paved the way for several clinical trials, including current trials of AAV9-mediated gene therapy via intrathecal delivery in children with CLN3 disease (clinicaltrials.gov NCT03770572) and CLN6 disease (NCT02725580), and a completed trial of enzyme replacement therapy by regular intracerebroventricular infusions of TPP1 (NCT01907087). Unfortunately, it seems unlikely, based on what we know from animal studies, that brain-directed treatments will greatly benefit the eye and therefore prevent retinal degeneration and vision loss in these children. Not only have brain-directed treatments administered via the cerebrospinal fluid (intracerebroventricular or intrathecal) failed to protect vision in most animal models, the cerebrospinal fluid does not come into contact with the retina so direct transduction of retinal cells is unlikely. Retrograde axonal transport of AAV's along the optic nerve to the eye is a possible mechanism of transduction and there is evidence of AAV vectors delivered systemically or into the cerebrospinal fluid leading to expression in retinal cells however these studies are predominantly in mice and this phenomenon has not been observed in larger animals (Taghian et al., 2020).

Ocular therapy for NCLs is still an emerging field and the optimal approach will vary depending on the NCL variant the treatment is for. As we have seen in animal models there are several options for the type of therapy employed, including enzyme replacement or gene therapy. For NCL variants where the function of the deficient protein is known, such as the lysosomal enzyme TPP1 in CLN2 disease, enzyme replacement therapy may be an option for treating retinal disease. However, as enzyme replacement requires regular dosing we need to consider whether it is clinically feasible to perform repeated injections to the eye in children. A treatment that involves a single dose, such as gene therapy, may be a preferable option in a clinical setting regardless of whether the function of the protein is known or not. Another factor for consideration is the route of administration. For ocular gene therapies there are several approaches to targeting the retina including intravitreal, sub-retinal, and suprachoroidal delivery. The approach used will depend on whether the deficient NCL protein is soluble or membrane-bound, and also which retinal cells are most vulnerable to disease. Deficiencies in soluble proteins may be more amenable to intravitreal therapies, whereas membrane-bound proteins will need a more targeted approach, for example direct delivery of therapy to the sub-retinal space if targeting outer retinal cells. In addition, higher doses are likely to be required for treating deficiencies in membrane-bound proteins to target as many cells as possible and counter the lack of cross-correction.

Given the success we have seen from brain-directed and eye-directed therapies separately, we believe we now need to move our focus to a dual route of administration approach. Ideally these treatments will be given at the same time, to reduce the number of anesthetic procedures required but also, in the case of AAV-mediated gene therapy, to allow the same therapeutic product to be delivered to the brain and eye without the body having a chance to develop neutralizing antibodies to the capsid or transgene. Optimizing a treatment strategy that targets both the brain and eye is the best way to ensure children with NCL are protected from neurological disease and also retain their vision. In doing so, we will achieve not only a longer life but, more importantly, an improved quality of life for NCL patients.

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Date of submission: July 12, 2021
Date of decision: August 13, 2021
Date of acceptance: September 8, 2021
Date of web publication: January 7, 2022

https://doi.org/10.4103/1673-5374.332148
How to cite this article: *Murray SJ, Mitchell NL (2022) Ocular therapies for neuronal ceroid lipofuscinoses: more than meets the eye. Neural Regen Res 17(8):1755-1756.*

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C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y