

# THE LANCET **Neurology**

## **Supplementary webappendix**

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Levin SW, Baker EH, Zein WM, et al. Oral cysteamine bitartrate and N-acetylcysteine for patients with infantile neuronal ceroid lipofuscinosis: a pilot study. *Lancet Neurol* 2014; published online July 3. [http://dx.doi.org/10.1016/S1474-4422\(14\)70142-5](http://dx.doi.org/10.1016/S1474-4422(14)70142-5).

## **SUPPLEMENTARY MATERIALS**

### **ONLINE METHOD**

#### **Electron microscopic analysis of GRODs**

We also sought to determine whether cysteamine bitartrate-N-acetylcysteine combination mediated the depletion of GRODs in peripheral WBCs from all of our INCL patients. Accordingly, we analyzed the GRODs in peripheral WBCs from all patients before and after several months of treatment by transmission electron microscopy (TEM). Briefly, WBCs from the blood were isolated by density gradient centrifugation using Lymphoprep (Nycomed Pharma, Oslo, Norway), and centrifuged at 800 x g for 20 min at room temperature. The cells were collected from the interface using a Pasteur pipette. The harvested cells were washed with normal saline once and then fixed in 2.5% glutaraldehyde in sodium phosphate buffer for 2 hrs. The fixed cells were washed once with Millonig's phosphate buffer and kept in the same buffer until further processing for TEM analysis. Ultra thin sections of the cell pellets were evaluated by TEM (JFE Enterprises, Brookeville, MD) performed using a LEO 912 electron microscope and photomicrographs were taken. Identification and counting of the GRODs in the patient lymphocytes from electron micrographs (30000x) were performed by two investigators working independently of each other. The results were averaged.

#### **Legends for Supplementary Tables and Figures:**

##### **Table S1: Relevant laboratory findings**

Relevant laboratory test results of all 9 INCL patients are presented in this table.

##### **Table S2. Findings on clinical ophthalmic examination.**

The results on fixation (as a measure of visual function), ocular motility (EOM balance), vascular attenuation, disc pallor, and pupillary light reaction are summarized. A decline in visual function is noted as the patients' age progressed. The majority of the patients presented with an ocular deviation. The vascular attenuation and disc pallor progressed with age. Pupillary light reactions became more sluggish and were totally lost as the disease progressed.

**Figure S1. Representative fundoscopic images (from Patient 8).**

**A.** Optic atrophy (age 47 m) progressing to **B.** chalky white optic nerve head (age 58 m).

**Figure S2. Changes in ERG amplitudes over study duration.**

ERG amplitudes of all the patients indicating an early progressive reduction approaching noise level between 48 and 60 months of age. Initial amplitudes for Patient 7 at around 10 months of age are close to the lower range of normal amplitudes.

**Figure S3. Electroencephalographs of an INCL patient.**

Representative EEGs of an INCL patient at 2.5 yrs (**A**), 3yrs (**B**) and 4yrs (**C**) of age. EEG channels were selected here to show progressive changes from generalized slowing, to appearance of multifocal epileptiform activity (arrow) and finally, virtual cessation of brain electrical activity. The EEG shown represents various electrodes and their corresponding placement on the head and an EKG lead. EEG done in the differential montage with y-axis showing electrodes corresponding to the exact placement on the head using the 10-20 international system of standardized electrode placement. The x-axis corresponds to time elapsed in seconds. The lowest electrode corresponds to the EKG lead. Electrodes displaying data and fields of interest are shown. Electrodes channels

with high impedance and erroneous signal were excluded. The numbers on the side of figure correspond to the EEG machine channel and were not numbered in continuity in the machine setting and labeling at the time of signal acquisition. More information about electrode placement techniques, electrode names, corresponding location and signal acquisition standards can be found in the supplemental attachments which include the American Clinical Neurophysiology Society Guidelines for Standard Electrode Position Nomenclature and the American Clinical Neurophysiology Society Guidelines for minimal technical standards for pediatric electroencephalography (EEG).

**Supplementary Table S1: Relevant Laboratory Results**

Patient #		Hgb (g/dl)	Hct (%)	WBC (k/ $\mu$ l)	BUN (mg/dl)	Creat (mg/dl)	Alk Phos (U/l)	ALT (U/l)	AST (U/l)	Bili (mg/dl)	T4 ( $\mu$ g/dl)	TSH ( $\mu$ IU/ml)	Prolactin ( $\mu$ g/l)	IGF-1 (ng/ml)
1	Visit #1 (n=4)	11.3	33.2	5.2	12	0.2	178	21	40	0.2	9	0.7	26	47
	follow-up mean	12.03	36.05	7.08	8.00	0.30	133.50	22.25	24.75	0.33	9.08	0.97	14.50	104.00
	follow-up SD	0.76	1.61	1.50	2.45	0.08	29.24	0.96	4.11	0.19	0.61	0.58	2.38	48.15
2	Visit #1 (n=8)	10.4	31.3	7.6	10	0.2	202	19	28	0.2	9.2	0.88	28	48
	follow-up mean	10.40	30.74	6.87	5.00	0.37	220.88	26.00	21.25	0.46	6.26	1.88	26.50	126.25
	follow-up SD	1.39	3.09	0.81	1.85	0.05	37.78	6.44	4.03	0.21	1.42	0.83	13.58	47.69
3	Visit #1 (n=4)	12.8	38.2	15.6	5	0.3	154	20	29	0.2	13.3	1.63	77	70
	follow-up mean	11.93	34.33	5.93	3.25	0.32	145.75	18.00	27.00	0.60	6.75	1.78	49.25	90.75
	follow-up SD	0.36	1.55	1.40	2.22	0.09	32.79	0.82	8.12	0.14	0.91	0.85	38.06	53.89
4	Visit #1 (n=4)	12.2	35.9	7.9	5.0	0.3	179.0	14.0	25.0	0.3	10.0	1.35	48.0	80.0
	follow-up mean	12.48	35.65	4.06	3.50	0.35	155.25	23.25	46.67	0.63	5.98	3.25	54.75	79.75
	follow-up SD	0.31	1.59	0.36	1.73	0.05	41.05	10.63	17.16	0.21	0.90	3.34	53.03	45.77
5	Visit #1 (n=3)	11.4	35.1	7.7	12	0.4	222	35	27	0.3	11.7	1.83	32	71
	follow-up mean	13.40	39.23	8.05	10.00	0.43	131.33	36.33	23.67	0.50	5.57	2.04	12.67	86.33
	follow-up SD	0.78	2.27	1.88	1.73	0.06	23.86	7.57	6.43	0.10	1.91	1.18	7.37	6.66
6	Visit #1 (n=3)	9.4	28.1	4.9	10	0.3	130	16	23	0.2	normal	normal	23	49
	follow-up mean	10.80	33.17	8.00	5.00	0.36	151.67	23.67	22.33	0.30	7.34	1.98	23.33	114.33
	f/u SD	0.46	2.01	3.48	2.00	0.05	31.88	5.03	2.08	0.17	1.45	0.51	12.06	18.01
7	Visit #1	11.7	34.2	10.2	8	0.3	217	19	44	0.6	9.7	2.48	32	33
	Visit #2	11.8	34.5	6.94	10	0.4	118	14	40	0.4	10.7	0.82	25	<25
8	Visit #1 (n=3)	12.8	37.4	11.1	9	0.32	270	19	24	0.7	8.7	2.13	19	105
	follow-up mean	13.07	37.83	7.53	6.67	0.24	288.33	26.67	16.67	0.43	6.65	3.52	14.70	322.67
	follow-up SD	0.38	1.89	0.95	1.53	0.02	25.70	7.51	5.51	0.23	0.44	0.90	15.29	148.81
9	Visit #1 (n=4)	12.7	37.8	11.9	8	0.36	192	20	30	0.6	8.6	1.55	18	79
	follow-up mean	12.28	35.53	6.29	10.00	0.29	219.25	21.75	17.00	0.25	6.13	3.18	30.60	152.50
	follow-up SD	0.57	2.07	1.49	2.16	0.07	35.56	4.03	4.24	0.10	1.18	2.10	25.14	31.40

Hgb, hemoglobin; Hct, hematocrit; WBC, white blood count; BUN, blood urea nitrogen; Creat, creatinine; Alk, alkaline phosphatase; Alt, alanine aminotransferase; Ast, aspartate aminotransferase; Bili, bilirubin; T4, thyroxine; TSH, thyroid stimulating hormone; IGF-1, insulin-like growth factor 1 . Laboratory results from the first visit (pre-treatment) and the Mean $\pm$ SD of those in subsequent visits (post-treatment) where n=number of follow-up visits.

## Supplementary Table S2

	Age (months)	Fixation	EOM Balance	Vascular Attenuation	Disc Pallor	Pupillary Light Reaction
Patient 1	28	NR	NR	1-2+	1-2+	normal
	34	no F&F	orthotropia	1-2+	1-2+	normal
	43	no F&F	12 pd XT	2-3+	2-3+	sluggish
	54	no F&F	orthotropia	3-4+	3-4+	sluggish
Patient 2	25	no F&F	20 pd ET	none	1-2+	normal
	31	no F&F	10 pd ET	1-2+	2-3+	normal
	39	no F&F	variable ET	2-3+	3+	sluggish
	45	no F&F	orthotropia	3+	3+	? Trace
	52	no F&F	orthotropia	4+ (sclerosed)	4+	nonreactive
Patient 3	27	frequent F&F	16 pd ET	1+	1+	normal
	37	no F&F	orthotropia	1-2+	2+	slow
	46	no F&F	orthotropia	2-3+	3+	sluggish
	59	no F&F	orthotropia	3+	3+	sluggish
	72	no F&F	orthotropia	3-4+	3-4+	sluggish
Patient 4	27	occasional F&F	orthotropia	1+	1+	normal
	37	no F&F	20 pd ET	1-2+	1+	sluggish
	46	no F&F	25 pd ET	2-3+	2-3+	sluggish
	59	no F&F	orthotropia	2-3+	3+	sluggish; almost tonic
	72	no F&F	orthotropia	3-4+	4+	nonreactive
Patient 5	33	no F&F	25 pd ET	1-2+	2+	normal
	44	no F&F	orthotropia	2-3+	3+	sluggish
	51	no F&F	orthotropia	3-4+	4+	sluggish; occasional hippus
	59	no F&F	orthotropia	NR	NR	NR
Patient 6	30	no F&F	orthotropia	1-2+	1+	normal
	36	no F&F	12 pd XT	2-3+	2+	sluggish
	50	no F&F	20 pd XT	3-4+	4+	sluggish; almost tonic
	59	no F&F	20 pd XT	4+	4+	sluggish; almost tonic
Patient 7	11	F&F	orthotropia	none	no pallor	normal
	19	F&F	orthotropia	none	1+	normal
Patient 8	31	no F&F	orthotropia	2-3+	3+	sluggish
	37	no F&F	orthotropia	3-4+	4+	sluggish
Patient 9	31	no F&F	12 pd XT	1-2+	1-2+	normal
	37	no F&F	ET 20-30 pd	2-3+	2-3+	sluggish

F&F: fix and Follow reflex

EOM: Extraocular muscle; pd: prism diopter; ET: esotropia; XT: exotropia

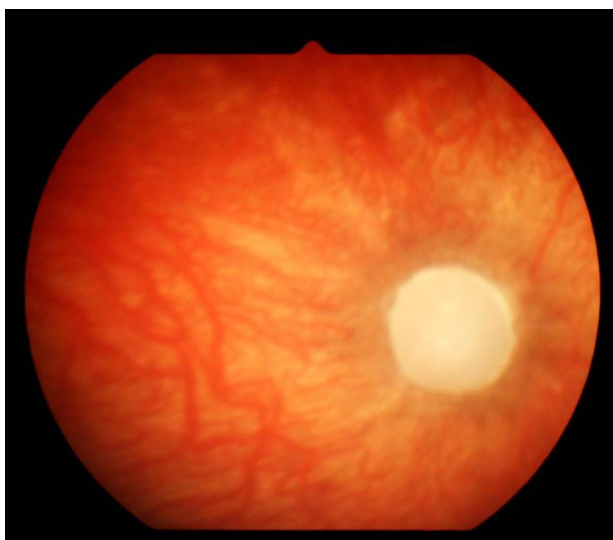
NR: Not recorded

Supplementary Figure S1

**11/2009**

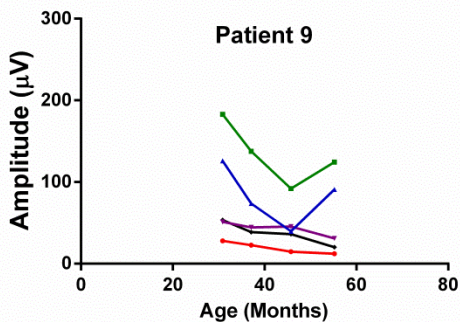
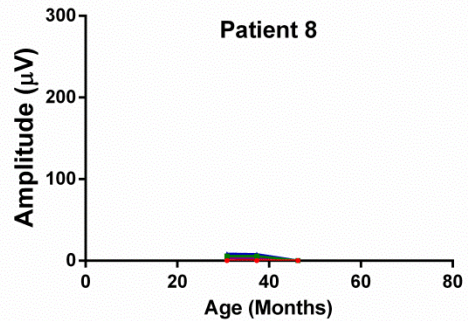
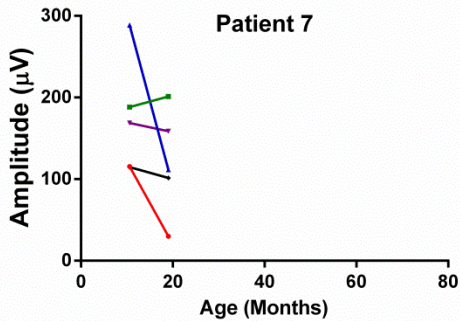
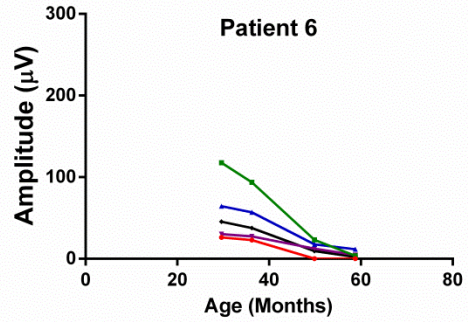
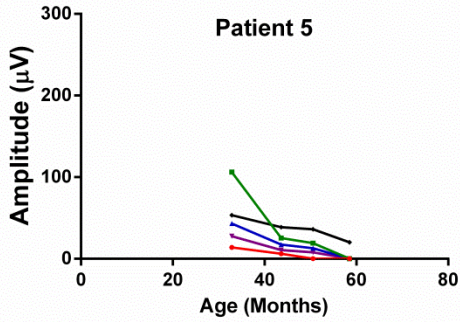
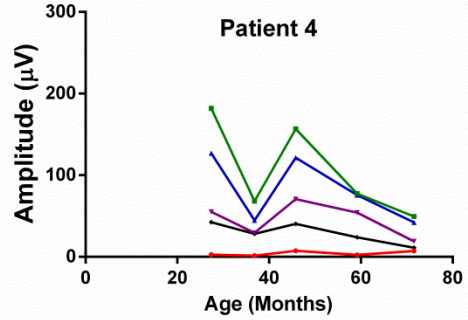
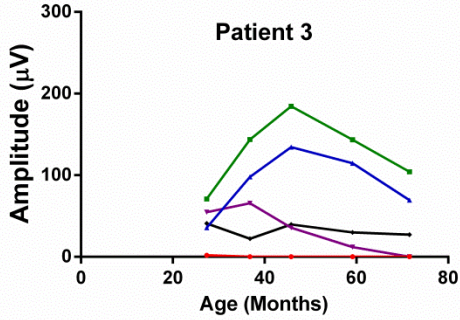
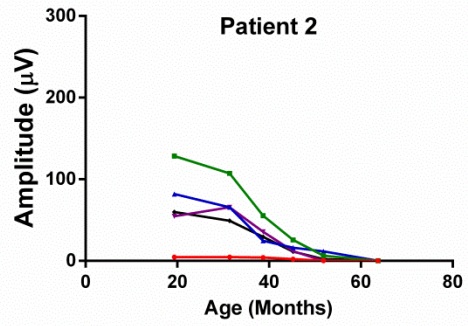
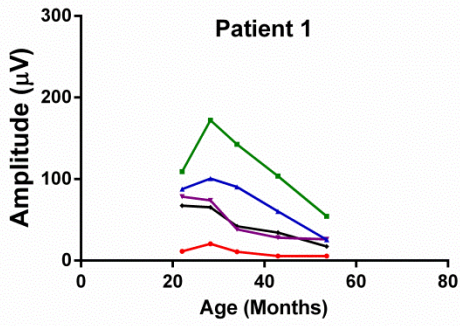


**10/2010**





# Supplementary Figure S2

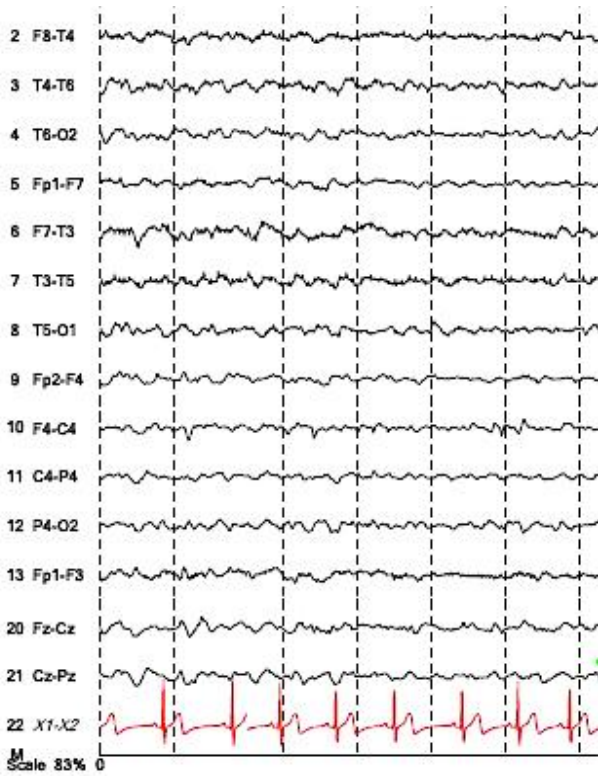


- Rod Response b-wave amplitude
- Combined (rod-cone) Response a-wave amplitude
- Combined (rod-cone) Response b-wave amplitude
- Cone Response b-wave amplitude
- 30 Hz Flicker

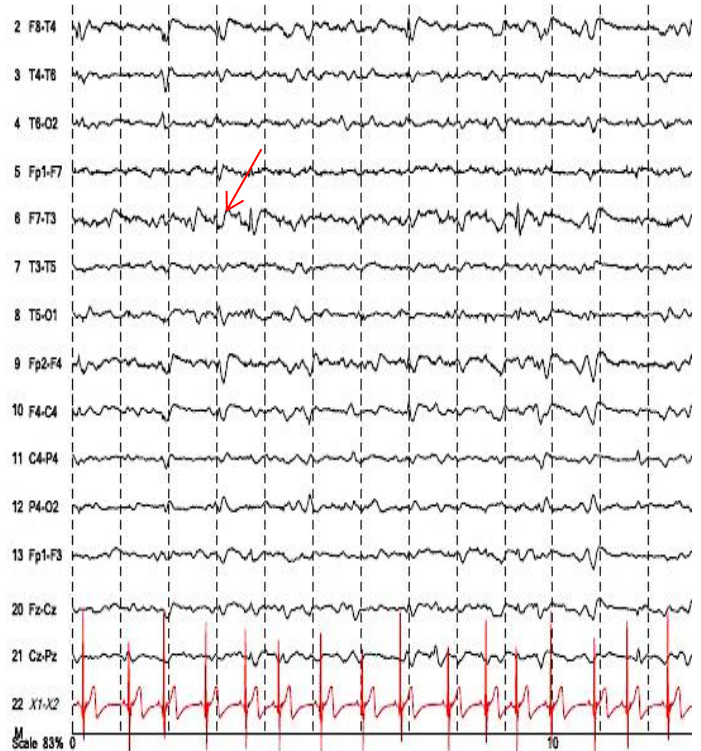


# Supplementary Figure S3

## A



## B



## C

