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Oral Cysteamine bitartrate and N-acetylcysteine combination for patients with infantile neuronal ceroid lipofuscinosis: a pilot study

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Summary

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Contributors

A.B.M. conceived the project and received a bench-to-bedside award from the Clinical Center of the NIH. The detailed study design was jointly performed by A.B.M., S.W.L., E.H.B., R.C.C., W.M.Z., Z.Z., Z.M.N.Q., and A.G. In-patient admissions and patient care were provided by K.J.G., N.M., S.B., SWL and ABM. Image processing and statistical analysis of the GRODs were performed by G.C., A. L. and Z.Z. R.C.C and W.M.Z. performed ERGs and analyzed the data. O.I.K analyzed EEGs. All authors had access to all the data. All authors contributed in preparing and revising the manuscript. All the authors reviewed and approved the final version of the manuscript and approved its submission for publication.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Background—Infantile neuronal ceroid lipofuscinosis (INCL) is a devastating neurodegenerative lysosomal storage disease caused by mutations in the *CLN1* gene encoding palmitoyl-protein thioesterase-1 (PPT1). PPT1-deficiency causes lysosomal ceroid accumulation leading to INCL pathogenesis. Previously, we reported that phosphocysteamine and N-acetylcysteine mediated ceroid depletion in cultured cells from INCL patients. We conducted a pilot study to determine whether a combination of cysteamine bitartrate and N-acetylcysteine is beneficial for these patients.

Methods—Patients (6-month to 3-years old) with any combination of 2 of the 7 most lethal *PPT1* mutations were admitted. All patients were recruited from physician referrals and the *PPT1* mutations were analyzed prior to admission. Patients were evaluated by electroretinography(ERG), brain MRI and MRS, electroencephalography (EEG), and electron microscopic analyses of leukocytes for granular osmiophilic deposits (GRODs). Patients received oral cysteamine bitartrate (60mg/kg/day) and N-acetylcysteine (60mg/kg/day) and were evaluated every 6 to 12 months until they showed isoelectric EEG attesting to a vegetative state or were too sick to travel. Outcomes were compared with the reported INCL natural history. In two cases, the disease progression was compared with that of a sibling who was above the age limit for inclusion into the protocol.

Findings—Between March, 2001, and June, 2011, we recruited 10 children with INCL but one was lost to follow-up after the first visit. Thus, a total of 9 patients (5 females and 4 males) were studied. At the first follow-up visit, peripheral leukocytes in all 9 patients showed virtually complete depletion of GRODs and 7 of 9 patients manifested less irritability and/or improved alertness based upon parental and physician observations. Evaluation by Denver scale showed acquisition of no new developmental skills and retinal function assessed by ERG progressively declined. Most notably, average time to isoelectric EEG (indicating vegetative state) was significantly longer in our patients compared to that previously reported. MRI studies demonstrated signal abnormalities similar to previous reports. Brain volume and NAA declined steadily, but no published quantitative MRI or MRS studies of INCL patients are available for comparison on these measures. There were no adverse events related to therapy other than a mild gastrointestinal discomfort in 2 of 9 patients, which was eliminated when the liquid preparation of cysteamine bitartrate was replaced with capsules.

Interpretation—The objectively demonstrated benefits in our study are the depletion of GRODs and delay of isoelectric EEG in all patients; in addition, several subjective benefits were suggested, all of which warrant further study. Nevertheless, this report systematically and quantitatively documents the natural history of 9 INCL patients with the most lethal *CLN1/PPT1* mutations and thereby provides a benchmark for evaluating future experimental therapies.

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Introduction

Background

The neuronal ceroid lipofuscinoses (NCLs), also known as Batten disease, constitute a group of the most common neurodegenerative lysosomal storage disorders (LSDs)¹⁻³. NCLs are both clinically and genetically heterogeneous although most are autosomal recessive diseases predominantly affecting children¹⁻³. Mutations in more than 10 different genes (*CLNs*) underlie various types of NCLs (<http://www.ucl.ac.uk/ncl>)⁴. Despite the age variability in the disease onset, the clinical features common to all NCLs include psychomotor retardation, seizures, visual loss, and premature death²⁻⁵. Pathological findings include rapidly progressive brain atrophy resulting from destruction of mostly cerebrocortical neurons and the presence of autofluorescent storage material both in neurons and in other cell types²⁻⁶.

Inactivating mutations in the *CLN1* gene encoding palmitoyl-protein thioesterase-1 (PPT1) underlie infantile NCL (INCL) pathogenesis⁷. Children with this disease are phenotypically normal at birth but by 6–18 months of age they manifest psychomotor retardation. By two years of age, these children undergo complete retinal degeneration and blindness. At around four years of age, an isoelectric electroencephalogram (EEG) attests to a vegetative state for several more years before eventual death²⁻⁷. These grim facts underscore an urgent need for developing effective therapeutic strategies for INCL and for that matter, for all the NCL types⁸.

Palmitoylation (also called S-acylation) is the only reversible lipid modification in which a long-chain saturated fatty acid (predominantly palmitate) is attached to cysteine residues in polypeptides via thioester linkage⁹. PPT1 cleaves this thioester linkage in palmitoylated proteins (constituents of ceroid) required for degradation by lysosomal hydrolases. Thus, PPT1-deficiency causes ceroid accumulation in lysosomes¹⁰ leading to INCL. In time, these ceroids organize to form granular osmiophilic deposits (GRODs)¹¹.

Objectives

Since the thioester linkage is labile¹² and susceptible to nucleophilic attack¹³, we reasoned that nucleophilic (electron donor) small molecules may have therapeutic potential for INCL. Previously, we used both *in vitro* and *in cellula* experimental models to demonstrate that phosphocysteamine, a nucleophilic and lysosomotropic drug with anti-oxidant properties, cleaves the thioester linkage in palmitoyl-CoA (a model substrate of PPT1) as well as in palmitoylated proteins and mediates the depletion of GRODs in cultured cells from INCL patients and suppresses apoptosis¹⁴. Moreover, N-acetylcysteine also cleaved thioester linkage and is a potent antioxidant, which has beneficial effects on neurodegenerative diseases including myoclonus epilepsy¹⁵. These results prompted us to conduct a pilot study to determine whether there are any positive effects of orally administering a combination of cysteamine bitartrate and N-acetylcysteine to INCL patients.

Methods

Study design and participants

This pilot study (www.clinicaltrials.gov; **NCT00028262**) was approved by the Institutional Review Board (IRB) of the *Eunice Kennedy-Shriver* National Institute of Child Health and Human Development (NICHD) at the National Institutes of Health (NIH). Continuous monitoring of this study for any adverse effects of the therapy was conducted by our institution's Data Safety Monitoring Committee, which is independent of the IRB. A written informed consent was read, understood and signed by a parent or guardian before any patient was enrolled into our protocol. In order to assure that the study population was homogeneous, we recruited only those patients who carried a combination of two of the following *CLNI/PPT1* mutations: L10X, R151X, R164X, W296X, R122W, c.169insA and E184K. These mutations were chosen because they have been reported to manifest a uniform phenotype¹⁶.

Initiation and discontinuation of Therapy

After initial evaluation (see below), oral cysteamine bitartrate therapy was initiated at a dose of 10 mg/kg/day in four divided doses given with food. The cysteamine bitartrate dose was gradually increased weekly, as tolerated, to a target dosage of 60 mg/kg/day. This dose was used because it has been previously reported that at this dose cysteamine is well tolerated, safe and efficacious in treating children with cystinosis¹⁷. After target dose of cysteamine bitartrate (60 mg/kg/day) was achieved, patients were then started on oral N-acetylcysteine titrated to 5% oral solution, with the dose incrementally escalated to a target dose of 60 mg/kg/day. N-acetylcysteine was also administered daily into four equal doses. Although N-acetylcysteine can be given in doses as high as 140mg/kg, we used a lower dose of because it is given in combination with cysteamine bitartrate, which is also an antioxidant. We used MRI and MRS evidence of advanced brain atrophy and/or isoelectric EEG attesting vegetative clinical status as the criteria to discontinue treatment.

Procedures

All patients were evaluated by brain MRI, MRS, ERG, visual evoked potential (VEP), EEG and transmission electron microscopic (TEM) analysis of the peripheral white blood cells (WBCs) for GRODs. Children also underwent physical and neuro-developmental assessments using the Denver scale¹⁸. The laboratory studies included blood counts, serum chemistries, prolactin, insulin-like growth factor (IGF-1) and thyroid function tests.

General anesthesia was required for most procedures for all patients except for some who were in the late stages of the disease in whom imaging and ERG examinations were performed without sedation. General anesthesia was administered by staff anesthesiologists as previously reported¹⁹. Upon completion of the procedures and emergence from anesthesia, children were observed in the post-anesthesia care unit until ready to be transferred to the pediatric ward.

Assessment of visual function and ocular motility were undertaken followed by recording a single channel flash visual evoked potential (VEP). Ganzfeld electroretinograms (ERGs)

were obtained while patients were anesthetized with continuous infusion of propofol. ERG responses were recorded following the standards set forth by the International Society for Clinical Electrophysiology of Vision (ISCEV)²⁰. All responses were recorded using a Gold Lens electrode and an Espion electroretinography console with a portable ColorBurst stimulator (Diagnosys, Lowell, MA). These responses were compared to a set obtained from age-matched controls who were children referred to the National Eye Institute for sedated ERG and had age-appropriate ERG responses. Additional specialized recordings included scotopic intensity-response function curves and photopic on off responses; data from these recordings will be presented in a separate publication. Follow-up recordings were obtained to study the rate of progression in individual patients. Cycloplegic refraction, funduscopy, and fundus photography were also performed at the end of the procedure.

All patients were serially evaluated with MRI and MRS of the brain. MRI consisted of sagittal spin-echo T1-weighted images, axial T1-weighted images, axial T2-weighted images, axial 3D T1-weighted images, and axial diffusion-weighted images. Single voxel MR spectroscopy was performed, with short echo time (25 or 35 ms) and PRESS technique. Voxels were located in the left centrum ovale, midline parietal grey matter, left thalamus, pons, and left cerebellar white matter.

We also analyzed the GRODs in peripheral WBCs from all patients before and during treatment by TEM at 30000x as previously described¹⁴. Identification and counting of the GRODs was performed by two investigators working independently of each other and the results were averaged.

Monitoring for Adverse Effects of the Therapy

Parents were instructed to document any adverse effect(s) of therapy. They were also given instructions on how to stop the medications and told to contact the PI and the local physician taking care of the patient if any adverse effects were apparent. All adverse effects were recorded and reported to the Data Safety Monitoring Committee.

Statistical analysis

Linear mixed effects models were used to investigate the change over time after treatment of the average number of GRODs per cell and the average area of the GRODs. For each of the 9 patients at each of the treatment time windows, the average number of GRODs per cell was obtained by dividing the total number of GRODs by the total number of cells under consideration, and the average area of a GROD was computed as the total area divided by the total number of GRODs. Log-transformation was applied to average number of GRODs per cell, the average area of a GROD, and the number of days from pre-treatment blood draws to post-treatment blood draws before fitting the linear mixed effects models assuming first order auto-regressive correlation structure among the longitudinal measures.

Role of the funding source

The funding sources had no role in the study design, data collection, data analyses, data interpretation, writing of the manuscript, or decision to submit for publication. The corresponding author along with all co-authors had full access to all the data in this study

and all authors reviewed the final draft of the manuscript prior to submission. The corresponding author (ABM) took final responsibility for the decision to submit the manuscript for publication.

Results

The natural history of our patient population was compared with that reported in the literature (Table 1). The duration of follow-up ranged from 8 to 75 months after initiation of therapy. The mean age of patients at the time of admission was 25.8 months. Of the nine subjects followed in this protocol, the average age of symptom onset was 12.7 months (range 4–18 months). Most of the subjects initially came to medical attention due to delayed acquisition of motor skills or developmental regression, with the exception of two patients whose INCL diagnosis was suspected because they had an affected older sibling. One of the patients (Pt#3) at 5 months of age developed strabismus before manifesting other developmental problems. In particular, four subjects demonstrated specific loss of ambulatory capability, and all subjects demonstrated language and/or cognitive decline as well as loss of other motor skills (Table 2). Only one subject presented with new onset seizures, although all patients had myoclonus. All subjects except patient 7 were on various anti-epileptic agents including sodium valproate, carbamazepine, benzodiazepines (nitrazepam, clonazepam) or levetiracetam prior to enrollment into the study.

Two of the nine children initially experienced mild gastrointestinal discomfort with liquid cysteamine bitartrate, which was eliminated when the liquid preparation was replaced with cysteamine bitartrate capsules, the contents of which was added to the food. No other untoward side effects of cysteamine bitartrate or N-acetylcysteine were observed throughout the treatment period. Notably, only one patient showed substantial interval weight loss (between admissions at age 4 and 2/12years and age 4 and 10/12 years). All nine patients showed normal height at the 10th to 75th percentile for age. Five of the nine patients showed appreciable decrease in head circumference to measure at or below the 5th percentile for age. Six of the patients underwent gastrostomy tube placement to ease difficulty with feeding due to progressive loss of oral motor skills and ability to protect the airway.

Routine laboratory studies were performed in all patients at each visit (Table S1). Laboratory parameters remained within the normal range over the entire course of our study with the exception of one patient, (Pt#1), who had a single elevated erythrocyte sedimentation rate (ESR) of 51 mm/hr at the time she had herpes simplex lesions on her skin (proven by viral DNA study and treated with acyclovir). Serum prolactin levels were occasionally elevated for 8 of 9 patients (range: low 30's to 109 µg/L) but otherwise within normal limits for all patients without persistently altered levels as has been previously reported in cystinosis patients treated with oral cysteamine²¹.

All anesthetic courses were uncomplicated and no patient displayed hemodynamic lability during or after procedures. During the course of anesthetics and imaging studies, two children developed hypothermia. In one of these cases, hypothermia was associated with bradycardia, which was successfully treated with atropine. The drop in temperature was observed despite the routine use of blankets during MRI and use of active warming

measures (forced-air warming blankets) during ERGs as well as in the recovery room²². All children returned to baseline status within a few hours after recovery from anesthesia.

Because eight of the nine patients admitted to this protocol clinically manifested considerable neurological impairment prior to the initiation of therapy, we expected the pre-treatment brain MRI scans to demonstrate cerebral atrophy. We did not obtain scans on normal children as part of this study, but for comparison we used previously reported normalized total brain volumes for children of various ages²³. Compared with this reference curve, our youngest (and only relatively asymptomatic) patient (Pt#7) had a total brain volume of +5% relative to the average volume for her age group at the time of admission to the study protocol. Follow-up measurements on this patient and all measurements on other patients were lower than the expected volume for the patient's age, ranging from 11–36% below average with a mean of 23% below average. Progression of atrophy was observed in all patients. In the early stages, the atrophy primarily involved the cerebral hemispheres but eventually progressed to involve the entire brain (Figure 1). Initially the atrophy primarily involved the cortical gray matter, but in the later stages cerebellar gray matter, white matter and deep nuclei were also affected. As has been reported in other studies^{22, 24} signal abnormalities were observed in the deep nuclei on T2 weighted images. Signal abnormalities were also observed on the T1 weighted images; the pattern we observed was that first the thalamus became hyperintense relative to the white matter, then the globus pallidus, and finally, the putamen and caudate nucleus.

In all patients the progression of the disease was also monitored by MRS. Representative MRS results from one patient (pt#8) are shown (Figure 2). The most striking abnormality is the decline in N-acetyl aspartic acid (NAA), at all five voxel locations. NAA is a metabolite found only in neurons, and is usually taken as a measure of functional neuronal mass. The earliest deficit of NAA was seen in the midline parietal gray matter, followed in succession by the left centrum semiovale, left thalamus, left cerebellar white matter, and pons. This corroborates the imaging observations that the earliest abnormalities are seen in the gray matter, followed by changes in the white matter and basal ganglia, with relatively late involvement of the cerebellum and brain stem.

Ophthalmological findings and the decline of visual function were previously reported in a cohort of INCL patients²⁵. All of our patients had a history of reduced visual function, nystagmus, or strabismus and for some, the ophthalmic manifestations were the initial findings that brought the patient to medical attention. The ophthalmic history and the findings on physical examination including those of fundoscopy (Figure S1) were documented, and clinical examination results are summarized in Table S2. On their initial ERG recordings, all patients demonstrated reduced b-wave amplitude on the dark-adapted rod and combined (rod-cone) response ERG. The combined (rod-cone) response b-wave amplitude (origin post-synaptic to photoreceptors with input from bipolar cells) was less than the a-wave amplitude (presumptive photoreceptor origin), which yielded an electronegative configuration. This was observed in all patients except for the initial recording of patient 7 who was admitted to our protocol at around 10 months of age and did not manifest this configuration. In contrast, initial a-wave amplitude in the 3.0 dark-adapted responses was normal in 4 cases; after age 2.6 years, a-wave amplitude was reduced in all

patients. A progressive decline in ERG amplitude with age was consistently observed (Figure S2). The decline was precipitous with most patients' ERG responses reaching noise level by 60 months of age (range 37 to 71 months). Some variability in amplitudes of scotopic ERG response was noted in four patients (Patient 1, 3, 4, and 9). In these patients, an increase in amplitude was noted on one visit following which the responses continued the trend of progressive decline (Figure 3). Eight out of 9 patients had noise level VEP responses through the treatment period. One patient (Pt#7) had a measurable VEP response that declined to noise level by the second visit. Cumulatively, our findings from all visits indicated poor visual function except for Patient #7, who continued to maintain a fix and follow response at 18 months of age. Progressively worsening retinal vascular attenuation and optic nerve head pallor were consistently documented for all patients.

EEG recordings were analyzed according to the criteria suggested for INCL diagnosis²². Only three of the nine patients developed new epileptiform foci during the course of our study. Representative EEG recordings from one patient (Pt#6) over several visits showed progressive deterioration (Figure S3). None of our nine patients displayed isoelectric EEG by three years of age as expected based on the previously reported natural history of INCL, and in fact, for 5 patients followed long enough to document isoelectric EEGs, the average age of patients with this finding was 59 ± 13 months.

The most striking effect of the combination therapy was observed in WBCs of cysteamine bitartrate-N-acetylcysteine-treated INCL patients. Prior to initiation of treatment, more than 50% of all WBCs in all patients contained readily identifiable GRODs in electron micrographs. Electron micrographs of peripheral WBCs with GRODs from one representative patient (before and after oral cysteamine bitartrate-N-acetylcysteine combination) are presented (Figure 4A, **arrows**). Notably, from the first post-treatment follow-up the number of GRODs per WBC declined in all nine patients (Figure 4B) as did the average cross-sectional area of the GRODs (Figure 4C). The analysis showed significant decrease after treatment in average number of GRODs (Beta=-0.3317, 95% CI=[-0.3943, -0.2691]; $P < 0.0001$), and in the average area of a GROD (Beta=-0.4379, 95% CI=[-0.5241, -0.3517]; $P < 0.0001$). In summary, subsequent to the first follow-up examination after initiation of treatment, GRODs were virtually undetectable in terms of the number as well as size, and remained so throughout the study period.

For several patients (#2, 3, 4, 5, and 9), myoclonic jerks seemed to have improved with cysteamine bitartrate-N-acetylcysteine combination, but this effect may have been confounded by other anti-epileptic medications that were also being given to decrease myoclonus. However, Patient #2 needed less medication to control myoclonic jerking than was needed for an older affected sibling at the same age. Two patients (#1 and #4) were reported by parents to resume attempts to roll over from back or side after initiation of cysteamine bitartrate-N-acetylcysteine combination. Improved alertness and spontaneous smiling were also noticed as was the clinical observation that the patients seemed less irritable (Table 3). This effect may be a substantial benefit since the patients with INCL typically manifest extreme irritability at around age 2 years up to age 4 years^{22, 23, 27}. The calming effects of oral cysteamine bitartrate and N-acetylcysteine combination were not

only reported by parents and other caregivers, but were also documented during developmental assessments.

Discussion

This study was conducted to determine whether a combination of oral cysteamine bitartrate and N-acetylcysteine is beneficial for INCL patients. We followed nine children with the most lethal *CLNI/PPT1* gene mutations who were receiving the two drug combination. This pilot study was limited by the inability to conduct a randomized protocol with such a small number of patients. A combination of INCL being a rare disease (1 in >100,000 births) and the stringent nature of the criteria for entry into this pilot study made it difficult to recruit a large number of patients in a reasonable period of time. Also, for humanitarian reasons, all affected patients received the combination regimen, and the effects were gauged based upon the previously published natural history of INCL.

The present study represents the first report of systematic, long-term follow-up and monitoring of several parameters to evaluate INCL disease progression. Such systematic follow-up has already provided new insights into INCL including subdural effusions in several of our patients, which were unrelated to the treatment²⁸. Thus, the results of our study, in addition to demonstrating some of the potentially beneficial effects of oral cysteamine bitartrate-N-acetylcysteine combination, provide additional comprehensive documentation of the progression of a subset of INCL patients with the most lethal *CLNI/PPT1* mutations under therapy, and may be useful for comparison in evaluating potential therapeutic regimens in the future.

One subjective outcome of this study is the apparent reduction of irritability in 7 of 9 patients, which may be important from the standpoint of management of these patients. The mechanism of irritability in INCL patients is not clearly understood although it has been reported that in other neurodegenerative diseases such as Parkinson and Alzheimer's disease, elevated levels of reactive oxygen species (ROS) mediates neuroinflammation and may contribute to irritability²⁹. Using postmortem brain tissues from an INCL patient as well as in those from *Ppt1*^{-/-} mice³⁰ that mimic INCL³¹, we previously reported elevation of superoxide dismutase (SOD) levels, most likely due to increased levels of ROS³². A recent report on gene expression profiling in *Ppt1*^{-/-} mice has shown that increased expression of genes related to inflammation occur in the brains of these mice³³. Consistent with this report, we also found that the receptor for advanced glycation end products (RAGE) signaling contributes to neuroinflammation in these mice³⁴. Since both cysteamine bitartrate and N-acetylcysteine are potent anti-oxidants and scavengers of ROS, these drugs may reduce ROS levels in the brain, thereby suppressing oxidative stress-mediated neuroinflammation, irritability in our patients. Recently, it has been reported that anti-oxidants have dual effects on neurodegenerative diseases, as these agents manifest direct neuroprotective effects against oxidative-stress and indirectly protect the neurons by suppressing inflammation mediated by activated glia³⁵.

A clear objective finding in the present study was the depletion of GRODs in peripheral WBCs of all 9 patients. At the first follow-up, both the fraction of WBCs containing GRODs

and the number of GRODs per WBC declined in all nine patients. Notably, by the second follow-up examination, the GRODs were completely undetectable and remained so for the duration of the study. The results of our present study are consistent with those of our previous *in vitro* and *in cellula* experiments¹⁴. These results may also suggest that cysteamine bitartrate-N-acetylcysteine combination mediates efficient depletion of ceroid *in vivo* ameliorating some of the deleterious effects of ceroid accumulation. Another objective finding of our combination therapy was that the extinction of EEG (i.e. isoelectric EEG) in our patients took significantly longer than reported in the literature (Table 1).

Our study provides the largest series of sequential electrophysiological findings (recorded using ISCEV standards) to date in INCL patients. Previous longitudinal studies in INCL patients have used Karpe's method of electroretinography (a technique that is no longer used) under general anesthesia³⁶. Weleber et al (2004)²⁵ have reported the results of electrophysiological findings from a single visit of INCL patients with clinical-pathological correlations. The results of our serial evaluations of 9 INCL patients are consistent with those of Weleber et al²⁵ that the onset of retinal degeneration in these patients occurs very early in life and has a rapid progression. Notably, the b-wave is affected much earlier than the a-wave in the dark-adapted combined (rod-cone) response indicating that the inner retinal responses are compromised earlier in life than those generated by photoreceptors³⁷. Scotopic responses were affected earlier than photopic responses as previously reported²⁵. Our data does not allow us to speculate on the natural course of the disease progression had these patients not received cysteamine bitartrate-N-acetylcysteine combination. However, the results of this study underscore the fact that like most neurodegenerative diseases, any therapeutic intervention must be instituted very early in life to prevent the progression of retinal degeneration and to preserve neuronal function in the retina.

Previously, MRI signal abnormalities in the basal ganglia of INCL patients have been described^{22, 24} and our findings concur with those reports. We observed that brain atrophy in all of our patients continued to progress even after initiation of the cysteamine bitartrate-N-acetylcysteine combination. However, due to the lack of quantitative details in the published reports on brain volume in INCL patients, we are unable to determine if the treatment mitigated progression of atrophy in any way. Similarly, we observed a progressive deficit in the NAA concentration at each of the anatomical locations of the brain that we studied by MRS. However, due to the lack of detailed quantitative reports in the literature, we are unable to determine if there was any mitigation in the decline of this marker of neuronal injury and loss. Nevertheless, the detailed measurements in our present study may serve as a quantitative biomarker to which future therapies for INCL can be compared and evaluated.

Cysteamine, a lysosomotropic drug, has been used for the treatment of cystinosis for more than 25 years¹⁷ and it also crosses the blood-brain barrier^{38, 39}. While both cysteamine bitartrate and N-acetylcysteine manifest nucleophilic properties, the efficiency of cleavage of the thioester linkage by cysteamine bitartrate may not be as efficient⁴⁰ as previously reported¹⁴. Nevertheless, in all of our patients a combination of these drugs mediated depletion of GRODs. It is possible that some of these effects are due to the combination of nucleophilic and anti-oxidant effects of these drugs.

Currently, several strategies are being tested to develop an effective treatment for INCL patients^{8, 41}. However, the blood-brain barrier (BBB) poses a formidable obstacle for macromolecular therapeutic agents to be transported from the blood to the brain. We previously reported that the BBB in *Ppt1*^{-/-} mice is disrupted^{42, 43}. Thus, it is possible that a macromolecular agent with therapeutic effectiveness may initially cross the leaky BBB, thereby allowing these agents to enter the brain. However, if these agents are effective in restoring the integrity of the BBB they may not reach the brain. Thus, non-toxic, thioesterase-mimetic small molecules may provide a more efficacious therapeutic strategy for INCL patients as these molecules would readily cross the BBB even when its integrity is restored. Towards this goal, we recently identified a small molecule, N-tert-(Butyl)-hydroxylamine, which protected neurons, and modestly extended lifespan in *Ppt1*^{-/-} mice⁴⁴.

Panel: Research in context

Systematic review—We searched PubMed for long-term studies of infantile neuronal ceroid lipofuscinosis (INCL) patients with a combination of cysteamine bitartrate and N-acetylcysteine to determine if this combination has any beneficial effects. We searched for reports published in the English language between May 1, 1980, and June 6, 2014, with the search terms “Infantile neuronal ceroid lipofuscinosis” AND “cysteamine bitartrate” AND “clinical trial” AND/OR “N-acetyl cysteine” OR “cysteamine bitartrate and N-acetylcysteine combination”. We did not identify any clinical studies meeting these criteria. We also searched using the terms “Infantile neuronal ceroid lipofuscinosis” AND “Cystagon” AND “clinical trial”. However, when we used the terms “infantile neuronal ceroid lipofuscinosis” AND “Cysteamine bitartrate” only one clinical study⁴⁵ was listed in which 4 patients with juvenile-onset INCL patients with mild *PPT1* genotypes who were above 3 years of age were given only cysteamine bitartrate at a dose of 50mg/kg body weight. In that study, the age of the patients was higher and the dose of cysteamine bitartrate was lower than those used in our study. In addition, only cysteamine bitartrate alone was used. The authors reported that disease progression may have been slower for patients taking cysteamine bitartrate, although it was not clear whether the slower progression was a continuation of an observation that began prior to the initiation of oral cysteamine bitartrate at a lower dose than that our patients received.

Interpretation—A pilot study using a combination of cysteamine bitartrate and N-acetylcysteine has shown that despite neurological deterioration prior to admission, this treatment had resulted in modest improvement in a homogeneous patient population carrying the most lethal *CLN1* mutations. It is recommended that any experimental therapy targeting INCL patients must be started early. It remains unclear if our treatment, started earlier, might have had a better outcome. Nevertheless, this report systematically documents the natural history of 9 INCL patients carrying the most lethal *CLN1* mutations and may provide a basis for evaluating future experimental therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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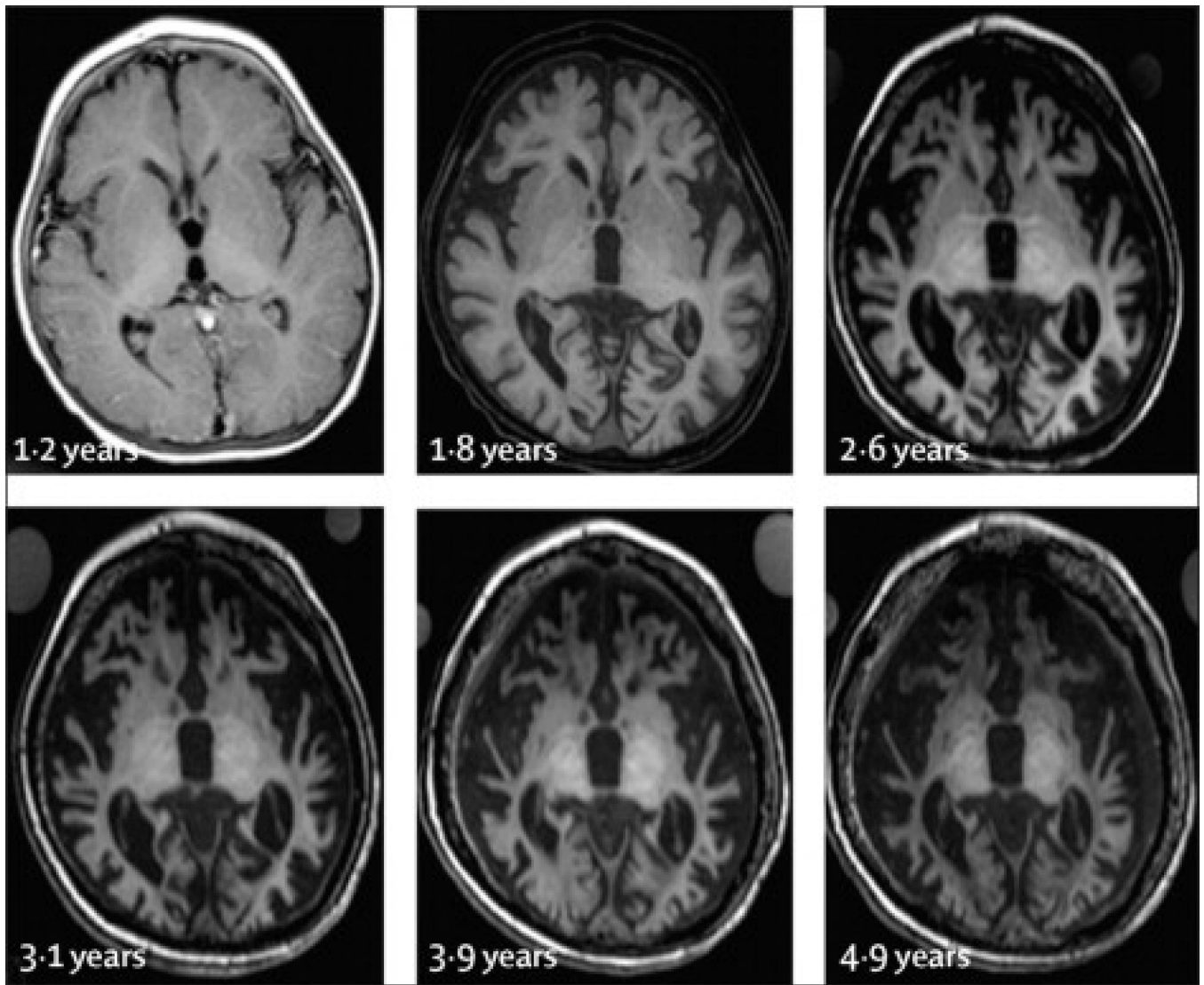


Figure 1. Progression of cerebral atrophy in a single subject (Patient 8)

All images are axial T1 weighted images acquired in the plane defined by the anterior commissure and posterior commissure. Age (in years) is noted adjacent to each image. Atrophy progressed rapidly between ages 1.2 yrs and 2.6 yrs, with gradual changes thereafter. This trajectory was typical for the patients in this study. Gray matter loss proceeded rapidly, and gray matter was not clearly identified after 2.6 yrs in this patient. Signal abnormalities in the deep nuclei progressed as follows: first the thalamus became hyperintense relative to white matter, then the globus pallidus, and finally the putamen and caudate.

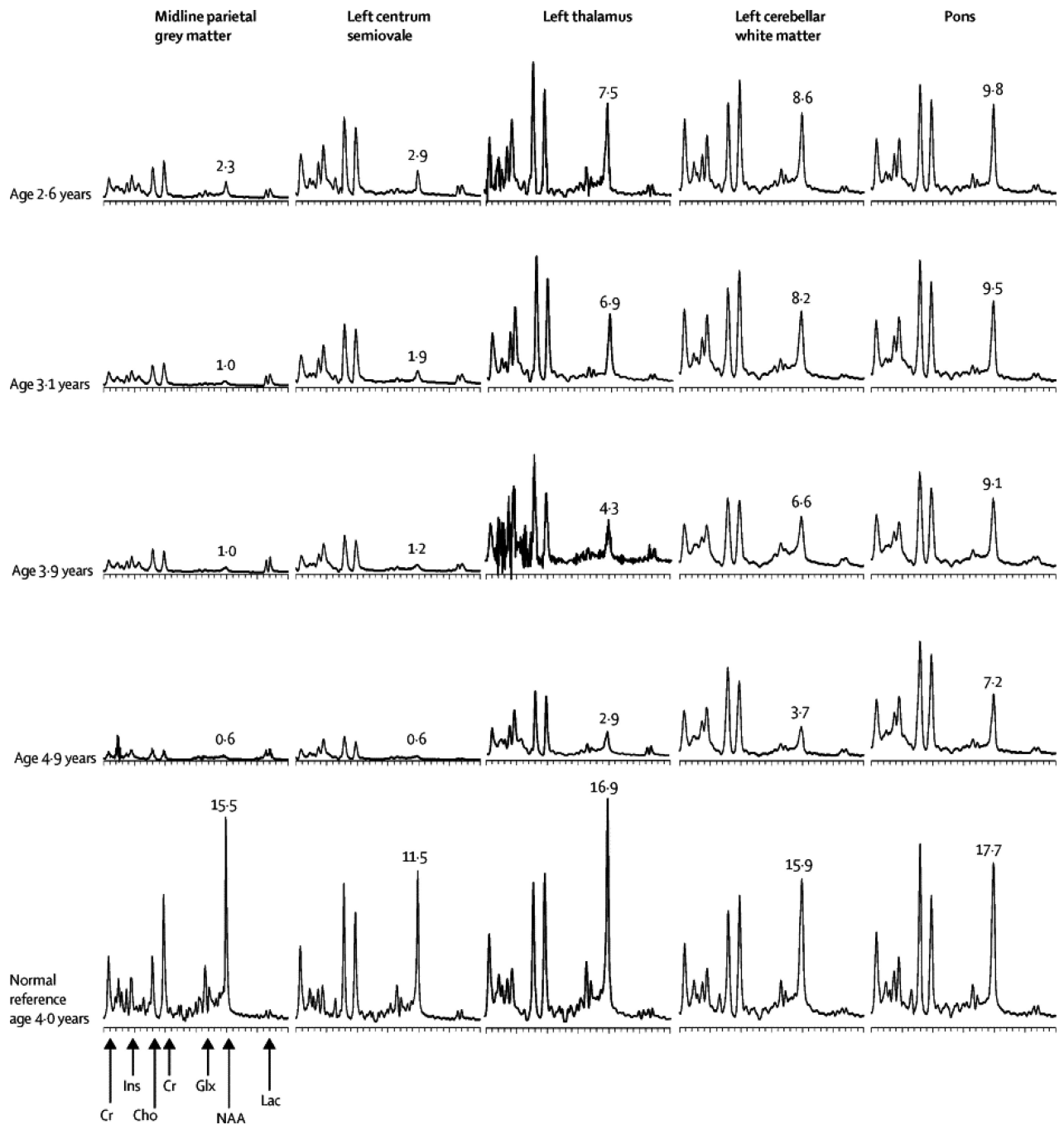


Figure 2. Progression of MRS abnormalities in a single subject (Patient 8)

All spectra are on the same scale (equal area for equal mM NAA), and have been corrected for T1 decay, T2 decay and CSF partial volume. NAA (mM) in tissue is noted next to the NAA peak. Cysteamine bitartrate-N-acetylcysteine combination treatment was initiated shortly after the examination at 2.6 yr. Abnormalities are most severe in the cerebral gray matter. Decline in the thalamus and cerebellum is slower than in the cerebrum. Progression of abnormalities is slowest in the pons. NAA demonstrates a steady decline at all locations.

Cr = creatine, **Ins** = myo-inositol, **Cho** = choline, **Glx** = glutamine+glutamate+GABA,
NAA = N-acetyl aspartic acid, **Lac** = lactate.

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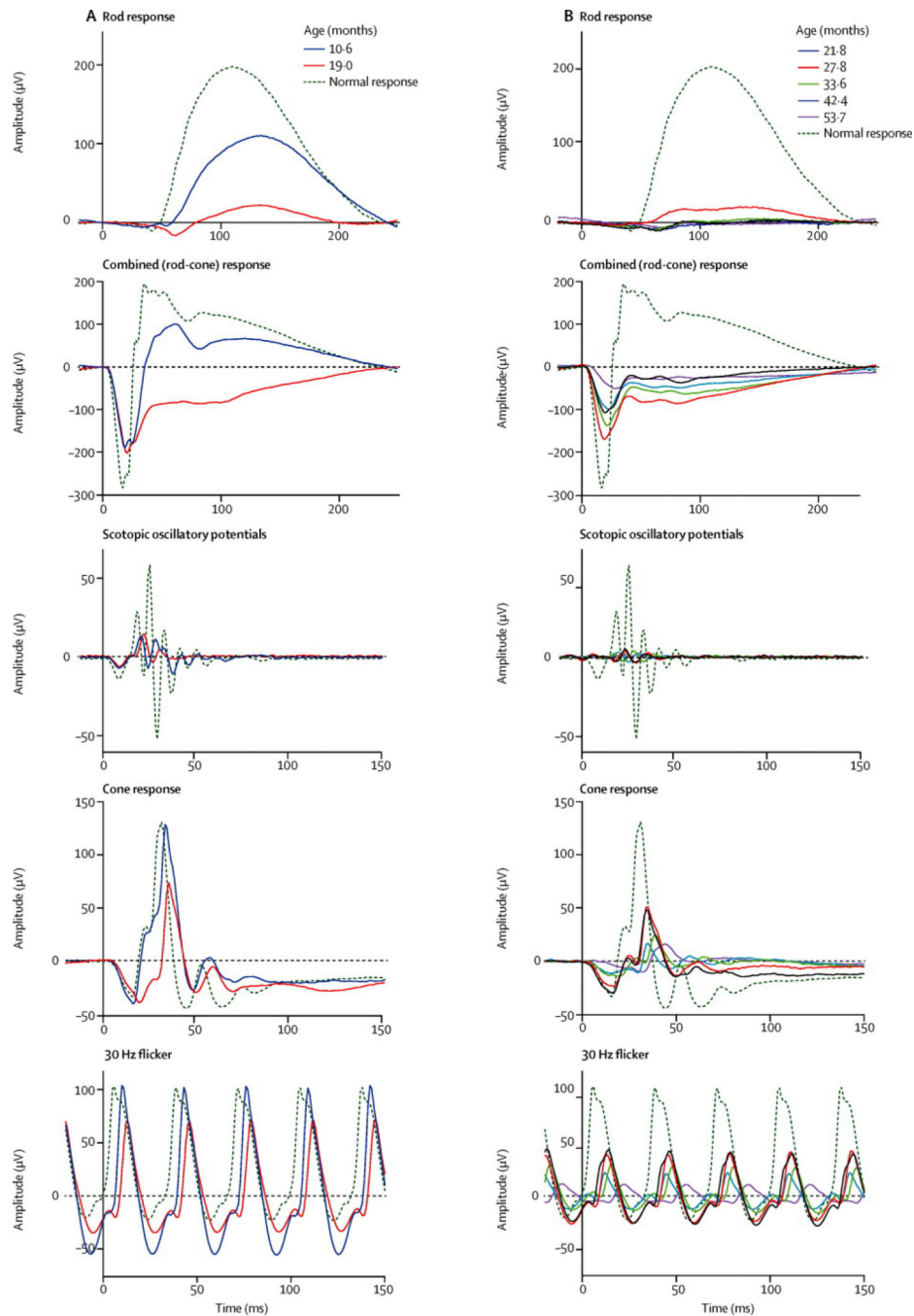


Figure 3. Electroretinographic recordings from two representative study patients

A. Patient 7 presented at the earliest age (10 m) and had the best preserved response amplitudes among all study patients at that visit. The follow-up visit for Patient 7 was at the age of 19.0 m and by that time her recordings showed the characteristic findings expected in INCL including significantly reduced b-wave amplitude on both the rod response and the scotopic combined (rod-cone) response. Note the electronegative waveform on the combined response at the 19.0 m follow-up visit (but not on initial presentation). **B.** Patient 1 had multiple study follow-ups with the ERG showing progressive reduction in amplitude

across all recorded responses. Note that, for all study patients, the scotopic responses were more severely affected and showed earlier changes than the photopic responses.

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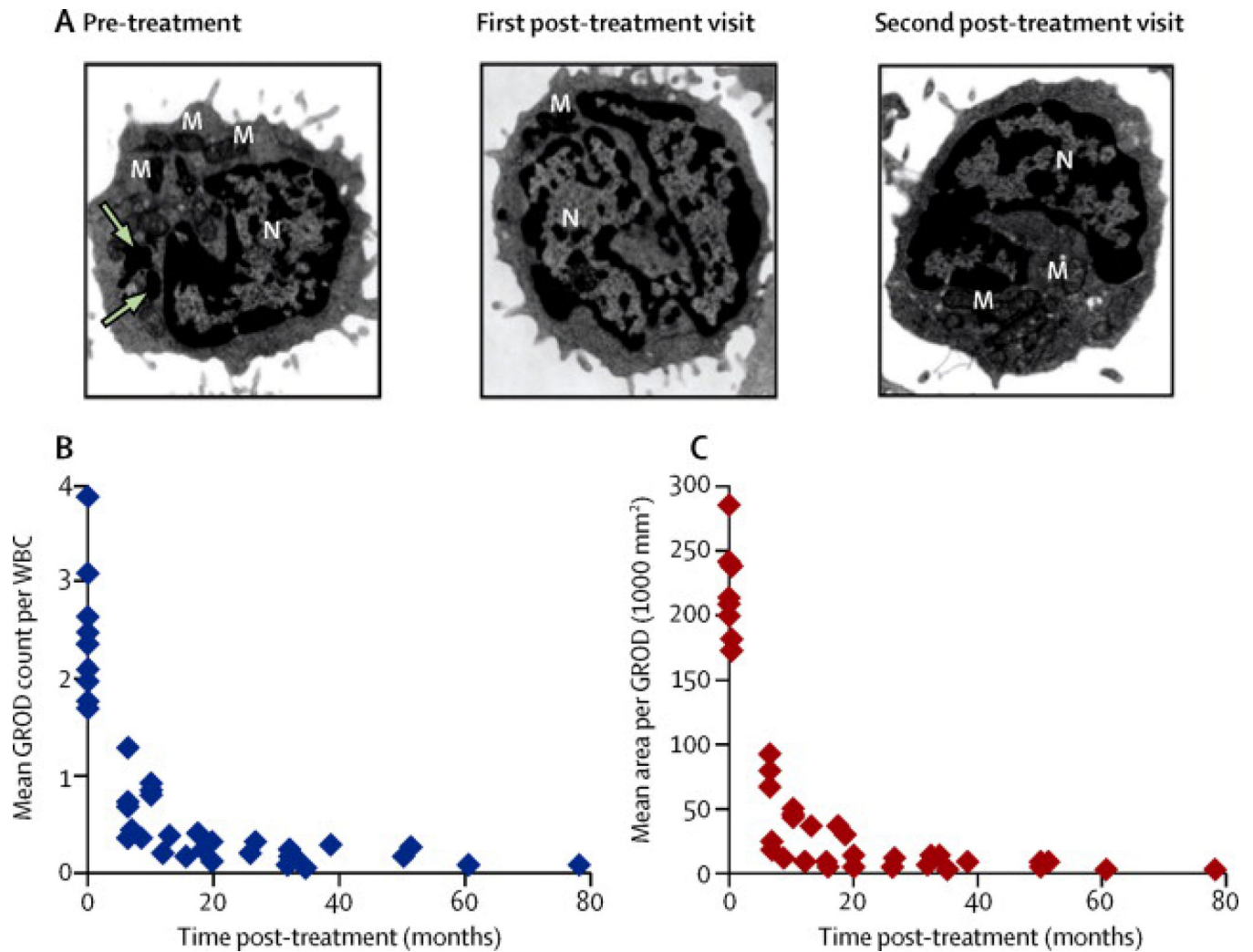


Figure 4. Electron microscopic analysis of GRODs in leukocytes before and after treatment
Transmission electron microscopic analyses of peripheral WBCs from all patients were performed at all patient visits, both pre-treatment and post-treatment. GRODs are irregularly-shaped dark extranuclear structures that are distinct from mitochondria, secretory granules, and other membrane-enclosed cellular organelles. Representative micrographs (A) from a patient are shown at 3 time points (pre-treatment and two post-treatment). Note that GRODs are readily detectable only in the pre-treatment image. **Arrows** indicate GRODs; **M**, mitochondria; **N**, nucleus. Number of GRODs per cell (B) and area of the GRODs (C) for all 9 patients together are shown as they evolved over time. For all patients, both the number and size of the GRODs declined over time, starting with the first visit after initiation of treatment.

Table 1

Natural History of Infantile Neuronal Ceroid Lipofuscinosis

	Per Literature (months)	Present Study (months)
Deceleration of head growth ⁴⁶	5	36 (CI 27.3 – 45.5)
Developmental regression ^{6, 26, 46, 46}	6 to 18	15 (CI 10.3–18.8)
Myoclonic jerks and seizures ^{6, 47}	12 to 24	23 (CI 18.8–27.8)
Loss of vision ^{6, 25, 47}	12 to 24	25 (CI 11.2–38.8)
Brain atrophy per head imaging ^{25, 49}	12 to 24	19 (CI 14.9 – 22.4)
Electroencephalogram and/or electroretinogram extinguished ^{26, 46, 48}	36	52 (CI 41.6 – 62.2)
Visual evoked potential extinguished ⁴⁸	48	48 (CI 38.6 – 56.4)

CI = confidence interval

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Table 2Protocol Patients and Their *CLNI/PPTI* Mutations

Patient	Age of Presentation	Presenting Symptoms	<i>CLNI/PPTI</i> Mutations
1	15 months	Verbal regression	364 A>T (p.R122W)
2	18 months	Stumbling & impaired vision	169 A>i (stop at 101)
3	5 months	Strabismus	451 C>T (p.R151X)
	22 months	Poor balance & myoclonus	
4	9 months	Increased tone, inability to bear weight	451 C>T (p.R151X)
5	18 months	Stopped walking, irritability	451 C>T (p.R151X) & 29 T>A (p.L10X)
6	12 months	Stopped crawling, decreased tone	451 C>T (p.R151X) & 29 T>A (p.L10X)
7	18 months	Stopped walking, impaired vision	451 C>T (p.R151X) & 29 T>A (p.L10X)
8	4 months	Delayed motor skills	451 C>T (p.R151X)
	16 months	Seizures	
9	15 months	Delayed motor skills	451 C>T (p.R151X) & 798+2T>C splice site

Table 3

Summary of Neurodevelopmental Findings[#]

Patient #	*CA at Start of Intervention	Skills at Start of Intervention	CA at Final Assessment	Skills at Final Assessment	Potential Improvements/Subjective Changes
1	22 months	8 months	53 months	Less than 2 months	Improved alertness and irritability, regained ability to roll from back or side, spontaneous smiling
2	25 months	8 months	100 months	Less than 2 months	Improved alertness and irritability, good growth without need for G-tube, less medications to control myoclonic jerking than were needed for older affected brother at same age
3	27 months	4 months	71 months	Less than 2 months	Reduced irritability and myoclonic jerking
4	27 months	3 months	71 months	2 months	Reduced irritability and myoclonic jerking, regained ability to roll from side, spontaneous smiling
5	32 months	4 to 7 months	58 months	3 months	Improved alertness, decreased myoclonic jerking and stable developmental status through age 58 months
6	29 months	4 months	58 months	Less than 2 months	Reduced irritability and spontaneous smiling
7	10 months	6 to 9 months	18 months	9 months increased irritability over time with skills worse than those of older affected brother at same age	None
8	30 months	4 months	58 months	Less than 2 months	None
9	30 months	4 months	65 months	Less than 2 months	Improved alertness and better sleeping, decreased

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Patient #	*CA at Start of Intervention	Skills at Start of Intervention	CA at Final Assessment	Skills at Final Assessment	Potential Improvements/Subjective Changes
					myoclonic jerking

* CA = chronological age;

According to Denver developmental screening test.