

# A canine model for neuronal ceroid lipofuscinosis highlights the promise of gene therapy for lysosomal storage diseases

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Patients with lysosomal storage diseases (LSDs) have abnormal lysosomal accumulation of waste products, due in most cases to enzyme deficiencies (1). The neuronal ceroid lipofuscinoses (NCLs), a subset of LSDs, are the most common childhood-onset neurodegenerative diseases and affect approximately 1 in 100,000 individuals globally (2). Children with this disease show a common set of clinical manifestations including seizures, progressive vision and motor control loss (ataxia), and accumulation of autofluorescent intracellular plaques called ceroid/lipofuscin in multiple tissues including the brain (3). The pattern of inheritance of NCLs is largely autosomal recessive, and mutations in any of 14 genes have been linked to the disease (4). Timing of disease onset correlates with which of these genes has been mutated. The two most studied and best-understood forms of NCL, Infantile NCL (INCL, onset at 6–24 months) and Late-infantile NCL (LINCL, onset at 2–4 years), are caused by mutations in the lysosomal enzymes Palmitoyl-protein thioesterase 1 (PPT1) and Tripeptidyl peptidase 1 (TPP1), respectively. There are no FDA-approved therapies for NCL, and although therapeutic treatments that manage disease symptoms are in use, the disease is inevitably fatal.

Relatively little is known about the normal function of the genes mutated in NCL. However, some NCL genes have orthologs in unicellular organisms such as yeast and the social amoeba *Dictyostelium discoideum*, allowing for characterization of these genes in tractable systems. Studies in unicellular organisms of the ortholog of the NCL gene *CLN3*, which encodes a multi-pass transmembrane protein that localizes to endosomes and lysosomes, have shown that the gene plays a role in vacuole homeostasis, pH regulation, and protein processing and secretion (5,6). Interestingly,

both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* lack a TPP1 orthologue, though studies of a *Dictyostelium* TPP1 orthologue suggest that TPP1 plays a role in maintaining cell viability under autophagic conditions (7).

Several clinical trials of NCL therapies have been performed or are currently ongoing and break down into four groups: (I) small molecules aimed at limiting neuroinflammation and/or clearing lysosomal storage material; (II) enzyme replacement therapies (ERTs), in which recombinant enzymes are delivered intracranially; (III) gene therapy by adeno-associated virus (AAV) delivery into the brain parenchyma or cerebrospinal fluid (CSF), or (IV) transplantation of neural stem cells that supply the lacking enzyme (8). Gene therapy in particular is an attractive potential therapy for the NCLs for multiple reasons. In contrast to ERT therapies, which can require invasive treatments as frequently as bi-weekly (ClinicalTrials.gov ID NCT01907087), gene therapy treatments would require less frequent or even single treatments. Further, the ependyma, the thin epithelial cell layer of the brain ventricular system, is readily transduced with certain AAV serotypes (9). Previous studies have shown that lysosomal enzyme-deficient cells can be rescued by being in the presence of cells with the wild-type enzyme (10), demonstrating that secreted lysosomal enzymes can be taken up by other cells. Thus, critical cells such as neurons might not themselves need to uptake the lacking gene to acquire the enzyme if they share extracellular fluid with transduced cells, and therefore transduction of the ependyma may be sufficient to supply the brain parenchyma with the lacking enzyme. Studies of gene therapy approaches in mouse models of INCL and LINCL (11) have shown that delivery of the lacking enzyme by gene therapy results in reduced autofluorescent deposits and reduction in disease

symptoms, suggesting that gene therapy may be an effective human therapy. However, further analysis of gene therapy for NCL treatment in large animal models has been lacking.

Large animal disease models can serve as an important bridge between murine studies and human therapies and are better suited for the development of invasive therapeutic technologies than mice given their closer size to humans. Further, given their inbred genetic history, laboratory mice can have short lifespans that limit the ability for long duration studies, and genetically engineered murine disease models sometimes fail to adequately recapitulate human genetic diseases. Thus the study of NCLs in larger animals may facilitate the development of translational therapies. Cases of NCL in canines have been reported since 1973 (12), and more recently a frame shift mutation in TPP1 was found to be responsible for NCL clinical manifestations in a population of dachshunds (13). These dogs show autofluorescent deposits in CNS tissue, neuronal depletion, seizures, and precocious death after progressive motor control decline. Analogous to LINCL patients, TPP1 mutant dogs show progressive worsening of both visual aptitude due to retinal dysfunction (14), and cognitive function as shown in a behavioral assay for learning and memory (15). Together, these findings indicate that the TPP1 mutant canine model is useful for the development of NCL therapies. Impressive progress has already been made on this front, as ERT treatment of TPP1 mutant dogs by bi-weekly enzyme delivery into the cerebrospinal fluid delays disease symptoms, reduces brain atrophy, and increases lifespan (16).

In a recent report in *Science Translational Medicine* (17), Katz *et al.* used gene therapy in the mutant TPP1 canine model. The authors used AAV, a popular gene therapy vector due to its lack of pathogenicity, its ability to infect non-replicating cells, and its low rate of host genome integration due to episomal localization (18). To target the CNS for TPP1 delivery, intraventricular injection of virus was performed to transduce ependymal cells, following similar successful approaches for gene therapy of CNS diseases in murine models (19). After testing several viral serotypes, the authors found that intraventricular injection of the rAAV2 serotype led to robust reporter gene expression in the ependyma. When a TPP1 transgene was delivered into TPP1 null dogs by this method, TPP1 levels in the CSF at 5 days post treatment were up to 30 times higher than those found in wild-type dogs, despite the fact that TPP1 null dogs normally show less than 1 percent of wild-type levels of TPP1 activity (13). However, TPP1 activity in treated TPP1 null dogs decreased to background levels by two months post treatment. This

decrease was likely due at least in part to the production of anti-TPP1 antibodies, despite the fact that dogs were being administered the immunosuppressant cyclosporine. To address this issue, the authors supplemented the gene therapy treatment with the immunosuppressant mycophenolate mofetil (MMF), which can suppress an immune response to extended gene therapy treatment (20). Addition of MMF to the treatment regimen increased the duration of TPP1 CSF activity when administered starting at 33 days post treatment or, more effectively, five days before treatment. For this reason, these MMF-treated dogs were used for further analysis.

Compared to TPP1 null dogs, dogs treated with this gene therapy approach showed delayed onset of a range of disease symptoms. For example, whereas cerebellar ataxia develops in TPP1 null dogs at an average of 7.3 months, treated animals had an average ataxia onset of 12.7 months with a range of 12 to 13.8 months in four animals. Similar delays were seen for impaired proprioception, loss of menace response, nystagmus, and pupillary light reflex abnormalities. Benefits of the gene therapy treatment were also observed in behavioral tests for memory and learning. Untreated TPP1 null dogs had to be euthanized due to disease progression at an average age of 10.4 months as compared with 17.5 months for treated dogs, with one treated dog performing well in learning and memory-based cognitive assays until 20 months. Although treated dogs did eventually succumb to the disease, these data make a strong case for the effectiveness of AAV-mediated expression of TPP1 in significantly delaying LINCL-like symptoms in a large animal model.

In treated animals, TPP1 was widely distributed throughout the brain parenchyma, indicating that TPP1 protein can spread through the CNS from the AAV-transfected ependymal cells. Interestingly, TPP1 staining was most evident in neurons and much less so in astrocytes or glia, although the cause of this difference is unknown. Autofluorescent lysosomal deposits, a hallmark of NCL, were sharply reduced in the spinal cord and occipital cortex in treated animals, and the degree of this autofluorescent deposit reduction in individual animals correlated with the effectiveness of treatment in slowing disease progression. Neuroinflammation is thought to play a role in NCL pathogenesis (21), and whereas TPP1 null dogs showed widespread reactive astrogliosis in the brain, this phenotype was reduced in treated animals. Slight increases in TPP1 activity were detected even in peripheral tissues such as the heart (although not in the liver or kidney), suggesting that this treatment approach could potentially ameliorate systemic effects of TPP1 deficiency outside of the CNS. However,

recent work indicates that in intraventricular gene therapy-treated dogs, TPP1 is not detectable in the retina, and retinal degeneration similar to that in untreated dogs is observed (22). It is therefore likely that localized gene delivery to the retina will be required to limit retinal degeneration in NCL patients. Fortunately, much progress has already been made on retinal delivery of gene therapy vectors (18).

This report, by demonstrating the effectiveness of AAV-mediated intraventricular gene therapy for LINCL in a large animal model, provides important reinforcement of the promise of this approach to treat human NCLs and, more broadly, other LSDs. Further, this work provides a model system for fine-tuning of NCL therapy approaches that is more analogous to humans than previously utilized murine models. However, there are significant questions and hurdles that remain to be addressed. The treatment regime in this report included MMF, an immunosuppressant employed to help reduce the emergence of neutralizing TPP1 antibodies. However, neuroinflammation itself is thought to play a role in NCL pathogenesis, and treatment of mouse models of NCL with MMF alone can reduce neuroinflammation and improve motor function (21), and a clinical trial of MMF for juvenile-onset NCL (ClinicalTrials.gov ID NCT01399047) has been performed. In the canine study, a single dog was treated with only MMF, and did not show the life span extension or cognitive improvements seen in gene therapy-treated animals. However, the interplay between MMF activity and TPP1 gene therapy in symptom delay is still somewhat unclear.

Despite the impressive success in the work described above and other efforts in delaying the onset of NCL by gene therapy or ERT, it has unfortunately been the case that treatments have managed to delay but not halt the onset of symptoms. Extension of symptom delay will be a critical component of the development of a true cure for the NCLs. As the authors address in this study, immunomodulation to prevent both the neutralization of TPP1 and the immune system-mediated suppression of AAV transduction is an important concern, especially if multiple AAV treatments are to be administered. Another concern is the reduction of TPP1 expression due to ependymal cell turnover. Studies in rats indicate that the ependyma has a turnover rate of approximately 140 days (23), although other studies indicate that ependymal cells are quiescent under normal circumstances (24), and that AAV-mediated transgene expression can be maintained for up to a year (19), so the degree of ependymal turnover is not entirely clear. However, there is evidence that ependymal cells can

function as neural stem cells that proliferate and give rise to neurons and astrocytes (25). An interesting possibility is that a genome-integrating virus-based approach could be used to target these stem cells for transduction, so that these TPP1-expressing stem cells could potentially repopulate some area of the brain with TPP1-expressing progeny.

Although there are no current FDA-approved therapies for NCL, there is reason to be optimistic about the development of future therapies for this devastating disease based on the successful implementation of therapeutic strategies in animal models such as the one described above. Continued refinements and technological developments in such models should pave the way for gene therapy-based treatments for patients with NCLs and other LSDs.

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### Footnote

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### References

1. Cheng SH. Gene therapy for the neurological manifestations in lysosomal storage disorders. *J Lipid Res* 2014;55:1827-38.
2. Santavuori P. Neuronal ceroid-lipofuscinoses in childhood. *Brain Dev* 1988;10:80-3.
3. Miley CE 3rd, Gilbert EF, France TD, *et al.* Clinical and extraneural histologic diagnosis of neuronal ceroidlipofuscinosis. *Neurology* 1978;28:1008-12.
4. Mole SE, Cotman SL. Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochim Biophys Acta*

- 2015;1852:2237-41.
5. Gachet Y, Codlin S, Hyams JS, et al. *btn1*, the Schizosaccharomyces pombe homologue of the human Batten disease gene CLN3, regulates vacuole homeostasis. *J Cell Sci* 2005;118:5525-36.
  6. Huber RJ, Myre MA, Cotman SL. Loss of Cln3 function in the social amoeba Dictyostelium discoideum causes pleiotropic effects that are rescued by human CLN3. *PLoS One* 2014;9:e110544.
  7. Phillips JE, Gomer RH. Partial genetic suppression of a loss-of-function mutant of the neuronal ceroid lipofuscinosis-associated protease TPP1 in Dictyostelium discoideum. *Dis Model Mech* 2015;8:147-56.
  8. Geraets RD, Yon Koh S, Hastings ML, et al. Moving towards effective therapeutic strategies for Neuronal Ceroid Lipofuscinosis. *Orphanet Journal of Rare Diseases* 2016;11:40.
  9. Davidson BL, Stein CS, Heth JA, et al. Recombinant adeno-associated virus type 2, 4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. *Proc Natl Acad Sci U S A* 2000;97:3428-32.
  10. Fratantoni JC, Hall CW, Neufeld EF. Hurler and Hunter syndromes: mutual correction of the defect in cultured fibroblasts. *Science* 1968;162:570-2.
  11. Sondhi D, Hackett NR, Peterson DA, et al. Enhanced survival of the LINCL mouse following CLN2 gene transfer using the rh.10 rhesus macaque-derived adeno-associated virus vector. *Mol Ther* 2007;15:481-91.
  12. Koppang N. Canine ceroid-lipofuscinosis-a model for human neuronal ceroid-lipofuscinosis and aging. *Mech Ageing Dev* 1973;2:421-45.
  13. Awano T, Katz ML, O'Brien DP, et al. A frame shift mutation in canine TPP1 (the ortholog of human CLN2) in a juvenile Dachshund with neuronal ceroid lipofuscinosis. *Mol Genet Metab* 2006;89:254-60.
  14. Whiting RE, Narfström 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;125:164-72.
  15. Sanders DN, Kanazono S, Wininger FA, et al. A reversal learning task detects cognitive deficits in a Dachshund model of late-infantile neuronal ceroid lipofuscinosis. *Genes Brain Behav* 2011;10:798-804.
  16. Katz ML, Coates JR, Sibigtroth CM, et al. Enzyme replacement therapy attenuates disease progression in a canine model of late-infantile neuronal ceroid lipofuscinosis (CLN2 disease). *J Neurosci Res* 2014;92:1591-8.
  17. Katz ML, Tecedor L, Chen Y, et al. AAV gene transfer delays disease onset in a TPP1-deficient canine model of the late infantile form of Batten disease. *Sci Transl Med* 2015;7:313ra180.
  18. Schön C, Biel M, Michalakis S. Retinal gene delivery by adeno-associated virus (AAV) vectors: Strategies and applications. *Eur J Pharm Biopharm* 2015;95:343-52.
  19. Yamazaki Y, Hirai Y, Miyake K, et al. Targeted gene transfer into ependymal cells through intraventricular injection of AAV1 vector and long-term enzyme replacement via the CSF. *Sci Rep* 2014;4:5506.
  20. Shin JH, Yue Y, Srivastava A, et al. A simplified immune suppression scheme leads to persistent micro-dystrophin expression in Duchenne muscular dystrophy dogs. *Hum Gene Ther* 2012;23:202-9.
  21. Seehafer SS, Ramirez-Montealegre D, Wong AM, et al. Immunosuppression alters disease severity in juvenile Batten disease mice. *J Neuroimmunol* 2011;230:169-72.
  22. Whiting RE, Jensen CA, Pearce JW, et al. Intracerebroventricular gene therapy that delays neurological disease progression is associated with selective preservation of retinal ganglion cells in a canine model of CLN2 disease. *Exp Eye Res* 2016;146:276-82.
  23. Chauhan AN, Lewis PD. A quantitative study of cell proliferation in ependyma and choroid plexus in the postnatal rat brain. *Neuropathol Appl Neurobiol* 1979;5:303-9.
  24. Carlén M, Meletis K, Göritz C, et al. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. *Nat Neurosci* 2009;12:259-67.
  25. Johansson CB, Momma S, Clarke DL, et al. Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* 1999;96:25-34.

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