

Long-Term Follow-Up After Gene Therapy for Canavan Disease

Paola Leone, Ph.D.

Associate Professor

Cell & Gene Therapy Center

Dept. Cell Biology

UMDNJ-SOM, NJ

Overview

Translational Gene Therapy Study

- Safety and Efficacy of a Phase I Study using AAV2-ASPA for Brain Gene Transfer in patients affected by Canavan Disease

HUMAN GENE THERAPY 13:1391-1412 (July 20, 2002)
© Mary Ann Liebert, Inc.

Clinical Protocol

Gene Therapy of Canavan Disease: AAV-2 Vector for
Neurosurgical Delivery of Aspartoacylase Gene (*ASPA*)
to the Human Brain

F.D.A.-I.N.D. #9119
N.I.H. (R.A.C.) #0001-381

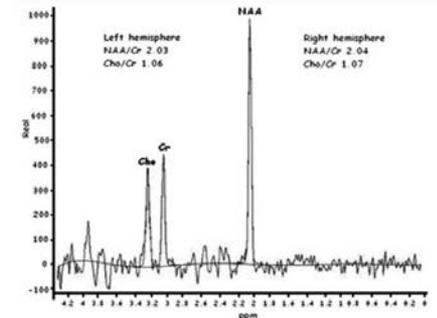
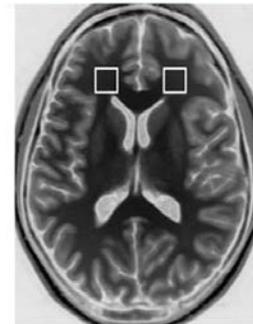
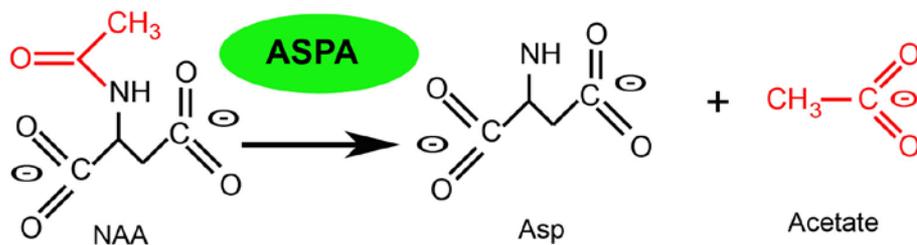
CHRISTOPHER JANSON, M.D.,^{1,*} SCOTT McPHEE, M.SC., LARISSA BILANIUK, M.D.,^{2,*}
JOHN HASELGROVE, PH.D., MARK TESTAIUTI, M.D.,¹ ANDREW FREESE, M.D., PH.D.,
DAH-JYUU WANG, PH.D., DAVID SHERA, D.SC., PETER HURH, B.SC., JOAN RUPIN, B.SC.,
ELIZABETH SASLOW, PH.D., OLGA GOLDFARB, M.D., MICHAEL GOLDBERG, M.D.,
GHASSEM LARIJANI, PHARM.D., WILLIAM SHARRAR, M.D., LARISA LIOUTERMAN, M.SC.,
ANGELIQUE CAMP, M.SC., EDWIN KOLODNY, M.D., JUDE SAMULSKI, PH.D.,^{3,*}
and PAOLA LEONE, PH.D.^{1,*†}

RAC Review : March 2000

FDA Approval : April 2001 *represented the first clinical use of AAV in the human brain*

Canavan Disease: Genetics

- Autosomal recessive, *ASPA* gene mapped to chromosome 17p13
- Loss of *aspa* function results in the accumulation of NAA to 8-14mM (normal range: 3-10mM).
- 1/45 to 1/60 Ashkenazi Jewish carry Canavan trait
- Different types of mutations possible in Jewish and non-Jewish populations
- Prevalence of Canavan Disease = 500
- Incidence of Canavan Disease = 50



Canavan Disease

Clinical Features & Neuropathology

- Patients present in infancy with hypotonia, lack of head control, macrocephaly, seizures, visual abnormalities, failure to thrive.
- Severe motor impairment, epilepsy, mental retardation, blindness.
- Spongy change of lower parts of cortex, subcortical white matter, cerebellar cortex,
- Microscopically – accumulation of fluid within myelin lamellae, disintegrating of myelin sheaths, swollen astrocytes, severe vacuolation



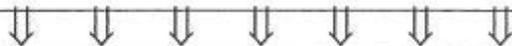
2 yrs

5 yrs

Clinical Phases

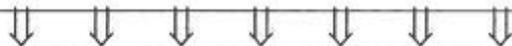
Pretreatment Phase (Total of 2-6 assessments)

1. Magnetic resonance spectroscopy (MRS) to assess relative NAA concentration
2. Magnetic resonance imaging (MRI) to assess neuroanatomical changes
3. Neurological examination (GMFM, Ashworth scales)
4. Neurodevelopmental testing (Mullen scales, PEDI, HELP-135)
5. CSF analysis to assess metabolite levels and levels of aspartoacylase gene (only in patients pre-implanted with Ommaya reservoir)
6. Biochemical analysis of urine for NAA
7. Serum hematology & chemistry, electrophoresis, anti-AAV2 antibody profile



Surgery & Gene Delivery

Single-arm clinical study with standard dose of 900 billion AAV-*ASPA* particles to each of 18 patients by intraparenchymal injections to the brain.



Post Gene Delivery — Serial Assessments

At two weeks, a complete physical and neurological exam will be conducted, including hematology & clinical chemistry. Brain MRI will be obtained in order to assess any delayed postoperative signal changes and to definitively rule out edema and bleeding.

(Time Points: 1, 3, 6, 9, 12, 18, 24 months)

1. MRS protocol as above
2. MRI protocol as above
3. Formal neurological assessments as above
4. Psychometric testing as above
5. CSF analysis as above
6. Urine and serum specimen analysis as above

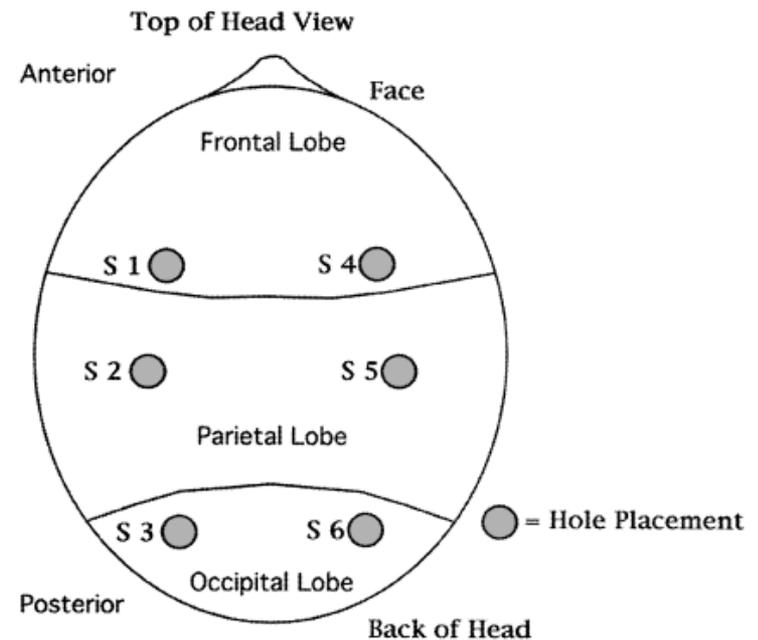


FIG. 2. Schematic diagram: surgical sites of delivery.

Enrollment Criteria

1. A definitive diagnosis of Canavan disease, based upon biochemical criteria and genetic (mutation) analysis.

Available online at www.sciencedirect.com

 ScienceDirect

Molecular Genetics and Metabolism 89 (2006) 156–163

 ELSEVIER

Molecular Genetics and Metabolism

www.elsevier.com/locate/ymgme

Rapid detection of three large novel deletions of the aspartoacylase gene in non-Jewish patients with Canavan disease

B.J. Zeng^a, Z.H. Wang^a, P.A. Torres^a, G.M. Pastores^a, P. Leone^b, S.S. Raghavan^a, E.H. Kolodny^{a,*}

^a Department of Neurology, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA
^b Division of Neurosurgery, Department of Surgery, University of Medicine and Dentistry of New Jersey, Camden, NJ, USA

J. Inher. Metab. Dis. 25 (2002) 557–570
© SSIEM and Kluwer Academic Publishers. Printed in the Netherlands.

Identification and characterization of novel mutations of the aspartoacylase gene in non-Jewish patients with Canavan disease

B. J. ZENG¹, Z. H. WANG¹, L. A. RIBEIRO^{1,2}, P. LEONE³, R. DE GASPERI¹, S. J. KIM¹, S. RAGHAVAN¹, E. ONG¹, G. M. PASTORES¹ and E. H. KOLODNY^{1,*}

¹Department of Neurology, New York University School of Medicine, New York; ²Department of Genetics, Sao Paulo State University, Sao Paulo, Brazil; ³Division of Neurosurgery, Department of Surgery, University of Medicine and Dentistry of New Jersey, Camden, New Jersey, USA

2. The patient must demonstrate clinical or radiologic findings consistent with Canavan disease, such as macrocephaly, hypotonia, developmental delay, seizures, or other positive findings.

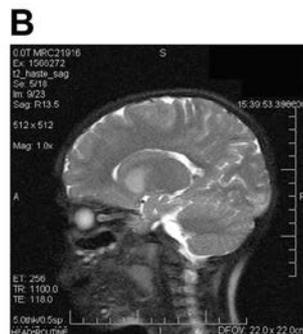
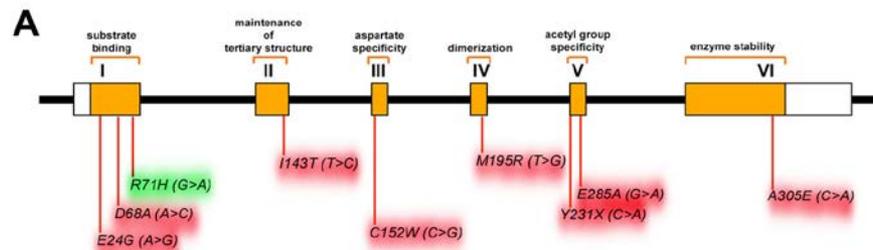
3. The patient must be between the age of 3 and 96 months.

ASPA

MTSCHIAEEHIQKVAI**I**FGG**T**H**G**NE**L**T**G**VFLVKHWLENGAE
IQRTGLEVKPFITNPR**A**VKKCTRYIDC**D**LNRIFDLENLKG
KMSEDLPEVRRRAQEINHFLGPKDSEDS**Y**DI**I**FDLHNTTS
NM**G**CTLIILEDNRNNFLIQMFHY**I**KTSLAPLPC**C**YVYLIEHP
SLKYAT**R**SIKYPVGI**E**VGP**Q**P**Q**G**V**LRADILDQ**M**RKMIK
HALDFIHHFNEGK**E**FPP**C**AIEVYKII**E**KVD**Y**PRDENG**E**IA
AII**H**PNLQ**D**QDWKPLHPGDPMFLTL**D**LGKTIPL**G**GDCTV**Y**P
V**F**V**N**E**A**Y**E**Y**K**KE**A**F**A**KT**T**KL**T**LN**A**K**S**IR**C**CL**H** (W)

substitution, truncation, addition

(insertion, deletion, frame shift not shown)



Atypical Canavan Disease

Mild-Onset Presentation of Canavan's Disease Associated with Novel G212A Point Mutation in Aspartoacylase Gene

Christopher G. Janson, MD,^{1,2} Edwin H. Kolodny, MD,³ Bai-Jin Zeng, PhD,³ Srinivasa Raghavan, PhD,³ Gregory Pastores, MD,³ Paola Torres, PhD,³ Mitra Assadi, MD,¹ Scott McPhee, PhD,¹ Olga Goldfarb, MD,¹ Beth Saslow, PhD,¹ Andrew Freese, MD, PhD,² D. J. Wang, PhD,⁴ Larissa Bilaniuk, MD,⁴ David Shera, ScD,³ and Paola Leone, PhD¹

jects could lead to a better understanding of Canavan's pathophysiology and improvements in *ASPA* gene therapy.

Ann Neurol 2006;59:428–431

Canavan's disease is an autosomal recessive childhood leukodystrophy¹ caused by defects in aspartoacylase.² Since a correlation between Canavan's disease and mutations in the aspartoacylase gene (*ASPA*) was demonstrated,³ more than 50 additional mutations have been identified. Loss of *ASPA* enzyme activity results in abnormal accumulation of *N*-acetyl-aspartic acid (NAA) and *N*-acetyl-aspartic-glutamic acid (NAAG) in brain white matter, leading to dysmyelination, spongiform degeneration, premature disability, and death. The leukodystrophy due to elevated NAA and NAAG appears

Clin Genet 2008; 73: 288–289
Printed in Singapore. All rights reserved

© 2007 The Authors
Journal compilation © 2007 Blackwell Munksgaard

CLINICAL GENETICS

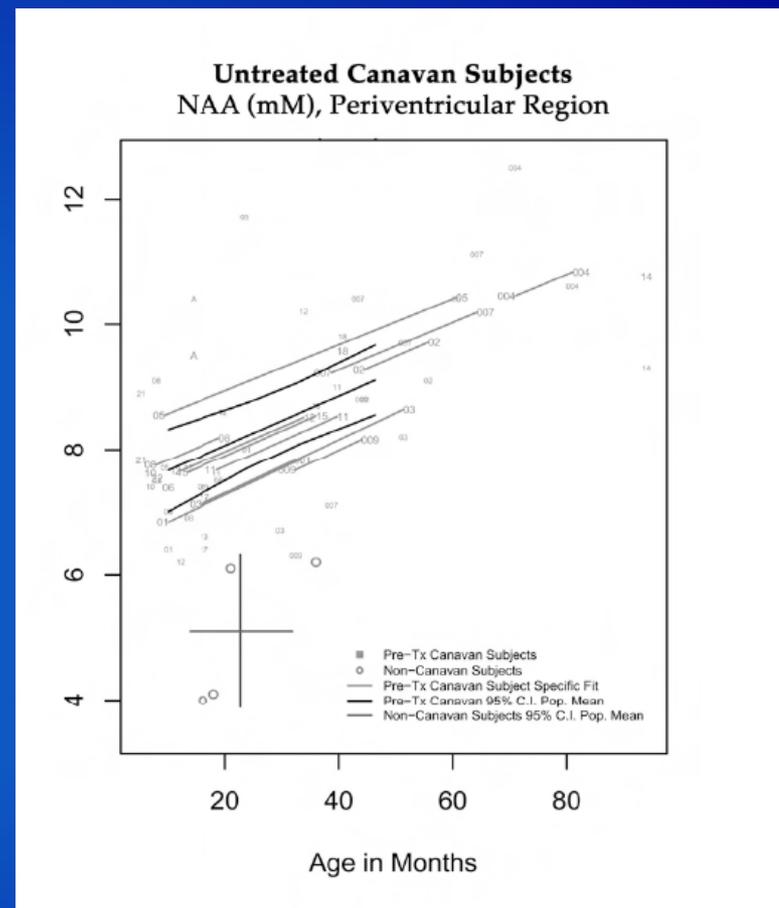
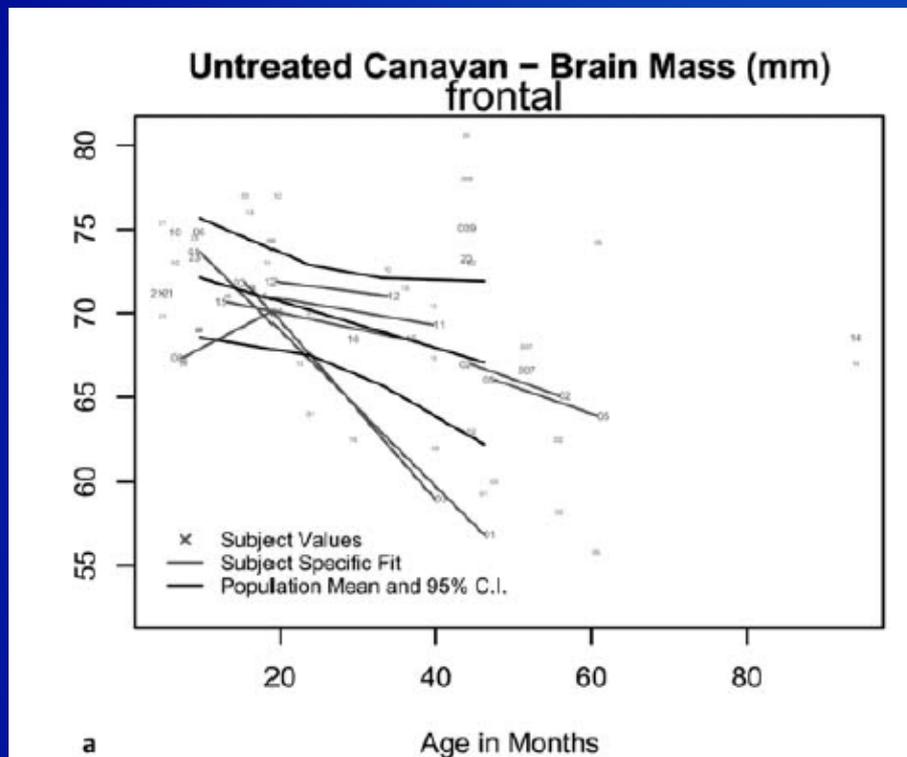
doi: 10.1111/j.1399-0004.2007.00934.x

Letter to the Editor

Homozygosity for mutation G212A of the gene for aspartoacylase is associated with atypical form of Canavan's disease

Velinov M et al.

Natural History of Canavan Disease



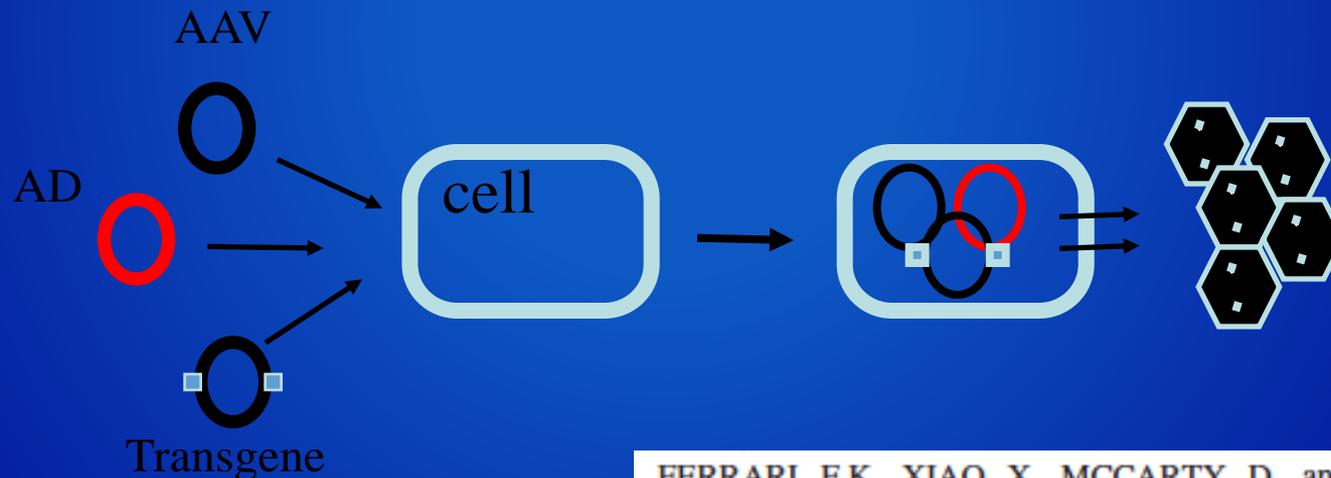
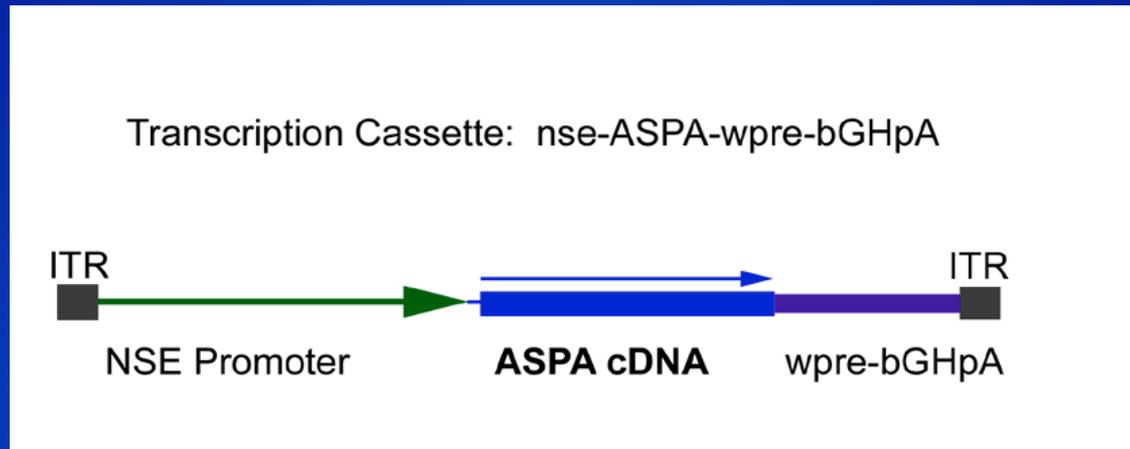
Strategy for analyzing longitudinally assessed measures was to fit a mixed effects model (Laird & Ware, 1983) with random intercept and age.

Neuropediatrics. 2006 Aug;37(4):209-21.

Natural history of Canavan disease revealed by proton magnetic resonance spectroscopy (1H-MRS) and diffusion-weighted MRI.

Janson CG, McPhee SW, Francis J, Shera D, Assadi M, Freese A, Hurh P, Haselgrove J, Wang DJ, Bilaniuk L, Leone P.

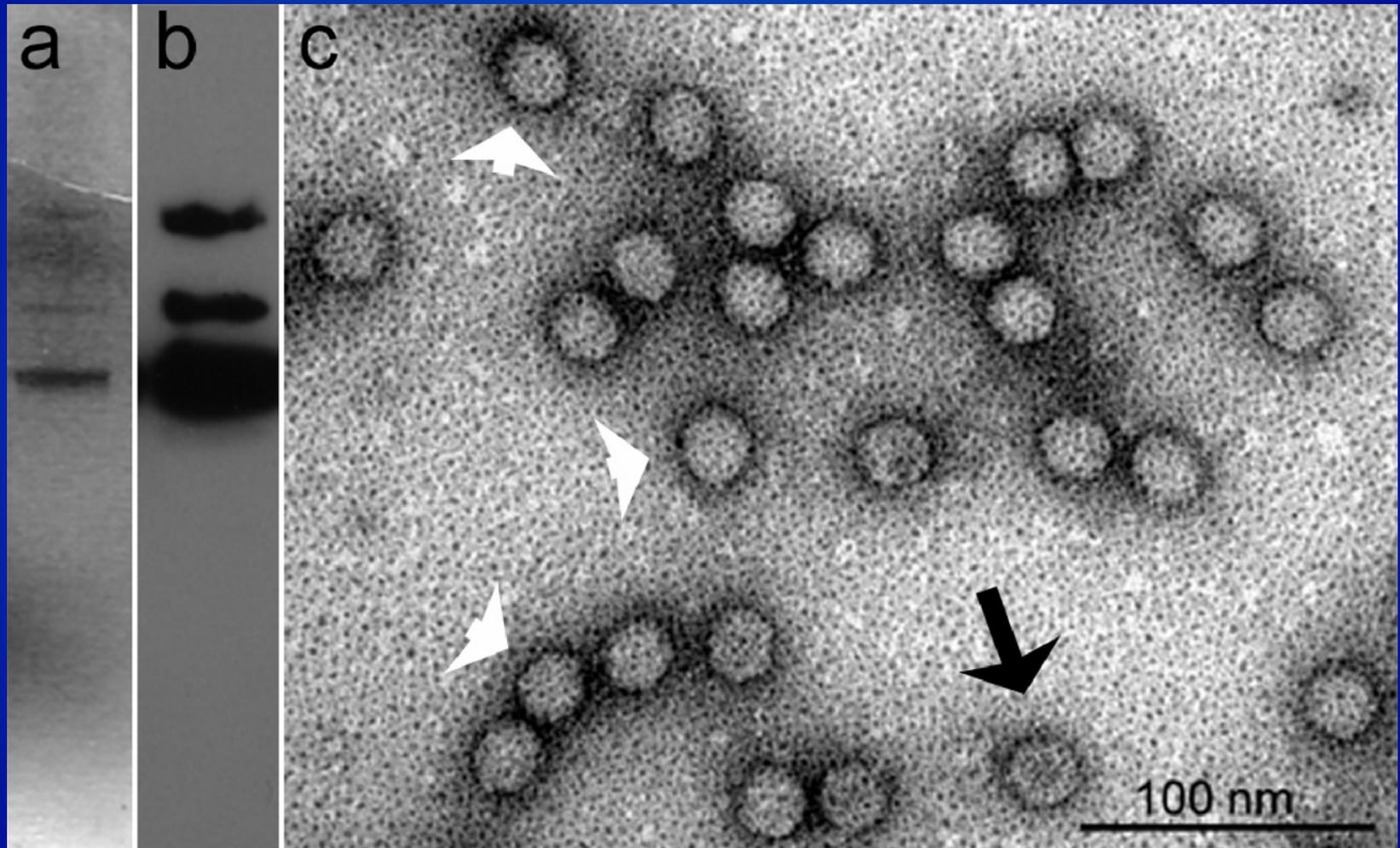
AAV-Based Gene Therapy



FERRARI, F.K., XIAO, X., MCCARTY, D., and SAMULSKI, R.J. (1997). New developments in the generation of Ad-free, high-titer rAAV gene therapy vectors. *Nat. Med.* **3**, 1295–1297.

XIAO, X., LI, J., and SAMULSKI, R.J. (1998). Production of high-titer recombinant adeno-associated virus vectors in the absence of helper adenovirus. *J. Virol.* **72**, 2224–2232.

Characterization of rAAV-ASPA



Study Outcomes:

Measuring Effects of Gene Transfer

- 1) Quantification of **NAA levels** in brain, urine, CSF, serum
- 2) **Magnetic Resonance** (i.e., DTI/Anisotropy/FA, T1/T2, brain atrophy)
- 3) **Serum analysis**: CBC, protein electrophoresis for a,b,g globulin & quantitative immunoglobulins, cytokines.
- 4) CSF analysis: cells, protein, electrolytes
- 5) **Anti-AAV2 neutralizing antibody** titers
- 6) **Clinical outcomes** (i.e., Canavan Neurological Exam, Gross Motor Function Measure/GMFM, HELP-135)
- 7) **Psychometric tests** (i.e., Mullen Scales of Early Learning, Pediatric Inventory of Disability Index/PEDI)
- 8) Data were analyzed fit **with longitudinal, mixed-effects models** (Laird and Ware, 1983) with subject-specific coefficients for intercept, pre-treatment slope, and post-treatment slope.

Viral Gene Transfer For Canavan Disease



First Patient June 5th, 2001

AAV2-ASPA administration in 13 patients (1×10^{12} g.t.)

Surgical Phase
2001

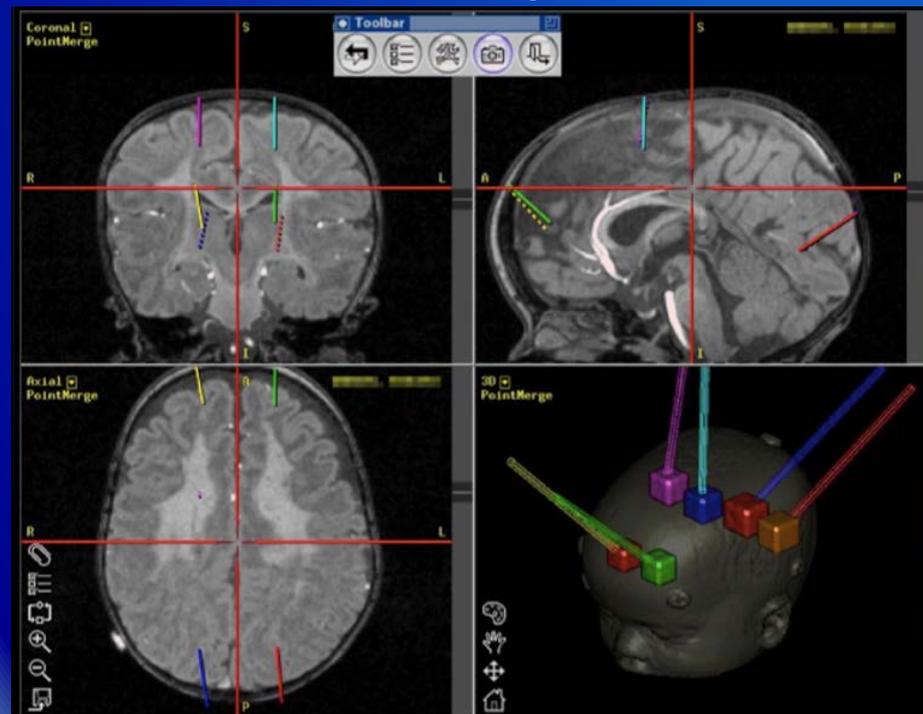
~ 60 minutes for **first** cohort of **3** patients
(Genomic Titer 8×10^{11} ml - 4.0ul/min.)

2003

~ 15 minutes for **second** cohort of **7** patients
(Genomic Titer 1×10^{12} ml - 2.0ul/min.)

2005

~ 60 minutes for the **third** cohort of **3** patients
(Genomic Titer 5×10^{12} ml - 1.5ul/min.)



Canavan Disease

Clinical Gene Therapy Development

Demographics-Enrolled

Subject ID	Sex	Age	ASPA mutations	Gene therapy	Baseline seizures
01-118-04	F	83	854A→C (E285A)693C→A (Y231X)	2001	+
01-118-09	M	45	854A→C (E285A)854A→C (E285A)	2001	+
01-118-07	M	65	854A→C (E285A)854A→C (E285A)	2001	+
01-118-12	F	22	859G→A (A287T) frameshift 10T→G; 11insG	2003	-
01-118-08	F	24	Splicing IVS1-2 A→T deletion	2003	-
01-118-02	F	56	854A→C (E285A)854A→C (E285A)	2003	++
01-118-15	F	36	640G→T (E214X)914C→A (A305E)	2003	++
01-118-01	M	47	914C→A (A305E)914C→A (A305E)	2003	+
01-118-05	M	59	914C→A (A305E)548C→A (P183H)	2003	++
01-118-03	F	53	854A→C (E285A)854A→C (E285A)	2003	+
01-118-33	F	4	914C→A (A305E) deletion	2005	-
01-118-27	F	21	854A→C (E285A)854A→C (E285A)	2005	+
01-118-24	M	23	548 C→A (P183H) deletion	2005	-

Canavan Disease

Clinical Gene Therapy Development

Demographics - Untreated

SUBJECT I.D.	SEX	AGE	ASPA MUTATIONS
01-118-11	M	19	914C→A (A305E) 47T→C (I16T)
01-118-16	M	29	244-245 del AT (codon 82 del) 244-245 del AT (codon 82 del)
01-118-17	F	16	914C→A (A305E) 815G→C (L272P)
01-118-18	M	41	502C→T (R168C) exon 2 deletion
01-118-21	M	5	914C→A (A305E) 362A→T (N121I)
01-118-22	M	7	914C→A (A305E) 914C→A (A305E)
01-118-23	M	9	733A→G (H244L) 733A→G (H244L)
01-118-25	M	20	914C→A (A305E) 640G→T (G214X)
01-118-26	M	8	854A→C (E285A) 854A→C (E285A)
01-118-20	M	8	854A→C (E285A) 854A→C (E285A)
01-118-19	M	10	914C→A (A305E) 914C→A (A305E)
01-118-10	M	7	914C→A (A305E) UNK
01-118-14	M	41	854A→C (E285A) 854A→C (E285A)
01-118-06	M	10	41T→G (V14G) 541A→C (P181T)
01-118-13	F	16	914C→A (A305E) 914C→A (A305E)

Toxicity/SAE

Table 4. Adverse events within 90 days of gene therapy. Adverse events in 13 Canavan disease subjects 90 days after AAV2-ASPA gene therapy are listed by category, with notation of serious adverse events (*). Because of

the small sample size and rarity of adverse events, analysis was performed with Fischer's exact method to calculate expected frequencies. For events with zero incidence, Agresti-Coulli intervals are provided.

Clinical finding	Group 1	Group 2	Group 3	Incidence (%)	95% CI
Fever (postoperative)	1/3*	4/7	1/3	46	0.192–0.749
Emesis (postoperative)	1/3	0/7	0/3	8	0.002–0.360
Seizures (postoperative)	1/3	0/7	0/3	8	0.002–0.360
Subarachnoid hemorrhage, mild	0/3	2/7	3/3	38	0.139–0.684
Subdural hemorrhage (<1 cm)	0/3	4/7	3/3	54	0.251–0.808
Soft tissue inflammation, extracranial	0/3	2/7	1/3	23	0.050–0.538
CSF leak	0/3	1/7	2/3	23	0.050–0.538
Hydrocephalus requiring shunt	0/3	0/7	0/3	–	0–0.247
Immune reaction, rash/urticaria	1/3	0/7	1/3	15	0.019–0.455
Immune reaction, other, local	0/3	0/7	1/3	8	0.002–0.360
Immune reaction, other, systemic	0/3	0/7	1/3	8	0.002–0.360
Superficial wound infection	0/3	0/7	0/3	–	0.002–0.360
Brain abscess, cerebritis, or meningitis	0/3	0/7	1/3*	8	0–0.247
Anemia, mild	2/3	0/7	0/3	15	0.019–0.455
Respiratory infection, mild	2/3	0/7	0/3	15	0.019–0.455
Pulmonary, other	0/3	0/7	0/3	–	0–0.247
Cardiovascular	0/3	0/7	0/3	–	0–0.247
Renal	0/3	0/7	0/3	–	0–0.247
Endocrine	0/3	0/7	0/3	–	0–0.247
Gastrointestinal	0/3	0/7	0/3	–	0–0.247
Genitourinary	0/3	1/7	0/3	8	0.002–0.360
Clinical chemistry, change from baseline	0/3	0/7	1/3	8	0.002–0.360

PT# 01-118-24 (SAE)

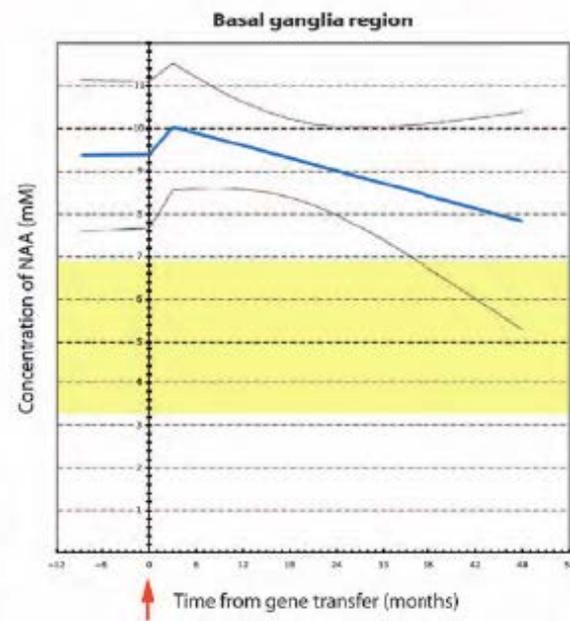
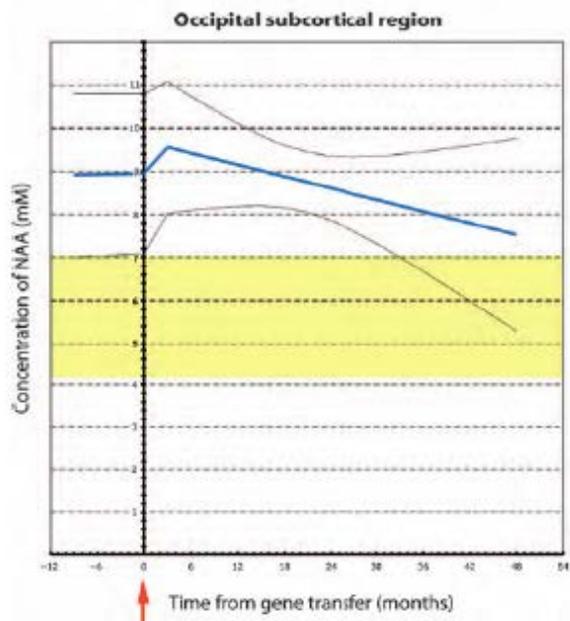
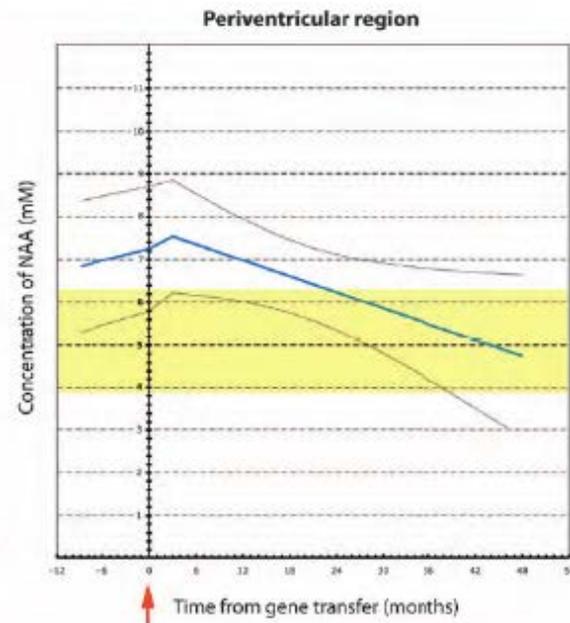
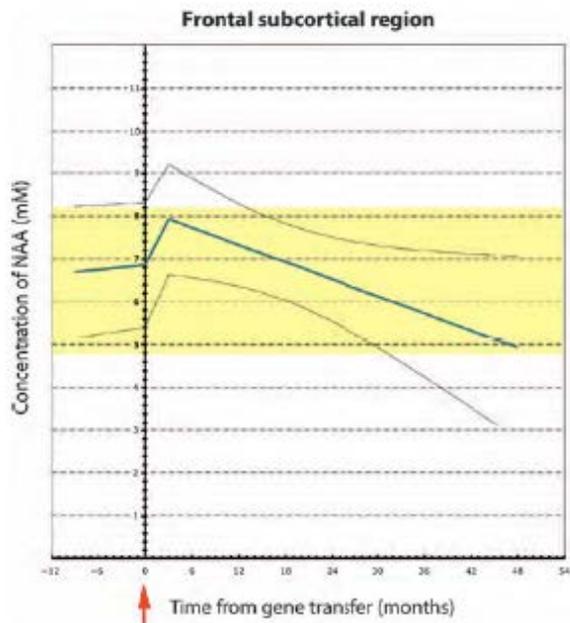


1 yr Post - Gene Therapy



8 yrs Post- Gene Therapy

NAA Changes after Gene Therapy



NAA Changes after Gene Therapy

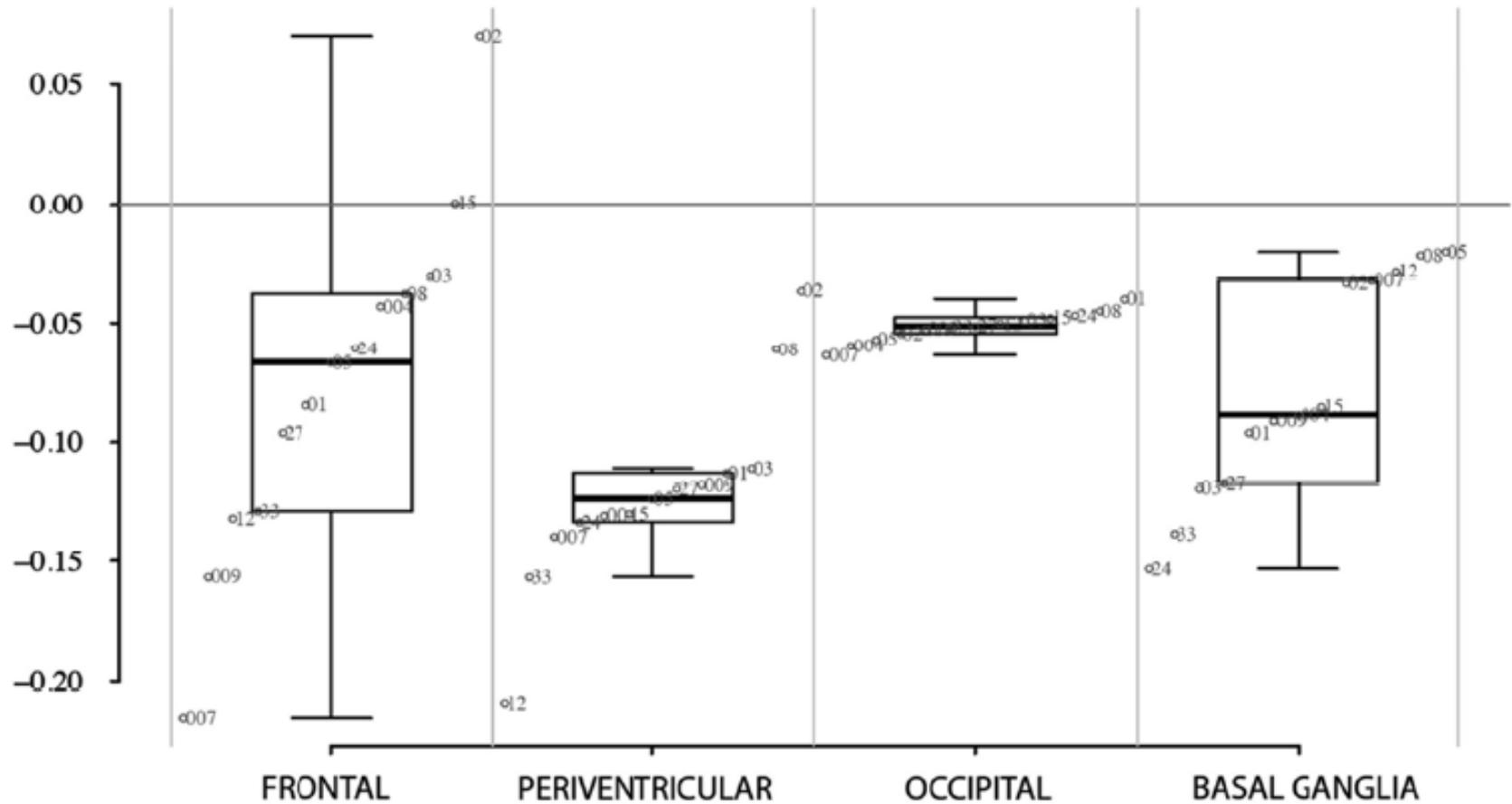
Brain region	Pre versus zero		Post versus zero		Post versus pre	
	Coefficient	P	Coefficient	P	Difference	P
Frontal	+0.022	0.049*	-0.040	0.0039**	-0.062	0.0008*
Periventricular	+0.050	0.000003**	-0.032	0.065	-0.082	0.00012**
Occipital	+0.014	0.126	-0.023	0.206	-0.037	0.065
Basal ganglia	+0.009	0.318	-0.067	0.0005**	-0.86	0.00049**
Frontal	+0.018	0.183	-0.066	0.025*	-0.084	0.025*
Periventricular	+0.046	0.002*	-0.062	0.043*	-0.108	0.003**
Occipital	+0.005	0.641	-0.045	0.255	-0.50	0.220
Basal ganglia	+0.002	0.880	-0.049	0.222	-0.51	0.235

Choline Changes after Gene Therapy

Choline, Table of Level Estimates & Contrasts (Mean & S.D. with T-tests)

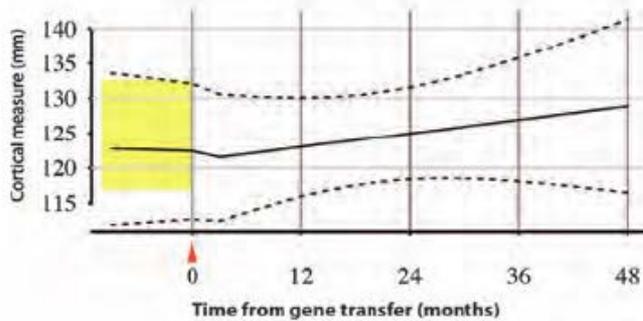
	Pre-treatment		Post-treatment		Post vs. Pre	
	mean	S.D	mean	S.D	difference	p-value
Frontal	0.550	0.308	0.627	0.244	+0.077	0.100
Periventricular	0.474	0.261	0.534	0.218	+0.060	0.138
Occipital	0.418	0.239	0.539	0.175	+0.121	0.0007
Basal ganglia	0.583	0.281	0.721	0.280	+0.138	0.003

Fig. S1. Distribution of individual NAA individual values by region.

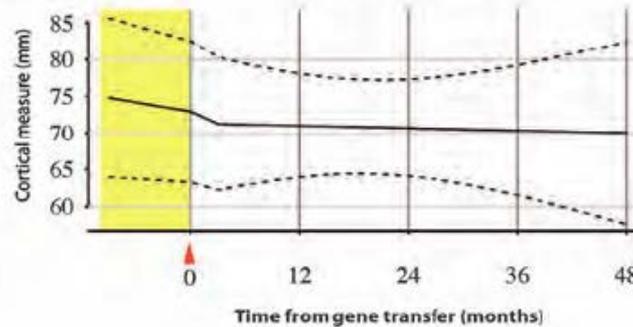


Brain Atrophy after Gene Therapy

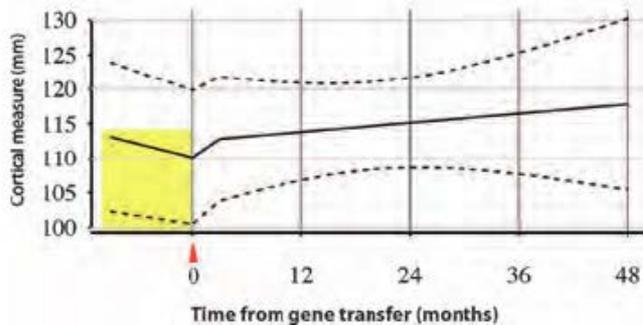
Central brain mass



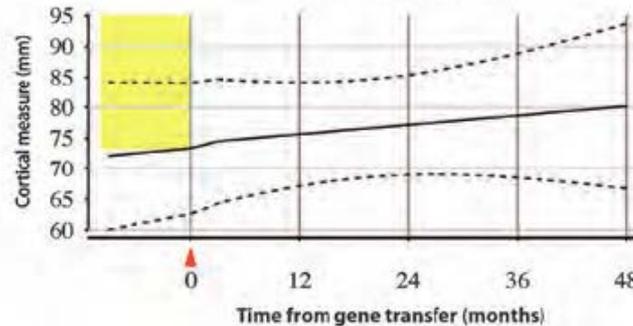
Frontal brain mass



Posterior brain mass



Cerebellum



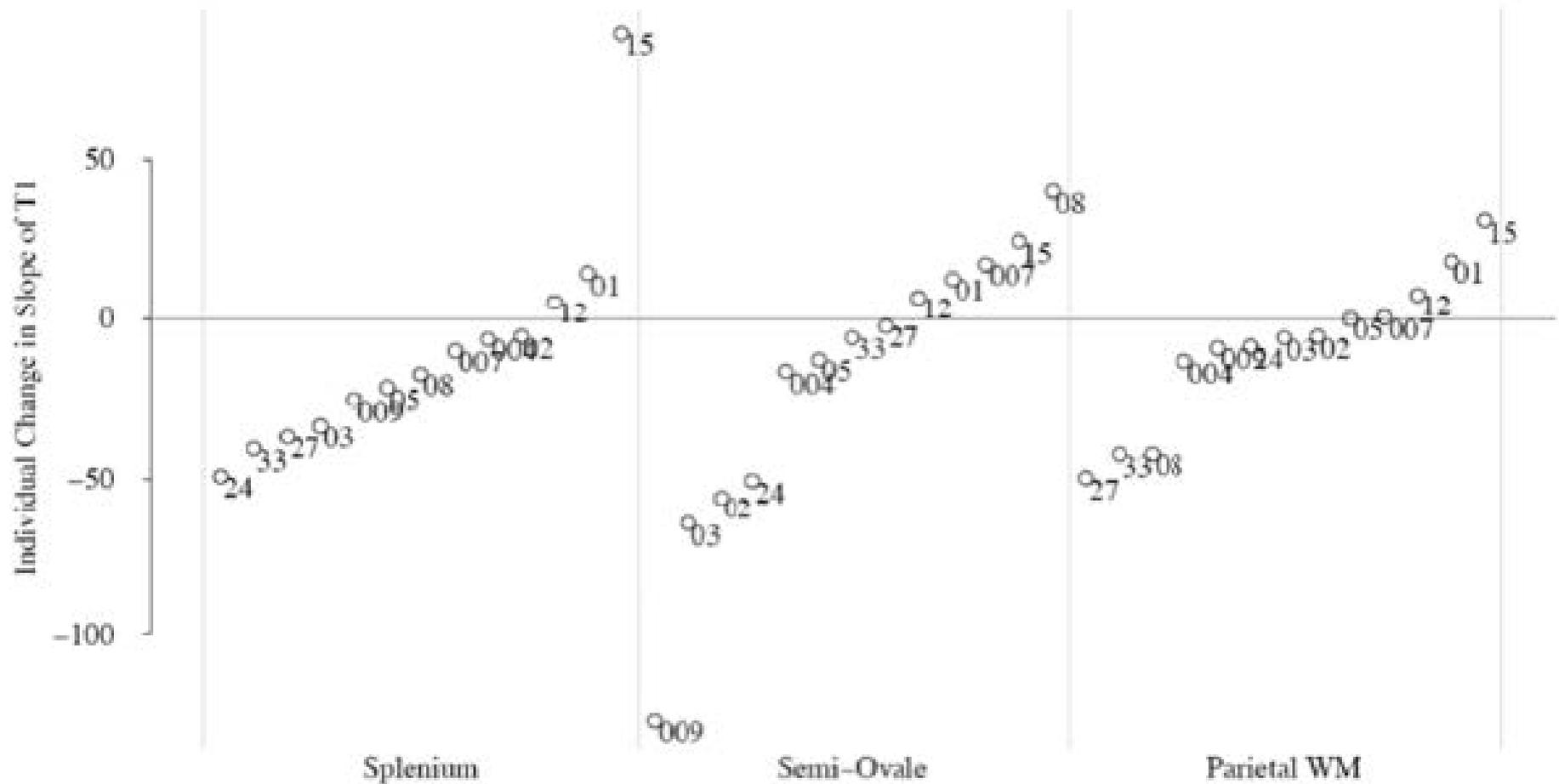
Brain region	Pre versus zero		Post versus zero		Post versus pre	
	Coefficient	P	Coefficient	P	Difference	P
Frontal	-0.221	0.0002**	-0.063	0.717	0.158	0.326
Central	-0.078	0.196	+0.142	0.412	0.221	0.171
Parietal	-0.345	<0.0001**	+0.070	0.684	0.415	0.010*
Cerebellar	+0.082	0.178	+0.104	0.549	0.022	0.890

Table S3. Quantitative T1 Values by Region.

T1 and anisotropy were exploratory outcome measures and were intended solely for further hypothesis generating. Although data are reported here for completeness, the study was underpowered for T1 comparisons across multiple brain regions. Regional values are fully quantitative, but summary results are semi-quantitative (without Bonferroni correction) due to the small number of subjects vs. independent comparisons. The individual trends for changes in slope are shown below the table for the three regions discussed in the main text.

	Pre vs. Zero		Post vs. Zero		Post vs. Pre	
	Coefficient	p-value	Coefficient	p-value	Difference	p-value
Internal Capsule, L	-4.05	0.025	+1.94	0.371	5.99	0.031
Internal Capsule, R	-4.31	0.016	+1.51	0.459	5.83	0.024
Parietal WM, L	-4.54	0.008	-2.83	0.533	1.71	0.732
Parietal WM, R	-3.52	0.040	-0.81	0.854	2.72	0.580
Occipital WM, L	-2.74	0.135	0.64	0.900	3.38	0.531
Occipital WM, R	-4.17	0.029	-1.89	0.712	2.27	0.683
Frontal U-fibers, L	-4.88	0.0004	+2.36	0.532	7.24	0.068
Frontal U-fibers, R	-6.53	<.0001	+1.30	0.700	7.83	0.026
Pons, L	-5.48	0.0003	+4.49	0.223	9.97	0.014
Pons, R	-5.25	0.0004	+6.24	0.118	11.49	0.009
Midbrain, L	-5.55	0.0001	-2.33	0.494	3.22	0.377
Midbrain, R	-4.42	<.0001	+1.07	0.716	5.49	0.090
Putamen, L	-1.53	0.075	+0.860	0.269	2.39	0.062
Putamen, R	-2.28	<.0001	+0.570	0.476	2.85	0.002
Thalamus, L	-6.73	<.0001	-1.56	0.510	5.16	0.063
Thalamus, R	-7.24	<.0001	-1.81	0.478	5.43	0.071
Globus pallidus, L	-9.52	<.0001	-8.09	0.018	1.43	0.708
Globus pallidus, R	-6.58	0.0018	-5.06	0.104	1.51	0.698
Caudate, L	-3.28	<.0001	1.90	0.232	5.18	0.003
Caudate, R	-3.78	<.0001	-0.47	0.778	3.31	0.068
Splenium	2.91	0.245	-5.44	0.063	-8.35	0.040
Region Semiovale	-0.64	0.799	-6.51	0.026	-5.87	0.149
Parietal White Matter	-3.07	0.221	-5.08	0.082	-2.02	0.620

T1 Slope (3 regions)



Stabilization of hydrocephalus

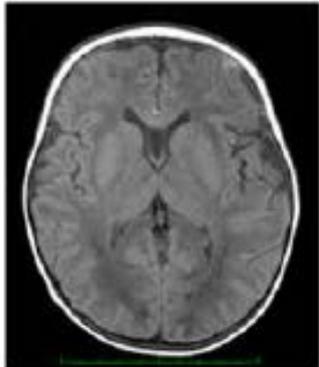


Age 23 months, -1 month GT Age 30 months, +6 months GT Age 42 months, +18 months GT

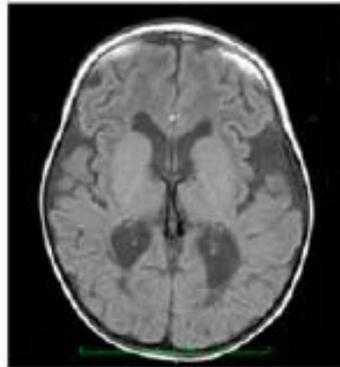
Fig. 4. Stabilization of brain atrophy in younger patients after gene therapy. Representative MRI images from Canavan disease patient 01-118-08 after AAV2-ASPA gene therapy. The MRI images of this younger subject, who did not have brain mass loss before gene therapy and was classified as a better responder, reveal stabilization of hydrocephalus in the frontal horn region (white arrow) and third ventricle (black arrow), as well as retention of brain mass in the frontal and posterior parietal regions.

Canavan Disease: Brain Atrophy

Subject 01-118-01



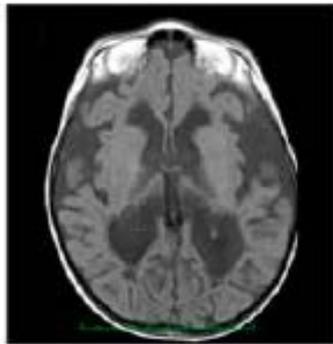
Age 10 months, -37 months GT



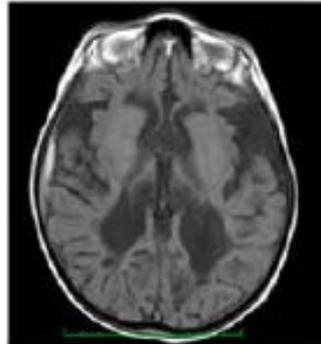
Age 22 months, -25 months GT



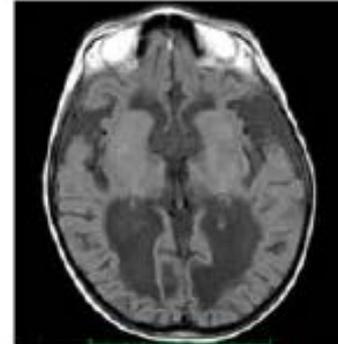
Age 33 months, -14 months GT



Age 47 months, +0 months GT



Age 57 months, +10 months GT



Age 64 months, +17 months GT

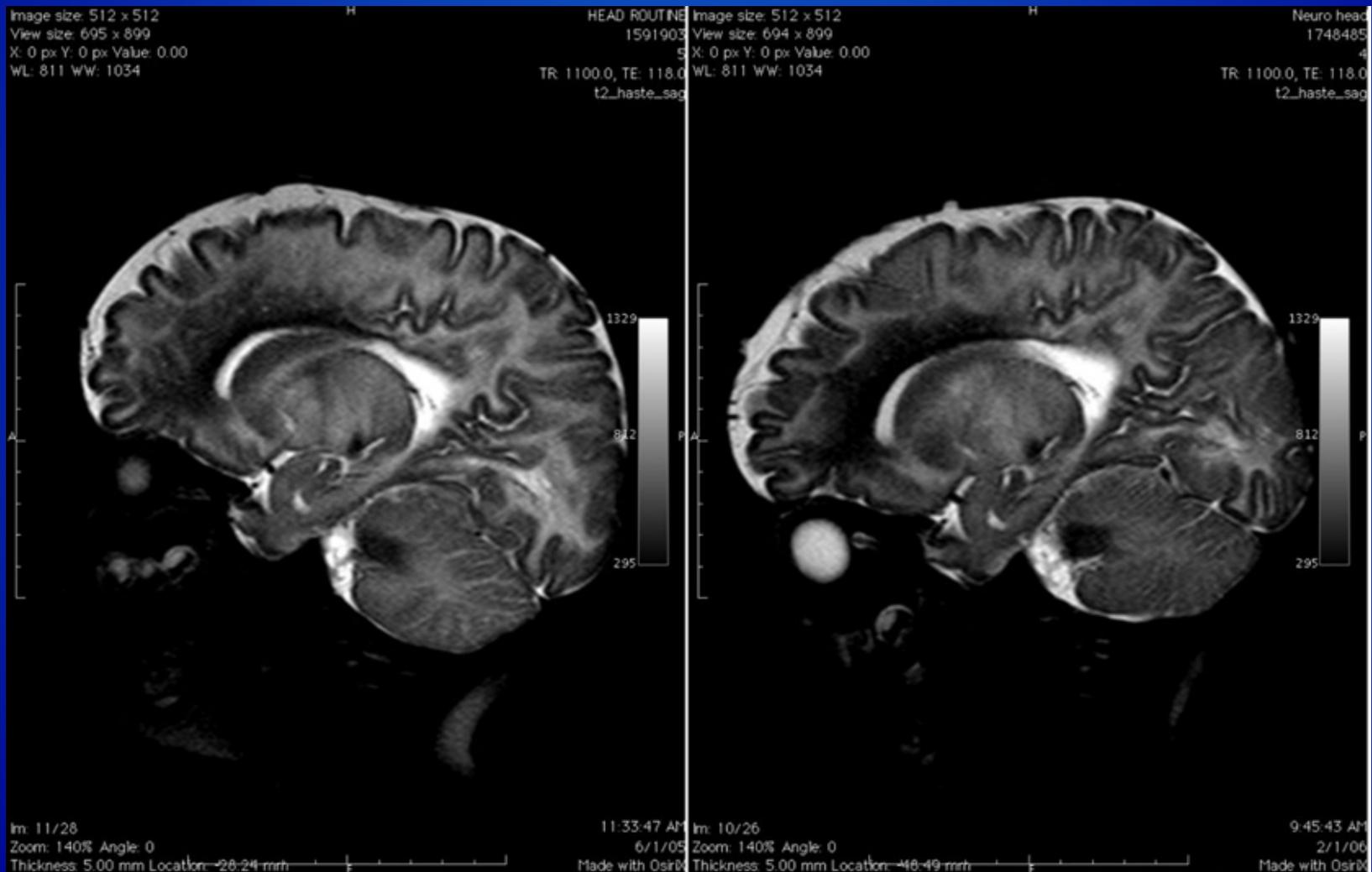
Montage #1: Progression of brain atrophy prior to treatment.

MR Studies: Conventional MR Analysis

Effect of gene therapy on MRI (T2 W/I)

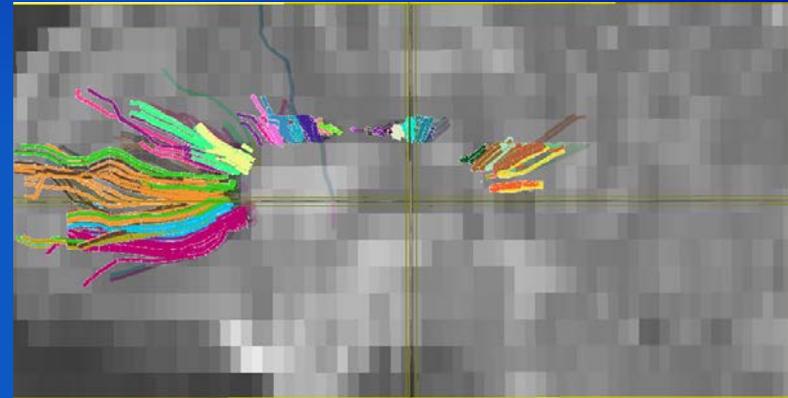
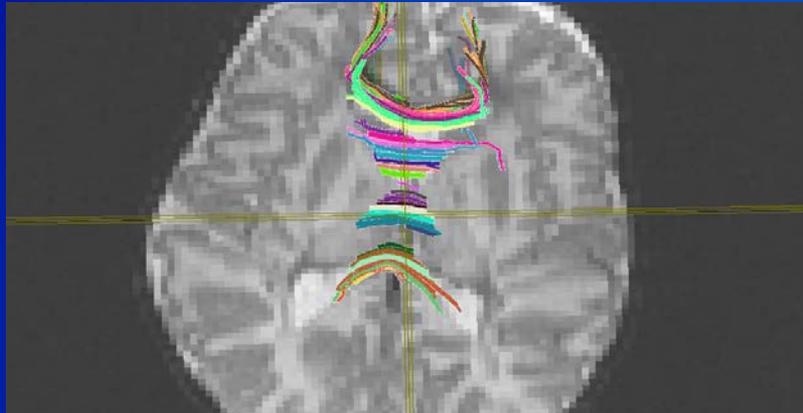
Pre-GT (18 months)

Post-GT (36 months)

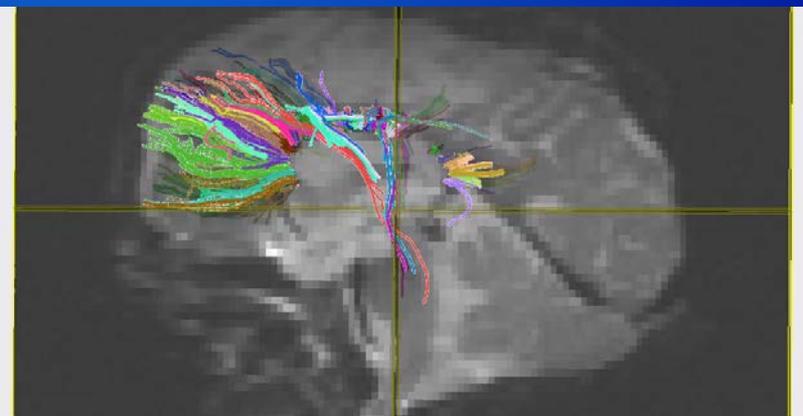
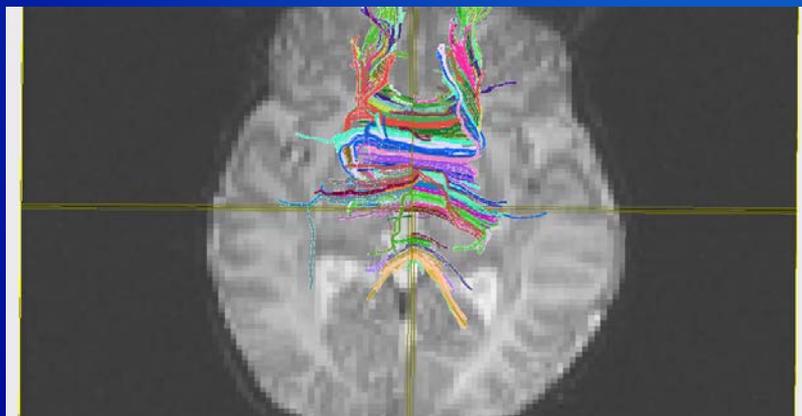


Diffusion Tensor Studies/Fractional Anisotropy

1 month Pre- Gene Therapy

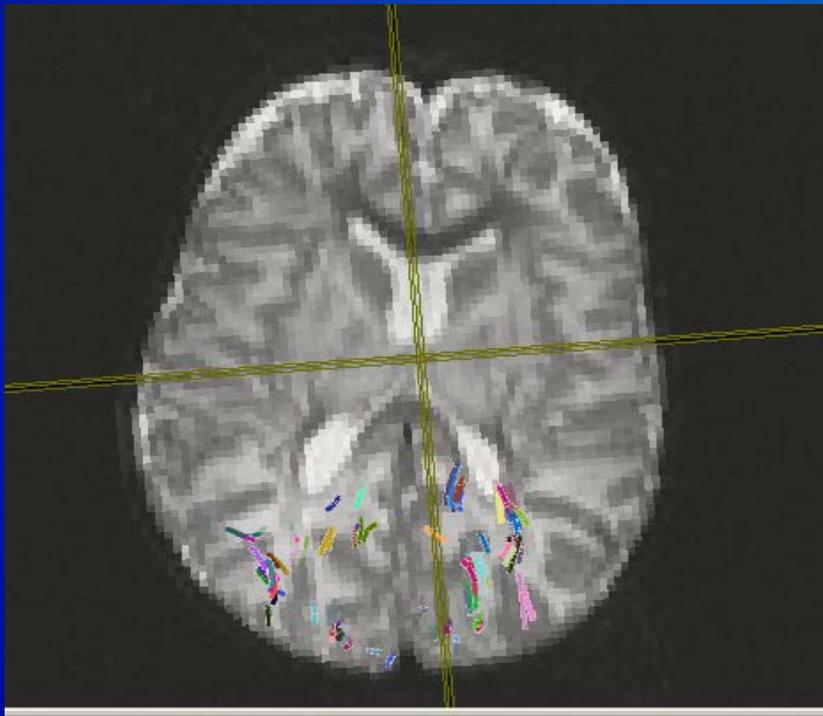


18-month Post - Gene Therapy

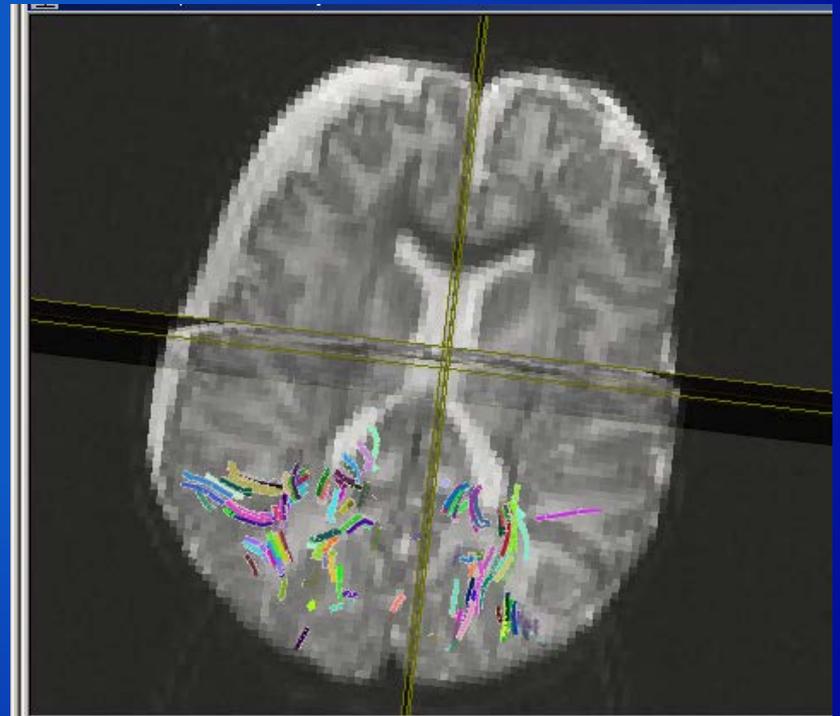


Fractional Anisotropy

Pre-GT (1 month)



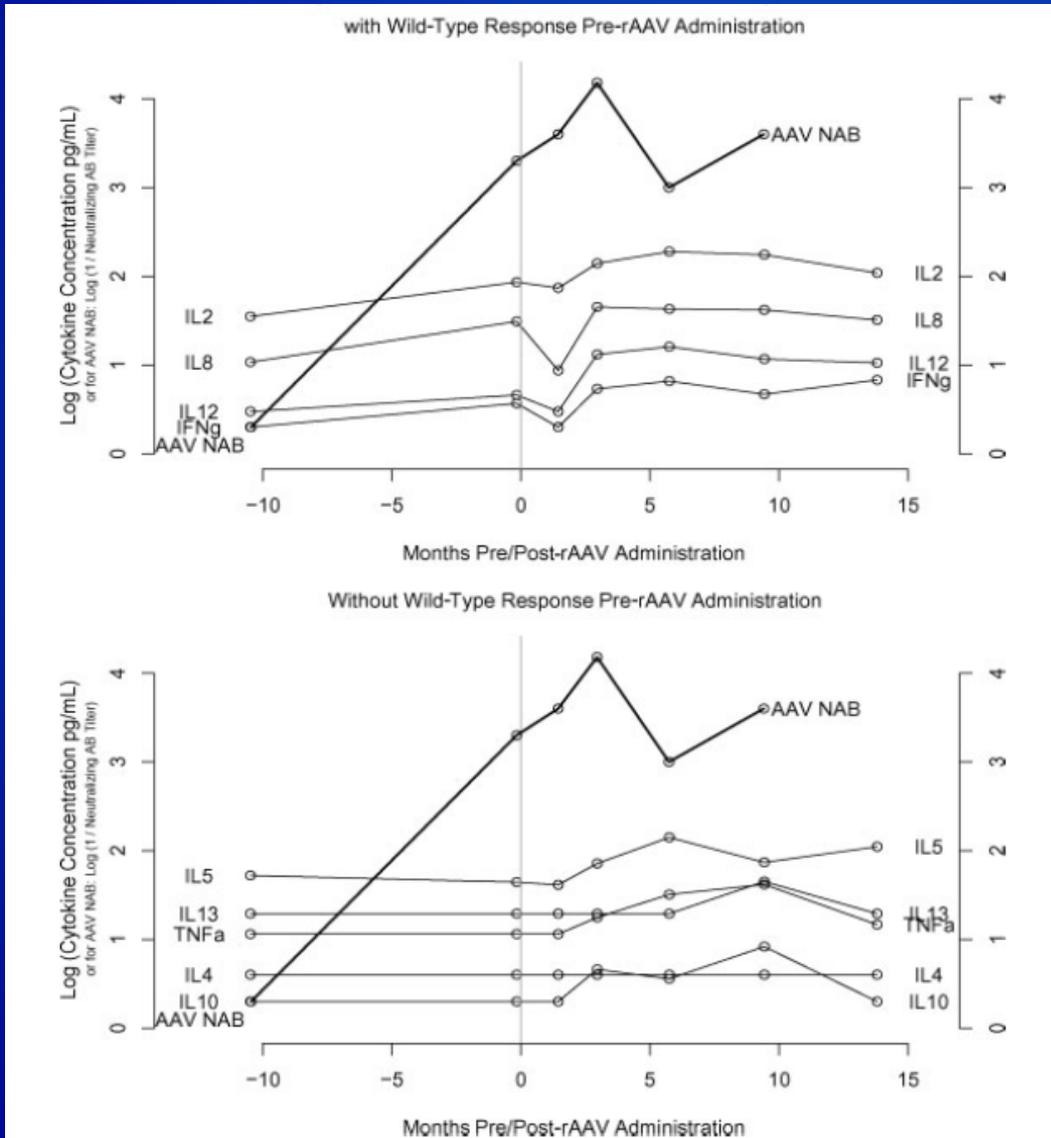
Post-GT (18 months)



AAV NAB titers pre- and post-rAAV2 administration to the CNS (cohort 1 + 2)

Subject ID	Timepoint				
	Baseline	1 months	3 months	6 months	9 months
Cohort 1					
A	1:2	1:2	1:8	1:2	1:2
B	1:1-1:2	1:40-1:400	1:10	1:8	1:4
C	1:2	1:8-1:200	1:8-1:200	NS	NS
Cohort 2					
D	1:4	NS	1:1-1:2	1:2	NS
E	1:2	NS	1:1-1:2	NS	1:2
F	1:2	1:4	1:1	NS	1:1
G	1:8	NS	1:4-1:20	1:4	1:4
H	1:2000	1:4000	1:10 000-1:20 000	1:1000	1:4000
I	1:1-1:2	NS	1:2	1:1	NS
J	1:2	NS	NS	1:2	NS

Cytokine and NAB Profiles in Pt. H



Adenovirus AB titers in sera & AAV AB Titers in CSF

Adenovirus Antibody Titers in Sera

ID	Baseline	Time-point after vector administration (months)		
		1m	3m	6m
D	<1:8	NS	NS	<1:8
E	<1:8	NS	<1:8	<1:8
H	<1:8	NS	1:32	<1:8
I	<1:8	NS	NS	<1:8

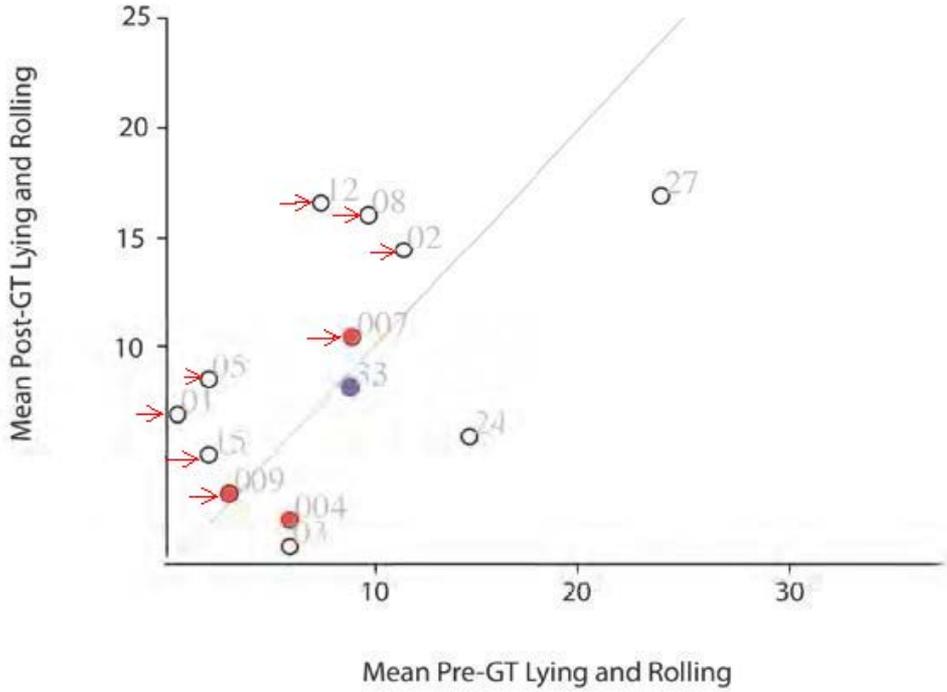
AAV Neutralizing Antibody Titers in CSF

ID	Time-point after vector administration (months)		
	1 month	3 month	6 month
A	NS	NS	1:1
B	NS	NS	1:1
C	1:2	1:4	NS

Effect of Gene Therapy on Neurodevelopment: Lying and Rolling

Fig. S3. Graph of pre-treatment vs. post-treatment GMFM.

This graph shows individual trajectories for the GMFM "lying and rolling" outcome, which was significant when the oldest subjects from the first treatment group (shown in red) were omitted. The mean magnitude of change in GMFM was variable among subjects. The youngest subject, shown in blue, had limited post-treatment values.



GMFM Normative Values (CD patients assessed 10-108 months post-GT)

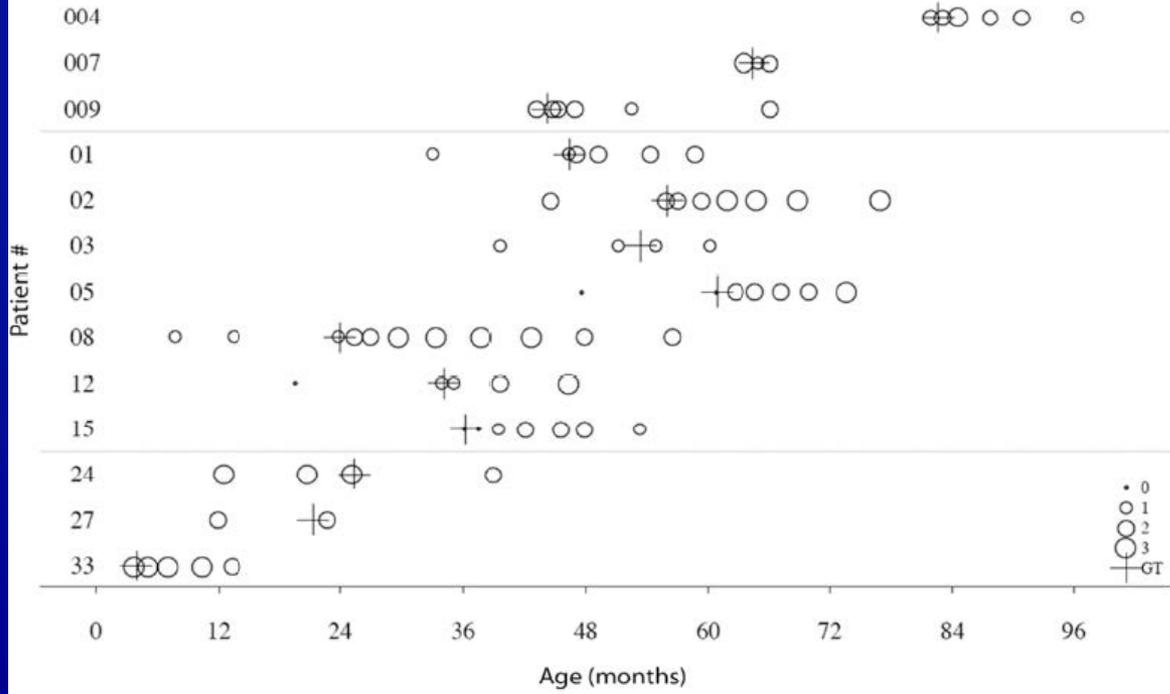
- 4-6 months - Gross-Fine Motor
- 2 months - Visual
- 9 months - Receptive Language
- 15-17mts-Expressive Language

Post-Treatment effect (18mts)
P = 0.04

Effect of Gene Therapy on Neurodevelopment: Alertness

Fig. S4. Canavan alertness scale.

This graph plots the pre- and post-treatment values for alertness scale versus patient age and time from gene therapy. Alertness was part of the Canavan clinical exam which was performed by the study neurologist.

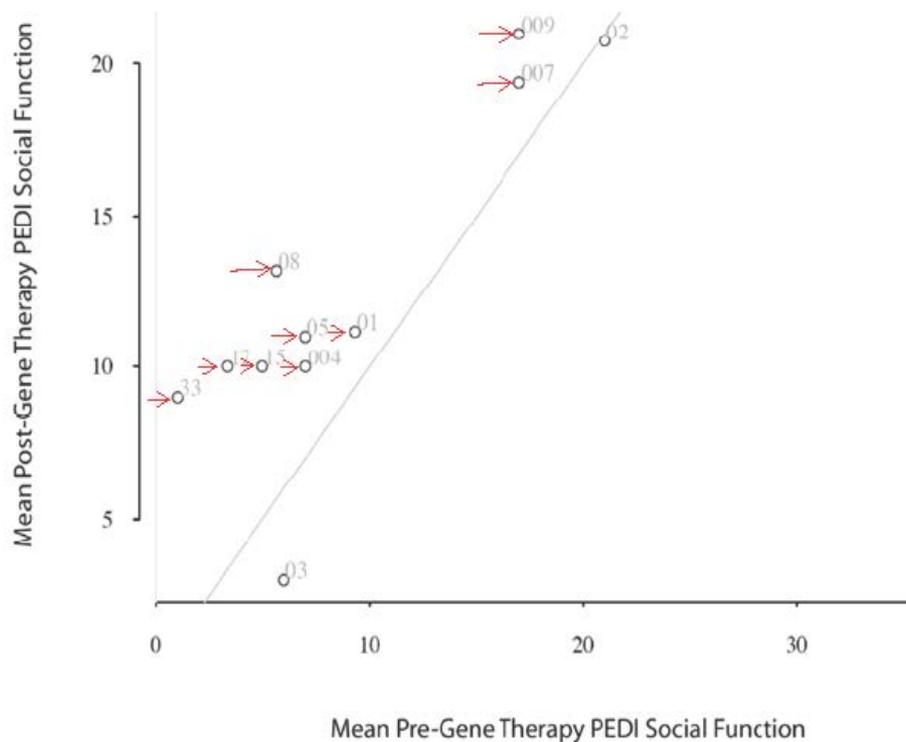


**Post-Treatment effect (60 mts)
P = 0.008 (- 1st Cohort)**

Effect of Gene Therapy on Neurodevelopment: Social Function

Fig. S6. PEDI social function, mean raw values plotted pre- vs. post-treatment.

Mean values on a per-patient basis for PEDI subscores through the 18 month time point, showing improvement or stable values in virtually all subjects.

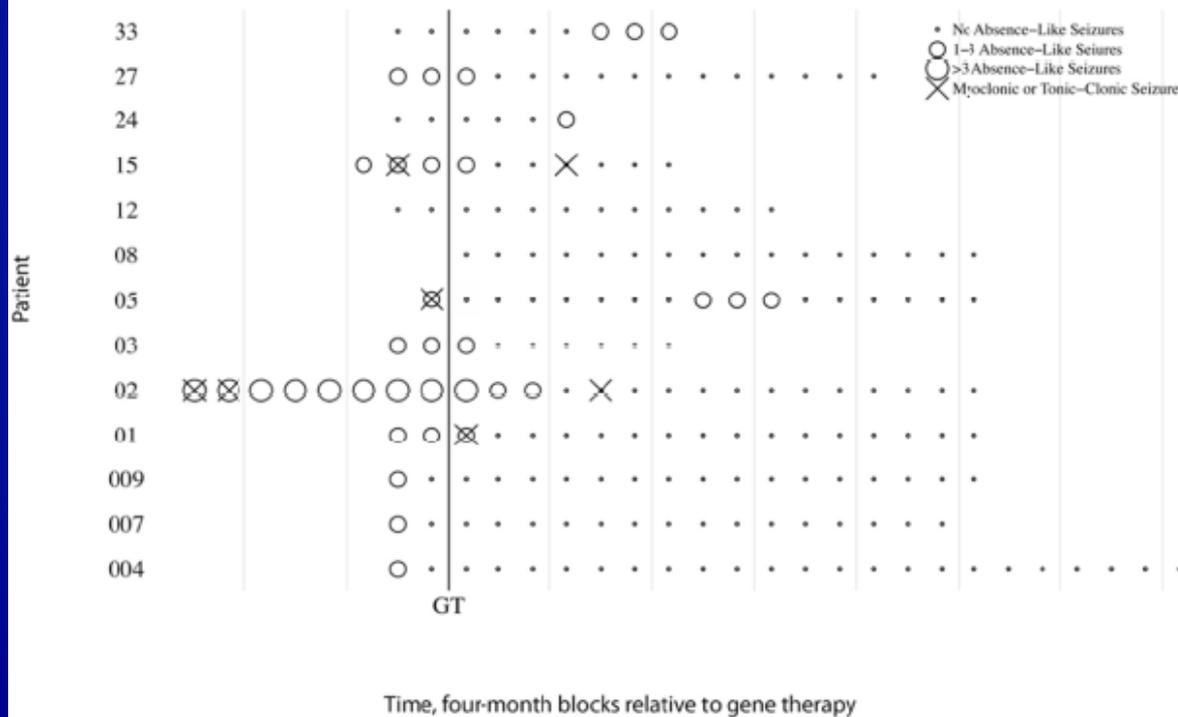


**Post-Treatment effect (18 mts)
P = 0.04**

Effect of Gene Therapy on Neurodevelopment: Seizure

Fig. S8. Seizure frequency.

Seizure frequency shown pre- and post-gene therapy, for various seizure types on a per-subject basis.



Post-Treatment effect (60 mts)
P = 0.001

Survival



Pt #118-33 – Youngest 3.7 months at GT



Pt #118-33 – Oldest 6.9 years at GT

Long-Term Follow-Up following AAV2 mediated Gene Therapy in the CNS

- 1) Intraparenchymal delivery of recombinant AAV2-ASPA was **well tolerated**
- 2) Intraparenchymal delivery of recombinant AAV2-ASPA **resulted in, a decrease in elevated NAA, in clinical stabilization and slowed progression of brain atrophy**
- 3) The **low levels of immune response** detected in this study, at this dose and with intraparenchymal administration, suggested that recombinant AAV2 is safe
- 4) **Early intervention** would be recommended to maximize benefit

Acknowledgments

RESEARCH ARTICLE

GENE THERAPY

Long-Term Follow-Up After Gene Therapy for Canavan Disease

Paola Leone,^{1*} David Shera,² Scott W.J. McPhee,³ Jeremy S. Francis,¹ Edwin H. Kolodny,⁴ Larissa T. Bilaniuk,⁵ Dah-Jyuu Wang,⁵ Mitra Assadi,⁶ Olga Goldfarb,⁶ H. Warren Goldman,⁶ Andrew Freese,⁷ Deborah Young,⁸ Matthew J. During,^{8,9} R. Jude Samulski,^{3,10} Christopher G. Janson^{7,11}

Sci Transl Med. 2012 Dec 19;4(165):



NINDS (RO1-NS42120)

Program Directors
Giovanna Spinella, M.D.
Dan Tagle, Ph.D.

