Current evidence suggests that the effects of lithium on metabolic and signaling pathways in the brain may vary depending on the specific clinical condition or disease model. For example, lithium increases levels of cerebral N-acetyl aspartate in patients with bipolar disorder but does not appear to affect N-acetyl aspartate levels in normal human subjects. Conversely, lithium significantly decreases whole-brain levels of N-acetyl aspartate in a rat genetic model of Canavan disease in which cerebral N-acetyl aspartate is chronically elevated. While N-acetyl aspartate is a commonly used surrogate marker for neuronal density and correlates with neuronal viability, grossly elevated whole-brain levels of N-acetyl aspartate in Canavan disease are associated with dysmyelination and mental retardation. This report describes the first clinical application of lithium in a human subject with Canavan disease. Spectroscopic and clinical changes were observed over the time period in which lithium was administered, which reversed during a 2-week wash-out period after withdrawal of lithium. This investigation reports decreased N-acetyl aspartate levels in the brain regions tested and magnetic resonance spectroscopic values that are more characteristic of normal development and myelination, suggesting that a larger, controlled trial of lithium may be warranted as supportive therapy for Canavan disease by decreasing abnormally elevated N-acetyl aspartate. © 2005 by Elsevier Inc. All rights reserved.
result from a combination of osmotic, metabolic, and cytotoxic insults [3,4,11-15].

One leading hypothesis for the Canavan pathophysiology relates to the role of NAA in osmolyte regulation [1,2,12] through its interaction with molecular water transport pumps. According to this hypothesis, dysmyelination found in Canavan disease is linked to faulty regulation of NAA transport from neurons to oligodendrocytes. Apart from possible deficiency of free acetate for myelin lipid production in oligodendrocytes [16] as proposed by the metabolic theory, the osmotic theory proposes that high levels of NAA in the vicinity of the axon leads to active degeneration of myelin and eventual compromise of other glia such as astrocytes. Increased cerebrospinal fluid pressure also has been speculated to contribute to spongiform pathology, although in our experience Canavan patients with cerebrospinal fluid shunts have done no better than those without shunts in place and spinal fluid opening pressures in Canavan patients are not elevated; therefore, it appears most likely that any effects of increased pressure act at the local level of the myelin sheath. Nevertheless, many Canavan patients have received acetazolamide, in part for its action in decreasing cerebrospinal fluid production as well as for its anti-epileptic properties.

In Canavan disease tremor rats [17] and the majority of human subjects [11] with Canavan disease, elevated NAA and NAAG leads to seizure activity [18,19] and is associated with hypomyelination and abnormal regulation of genes affecting myelination (Dr. J. Francis, personal communication). NAA contributes to myelination as a source of acetate for lipid synthesis [3,4,7], and although it is primarily synthesized and localized within neurons, NAA is also found in oligodendrocyte and astrocyte precursors [20]. With respect to the efflux of elevated NAA from the brain in Canavan disease, in addition to its hydrolysis by aspartoacylase, NAA may be taken up by astrocytes [21] that are contiguous with the vasculature through their foot processes. In this context, Baslow and other investigators have looked at the possibility of blood-brain barrier disruption in the treatment of elevated brain NAA [22], in order to increase clearance from the brain, but so far there have been no clinical applications. The compartmentalization, transport, and utilization of NAA in the brain is complex, and aspartoacylase is present not only in oligodendrocytes and O2A progenitor cells at the site of myelin synthesis [23-25] but also may be located in type-2 astrocytes, neurons, and microglia [26,27]. Baslow has examined the effects of lithium chloride in the tremor rat model of Canavan disease and found a statistically significant 13% reduction of whole-brain NAA after acute treatment with 300 mg/kg/day over 4 days [28]. A previous study by O’Donnell et al. [29] also demonstrated a statistically significant 9% drop in brain NAA in wild-type rats after administration of lithium chloride for 2 weeks at a dose of 170 mg/kg/day. These data suggest that the Canavan disease rat, which has severely elevated whole-brain NAA in the range of 12-18 mM [30], may be particularly susceptible to effects of lithium in lowering whole-brain NAA.

Previous studies in adult humans have reported variable results in the ability of lithium to affect levels of brain NAA, depending on the underlying disease of subjects. For example, Moore et al. reported that 74% of a mixed population of bipolar and normal adults had a 5% increase in NAA after treatment for 4 weeks with lithium, but 26% had a decrease or no change [31]. Recent studies reported a decrease or absence of change in brain NAA in normal, healthy individuals after administration of lithium [32]. These data suggest that disease processes such as depression or mania may affect the activity of lithium with respect to NAA, because one group reported that chronic lithium paradoxically decreased brain NAA in healthy rats [29] but increased NAA in human bipolar patients [33]. The relevance of these other studies to Canavan disease is that there may be effects of lithium on NAA levels that are relatively disease-specific in the context of grossly elevated NAA. Effects of lithium in increasing NAA may be idiosyncratic with respect to bipolar disorder, whereas in Canavan disease the opposite effects are observed. This paradoxical effect of lithium on NAA levels could be the result of pleiotropic effects of lithium (i.e., the drug affects many different pathways) together with a unique metabolic milieu in different disorders. Until this report using lithium in Canavan disease, to our knowledge no one has examined the effect of lithium on NAA in pediatric patients, either normal or otherwise.

Apart from its potential benefit in directly lowering NAA levels in the extracellular fluid compartment, another possible rationale for lithium use in Canavan disease is its putative neuroprotective effect against glutamatergic excitotoxicity [34] which may be relevant to the cytotoxic hypothesis for Canavan disease [18,19]. In addition, another mechanism which is implicated in lithium’s therapeutic effects in bipolar disease, which may or may not be relevant in Canavan disease, is the depletion of inositol monophosphates [35]. This decrease leads to a shift in cell signaling mediated by the phosphatidylinositol (PIP2) secondary messenger pathway, mediated through pathways such as Akt-1 [36]. Effects of lithium on other membrane phospholipids also have been reported, which may be relevant to myelin synthesis [37]. Diverse molecular effects of lithium have been documented, including blocking apoptosis through effects on genes (e.g., p53, Bax, Bcl-2) [38] and neuroproliferative effects mediated through inhibition of glycogen synthetase kinase-beta and potentiation of Wnt signaling [39,40]. Moreover, there is recent evidence to suggest that lithium may induce both proliferation and neuronal differentiation of progenitor cells in the brain [41-43] which may be mediated through brain-derived neurotrophic factor/tyrosine kinase B (BDNF/trkB) [44]. Similarly, lithium may have effects on oligodendrocyte development through neurotrophin-mediated, axon-derived signals [45-47]. The extent to which
any of these mechanisms of lithium may act in Canavan disease or other leukodystrophies remains to be explored. The present study examined the end effects of lithium on clinical status and quantitative changes in brain NAA and T1, without investigating any specific mechanisms of its action.

**Methods**

At the time of initial treatment, the patient was an 18-month-old female with Canavan disease. During her early infantile period, she exhibited poor neck control, difficulty with visual fixation and tracking, and abnormal posturing of her extremities. Diagnosis of Canavan disease was confirmed by NAA acedia and reduced ASPA levels in skin fibroblasts. She did not carry any known Jewish mutations in the ASPA gene, and a novel mutation was later identified as IVS 1/2 A→T. Brain magnetic resonance imaging documented diffuse signal abnormality in the supratentorial white matter, indicating severe lack of myelination. Other laboratory abnormalities included mild hyperchloremic metabolic acidosis and hypercalcemia related to Type IV renal tubular acidosis. Previously she had been symptomatically treated by her consulting pediatrician with sodium carbonate, sodium acetate, furosemide, acetazolamide, and l-carnitine. The rationale for prescribing sodium acetate was as a substitute for calcium acetate given the patient’s underlying mild hypercalcemia before lithium treatment, which otherwise could have been exacerbated by calcium acetate. Canavan patients on calcium acetate (PhosLo) for treatment of hyperphosphatemia were previously observed to improve symptomatically [11], and it is speculated that the beneficial clinical effects observed by Dr. Edwin Kolodny may be derived from an increase in bioavailable acetate for myelin synthesis. The loop diuretic Lasix was used to treat mild hyperkalemia from her Type IV renal tubular acidosis (RTA), and the carbonic anhydrase inhibitor acetazolamide was prescribed both for its effects as an antiepileptic for absence seizures as well as for theoretical effects on lowering cerebrospinal fluid production. Patients taking acetazolamide are routinely monitored for their serum bicarbonate levels and may receive sodium carbonate, as did this patient. Dietary supplementation of l-carnitine (Carnitor) was recommended by the patient’s pediatrician for symptomatic treatment of muscle wasting, but there are no specific indications for Canavan disease. However, it has been proposed that l-carnitine might have beneficial effects on mitochondrial function or other metabolic pathways [48,49].

After obtaining institutional approval (institutional review board protocol 2097) and written consent, the patient was begun on lithium citrate 20 mg/kg/day for the first week which was escalated to 45 mg/kg/day. She received 12 mEq per day of oral lithium citrate (2.44 mL three times daily @ 8 mEq/5 mL) over a course of 3 months, equivalent to 450 mg/day of lithium carbonate. The serum lithium levels were maintained in the therapeutic range between 1.2-1.5 mg/dL. This dose was tapered during a 2-week wash-out period at the fourth month. During the treatment period, serum lithium levels, electrolytes, liver and renal indices, and thyroid studies were performed every 2-4 weeks to ensure that the patient remained in the therapeutic range and did not experience any complications. The patient was closely monitored by a pediatric nephrologist, and her renal and liver function studies remained within normal limits. Her mild acidosis remained unchanged during the study. Thyroid function and blood counts were also normal during the follow-up period. The patient did not experience any adverse effects and tolerated the medication well.

Clinical evaluations were completed by a neurologist at baseline and at 3 and 18 weeks. Five months before treatment, the patient’s head circumference was 50.5 cm. At the onset of treatment with lithium, the head circumference was 51.5 cm, weight was 9.0 kg, and length was 74 cm. After 4 months of treatment with lithium, the head circumference was 52 cm, weight was 9.6 kg, and length 82 cm. The clinical examinations included the Gross Motor Function Measure and Canavan neurologic exam, a quantitative scoring system which was developed as an assessment tool in a clinical trial for gene therapy of Canavan disease [50]. In addition, the Pediatric Evaluation of Disability Index (PEDI), a quality of life measurement, and the Mullen Scales of Early Learning were administered by a clinical psychologist at 3 and 18 weeks. It was not possible to blind the evaluators to the patient’s diagnosis of Canavan disease or use of lithium, which may represent a source of observer bias in clinical examinations. However, prior use of these formal tests in the setting of Canavan disease suggests that they are sensitive to changes in clinical status in patients with Canavan disease.

Quantitative magnetic resonance studies, which provide noninvasive information about the biochemical state of the brain, were performed before initiation of treatment and at 3, 12, 19, and 26 weeks. Conventional T1-weighted and T2-weighted images have been used by many groups to study the development of the brain during infancy [51]. The relative change in contrast between the white and gray matter gives rise to characteristic appearances of these images. Standard magnetic resonance imaging scans for Canavan disease include a T1-weighted spin-echo sequence (TR/TE = 700/14 ms, flip angle 70°, 23 slices; slice thickness 5 mm, gap 1.25 mm, field of view ~200 mm, matrix 144*256, rectangular field of view 6/8). Two such acquisitions are obtained in the axial and coronal planes. In addition, we typically acquire proton-density T2-weighted sequence (double-spin-echo TR/TE = 2500/20 and 2500/80 ms, 21 slices; slice thickness 5 mm, gap 1.25 mm, field of view approximately 200 mm (as required), matrix 192*256, rectangular field of view 6/8). Fluid-attenuated inversion-recovery and diffusion tensor imaging are also performed, as time permits, to obtain information on fluid shift.

Although such images do provide exquisitely high-resolution images with which to assess general development of brain anatomy, they do not provide a quantitative assessment required for longitudinal studies of brain condition. For such purposes, quantitative measurement of the longitudinal relaxation time (T1) is the preferred method [52,53]. It is well-known that the longitudinal relaxation time (T1) is influenced by a variety of factors, of which the dominant ones in the brain of young children are myelination and water content [54]. Although it is not appropriate to use the relaxation time as a measure of myelin content per se, it is common practice to use it to track the changes in the physiologic status of the brain in situations where myelin content is believed to be changing, such as brain development after birth.

T1 values were measured in selected white and gray matter regions in each brain hemisphere. T1 is considered a useful surrogate marker for normal myelination, in the sense that T1 values change predictably from birth as myelination occurs. Specifically, as myelin is formed over the first 2-4 months of life, white matter T1 values drop exponentially in normal pediatric subjects over this period [55]. In patients with Canavan disease, however, we have modeled the same T1 changes and found that the decrease in T1 is severely attenuated and drops linearly over the same time period, concomitant with lack of proper myelination. Therefore, any intervention that creates a substantial drop in T1 values would fit with a more age-appropriate pattern of myelination. Recently a protocol has been developed by which T1 values can be measured with >1% accuracy and 4% precision over the whole brain (i.e., 15 slices) in under 2 minutes [56]. The resolution of 2 × 2 mm in-plane is slightly less than that of conventional clinical images but is still sufficient to analyze different regions of the brain. Data for quantitating T1 are acquired using an inversion-recovery sequence (field of view = 256 mm, matrix 96*128, slice thickness 5 mm, gap 5 mm, 15 slices). Six images are acquired from each slice with different inversion times of 50, 400, 800, 1200, and 2000. The data are downloaded onto a SUN workstation where T1 values for each pixel are calculated using processing routines written for this purpose in IDL (Interactive Data Language, Research Systems, CO). T1 values (mean and standard deviation) are measured from each of 17 white or gray matter regions: frontal, parietal, and occipital central white matter; frontal, parietal, and occipital U fibers; semi-ovale; globus pallidus; caudate; putamen; thalamus; internal capsule; genu and splenium of the corpus callosum; midbrain;pons; dentate nucleus.

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Proton magnetic resonance spectroscopy also provides a method of performing quantitative and semiquantitative measurements of the brain. Of particular interest to Canavan disease is the fact that NAA levels may be measured noninvasively and accurately [57] and this technique provides quantitative serial measurements of NAA, the chief pathologic molecule in Canavan disease. Magnetic resonance imaging/magnetic resonance spectroscopy was performed at the same time as T1 measurements at Children’s Hospital of Philadelphia using a 1.5-T whole-body Siemens Vision system with a conventional head coil. Proton magnetic resonance spectroscopy examinations were performed using a single voxel stimulated-echo acquisition mode (STEAM) method (TR/TM/TE = 1600/30/20 ms, voxel size $2 \times 2 \times 2$ cm$^3$, 220 averages). The voxel positioning was guided using the graphic interface feature of the scanner. After the conventional water-suppressed measurement, the H$_2$O signal from the same voxel was measured with TR = 5000 ms. Data were acquired in four regions of interest: right frontal lobe (mostly white matter), right fronto-parietal lobe (mostly white matter), occipital cortex (mostly gray matter), and right basal ganglia (mostly gray matter). Signal calibration was performed using a phantom replacement technique [58] in which the metabolite levels in the spectra are obtained by fitting the in vivo brain absorption spectra as linear combinations of individual metabolite spectra. Phantom solutions of major brain metabolites with known concentration are measured, and the absorption spectrum of each metabolite is obtained and used as a basis function. Software developed by John Haselgrove, Ph.D. at Children’s Hospital, Department of Medical Physics, was used to quantify the prominent NAA peak. Metabolite levels of the in vivo brain spectra were obtained from a least-squares estimation routine written in IDL (Interactive Data Language) using the Provencher Linear Combination or LC-model approach [59]. The signal intensity was calibrated by the absolute reference method, in which the water signal from a standard phantom is used to calibrate the metabolite signal [60].

The strategy for analyzing longitudinally assessed measures such as NAA and T$_1$ was to fit a mixed effects model [61] with random intercept, age, and time posttherapy effects. Natural history data from this subject and 20 naive age-matched Canavan subjects were used to establish a range of predicted values for NAA effects over the 4-month treatment period with lithium. NAA data were modeled as a mixed effects model with random intercept and slope for each of the subjects, thereby matching all subjects on age. To study the posttreatment effect of lithium citrate on the treated patient, we included it as a piecewise-linear, time-varying covariate for the patient who received it. Statistical significance of each coefficient was evaluated by examining the Wald statistic, the estimate of the coefficient divided by its estimated standard error. A

Figure 1. Brain NAA by region. All brain regions tested manifested a relative decrease in NAA over the time course of treatment (nominal mM units), which deviated from the expected model based on the patient’s pretreatment values and values from age-matched Canavan subjects enrolled in another unrelated study. Because this lithium treatment was limited to a single patient, for statistical significance these effects require validation in additional subjects. Note that normal non-Canavan NAA values at this age would typically vary between 3-7 mM [cf. Ref. 55].
similar model was fit for $T_1$ scores of white matter (average of 18 regions) and gray matter (average of 16 regions). Although we were able to generate predicted values over the treatment period based upon baseline values for this subject and values extrapolated from other naive Canavan subjects, statistical analysis is complicated by the fact that this report describes treatment effects only in a single treated subject.

**Results**

The effect of lithium appears to be a strong decrease in NAA in all four regions tested over the 4-month treatment period (Fig 1). Although the trend of decreasing NAA was evident in all regions tested, a statistically significant lithium effect on NAA was found only in the frontal region ($P < 0.04$) using the mixed model approach [61], with the periventricular and occipital regions having $P$ values of 0.28 and 0.06 respectively. There were also highly statistically significant results for overall age effects in the periventricular ($P < 0.0001$) and occipital regions ($P < 0.005$). As noted above, the small sample size and lack of sufficient independent observations during the lithium treatment make it difficult to assess statistical significance. Studies in a series of patients with Canavan disease will allow us to clarify whether the NAA changes after lithium treatment are statistically significant in other brain regions.

After treatment with lithium, it is noteworthy that the patient’s $T_1$ values decreased in white matter regions, with a particularly dramatic drop in the genu of the corpus callosum. This brain region is in the vicinity of the periventricular region, which also revealed a decrease in cerebral NAA at 18 weeks of treatment. Gray matter regions disclosed a similar treatment effect (Fig 2). When all white matter regions were pooled for analysis, a treatment effect of lithium appears likely,
compared with the theoretical $T_1$ values obtained from modeling age-matched Canavan patients which are built into the statistical ramp function. Moreover, this pattern of $T_1$ changes was more similar to age-matched values which have been obtained using a model based upon normal pediatric subjects in this age range [55]. All nine white matter regions tested were statistically significant for lithium effect on $T_1$ (genu corpus callosum, $P < 0.001$; splenium corpus callosum, $P < 0.01$; internal capsule, $P < 0.08$; frontal/parietal/occipital white matter, $P < 0.002$; frontal/parietal/occipital u-fibers, $P < 0.005$). Although the effect of lithium treatment also appears strong for $T_1$ measurements in the eight gray matter regions tested (coefficient $= -89.7$ per month), proof of statistical significance would require more observations or subjects.

Magnetic resonance imaging results over the treatment period (Fig 3) yielded changes that were consistent with myelination on $T_1$-weighted images. The top panel (A) is at the initiation of treatment with lithium citrate. The bottom panels are at 3 months (B) and 4 months (C) of treatment. After 4 months, there is emergent myelination in genu of corpus callosum and periventricular regions, in association with a decrease in quantitative $T_1$ values in those regions. There also appears to be an increase in ventriculomegaly over the treatment period, which is
typical for the natural history of Canavan disease and was not retarded by lithium.

Clinical data obtained over the 4-month treatment period suggested that lithium produced mild symptomatic improvement. Before treatment, physical examination was notable for macrocephaly, poor head control, visual impairment, truncal hypotonia, and limb spasticity. Electrocardiogram and thyroid function studies were normal at baseline. Neurologic examination was performed once before treatment and once at the 18-week timepoint. At the later timepoint, it was observed that the patient initiated and made an attempt to grasp objects with her dominant hand, an improvement since the prior exam. In addition, the patient appeared more alert and aware of her surroundings since the baseline examination and vocalized intermittently, which represented a new skill. The Gross Motor Function Measure was given at 3 and 18 week timepoints, which did not detect statistically significant changes in lying and rolling over this limited 4-month follow-up period. However, the Pediatric Evaluation Disability Index, a semiquantitative quality-of-life instrument, revealed a highly statistically significant difference ($P < 0.0002$) in performance compared with age-matched data from the untreated Canavan reference group. Finally, the Mullen psychometric test suggested that the patient was stable over this 4-month time period with minor gains in receptive language, expressive language, and gross motor categories, which were also statistically significant ($P < 0.01$) compared with the expected age-matched performance level for Canavan patients.

**Discussion**

Janson et al. have recently described a clinical protocol using adeno-associated virus for transferring the *ASPA* gene into the brains of patients with Canavan disease [50]. This intervention has been demonstrated to significantly lower the level of NAA in at least one brain region tested and in some subjects was associated with changes in T1 signal (i.e., index of myelination and brain water diffusion) and overall clinical status (Dr. Paola Leone, manuscript submitted). We propose that simultaneous use of lithium citrate might be a noninvasive adjunctive treatment method for these patients. The possibility of a limiting therapeutic effect of lithium also needs to be addressed using a longer-term treatment period. There have been some reports suggesting that chronic lithium treatment may not be as benign as commonly assumed [62,63], and the costs and benefits of lithium in the setting of Canavan disease also should be explored in more detail.

Because these exploratory results are based on a single lithium-treated subject, modeled in conjunction with natural history data from untreated age-matched Canavan patients, there is a lack of statistical power for a conclusive test of treatment effects. Nevertheless, the results are consistent with a hypothesis associating improvement in pathologically elevated brain NAA with lithium, which warrants further investigation of lithium as a treatment strategy. In our experience, the standardized psychometric and neurologic tests we have chosen for clinical assessments are a sensitive and reliable index of clinical changes in the setting of Canavan disease, but future studies should be blinded with respect to the presence or lack of lithium to minimize observer bias. Because of the small number of patients with Canavan disease and the distinctive phenotype, it is currently not possible to keep evaluators unaware of the patient’s diagnosis.

Although the mechanism of lithium’s action in lowering NAA in the setting of Canavan disease is unknown, one possible and largely unstudied mechanism of action for lithium might be to prevent efflux of NAA from neurons to the extracellular fluid compartment where it is thought to have its toxic effects. Alternatively, lithium could have effects at the blood-brain barrier in facilitating the passage of NAA from the brain to the blood, or perhaps also at the level of renal excretion of NAA. As explained earlier, diverse effects of lithium are possible on the molecular level, and additional studies with lithium in Canavan disease will be required to test these hypotheses and to ascertain the magnitude of clinical effects possible. It is therefore proposed that a case series or controlled clinical trial of lithium in Canavan disease is indicated at this time. It is especially important to determine if the effects of lithium in lowering NAA will continue over a longer treatment period, and also if other brain regions will be affected as much as the frontal region. Because an increasing frontal to occipital gradient of NAA is typical of Canavan disease, a greater treatment effect may be required to decrease NAA in brain regions where a higher basal amount exists, and more subjects would need to be evaluated over a longer time period to observe this possible effect.

**References**


