Revoking the Seize Order: Preventing Spontaneous Seizures With AAV in a CLN2 Mouse Model

Gene Therapy Ameliorates Spontaneous Seizures Associated With Cortical Neuron Loss in a *Cln2^{R207X}* Mouse Model

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Although a disease-modifying therapy for classic late infantile neuronal ceroid lipofuscinosis (CLN2 disease) exists, poor understanding of cellular pathophysiology has hampered the development of more effective and persistent therapies. Here, we investigated the nature and progression of neurological and underlying neuropathological changes in *Cln2^{R207X}* mice, which carry one of the most common pathogenic mutations in human patients but are yet to be fully characterized. Long-term electroencephalography recordings revealed progressive epileptiform abnormalities, including spontaneous seizures, providing a robust, quantifiable, and clinically relevant phenotype. These seizures were accompanied by the loss of multiple cortical neuron populations, including those stained for interneuron markers. Further histological analysis revealed early localized microglial activation months before neuron loss started in the thalamocortical system and spinal cord, which was accompanied by astrogliosis. This pathology was more pronounced and occurred in the cortex before the thalamus or spinal cord and differed markedly from the staging seen in mouse models of other forms of neuronal ceroid lipofuscinosis. Neonatal administration of adeno-associated virus serotype 9–mediated gene therapy ameliorated the seizure and gait phenotypes and prolonged the life span of *Cln2^{R207X}* mice, attenuating most pathological changes. Our findings highlight the importance of clinically relevant outcome measures for judging preclinical efficacy of therapeutic interventions for CLN2 disease.

Commentary

Targeted gene therapies are becoming more common, with many genetic diseases, including genetic epilepsies, being amenable to gene therapies.[1] Neuronal ceroid lipofuscinosis (NCL) is a class of genetic diseases that may benefit from development of gene therapies. These are a group of lysosomal storage diseases that affect children through young adults.[2] Several forms, including the late-infantile form (CLN2), prominently feature epilepsy, along with other features including ataxia, speech delay, psychomotor retardation, and early mortality.[2] CLN2 is caused by deficiency of the enzyme tripeptidyl peptidase 1 (TPP1).[2] A TPP1 replacement therapy is available which helps to some extent, but it is not curative and necessitates biweekly injections.[3] Targeted gene therapies to restore the dysfunctional gene have the potential to be curative. One limitation to development of gene therapies is the lack of animal models that recapitulate disease features in which to test the putative therapy.

Here, Takahashi and colleagues report a new mouse model carrying a common gene mutation found in patients with CLN2, *Cln2^{R207X}*.[4] First, to assess for behavioral phenotypes,

Cln2^{R207X} mice and wildtype (WT) littermates were implanted with EEG electrodes and longitudinal EEG data collected. Nine of 10 *Cln2^{R207X}* mice displayed spontaneous seizures between 14 and 19 weeks. These mice all died following a seizure. The one that did not have a seizure died at 17 weeks. No WT mice had seizures or died. A separate cohort of *Cln2^{R207X}* and WT mice underwent gait assessment at 1, 2, 3, and 4 months of age. When compared to WT mice, *Cln2^{R207X}* mice developed gait abnormalities during the fourth month. Seizures, early death, and late-onset gait abnormalities are all consistent with CLN2 in patients.

Second, to assess for neuroanatomical changes, groups of *Cln2^{R207X}* and WT mice were euthanized at 1, 2, 3, and 4 months of age, brains extracted, and somatosensory cortex, thalamus, and spinal cord histologically assessed. There was progressive loss of pyramidal neurons in cortex and thalamus. There was also progressive loss of parvalbumin (PV)-, calbindin (CB)-, somatostatin (SS)-, and calretinin (CR)-positive GABAergic interneurons in the cortex, loss of PV+ interneurons in the thalamus, and loss of SS+ interneurons in the hippocampal hilus. Thus, increased excitability and seizure risk were likely due to inhibitory interneuron loss.

Third, since a hallmark of CLN2 is accumulation of storage material in lysosomes, groups of $Cln2^{R207X}$ and WT mice were euthanized, and brains collected and assessed for storage material accumulation at postnatal day 1 (P1), and 1, 2, 3, and 4 months of age. Threshold analysis of immunostaining for subunit c of mitochondrial ATPase (SCMAS) was employed for this assessment since SCMAS is a prominent protein comprising storage material. $Cln2^{R207X}$ mice showed progressive lysosomal storage material accumulation across the 8 sites in the CNS, including cortical, subcortical, and spinal cord regions, consistent with NCL.

Microglial activation and astrogliosis are features of CLN2, but these had previously only been documented late in disease. Therefore, to assess development of these inflammatory changes, $Cln2^{R207X}$ and WT mice were euthanized, and brains collected and assessed for microglial activation and astrogliosis at 1, 2, 3, and 4 months of age. In all CNS sites examined, there was early and progressive microglial activation. Astrogliosis was also present in all CNS sites evaluated, but it occurred at later ages. Assessment of a range of cytokines and chemokines at the same timepoints revealed distinct neuroinflammatory changes especially at later ages.

Finally, to assess whether behavioral and anatomical phenotypes could be rescued in $Cln2^{R207X}$ mice, a single intracerebroventricular injection of an adeno-associated virus 9 (AAV9)-mediated gene therapy (AAV9.hCLN2)[5] was made at P1. This single application of the AAV9-mediated therapy increased TPP1 activity by 28-fold in the forebrain and 15-fold in the spinal cord. Such treatment also attenuated neuropathological changes by reducing neuron loss, microglial activation, and astrogliosis, and by completely preventing storage material accumulation. Epileptiform activity was reduced, and spontaneous seizures were absent in 9 of 10 animals up to 20 weeks after treatment. Only 2 of the 10 treated mice died before 20 weeks, whereas all vehicle-treated and untreated mice died before 20 weeks. Six of the 8 treated $Cln2^{R207X}$ mice survived up to 48 weeks, a nearly 3-fold improvement in life expectancy compared to vehicle-treated or untreated mice. Gait was also greatly improved in treated $Cln2^{R207X}$ mice.

The use of AAV vectors to mediate genetic manipulations is widespread in basic, preclinical research, and in clinical trials.[1;6] This is an exceedingly useful tool to knock in disease-causing mutations found in patients, express/delete proteins of interest, cause cell-type specific lesions, trace neuronal connections, and so on. In some genetic diseases, antisense oligonucleotides can be useful to silence the mutated allele. Such therapies exist for some diseases and are under development for others.[7] In other cases, such an approach is insufficient to effect changes, but instead measures to restore gene function are required. This manuscript provides proof of principle that such an approach can work. That they had such extensive effect with a single injection just after birth is intriguing. If such a therapeutic approach could be employed in patients, this would be a radical improvement from existing therapies. For one, this appears to be nearly curative for most phenotypes. Second, this is just a single injection early in life,

not biweekly for life. It would be interesting to know whether this abolishes the early mortality normally associated with the disease. While deaths generally occurred following seizures, it is not clear if these are seizure-related deaths consistent with sudden unexpected death in epilepsy (SUDEP).[8] As they demonstrated, there are many progressive neuroanatomical changes in these mice that could contribute to increased mortality.[4] More work to dissect factors associated with mortality in this model will be needed. Perhaps taking a page from the study of SUDEP in preclinical models and measuring cardiorespiratory physiology in these animals will be useful.[9]

There, of course, are many challenges to overcome before these findings can be translated to patients. For instance, with AAV9.hCLN2 application there was somewhat unexpected regional variability in gene expression. There was also a distinct immune response to the overexpression of TPP1, and there was rescue of CB+, but not PV+ interneurons. Recombinant AAV-mediated therapies are in use for some diseases, but more work is needed to address curiosities such as these and others that may arise with AAV-mediated expression of specific genes before they can be employed more widely. When would be the ideal time to make the injection? Does it need to be on day one of life, or can it be later? How will it be known that the soon to be born baby has the mutation of interest? Will this require whole exome or whole genome sequencing during gestation? Ideally, some biomarker would be developed to detect the disease/mutation and identify individuals in which to deploy the therapy. Nevertheless, this is an impressive effect of the gene therapy in this mouse model. It will be interesting to learn how widely such AAV-mediated gene therapies can be applied to other genetic epilepsies.

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Declaration of Conflicting Interests

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