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# Enzyme Replacement Therapy Attenuates Disease Progression in a Canine Model of Late-Infantile Neuronal Ceroid Lipofuscinosis (CLN2 Disease)

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Using a canine model of classical late-infantile neuronal ceroid lipofuscinosis (CLN2 disease), a study was conducted to evaluate the potential pharmacological activity of recombinant human tripeptidyl peptidase-1 (rhTPP1) enzyme replacement therapy administered directly to the cerebrospinal fluid (CSF). CLN2 disease is a hereditary neurodegenerative disorder resulting from mutations in *CLN2*, which encodes the soluble lysosomal enzyme tripeptidyl peptidase-1 (TPP1). Infants with mutations in both *CLN2* alleles develop normally but in the late-infantile/early-childhood period undergo progressive neurological decline accompanied by pronounced brain atrophy. The disorder, a form of Batten disease, is uniformly fatal, with clinical signs starting between 2 and 4 years of age and death usually occurring by the early teenage years. Dachshunds homozygous for a null mutation in the canine ortholog of *CLN2* (*TPP1)* exhibit a similar disorder that progresses to end stage at 10.5–11 months of age. Administration of rhTPP1 via infusion into the CSF every other week, starting at approximately 2.5 months of age, resulted in dose-dependent significant delays in disease progression, as measured by delayed onset of neurologic deficits, improved performance on a cognitive function test, reduced brain atrophy, and increased life span. Based on these findings, a clinical study evaluating the potential therapeutic value of rhTPP1 administration into the CSF of children with CLN2 disease has been initiated. 

**Key words:** Batten disease; tripeptidyl peptidase-1; *TPP1*; Dachshund; lysosomal storage disease; cerebrospinal fluid; NCL; CLN2

Classical late-infantile neuronal ceroid lipofuscinosis (CLN2 disease) is an autosomal-recessive inherited, progressive neurodegenerative disorder that results from mutations in the *CLN2* gene (Sleat et al., 1997; Warrier et al., 2013). Children with this disorder exhibit apparently normal development until 2–4 years of age, after which they begin a progressive neurological decline that culminates in death, usually in the early to middle teenage years (Mole et al., 2011). Neurological signs of CLN2 disease include progressive cognitive decline, ataxia, seizures, myoclonus, and vision loss. As the disease progresses, the children lose the ability to communicate and voluntary and involuntary muscle control. There are no disease-altering treatments for the CLN2 disorder, only symptomatic relief with antiepileptics.

A Dachshund model of CLN2 disease with a spontaneous null mutation in tripeptidyl peptidase-1 (*TPP1*) has been developed and characterized (Awano et al., 2006; Katz et al., 2008; Sanders et al., 2011). Affected dogs exhibit many of the same signs that are seen in children with the CLN2 disorder and therefore offer a good model for testing the pharmacologic activity of potential therapeutic interventions for this disease.

The TPP1 protein is a soluble lysosomal enzyme that plays an important role in protein catabolism. TPP1 is synthesized as a 66-kDa, 563-amino-acid proenzyme that is activated in the lysosome by cleavage of a 20-kDa peptide to yield the 46-kDa active enzyme (Chang et al.,

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**TABLE I. Experimental Groups**

| Genotype | Treatment | No. of dogs |
|---|---|---|
| *TPP1*$^{+/+}$ | Vehicle | 4 |
| *TPP1*$^{+/+}$ | 4 mg rhTPP1 | 3 |
| *TPP1*$^{+/+}$ | 16 mg rhTPP1 | 3 |
| *TPP1*$^{-/-}$ | Vehicle | 3 |
| *TPP1*$^{-/-}$ | 4 mg rhTPP1 | 3 |
| *TPP1*$^{-/-}$ | 16 mg rhTPP1 | 3 |
| *TPP1*$^{-/-}$ | 48 mg rhTPP1 | 1* |

*Initially, three dogs were in this group, but two had to be excluded early because of complications unrelated to the disease or to reactions to rhTPP1.

2008). Like many soluble lysosomal enzymes, TPP1 not only is localized within lysosomes but also is secreted from cells and taken up via cation-independent mannose-6-phosphate receptors that recognize the mannose 6-phosphate moieties common to many lysosomal enzymes. Thus, recombinant human tripeptidyl peptidase-1 (rhTPP1) enzyme-replacement therapy (ERT) to the brain should result in cellular uptake and trafficking to the lysosomes. Because large molecules such as TPP1 cannot cross the blood–brain barrier, enzyme delivery to the brain has been achieved only by direct CNS administration of rhTPP1 to the cerebrospinal fluid (CSF; Passini et al., 2006; Chang et al., 2008; Vuillemenot et al., 2011). With the Dachshund CLN2 disease model, we previously demonstrated that this route of administration of rhTPP1 results in widespread distribution and uptake of the active enzyme into many structures of the brain and in reduction of the accumulation of neuronal lysosomal storage material that is characteristic of this disease (Vuillemenot et al., 2011). Experimental treatment was therefore conducted to determine whether long-term administration of rhTPP1 via periodic infusion into the CSF of dogs with the CLN2 disorder would delay disease onset and/or attenuate the progression of the clinical signs.

## MATERIALS AND METHODS

### Study Design

Studies were undertaken to assess the pharmacodynamic effects of CSF administration of rhTPP1 in ameliorating the progression of neurological decline and brain atrophy in a Dachshund model of the CLN2 form of neuronal ceroid lipofuscinosis. Dogs that were homozygous for a null mutation in *TPP1* received CSF infusions of either rhTPP1 or vehicle starting prior to the onset of neurological disease signs and were monitored for the onset and progression of these signs. The progression of brain atrophy was assessed with quantitative measurements of brain ventricular volume. All procedures performed were approved by the University of Missouri Institutional Animal Care and Use Committee.

### Dog Breeding and Husbandry

Long-haired Dachshunds, one male and one female, that were both heterozygous for a null mutation in the *TPP1* gene (Awano et al., 2006) were bred to initiate development of a research colony. The descendants of these founder dogs and of unrelated, homozygous, normal long-haired Dachshunds were bred by using a strategy to minimize inbreeding while maximizing the yield of homozygous affected dogs. Breeding was performed by a combination of natural mating and artificial insemination. All puppies were genotyped at the *TPP1* locus by using an allelic discrimination assay with a real-time PCR instrument (Applied Biosystems, Carlsbad, CA). Dogs that were either homozygous for the mutant *TPP1* allele or homozygous for the normal allele were utilized in these studies. All dogs were implanted with microchips for identification prior to genotyping.

Dogs were housed in kennels maintained by the University of Missouri Office of Animal Resources (OAR). In addition to routine care and husbandry provided by the OAR, the dogs were socialized on a daily basis throughout the study.

### rhTPP1 Formulation and Administration

rhTPP1 was synthesized in Chinese hamster ovary cells and was purified by column chromatography (Lin and Lobel, 2001). rhTPP1 was formulated in an artificial CSF vehicle (aCSF; 216.5 mM NaCl, 0.8 mM $MgSO_4$, 3.01 mM KCl, 1.4 mM $CaCl_2$, 0.8 mM $Na_2HPO_4$, 0.2 mM $NaH_2PO_4$, pH 7.3) to concentrations of either 3.33 mg/ml or 13.33 mg/ml. aCSF was administered alone to normal control (*TPP1*$^{+/+}$) and CLN2 affected (*TPP1*$^{-/-}$) dogs. Endotoxin was less than 0.06 EU/ml in rhTPP1 and vehicle preparations.

At approximately 2 months of age, two catheters were implanted in each dog. One catheter terminated in a lateral ventricle of the brain (ICV catheter) and the other terminated in subarachnoid space at L5 (ITL catheter). The catheters were connected to subcutaneous titanium access ports anchored in the fascia of the muscle and subcutaneous tissues of the paracervical (ICV) and paralumbar (ITL) vertebral column. Detailed descriptions of the catheter and port implantation procedures are provided in the Supporting Information.

Beginning 2 weeks after the catheter implantation surgeries, the test substances were administered via infusion through the ICV or ITL catheters or directly into the cerebellomedullary cistern (CM) once every other week (see Supp. Info.). The treatment groups are listed in Table I. Three dogs were initially assigned to each treatment group. Two of the dogs assigned to the 48 mg treatment group developed meningitis and obstructive hydrocephalus early in the course of treatment and had to be euthanized; they were therefore excluded from the study. Each infusion of vehicle or 4 mg rhTPP1 was performed over a 2 hr period. Infusions of 16 mg rhTPP1 were initially conducted over a 2 hr period, but the duration was increased to 4 hr to mitigate systemic infusion-associated reactions. For the dog that received the 48 mg dose, all infusions were given over a 4 hr period. During the third infusion, the dog exhibited an anaphylactic reaction. To mitigate this adverse response, the subsequent dose was reduced to 2 mg rhTPP1. The dose was then gradually increased over the course of the 15 subsequent biweekly infusions until a dose of 48 mg had been reached. For all subsequent infusions, the dog received 48 mg rhTPP1, which was well tolerated. The 2 and 4 hr infusion rates were 0.6 or 0.3 ml/hr, respectively.

Detailed descriptions of the rhTPP1 administration procedures are provided in the Supporting Information. Dogs were sedated during ITL or ICV infusions of rhTPP1 as previously described (Vuillemenot et al., 2011). Vital parameters and clinical signs of infusion-associated reactions (hypotension, hyperpnoea, angioedema, discolored mucous membranes, etc.) were monitored during and after the rhTPP1 administration. Physical and neurologic examinations were performed between each infusion and injection procedure.

After 4 months of age, prior to each infusion, the location within the lateral ventricle and patency of the ICV catheter was assessed by computed tomography of the brain after infusion of 0.1 ml of the nonionic contrast agent Iohexol. Over time, either the ICV catheter became occluded or the intraventricular portion migrated into the brain parenchyma as the result of growth of the cranial cavity. When this occurred, the infusion site was switched from the ICV to the ITL catheter. The ITL catheters often became occluded as well. When this occurred, the treatment administration was continued by bolus injection of the agent into the subarachnoid space at the CM over a 2-min period. For the bolus injections the dogs were anesthetized as previously described (Vuillemenot et al., 2011).

### Neurologic and Physical Examination

Dogs underwent clinical assessments that encompassed physical and neurologic examinations on a weekly basis. Body weights were recorded in conjunction with physical examinations of all dogs prior to the first dose to establish baseline values and were recorded weekly thereafter. A neurologic examination was performed weekly throughout the course of the study. Signs of neurologic dysfunction were subjectively monitored by a standardized clinical neurologic examination (Lorenz et al., 2011). Components of the neurologic examination included observation of mentation, posture, and gait; testing of cranial nerves; evaluation of postural reactions (proprioceptive placement, paw replacement, hopping, wheelbarrow, tactile placement, and extensor postural thrust); spinal reflexes (myotatic and flexor); and sensory testing. Gait evaluation was assessed as normal or abnormal with presence of ataxia (cerebellar, general proprioceptive, vestibular) and paresis (ambulatory, nonambulatory). Postural reactions, spinal reflexes, cranial nerve tests, and sensation were assessed as intact, decreased, or absent. Dogs were also evaluated for abnormal movement and seizure activities. Age at onset was recorded for the following neurologic deficits: menace response deficits (unilateral and bilateral), visual tracking, intention tremor, head tremor, myoclonic jerks, proprioceptive placement of pelvic and thoracic limbs, ataxia, and circling.

### Cognitive Function Testing

Memory and reversal learning were tested objectively in the Dachshunds by using a T-maze apparatus and testing protocol developed by CanCog Technologies (Toronto, Ontario, Canada), with slight modifications. Detailed descriptions of the testing apparatus and protocol were published previously (Sanders et al., 2011). After an initial training period from 3 to 4 months of age, each dog was tested for its ability to achieve a performance criterion in the T-maze on a monthly basis. The T-maze consisted of a start box, a runway, and left and right reward arms. Monthly testing consisted of three phases, preference determination, preference reinforcement, and reversal learning. In the preference determination phase a food reward was placed at the end of each reward arm of the maze, and the dogs were allowed to navigate the maze nine times. Which side the dog chose most frequently was designated the preferred side. After determination of the preferred side, a food reward was placed only on this side, and the dog was allowed to navigate the maze 10 times each day until it reached criterion performance (eight correct choices on each of 2 consecutive days or nine or 10 correct choices on a single day). Once this criterion had been reached, the dog entered the reversal learning phase of the test. In this phase, the food reward was switched from the preferred/reinforced side to the opposite reward arm of the maze. The dog was then allowed to navigate the maze 10 times each day, and each choice was recorded as correct or incorrect. This procedure was repeated on a daily basis until the dog's performance reached the same criterion as for the reinforcement phase. The food reward was then switched to the opposite arm of the maze and the test was continued until the dog reached criterion with the food on this side of the maze. Finally, the location of the food reward was again switched to the opposite arm of the maze and the month's trial was completed when the dog had reached criterion performance with the reward on this side of the maze. Each dog was then scored for the number of incorrect choices it made in order to reach criterion performance in the reversal learning phase of the test.

### MRI Analysis of Brain Atrophy

The brains of all dogs were examined by magnetic resonance imaging (MRI) prior to ICV catheter placement to locate the ventricles. Subsequent MRI of the brains was performed during the weeks when doses 10 and 20 were administered and then every 6 weeks thereafter if the dogs continued in the study past dose 20. Dogs were placed under general anesthesia for MRI acquisition. Imaging was performed with a 1.5-Tesla instrument (Signa; General Electric Healthcare, Milwaukee, WI). Pulse sequences were selected to obtain T2-weighted and FLAIR sequences in three planes. In addition, a 3D isotropic voxel T1W fast spoiled gradient-echo sequence was obtained for surgical planning of ICV placement and volumetric studies.

Brain ventricular volume determinations to assess brain atrophy were performed by analyzing the MRI images in Brainsight software (Rogue Research, Montreal, Quebec, Canada). The image of each MRI slice through the brain was manipulated to isolate the ventricles. The software then automatically assembled the ventricular images from each slice into a three-dimensional image of the ventricular system. Each image was examined prior to volumetric determination, and manual adjustments to the image were made if necessary to correct for any errors made by the program in identifying the boundaries of the ventricles. The total ventricular volume was then calculated by the software from the composite three-dimensional image.

### Survival

As with human late-infantile neuronal ceroid lipofuscinosis (NCL, CLN2 form), CLN2 disease in Dachshunds is a

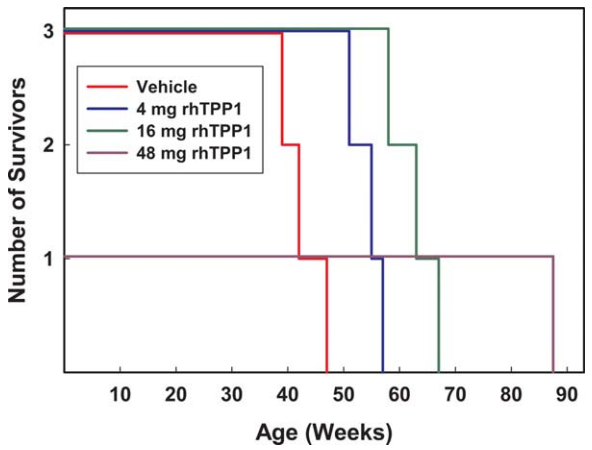


Fig. 1. Survival times of $TPP1^{-/-}$ Dachshunds in the four treatment groups. Two of the dogs given the 16 mg dose were euthanized prior to reaching end-stage disease because they had developed meningitis or obstructive hydrocephalus. The dog receiving the 48 mg dose was euthanized before reaching end-stage disease because it had developed obstructive hydrocephalus. All remaining dogs were euthanized when they reached end-stage disease.

progressive neurodegenerative disease that is ultimately fatal. For humane reasons, euthanasia was performed by using a uniform criterion for defining end-stage disease. End-stage disease criteria consisted of loss of cognition, severe mentation abnormalities, loss of visual tracking, medication-refractory myoclonic jerks, and inability to eat without significant assistance. Survival time was defined as the time from birth to euthanasia regardless of the clinical signs that led to decisions to euthanize.

### Statistical Analysis

Survival times, the times of onset of neurological signs, and brain ventricular volumes of $TPP1^{-/-}$ dogs in the vehicle, 4 mg and 16 mg rhTPP1 treatment groups were compared with one-way analysis of variance (ANOVA). Some of the affected dogs in the 16 mg rhTPP1 group did not exhibit all of the neurological signs by the time of euthanasia. In these cases, the onset of the specific sign not observed was set, conservatively, to the age at death for the purposes of statistical analysis. To compare performance on the reversal learning task among the $TPP1^{-/-}$ treatment groups, data from the 7 and 8 month time points were pooled, and the treatment groups were compared by using one-way ANOVA. Pairwise comparisons among the treatment groups for all parameters were performed by using the Holm-Sidak method. For the brain ventricular volume data, the data from the vehicle- and rhTPP1-treated $TPP1^{+/+}$ groups were combined for the initial ANOVA and Holm-Sidak analyses. After the initial analysis, in which the $TPP1^{-/-}$ groups were considered separately, subsequent ANOVA and Holm-Sidak analyses were performed with the data from all three rhTPP1-treated $TPP1^{-/-}$ groups pooled. This pooling of the data was justified by the fact that the initial analysis did not detect a significant difference among the groups receiving the different doses of rhTPP1.

## RESULTS

### Survival is Prolonged by rhTPP1 Infusion

$TPP1^{-/-}$ dogs were maintained until disease had progressed to the defined end stage unless complications unrelated to disease progression necessitated early euthanasia. In a previous study, survival time of eight untreated affected dogs to disease end stage was $44 \pm 3$ weeks. In the current study, the three $TPP1^{-/-}$ dogs that received vehicle reached end-stage disease requiring euthanasia between 39 and 47 weeks of age (Fig. 1). Dogs that received 4 mg rhTPP1 every other week reached end-stage disease requiring euthanasia between 51 and 57 weeks of age. The dogs that received a 16 mg dose of rhTPP1 survived to between 57 and 67 weeks of age (Fig. 1). However, two of these dogs had to be euthanized at 57 and 63 weeks of age, prior to reaching disease end stage, as the result of meningitis and obstructive hydrocephalus, likely related to foreign body reactions to the CNS delivery catheters after they had been in place for long periods (Butt, 2011).

A dog that received a 48 mg dose of rhTPP1 had not reached end-stage disease by 87 weeks of age but was euthanized at this time because of the development of obstructive hydrocephalus that had an acute onset between 83 and 87 weeks of age. This dog was not exhibiting clinical signs of hydrocephalus at this time, but a dramatic asymmetric enlargement of the ventricle was observed during a planned MRI, and the decision was made to euthanize. Upon necropsy, the ventricular enlargement was found to be associated with a large lesion that had developed along the track of the ICV delivery catheter. The survival times of the rhTPP1-treated groups of affected dogs were significantly longer than those of the vehicle-treated affected dogs ($P < 0.05$). The survival times of the dogs treated with the 16 mg dose of rhTPP1 were also significantly longer than those of the dogs treated with the 4 mg dose. Three dogs were initially enrolled in a 48 mg group, but two were euthanized early because they had developed meningitis and obstructive hydrocephalus, which appeared to result from foreign body reactions to the CNS catheters; these dogs were therefore excluded from the survival extension analysis. Among the nine treated dogs, five were terminated prior to reaching disease end stage because of complications related to the catheters or to the dose administration procedures and not to either disease progression or toxicity of the rhTPP1.

### Neurological Signs of NCL Delayed by rhTPP1 Infusion

To determine whether administration of rhTPP1 to the CSF delayed the onset and progression of clinical disease signs, dogs in this study were assessed for neurological abnormalities on a weekly basis. $TPP1^{+/+}$ dogs all remained neurologically normal throughout the duration of the study. Among vehicle-treated $TPP1^{-/-}$ dogs, an array of neurological deficits became apparent starting

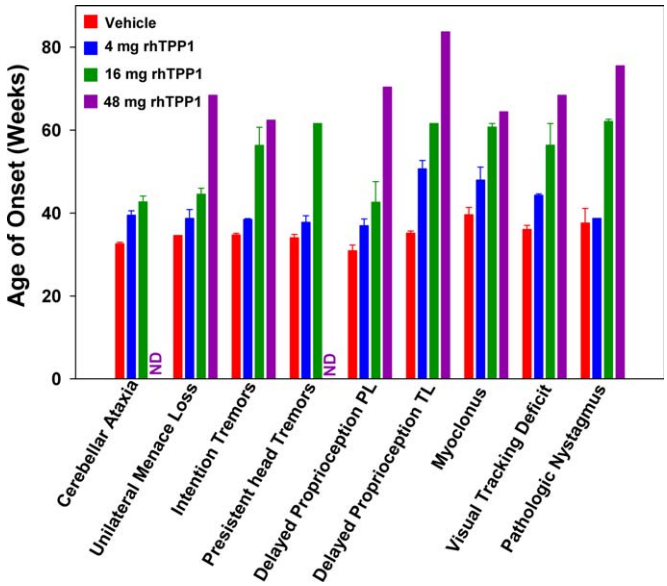


Fig. 2. Ages of onset of neurological signs in *TPP1*$^{-/-}$ dogs. For dogs treated with the 4 or 16 mg doses, bars indicate mean and SEM for the three dogs in each group. When no error bars are present, only one of the three dogs in the treatment group exhibited the indicated sign. Data from only one dog that received the 48 mg dose of rhTPP1 are shown. TL, thoracic limb; PL, pelvic limb; ND, sign did not appear prior to euthanasia.

between 32 and 38 weeks of age (Fig. 2). These deficits worsened and eventually became so severe that euthanasia was necessary. Euthanasia was performed at the same disease stage based on neurological status in all dogs except when dogs developed complications unrelated to the disease that necessitated early euthanasia as described above.

Treatment with infusions of rhTPP1 into the CSF significantly delayed the onset of most of the neurological signs, in a dose-dependent manner (Fig. 2, Table II). Despite these delays in the development of neurological signs, the disease did eventually progress to end stage in dogs that received 4 mg or 16 mg doses of rhTPP1 every other week. Exceptions were the two *TPP1*$^{-/-}$ dogs treated with the 16 mg dose that were euthanized because of meningitis and obstructive hydrocephalus, likely related to the CNS delivery devices. In addition, the dog treated with the 48 mg dose also had to be euthanized prior to reaching disease end stage. Within the *TPP1*$^{-/-}$ treatment groups that received rhTPP1, some neurological signs were not observed in every dog by the time of euthanasia, even among those that reached end-stage disease (Fig. 2). For example, only one of the dogs receiving the 16 mg dose of rhTPP1 exhibited delayed proprioception prior to euthanasia, and the dog receiving the 48 mg dose never exhibited cerebellar ataxia or persistent head tremors. The *TPP1*$^{-/-}$ dogs that received vehicle were neurologically debilitated by 35–40 weeks of age, whereas those that received rhTPP1 retained almost normal neurological function well beyond this age. Video recordings illustrating the effects of rhTPP1 in delaying the onset and progression of neurological signs are included in the Supporting Information.

**TABLE II. Statistical Comparisons of Neurological Deficits Among Treatment Groups***

| | Difference significant at $P < 0.05$ | | |
|---|---|---|---|
| Neurological deficit | Vehicle vs. 4 mg | Vehicle vs. 16 mg | 4 mg vs. 16 mg |
| Persistent head tremor | No | Yes | Yes |
| Intention tremor | No | Yes | Yes |
| Myoclonus | Yes | Yes | Yes |
| Cerebellar ataxia | No | Yes | Yes |
| Visual tracking | Yes | Yes | Yes |
| Delayed proprioceptive placement (TL) | Yes | Yes | Yes |
| Delayed proprioceptive placement (PL) | No | No | No |
| Menace response deficit | No | Yes | No |

*The dog that received the 48 mg dose was not included in the statistical analysis.

## Cognitive Function Preserved by rhTPP1 Infusion

Assessments of cognitive function were performed by using a T-maze test of spatial learning and memory (Sanders et al., 2011). After the initial training period in the T-maze, the dogs were evaluated monthly for performance of the reversal learning tasks, starting at 4 months of age. The mean number of errors made until reaching criterion at 4 months of age within each study group ranged from seven to nine (Fig. 3). Except for the vehicle-treated *TPP1*$^{-/-}$ dogs, the performance of the dogs improved progressively at subsequent monthly testing sessions. There were no significant differences in performance between the rhTPP1-treated affected dogs and the homozygous normal dogs at any of the time points. In contrast, the *TPP1*$^{-/-}$ dogs that received vehicle showed no improvement in T-maze performance. In fact, the performance of these dogs deteriorated sharply after 7 months of age. Two of the three dogs in this group were able to complete the T-maze testing only through 8 months of age. At 7–8 months of age, the performance of the *TPP1*$^{-/-}$ dogs that received both the 4 and 16 mg doses of rhTPP1 was significantly better that of the affected dogs that received vehicle ($P < 0.05$; Fig. 3). A dog that had received 48 mg doses of rhTPP1 was able to complete the task with fewer than two errors until 15 months of age and was able to continue performing this test until 18 months of age, with only a small decline in performance between 15 and 18 months (Fig. 3).

Evaluation of T-maze performance for all of the *TPP1*$^{-/-}$ dogs was discontinued before the dogs reached end-stage disease status because of both behavioral and motor problems. As they approached end-stage disease, some dogs refused to run the maze. Others attempted to run the maze but because of ataxia, tremors, and myoclonus were unable to do so.

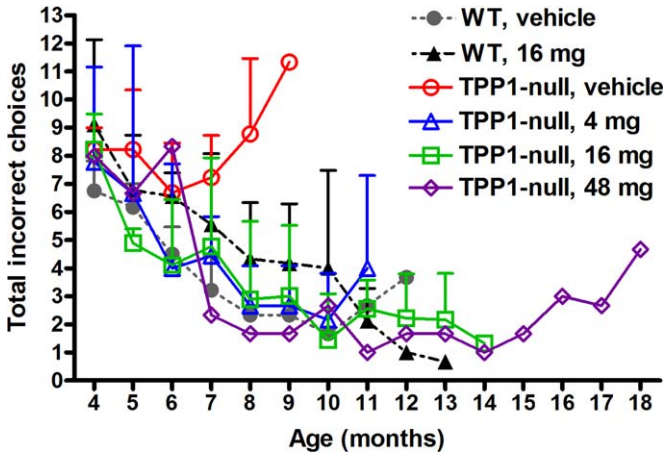


Fig. 3. T-maze performance of dogs in the different treatment groups. Data show the average number of incorrect choices. Vertical bars indicate SD when more than one dog was included in the data point. All groups started with three dogs, except one dog was included at the 48 mg-dose level. Among the $TPP1^{-/-}$ dogs, some could not complete the T-maze test at the later time points, and testing of the wild-type dogs was suspended after 12–13 months. Among the $TPP1^{-/-}$ dogs, in addition to the dog that received the 48 mg dose, data points representing fewer than three dogs are as follows: vehicle 9 months, n = 1; 4 mg 8–11 months, n = 2; 16 mg 13 months, n = 2; 14 months, n = 1. Error bars represent SD and are not shown for data points represented by only one animal.

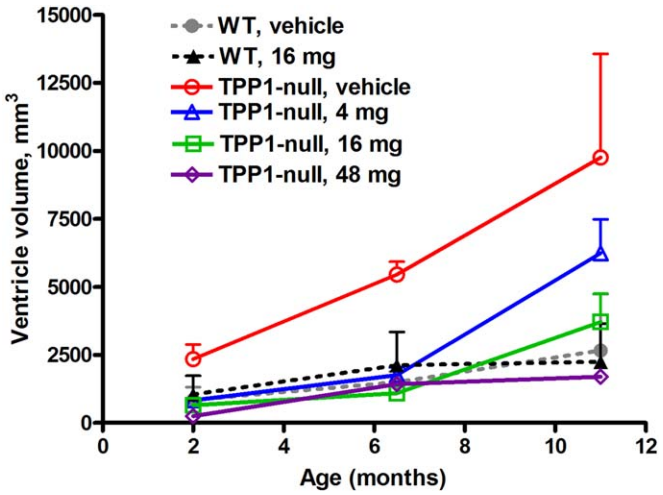


Fig. 4. Effect of rhTPP1 infusion on brain ventricular volume. There was a dose-related attenuation of ventricular enlargement in affected animals treated with rhTPP1 (n = 3/data point). Error bars represent SD. WT, wild type.

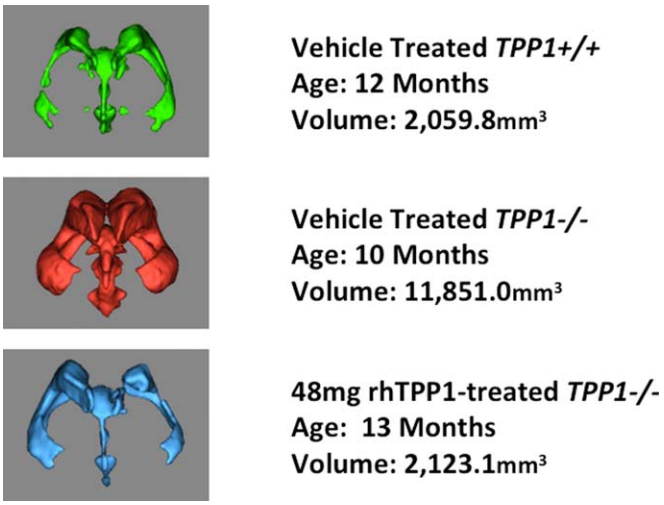


Fig. 5. Representative three-dimensional images of brain ventricles reconstructed from MRI. The disease-related ventricular enlargement was inhibited by the TPP1 treatments.

### Brain Atrophy is Inhibited by rhTPP1 Infusion

Progressive brain atrophy that characterizes CLN2 disease was measured by assessing the increase in brain ventricle volume as estimated by MRI. Brain ventricular volumes were determined in the dogs at approximately 2, 6.5, and 11 months of age (Figs. 4, 5). Among the $TPP1^{+/+}$ dogs, there were only modest increases in ventricular volumes over this age range as the dogs matured (mean increase in ventricular volume for all of the $TPP1^{+/+}$ dogs was 946 $mm^3$). Among the $TPP1^{-/-}$ dogs that received vehicle, mean ventricular volume increased by almost 7,500 $mm^3$ over this age range ($P < 0.01$ relative to the $TPP1^{+/+}$ dogs). CSF infusion of rhTPP1 significantly reduced the disease-related ventricular enlargement. Relative to vehicle-treated $TPP1^{-/-}$ dogs, the group that included all affected dogs treated with rhTPP1 exhibited a significant reduction in age-related ventricular enlargement ($P < 0.01$; Figs. 4, 5). There was a trend of greater inhibition of ventricular enlargement with higher doses of rhTPP1 (Fig. 4), but the samples were not large enough to determine whether this dose-level effect was significant. For all of the rhTPP1-treated $TPP1^{-/-}$ dogs combined, ventricular volume increased by a mean of 4,864 $mm^3$ between 2 and 11 months of age ($P < 0.05$ compared with the vehicle-treated $TPP1^{-/-}$ dogs). Representative three-dimensional reconstructions of the ventricles of vehicle-treated $TPP1^{+/+}$, vehicle-treated $TPP1^{-/-}$, and 48 mg-rhTPP1-treated $TPP1^{-/-}$ dogs are shown in Figure 5.

## DISCUSSION

No effective treatment has been developed for CLN2 disease; every child affected with this disorder will experience a progressive and profound loss of neurological functions that ends in death. We have demonstrated that direct rhTPP1 enzyme replacement to the brain by administration to the CSF attenuates disease progression, improves neurological function, and increases life span in a dog model of CLN2 disease. The clinical benefits occurred in a dose-dependent fashion. Although disease progression was not completely prevented in the dogs, the finding that the delay in disease progression increased with increasing dosages of rhTPP1 suggests that at a

sufficiently high dose it may be possible to prevent or stabilize neurological decline completely.

We previously demonstrated that rhTPP1 administered into the CSF results in enzyme delivery to the brain (Vuillemenot et al., 2011). This suggests that CLN2 disease is likely to be amenable to ERT. Systemically administered ERTs have been shown to be highly efficacious in treating lysosomal storage diseases without a significant neurological component, such as the mucopolysaccharidoses (Morel and Clarke, 2009; Grubb et al., 2010; Lachmann, 2011; Valayannopoulos and Wijburg, 2011). Treating the CLN2 disorder with ERT presents a particular challenge, because the disease results almost exclusively from degenerative changes in the CNS. Large molecules such as the rhTPP1 protein cannot cross the blood–brain barrier; thus, systemic administration of the enzyme would be unlikely to be efficacious in treating the neurological symptoms of this disease.

To deliver TPP1 to the CNS, we took advantage of the fact that the entire brain and spinal cord are bathed in the circulating CSF. Our previous *TPP1*$^{-/-}$ dog study indicated that the rhTPP1 is distributed to brain cells and activated after administration into the CSF (Vuillemenot et al., 2011). Activation of the proenzyme takes place within the acidic environment of the lysosome, so the presence of the active form in the brains of treated dogs indicates that the protein has reached the intracellular sites where it normally functions. The CNS pharmacokinetic and distribution analyses of the rhTPP1 infusions that are described elsewhere (Vuillemenot et al., 2014) indicate that high levels of CSF rhTPP1 exposure and widespread brain distribution were achieved with administration of the protein into the CSF.

Delivery of the rhTPP1 enzyme to the CNS resulted in a significant attenuation of the cognitive deficits of CLN2 disease, reduced brain atrophy, and led to significant delays in onset and progression of neurological disease signs. Although widespread distribution of TPP1 activity was observed in all dogs that received rhTPP1 via CSF infusion (Vuillemenot et al., 2011, 2014), the slowing in disease progression was greatest in the dogs that received the highest doses. Disease progression appeared to be inhibited to the greatest extent in a dog that received a 48 mg dose of rhTPP1. Other than clinically manageable infusion-associated reactions to the initial infusions, this dog exhibited no apparent adverse effect from the rhTPP1 itself. Asymmetric hydrocephalus developed in this dog late in the study, most likely the result of a foreign body reaction to the ICV catheter (Butt, 2011). The more pronounced therapeutic effects of the higher doses may be due to the enzyme reaching a larger number of cells in the brain and/or to higher enzymatic activity per cell. Our data to date do not enable us to distinguish between these possibilities. However, the fact that the treatments slowed the progression of some neurological deficits more than others is consistent with the finding that the distribution of active enzyme in the treated dogs was not uniform among different brain regions (Vuillemenot et al., 2014). It is likely that the greater therapeutic efficacy observed at the higher doses is due at least in part to more widespread distribution of the enzyme throughout the CNS.

The results of this study indicate that regular infusion of rhTPP1 proenzyme into the CSF is very promising as a potential therapy for children suffering from CLN2 disease. At present, there is no effective treatment for this progressive and uniformly fatal disorder. Based on the functional effects seen in the dog model, rhTPP1 ERT to the CNS appears likely to benefit children with the CLN2 disorder. A clinical trial in patients early in the progression of CLN2 disease with biweekly rhTPP1 infusion to the lateral ventricle is currently underway.

Although clinical signs of CLN2 disease and the other neuronal ceroid lipofuscinoses are primarily neurological, and death usually occurs as a result of neurological impairment, TPP1 expression is not restricted to the CNS, and the disease-related accumulation of autofluorescent lysosomal storage material occurs in many tissues outside of the nervous system (Kida et al., 2001; Kurachi et al., 2001). Thus, it is quite possible that, if the neuropathology associated with CLN2 disease can be prevented by infusion of recombinant TPP1 into the CSF, pathology in other organs that are less sensitive to TPP1 deficiency may become apparent. Indeed, we found that all of the affected dogs exhibited progressive increases in serum levels of troponin, an indicator of heart damage (Adamcova et al., 2005). In normal dogs, serum troponin levels are less than 0.03 ng/ml. In the later stages of disease, troponin levels were elevated many fold above this, including in the dogs that received CSF infusions of rhTPP1. By end-stage disease, serum troponin levels in the affected dogs ranged from 0.9 to 2.0 ng/ml. This suggests that optimal therapy for CLN2 disease may require infusion of TPP1 not only into the CSF but into the peripheral circulation as well. At least one case of progressive cardiac functional impairment has been reported in a human subject with the CLN2 disease (Fukumura et al., 2012), consistent with disease effects on visceral organs. As with children with the CLN2 disorder, *TPP1*$^{-/-}$ Dachshunds suffer from progressive vision loss resulting from retinal degeneration (Katz et al., 2008; Whiting et al., 2013). Retinal function and structure were evaluated in all of the dogs in this study by using electroretinography and morphological techniques, respectively. CSF infusion of rhTPP1 was not effective in preserving retinal structure and function (Whiting et al., 2014). Therefore, if this treatment is effective for preserving neurological function and inhibiting CNS atrophy in children with CLN2 disease, an adjunct treatment will most likely be required to preserve vision.

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