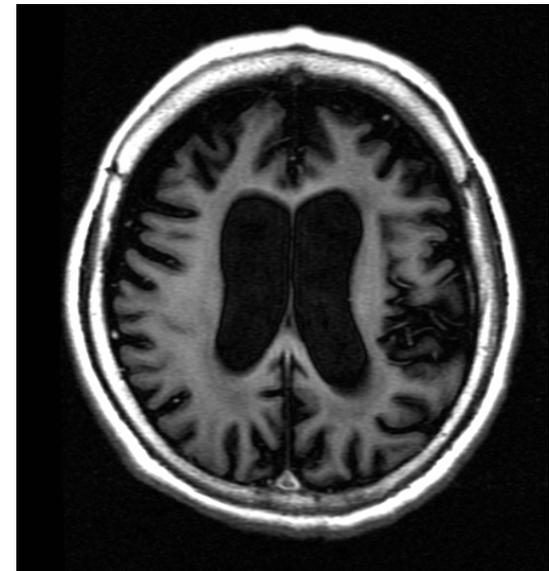


**Direct CNS Administration of a Replication  
Deficient Adeno-associated Virus Gene  
Transfer Serotype rh.10 Expressing the Human  
CLN2 cDNA to Children with Late Infantile  
Neuronal Ceroid Lipofuscinosis  
NIH-OBA # 0904-977**

R. Crystal  
Department of Genetic Medicine  
Weill Cornell Medical College  
6-18-09

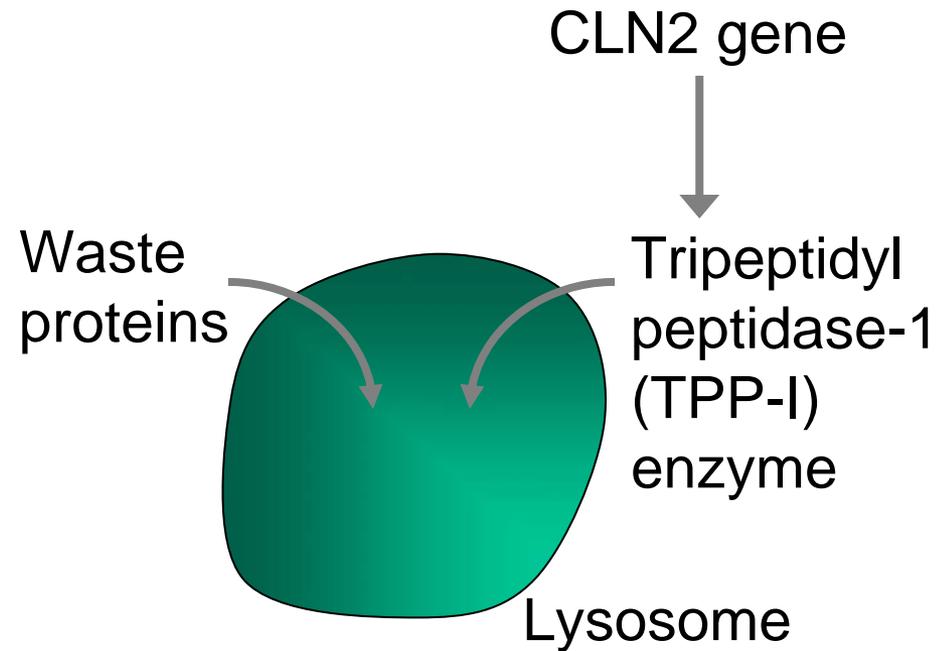
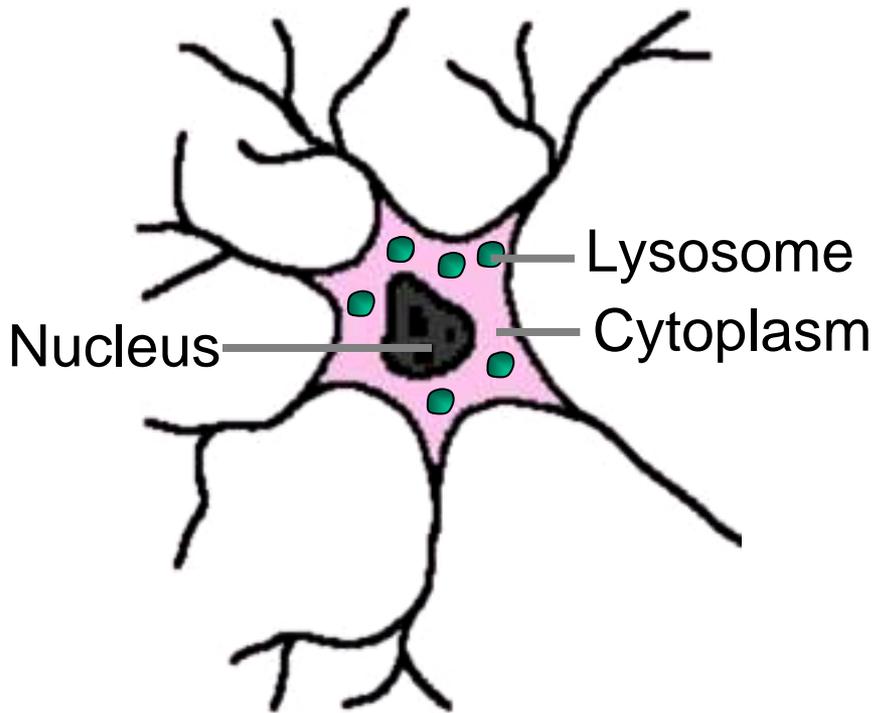
# Late Infantile Neuronal Ceroid Lipofuscinoses (LINCL, Batten Disease)

- Autosomal recessive, 1 / 2 million births, ~ 200 cases worldwide
- Disease onset ages 2-4
- Cognitive impairment, visual failure, seizures, and deteriorating motor development, leading to a vegetative state and death by ages 8-12

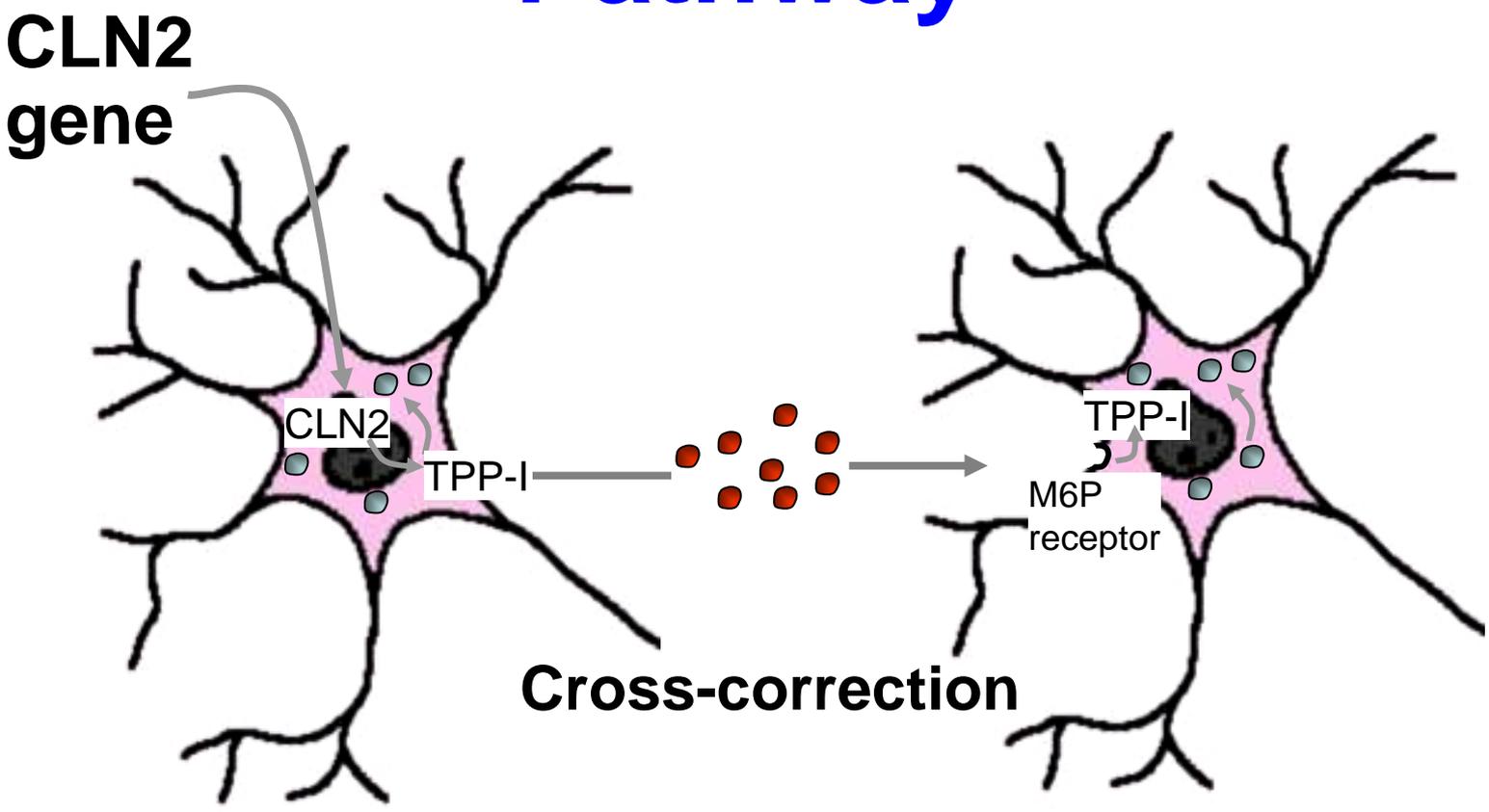


# LINCL Is Caused by Mutations in the CLN2 Gene

## Neuron



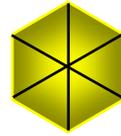
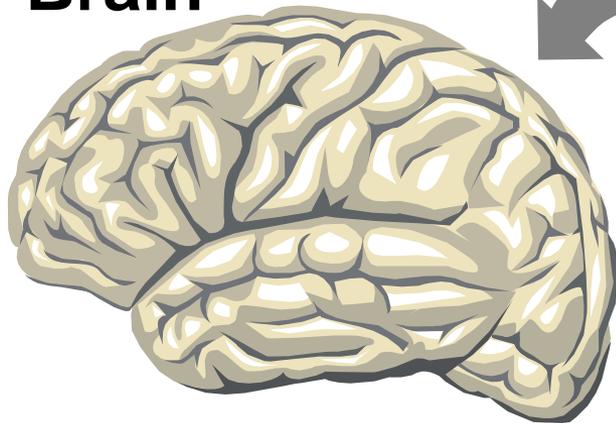
# Cross-correction of CNS Cells via the Mannose-6-phosphate Pathway



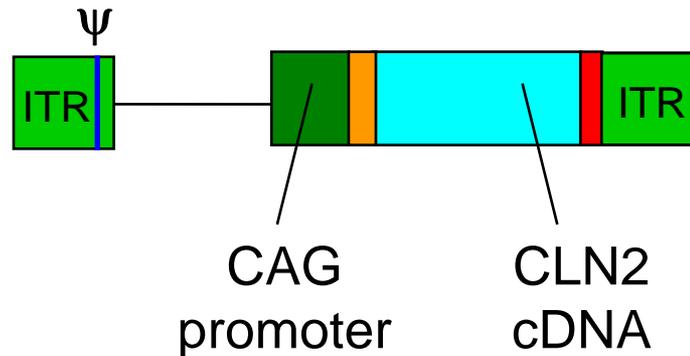
# CNS Gene Therapy for LINCL

Adenoassociated  
virus serotype 2

Brain

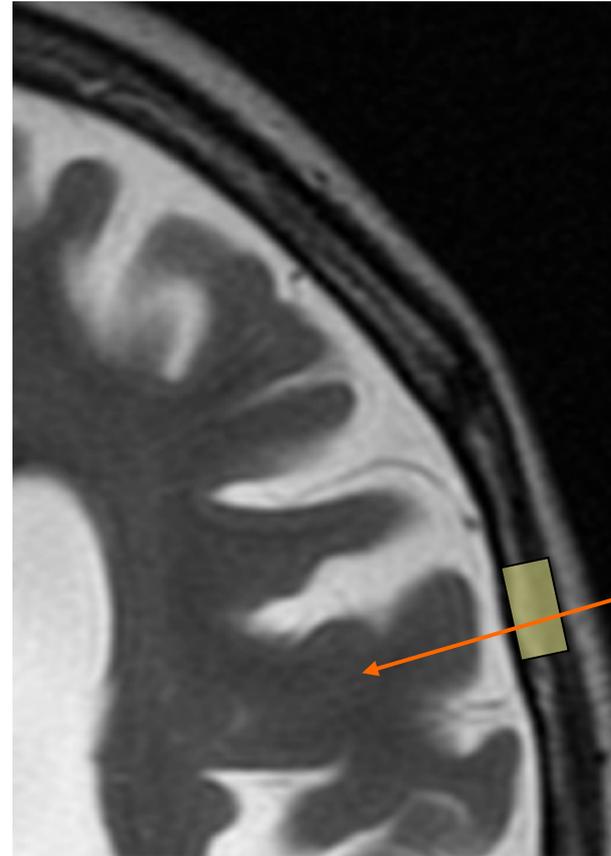
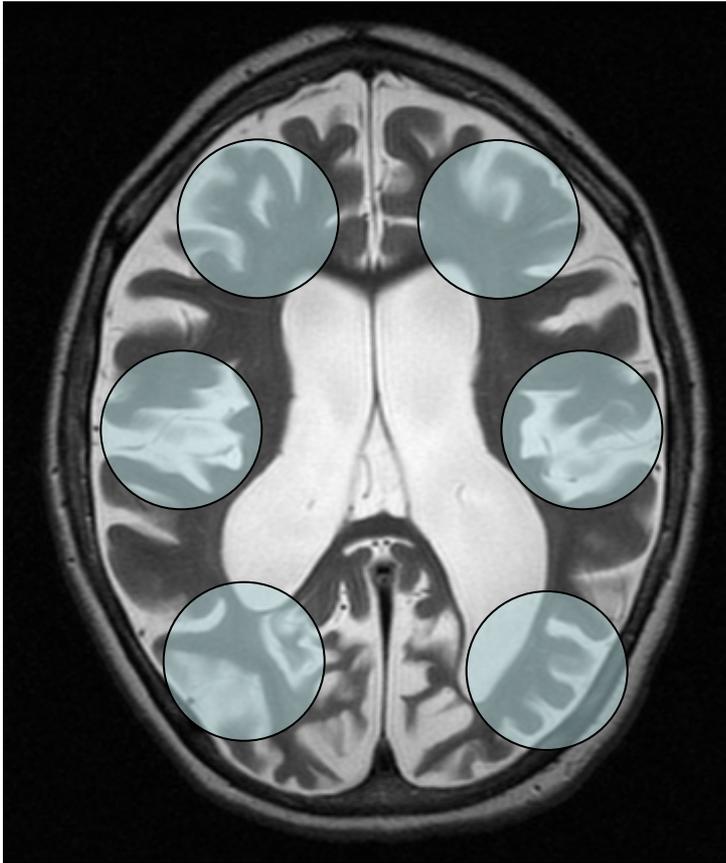


4.5 kb genome



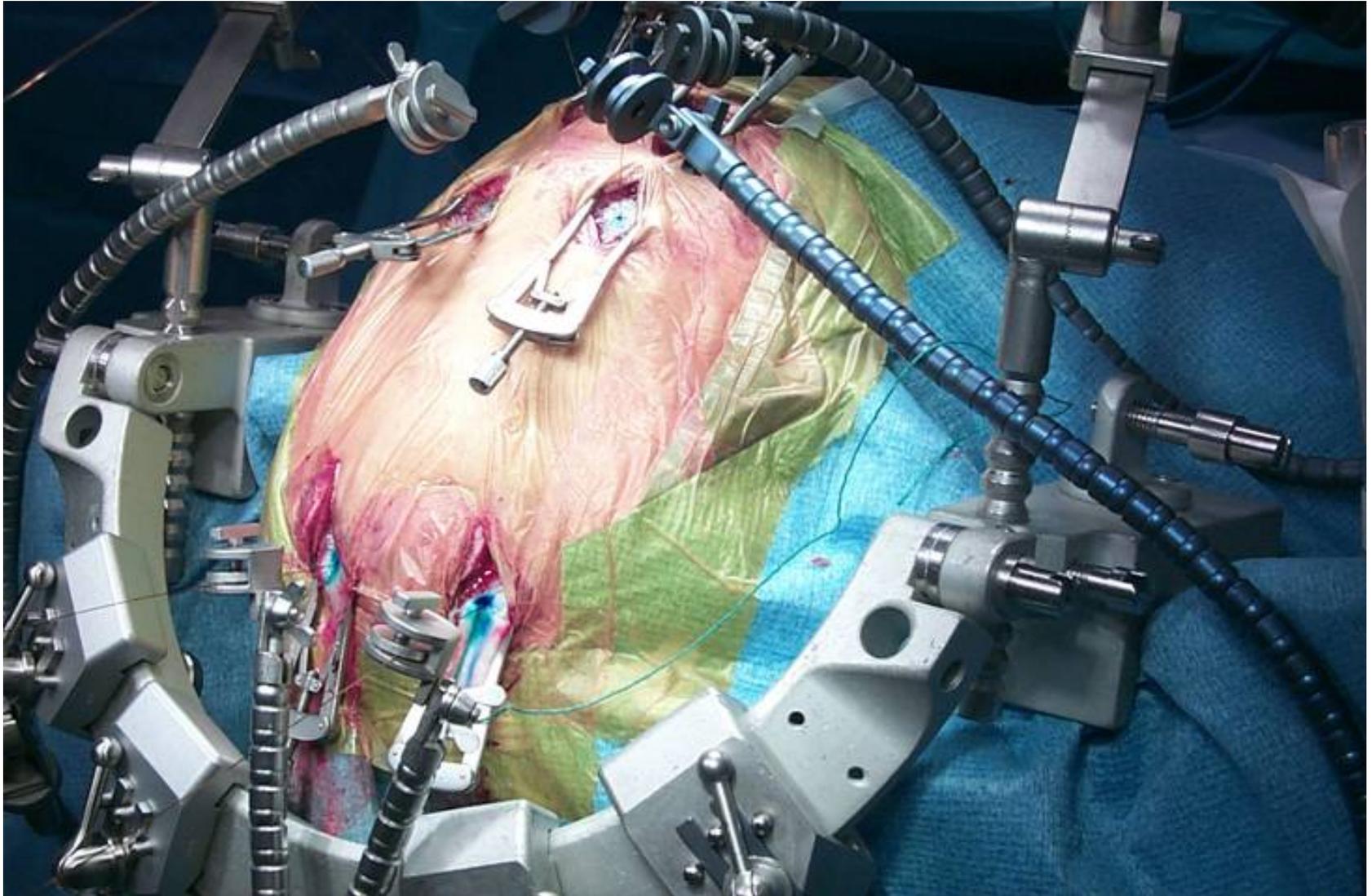
- CAG promoter – human cytomegalovirus immediate/early enhancer, splice donor and left hand intron from chicken  $\beta$ -actin, splice acceptor from rabbit  $\beta$ -globin

# Targets for Therapy



- Dose  $1.7 \times 10^{11}$  gc/site;  $2 \times 10^{12}$  gc total dose
- 6 burr holes, 2 injections (different levels) per burr hole,  $150 \mu\text{l}$  flexible glass catheters,  $2 \mu\text{l}/\text{min}$ ,  $150 \mu\text{l}$  at each level, 3 hr total infusion time

# Vector Administration

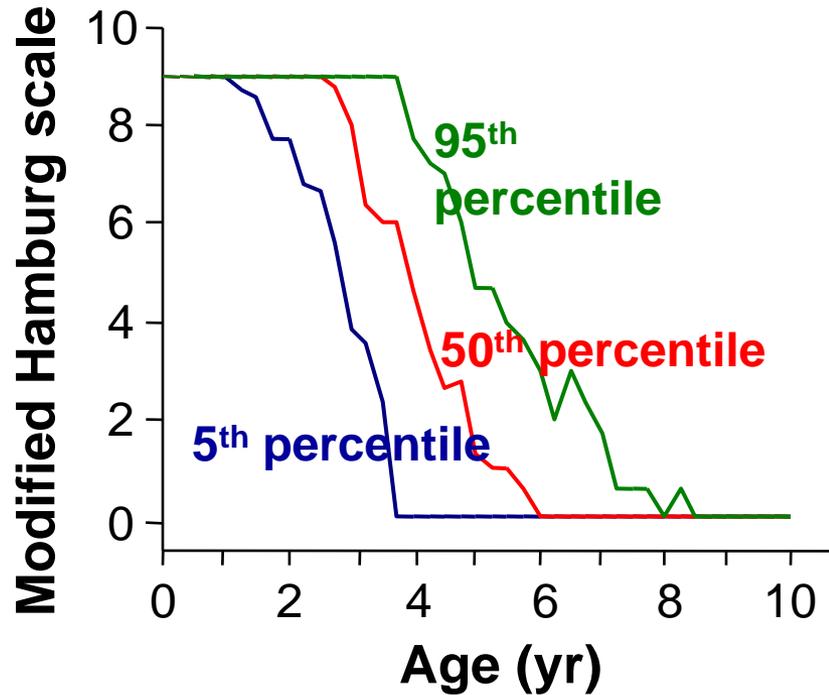


# Study Design

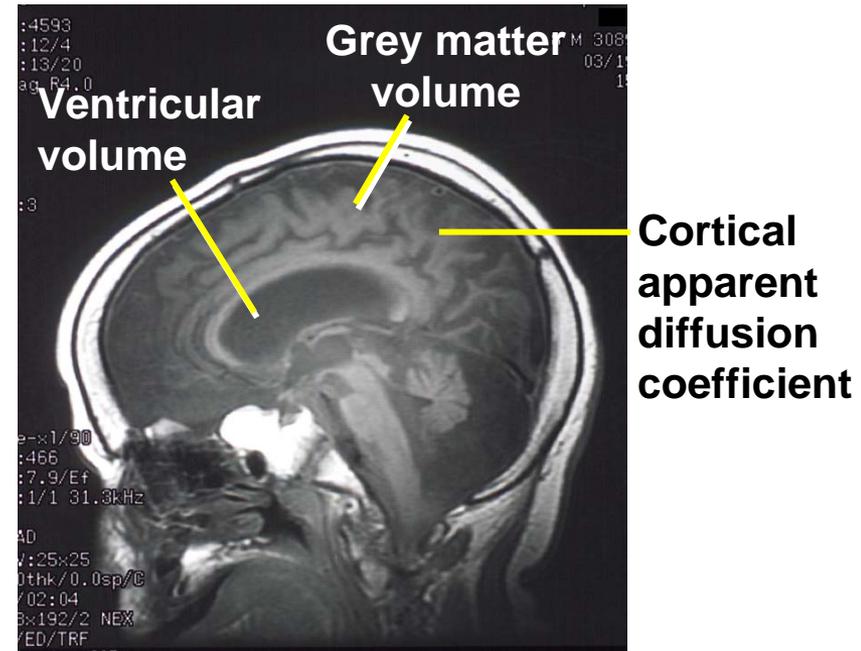
- n=10 children with LINCL
- 6 male, 4 female, ages 3-9 yr
- 5 severe, 5 moderate
- Assessment pre-therapy, post-therapy 1, 6, 12, 18 months

# 1° and 2° Outcome Variables

## 1° - Modified Hamburg clinical rating scale\*



## 2° - Quantitative MRI



\* Motor function, seizures, language

# Safety

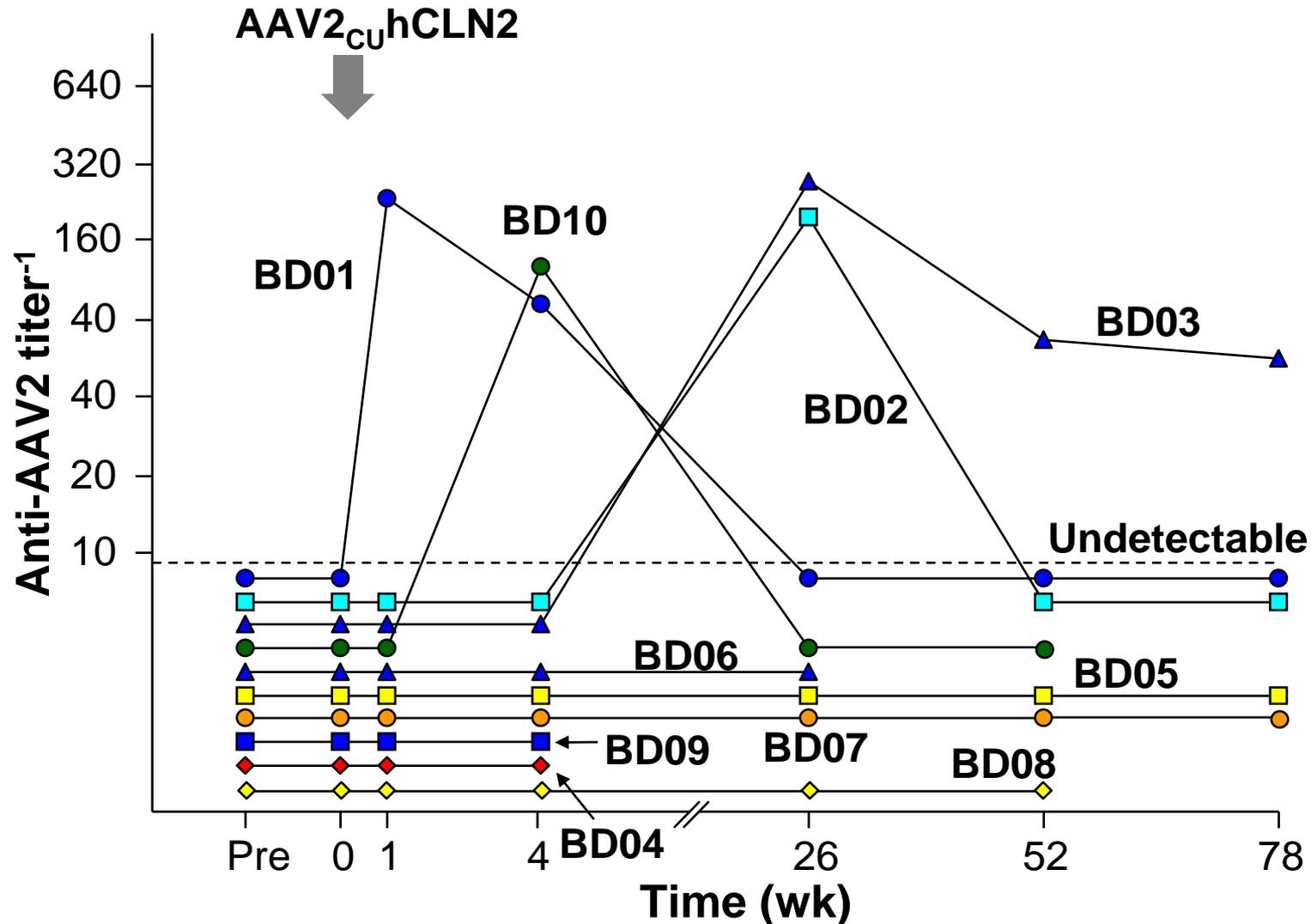
## Within the 18 month study period

- No unexpected serious adverse events
- 1 death 49 days post-rx, uncontrollable seizures

## Post-study follow-up

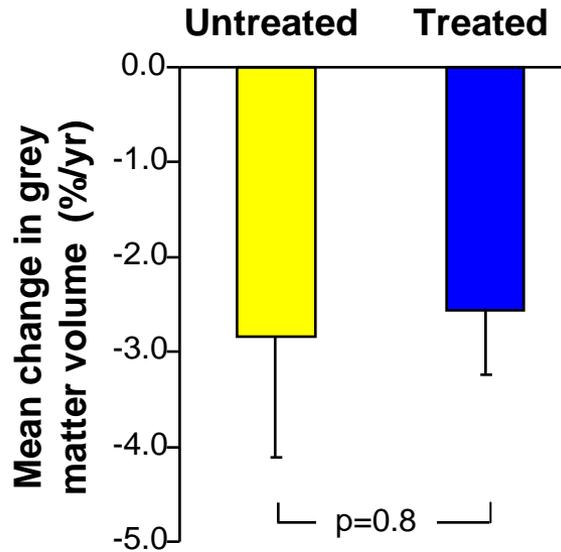
- 1 additional death 23 months post-therapy
- As of 6-15-09, of the 8/10 surviving children, average survival post-therapy  $50 \pm 9$  months (range 39 - 60 months)

# Anti-vector Neutralizing Antibodies Following CNS Administration of AAV2<sub>CU</sub>hCLN2

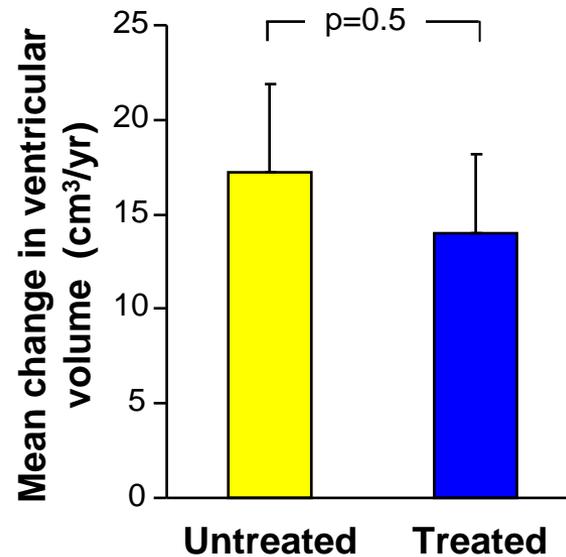


# 2<sup>o</sup> Variables – Quantitative MRI

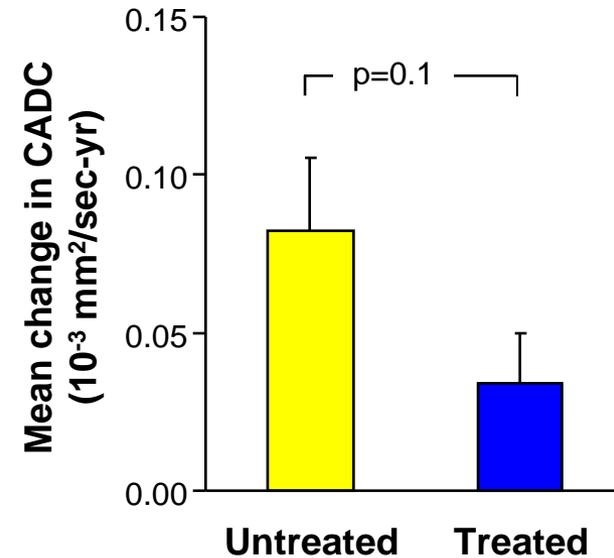
A.



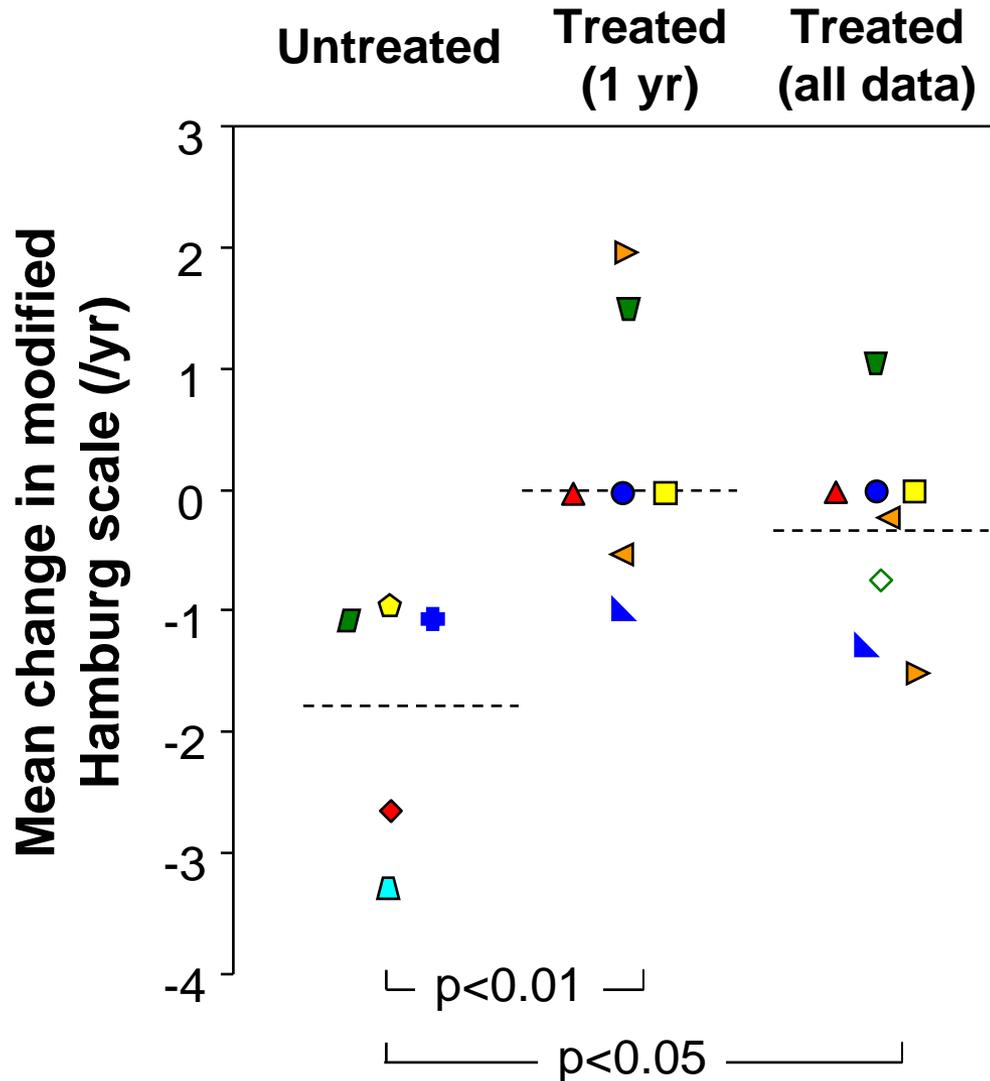
B.



C.



# 1<sup>0</sup> Variable – Modified Hamburg LINCL Scale



# Summary of AAV2<sub>CU</sub>hCLN2 Gene Therapy Clinical Trial for LINCL

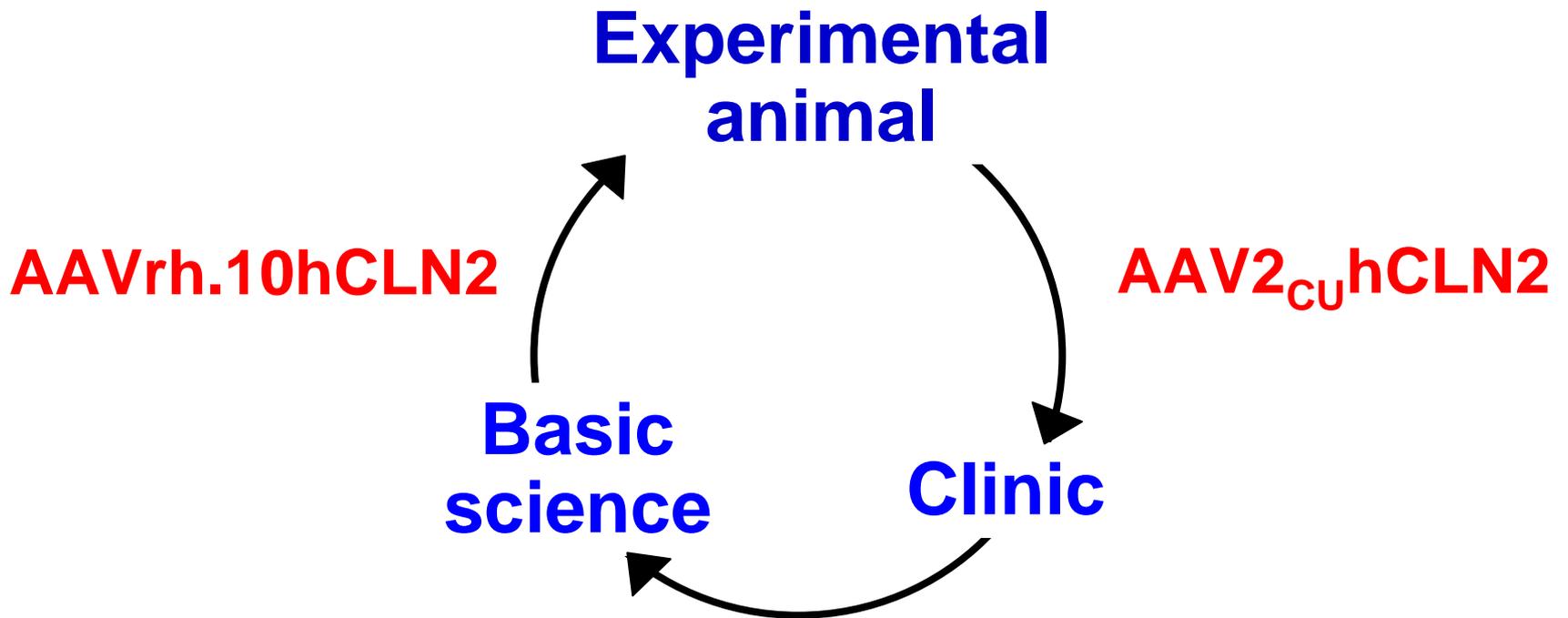
## Safety

- Acceptable for a universally fatal disorder for which there is no therapy

## Efficacy

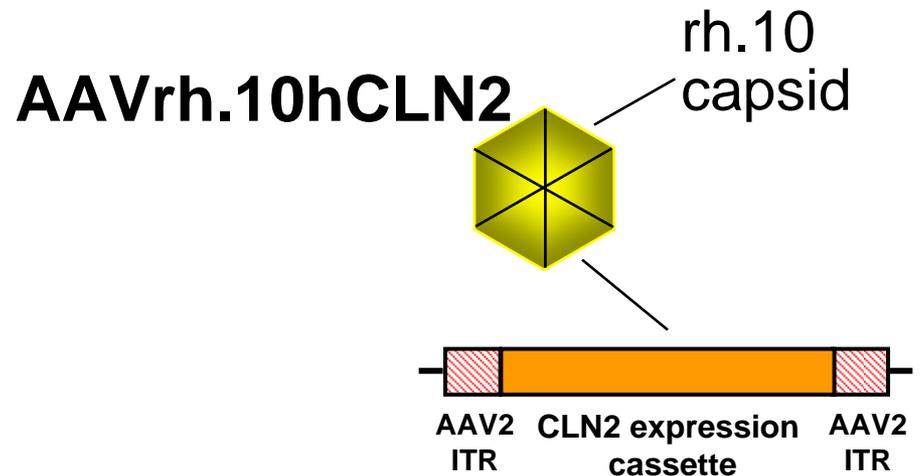
- Although the trial is not matched, randomized, or blinded, and lacked a contemporaneous placebo/sham control group, assessment of the primary outcome variable suggests a slowing of progression of LINCL in the treated children

# How Do We Achieve Success with Genetic Medicines?



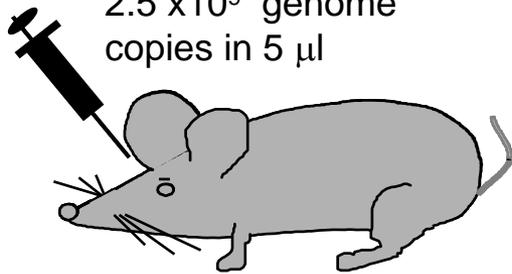
# AAVrh.10hCLN2

- Clade E AAV, derived from Rhesus macaque
- Mediates high levels of protein expression in multiple organs
- AAV2 ITRs with expression cassette, pseudotyped with rh.10 capsid



# AAVrh.10hCLN2-mediated TPP-I Expression in the Rat Brain

- AAV2hCLN2
  - AAV5hCLN2
  - AAV8hCLN2
  - AAVrh.10hCLN2
  - Naive controls
- 2.5 x 10<sup>9</sup> genome copies in 5  $\mu$ l



n = 3/group

Fischer 344 rats

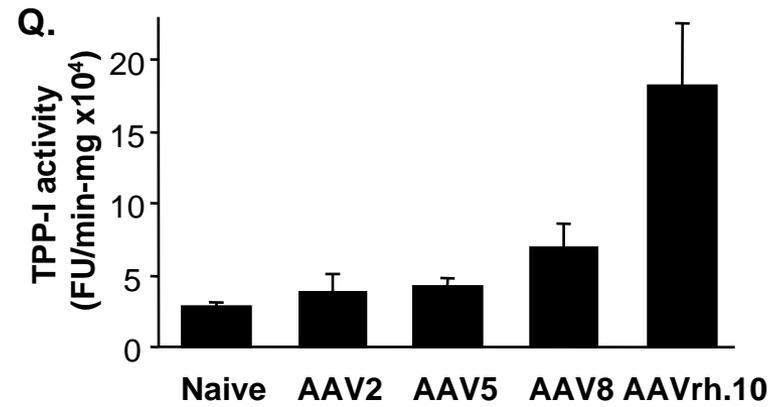
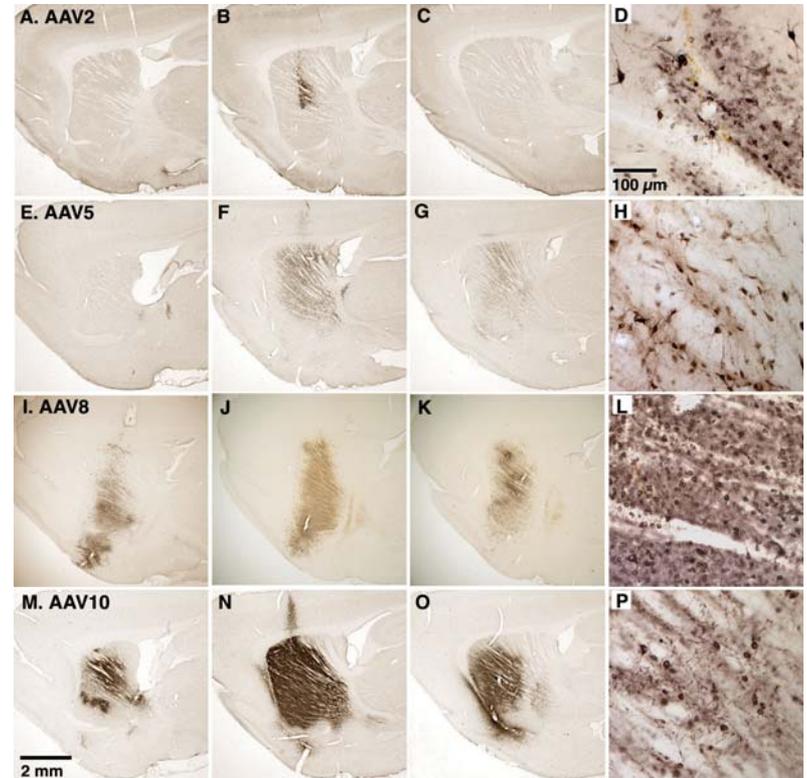
4 wk

2 mm

coronal sections

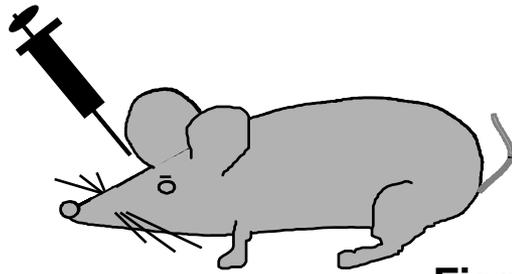
## Evaluate

- TPP-I distribution by immunohistochemistry
- TPP-1 activity



# TPP-I Accumulation in Neurons Following AAVrh.10hCLN2 Gene Transfer to Striatum

- AAVrh.10hCLN2  
2.5 x10<sup>9</sup> genome  
copies in 5  $\mu$ l



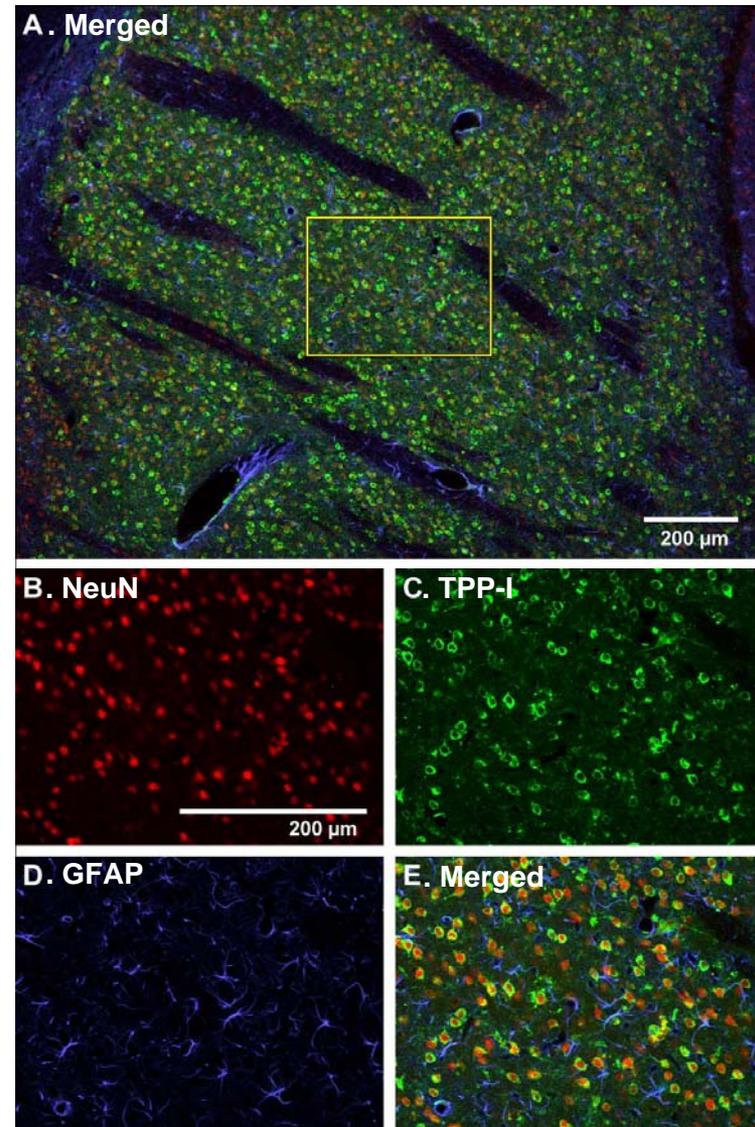
n = 3

Fischer 344 rats

4 wk

## Evaluate

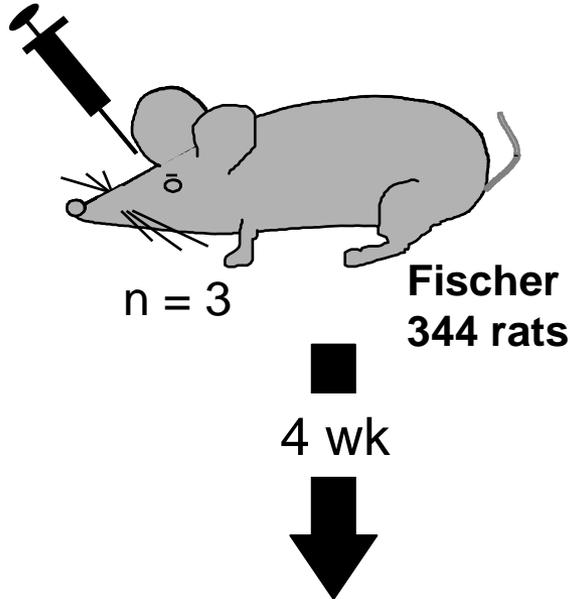
- TPP-I expression by  
immunofluorescence



NeuN – neuron specific marker, GFAP – glia specific marker

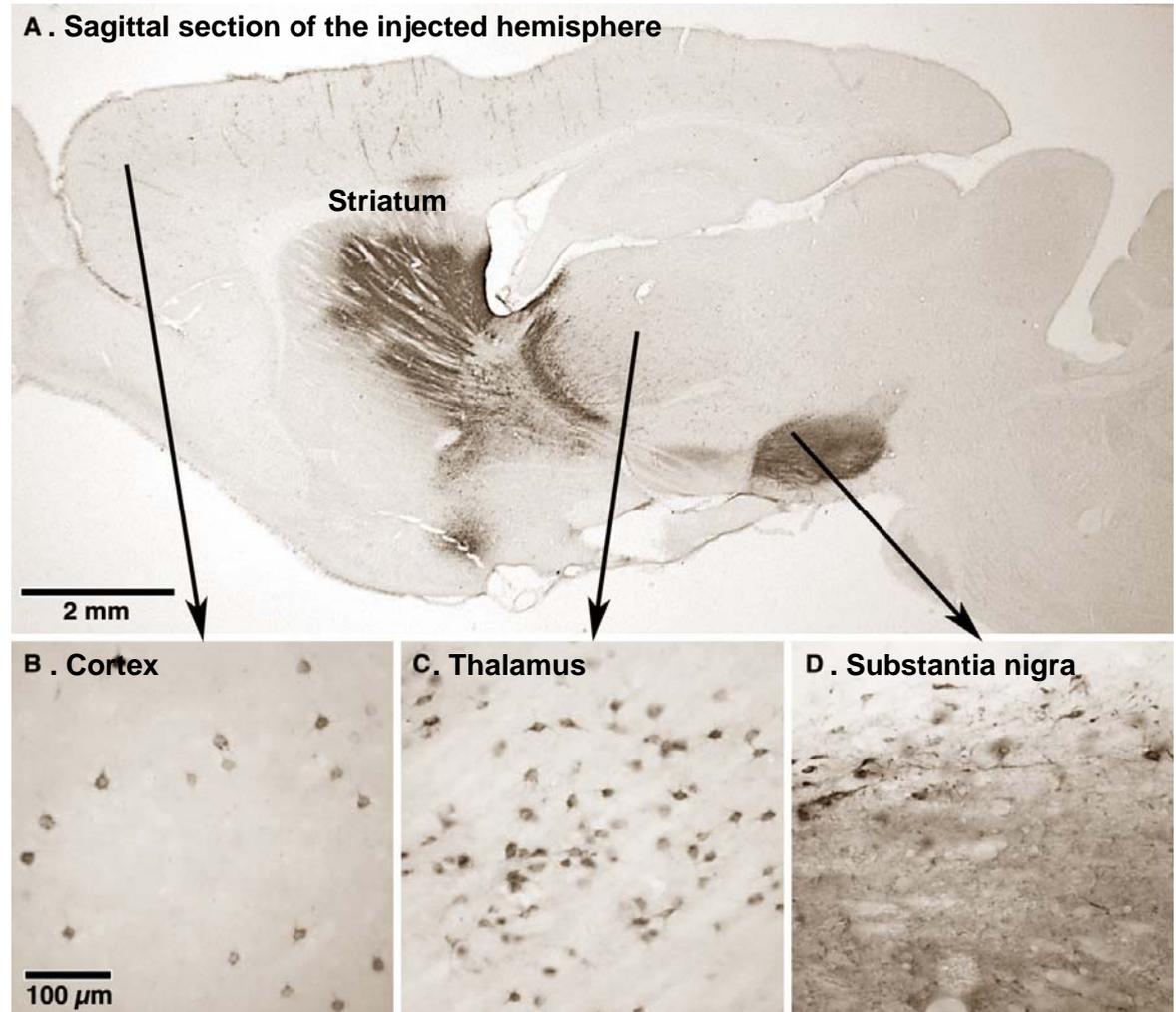
# Detection of TPP-I Outside the Striatum Resulting from AAVrh.10hCLN2 Gene Transfer to Striatum

- AAVrh.10hCLN2  
2.5 x10<sup>9</sup> genome  
copies in 5  $\mu$ l



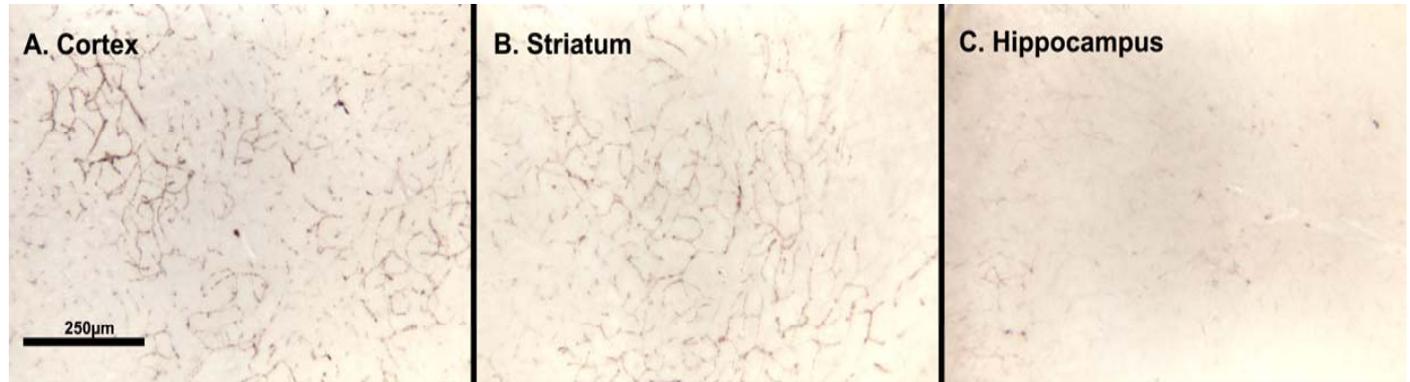
## Evaluate

- TPP-I expression by  
immunohistochemistry

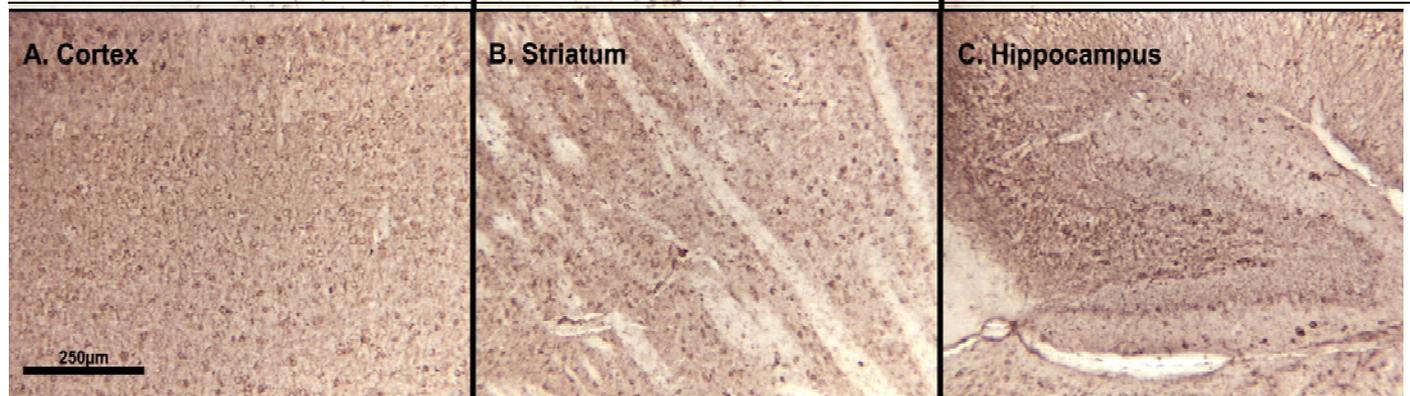


# CNS TPP-I Expression Following AAVrh.10hCLN2 Gene Transfer to Day 2 CLN2 -/- Mice and Assessment at 1 yr

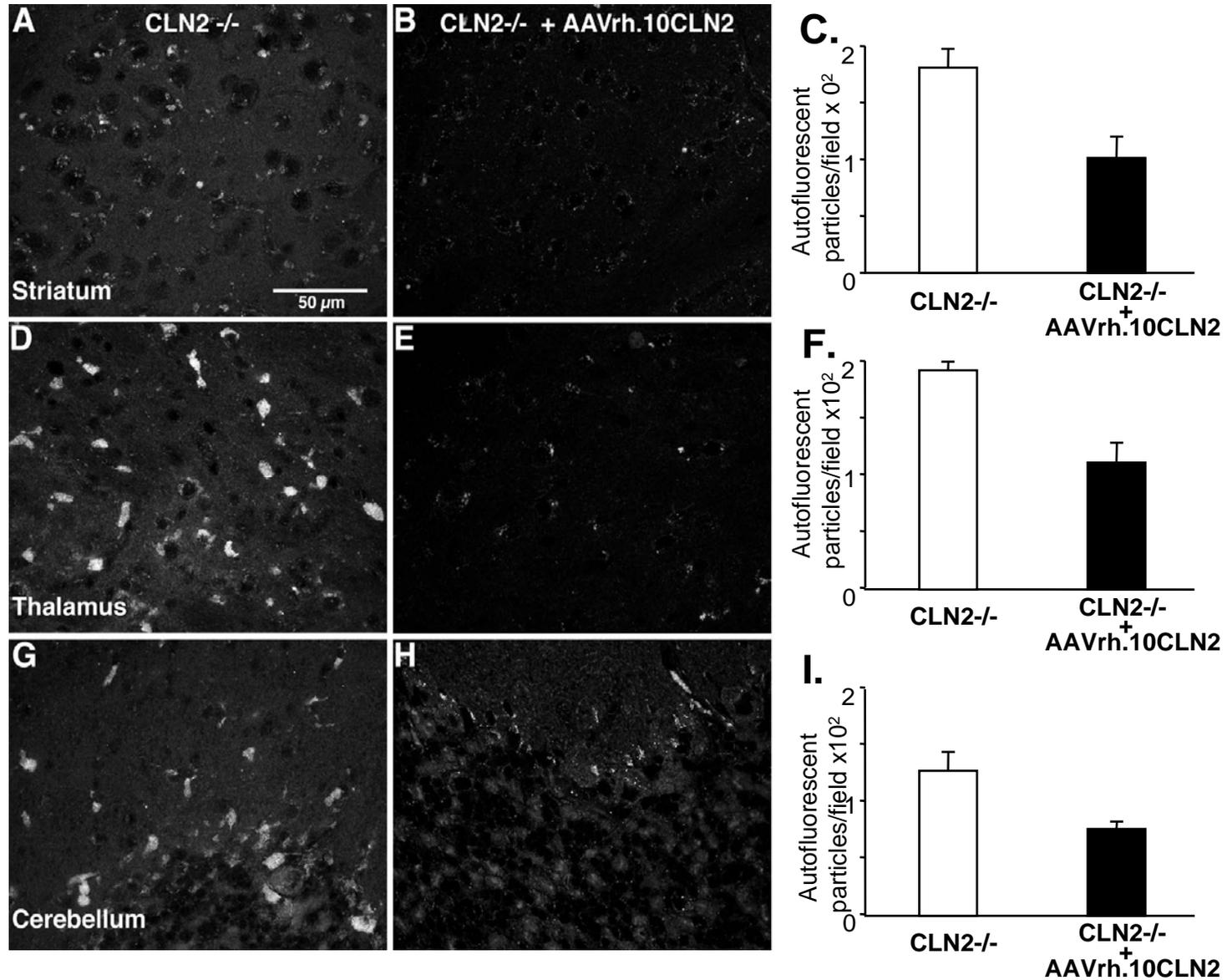
PBS control



AAVrh.10hCLN2



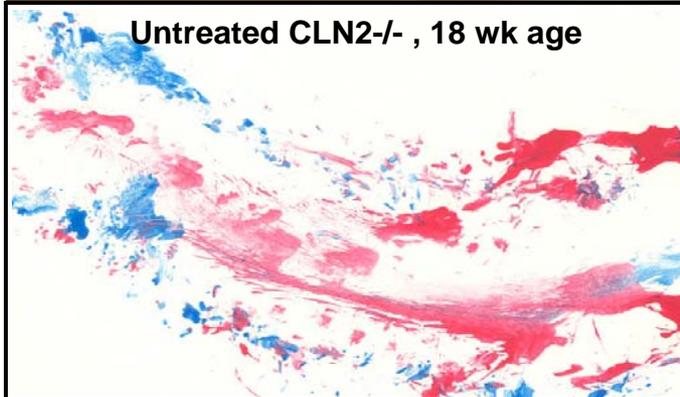
# AAVrh.10CLN2-mediated Reduction in CNS Storage Granules



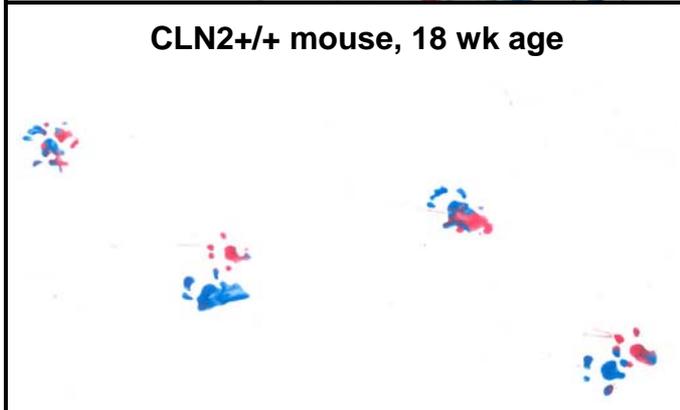
# Gait Analysis of CLN2<sup>-/-</sup> Mice Treated AAVrh.10hCLN2



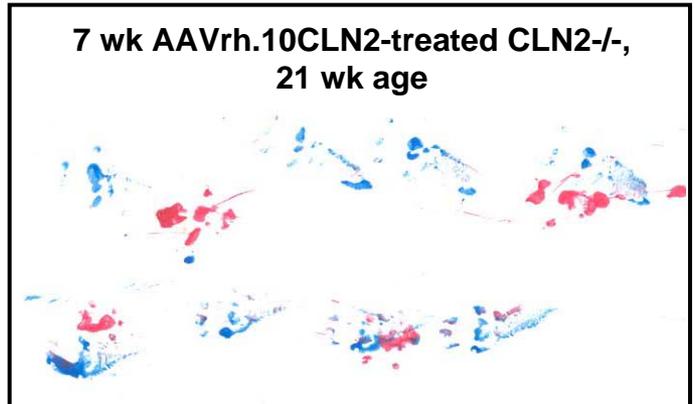
Untreated CLN2<sup>-/-</sup> , 18 wk age



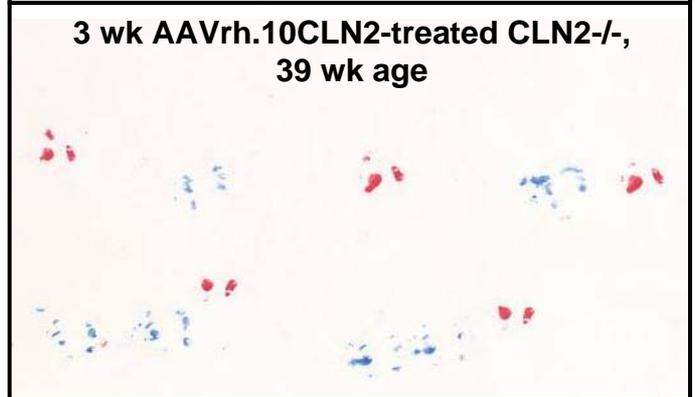
CLN2<sup>+/+</sup> mouse, 18 wk age



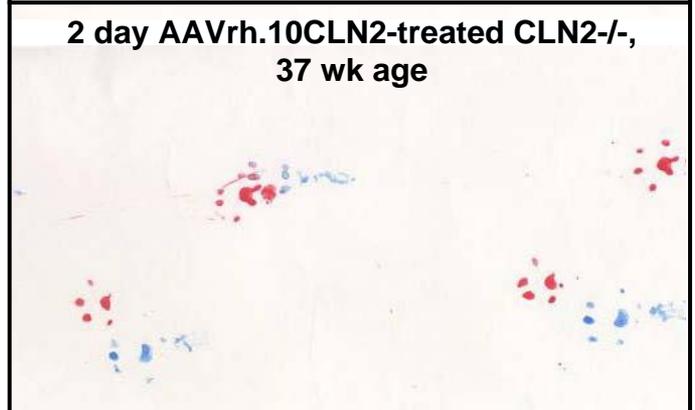
7 wk AAVrh.10CLN2-treated CLN2<sup>-/-</sup>,  
21 wk age



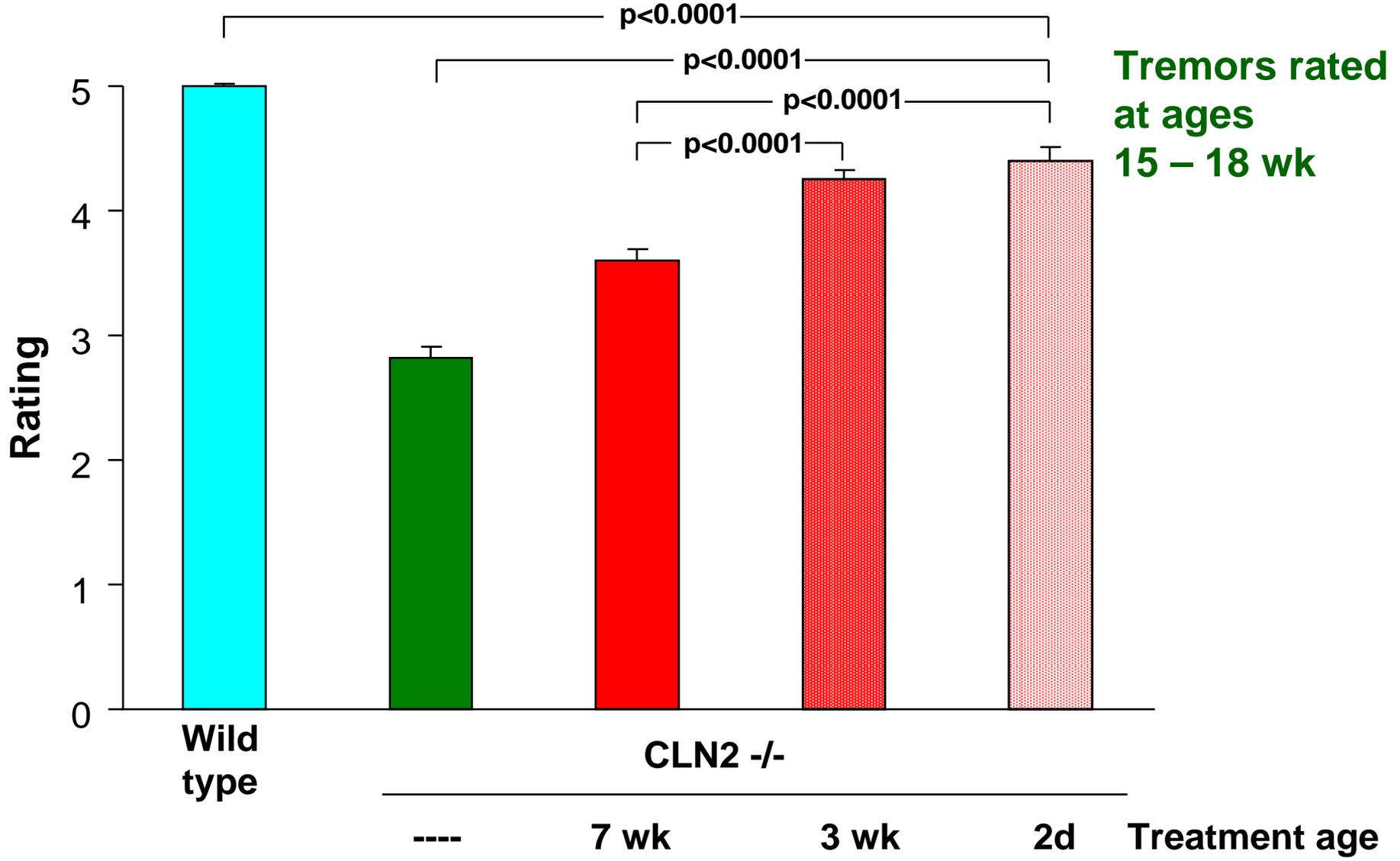
3 wk AAVrh.10CLN2-treated CLN2<sup>-/-</sup>,  
39 wk age



2 day AAVrh.10CLN2-treated CLN2<sup>-/-</sup>,  
37 wk age

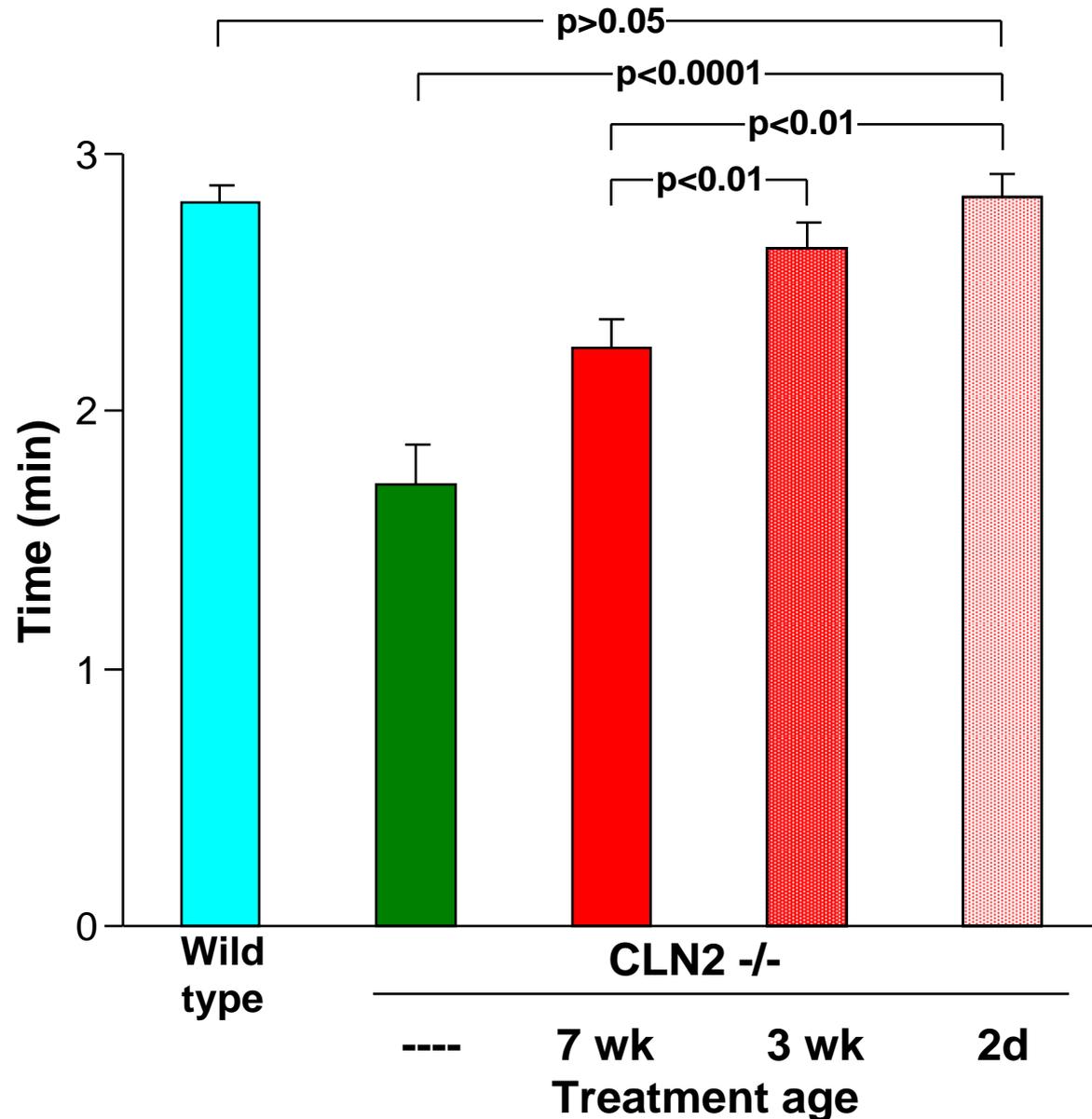


# Impact of AAVrh.10hCLN2 Treatment on Tremors



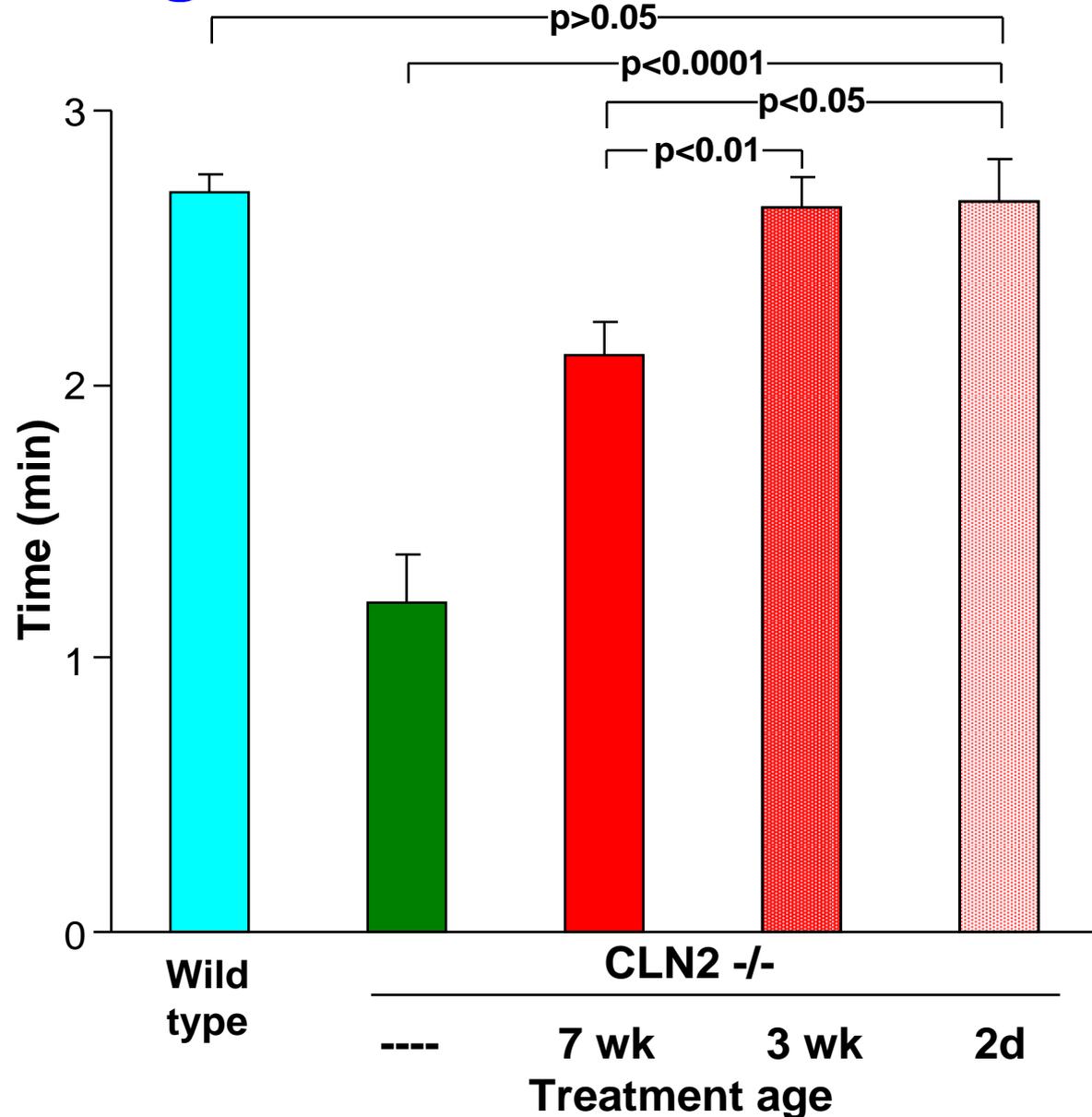
# Impact of AAVrh.10hCLN2 Treatment on Balance Beam Performance

Balance beam assessed at age 15 to 18 wk

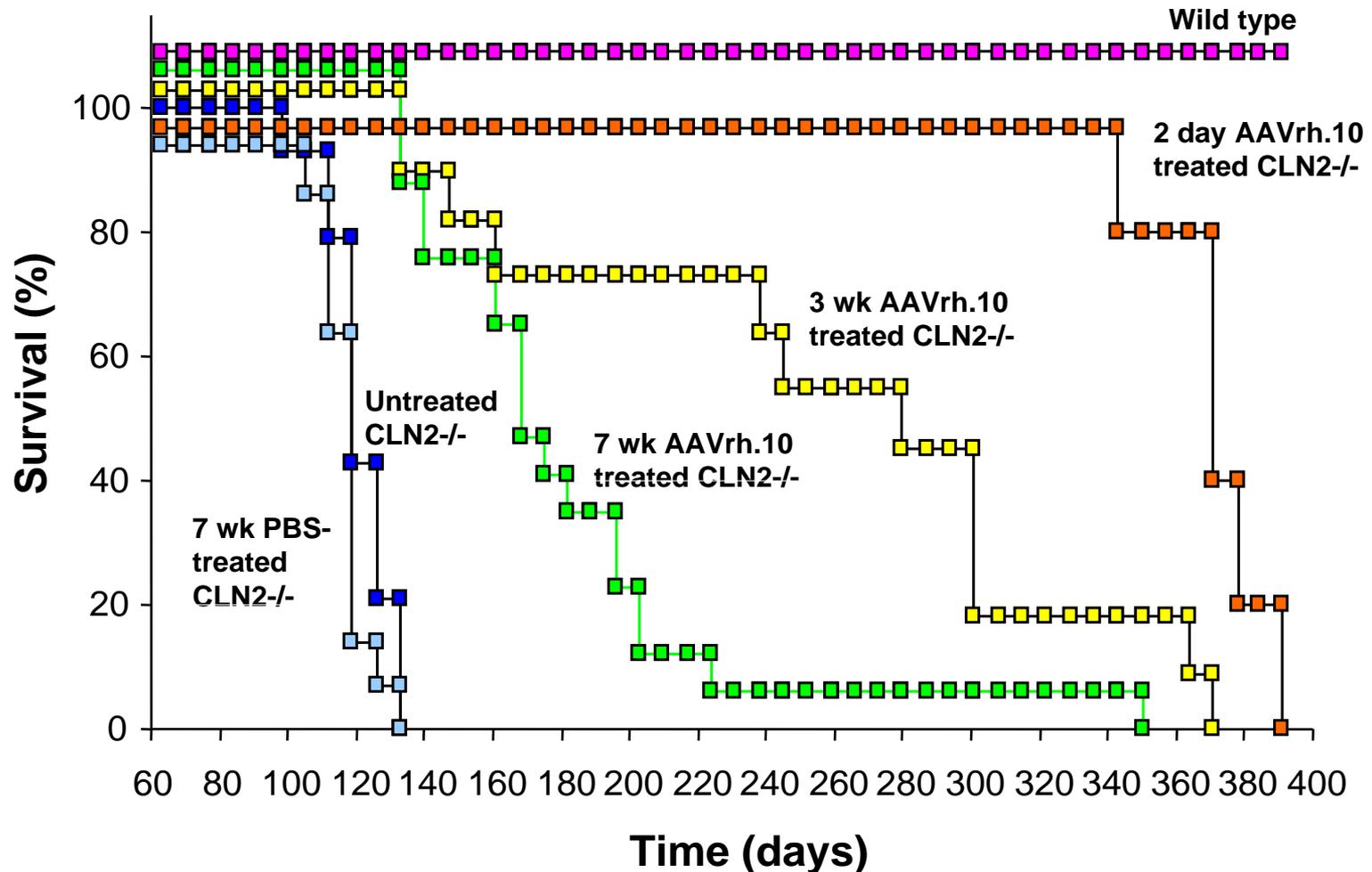


# Impact of AAVrh.10hCLN2 Treatment on Grip Strength Performance

Grip strength assessed at age 15 to 18 wk

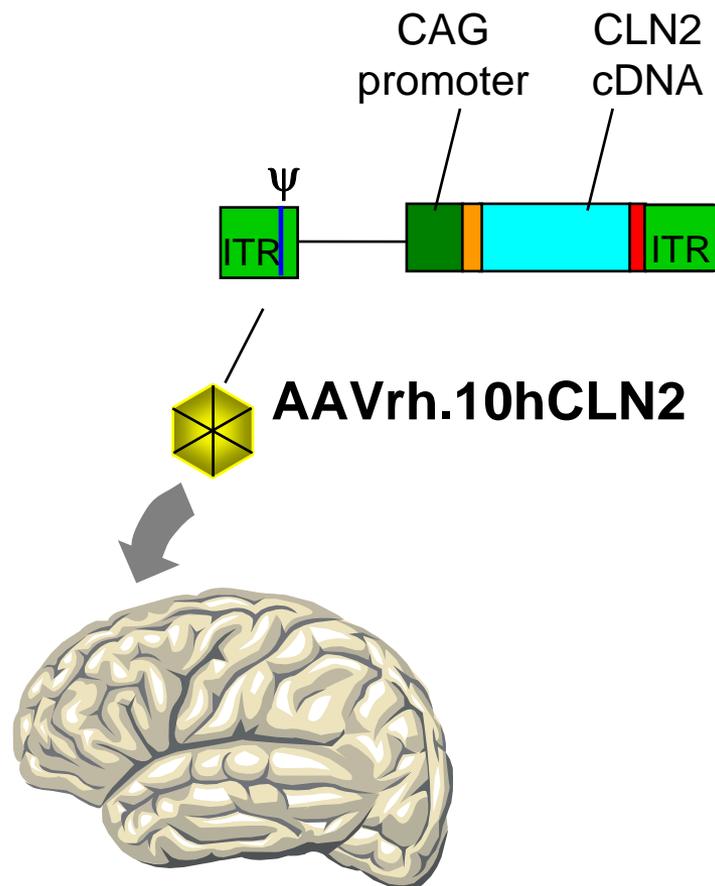


# Survival of CLN2<sup>-/-</sup> Mice Treated at Different Times with AAVrh.10hCLN2



# Toxicology of AAVrh.10hCLN2 in the Rat and Non-human Primate CNS

- Produce the AAVrh.10hCLN2 vector under GMP conditions
- Assess acute (7 days) and chronic (90 days) toxicology
- Assess expression of TPP-I in the CNS



# Toxicology Assessment of AAVrh.10hCLN2 Administration to the CNS of Rats 7 and 90 days

- Bilateral administration to striatum  $5 \times 10^{10}$  genome copies/site; total  $1 \times 10^{11}$  genome copies
- No abnormalities of hematologic, serum chemistry or histopathology of 18 organs
- CNS histology
  - 7 days – minor trauma localized to site of administration
  - 90 days – mild-moderate inflammation localized to site of administration
- No abnormalities in behavior (daily for 7 day group; 3x/wk for 90 day group)
  - respiratory, alertness, reflex responsiveness, excretory system function, healing, overall health

# Assessment of Local CNS Inflammation in 7 Day Rats

## Rat toxicology with AAVrh.10hCLN2: 7day-PBS

(n=10)

Parameters	Frontal cortex	Striatum	Caudal diencephalon	Cerebellum
Lymphoplasmacytic perivascular cuffing	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gliosis	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Swollen microvesiculated neurons	0 ± 0	0 ± 0	0 ± 0	0 ± 0

## Rat toxicology with AAVrh.10hCLN2: 7day-Vector

(n=10)

Parameters	Frontal cortex	Striatum	Caudal diencephalon	Cerebellum
Lymphoplasmacytic perivascular cuffing	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gliosis	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Swollen microvesiculated neurons	0 ± 0	0 ± 0	0 ± 0	0 ± 0

**Note:** Values represent average scores from a scale from 0-4 ± standard deviation  
Scale: 0-Normal, 1-Minimal, 2-Mild, 3-Moderate, 4-Marked

# Assessment of Local CNS Inflammation in 90 Day Rats

Rat toxicology with AAVrh.10hCLN2: 90 day-PBS

(n=10)

Parameters	Frontal cortex	Striatum	Caudal diencephalon	Cerebellum
Lymphoplasmacytic perivascular cuffing	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gliosis	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Swollen microvesiculated neurons	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Rat toxicology with AAVrh.10hCLN2: 90 day-Vector

(n=8)

Parameters	Frontal cortex	Striatum	Caudal diencephalon	Cerebellum
Lymphoplasmacytic perivascular cuffing	0 ± 0	1.9 ± 1.0	0.6 ± 0.7	0 ± 0
Gliosis	0 ± 0	2.0 ± 1.1	0.3 ± 0.5	0 ± 0
Swollen microvesiculated neurons	0 ± 0	2.4 ± 0.9	0.3 ± 0.5	0 ± 0

**Note:** Values represent average scores from a scale from 0-4 ± standard deviation  
Scale: 0-Normal, 1-Minimal, 2-Mild, 3-Moderate, 4-Marked

# Toxicology Assessment of AAVrh.10hCLN2 Administration to the CNS of Non-human Primates (African Green Monkeys; 7 and 90 days)

- Bilateral administration to 12 sites (identical to human study);  $1.5 \times 10^{11}$  gc/site,  $1.8 \times 10^{12}$  gc total dose
- No abnormalities of hematologic, serum chemistry or histopathology of 25 organs
- CNS histology
  - 7 days – none except minor trauma localized to site of administration
  - 90 days – mild-moderate inflammation localized to site of administration
- Extrapolation to human, 0.15% of total brain volume
- No change in behavior observed 7, 16, 30, 60 and 90 days post-administration

# Assessment of Local CNS Inflammation in 7 Day Non-Human Primates

## NHP toxicology with AAVrh.10hCLN2: 7day-PBS (n=1)

Parameters	Rostral: cerebrum, caudate, lateral ventricle, choroid plexus	Caudal: cerebrum, hippocampus, lateral ventricle, choroid plexus	Cerebellum and brainstem
Lymphoplasmacytic perivascular cuffing	0	0	0
Gliosis	0	0	0
Swollen microvesiculated neurons	0	0	0

Note: Only one NHP injected with PBS, therefore standard deviation was not applicable.

## NHP toxicology with AAVrh.10hCLN2: 7day-Vector (n=4)

Parameters	Rostral: cerebrum, caudate, lateral ventricle, choroid plexus	Caudal: cerebrum, hippocampus, lateral ventricle, choroid plexus	Cerebellum and brainstem
Lymphoplasmacytic perivascular cuffing	0 ± 0	0 ± 0	0 ± 0
Gliosis	0 ± 0	0 ± 0	0 ± 0
Swollen microvesiculated neurons	0 ± 0	0 ± 0	0 ± 0

**Note:** Values represent average scores from a scale from 0-4 ± standard deviation  
Scale: 0-Normal, 1-Minimal, 2-Mild, 3-Moderate, 4-Marked

# Assessment of Local CNS Inflammation in 90 Day Non-Human Primates

## NHP toxicology with AAVrh.10hCLN2: 90 day-Vector (n=4)

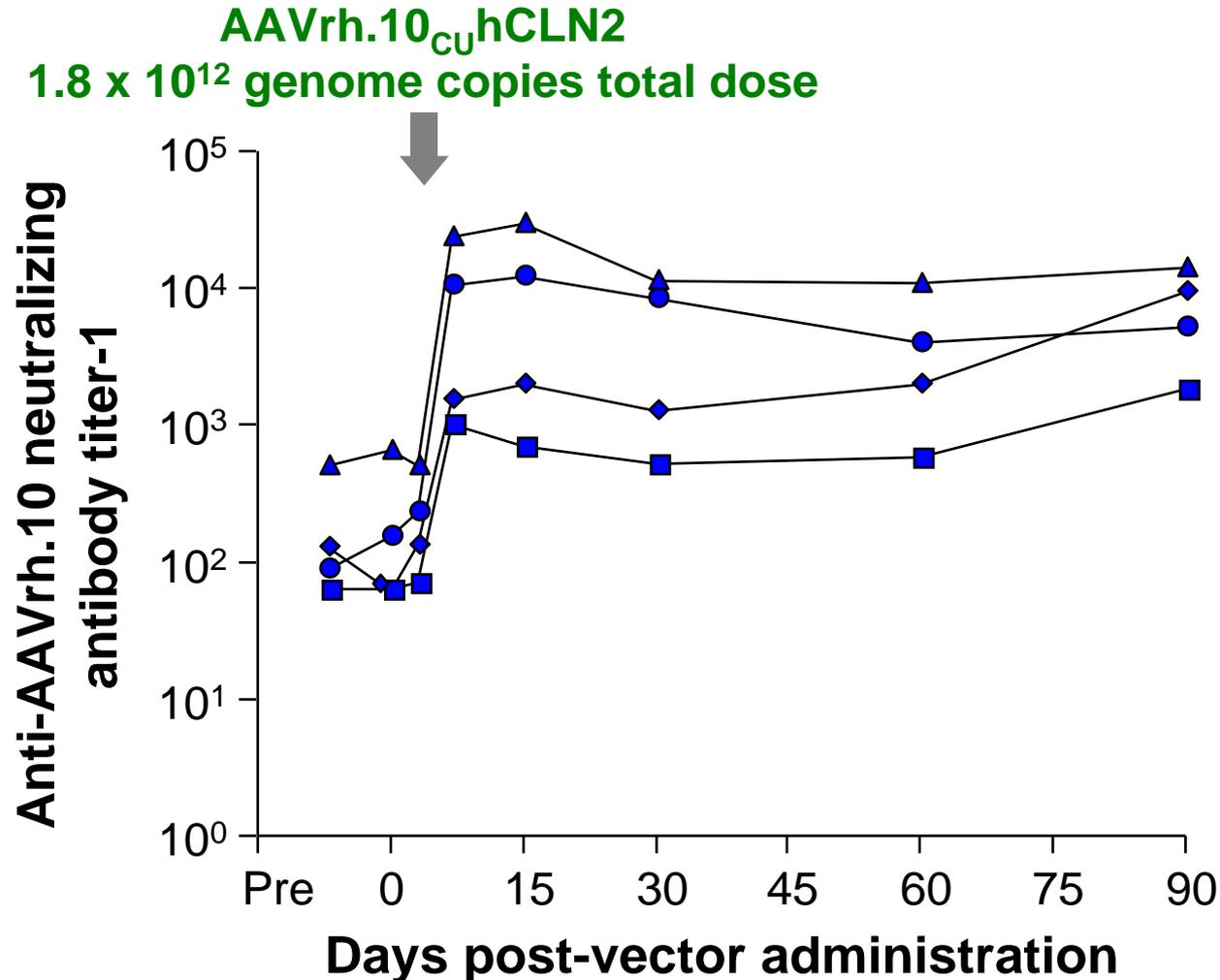
Parameters	Rostral: cerebrum, caudate, lateral ventricle, choroid plexus	Caudal: cerebrum, hippocampus, lateral ventricle, choroid plexus	Cerebellum and brainstem
Lymphoplasmacytic perivascular cuffing	2.8 ± 1.0	1.5 ± 1.0	0 ± 0
Gliosis	2.5 ± 0.6	1.5 ± 1.0	0 ± 0
Swollen microvesiculated neurons	0 ± 0	0 ± 0	0 ± 0

**Note:** Values represent average scores from a scale from 0-4 ± standard deviation  
Scale: 0-Normal, 1-Minimal, 2-Mild, 3-Moderate, 4-Marked

# Clinical Assessment of Non-human Primates Treated with AAVrh.10CLN2

- Blinded assessment of videotaping pre, 7, 16, 30, 60 and 90 days
- 18 parameters of behavior
- No differences from controls (n=8 pre-therapy; current, n=35 pre-therapy historic)

# Anti-AAVrh.10 Neutralizing Antibodies Evoked by CNS Administration of AAVrh.10hCLN2 to Non-human Primates

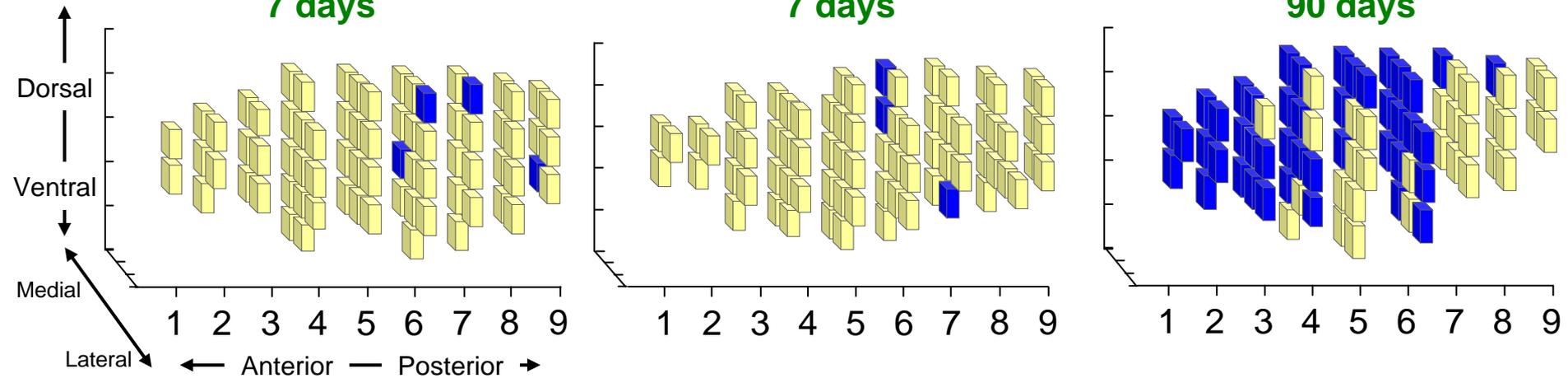


# Distribution of TPP-I Activity in the CNS of Non-human Primates Following CNS Administration of AAVrh.10hCLN2

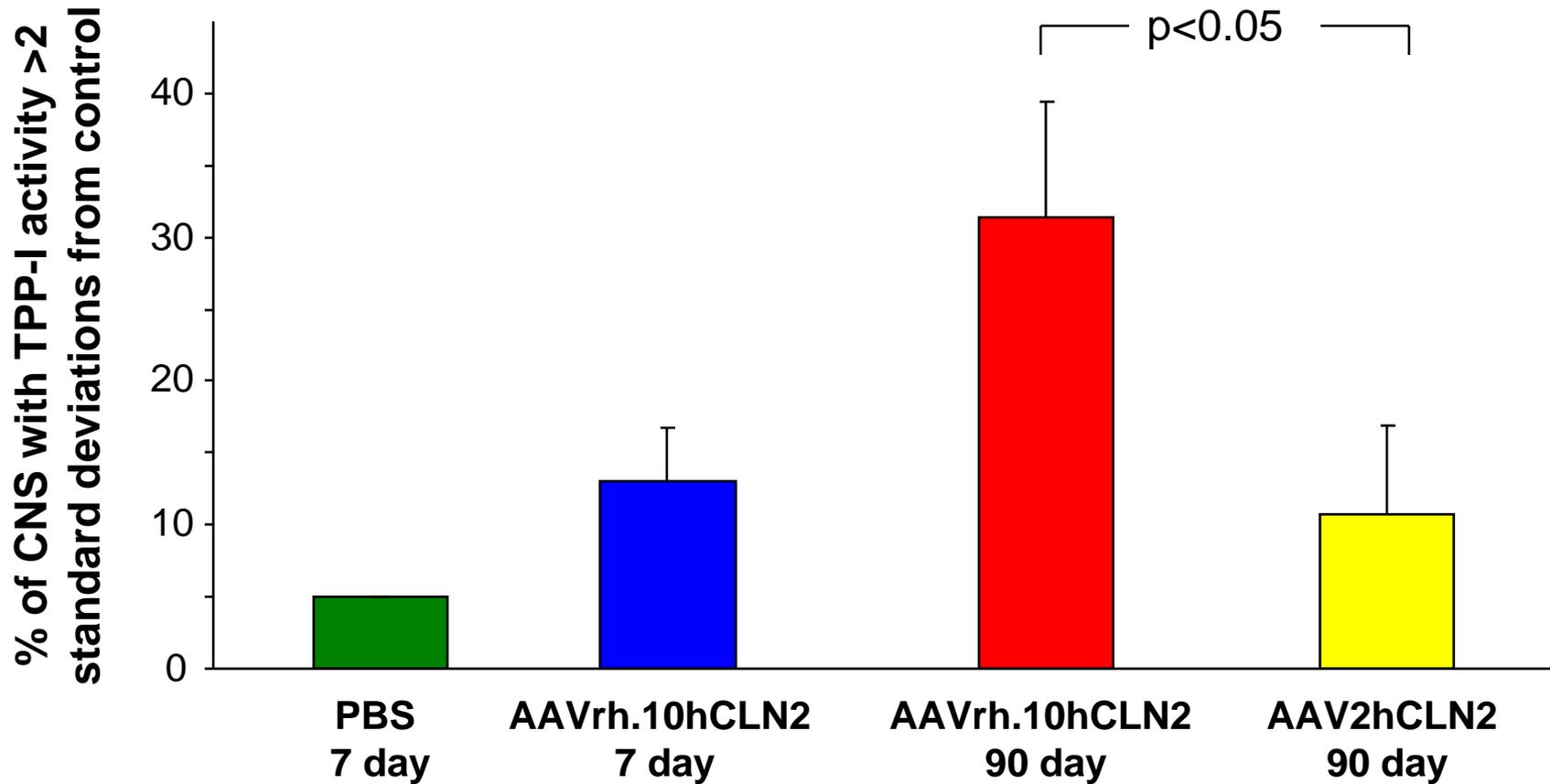
NHP Y132  
PBS  
7 days

NHP 066  
AAVrh.10hCLN2  
7 days

NHP 029  
AAVrh.10hCLN2  
90 days



# Distribution of TPP-I Activity in the CNS of Non-human Primates Following CNS Administration of AAVrh.10hCLN2



# Challenge - Choice of Dose

## Efficacy vs Risk

- Efficacy - requires maximum number of genetically modified neurons
- Risk – localized inflammation
- Context – progressive fatal disorder for which no therapy is available

# Doses of AAVrh.10hCLN2

Species	Dose/site (gc)	Total dose (gc)
Rat	$5.0 \times 10^{10}$	$1.0 \times 10^{11}$
Non-human primate	$1.5 \times 10^{11}$	$1.8 \times 10^{12}$
Human – original		
1 <sup>st</sup> n = 8	$1.5 \times 10^{11}$	$1.8 \times 10^{12}$
2 <sup>nd</sup> n = 8	$7.5 \times 10^{11}$	$9.0 \times 10^{12}$
Human – new		
1 <sup>st</sup> n = 8	$7.5 \times 10^{10}$	$9.0 \times 10^{11}$
2 <sup>nd</sup> n = 8	$1.5 \times 10^{11}$	$1.8 \times 10^{12}$

# Challenge – Controls for Studies of Treatment of Fatal, Rare Childhood Disorders for Which There is No Therapy

## Ethics

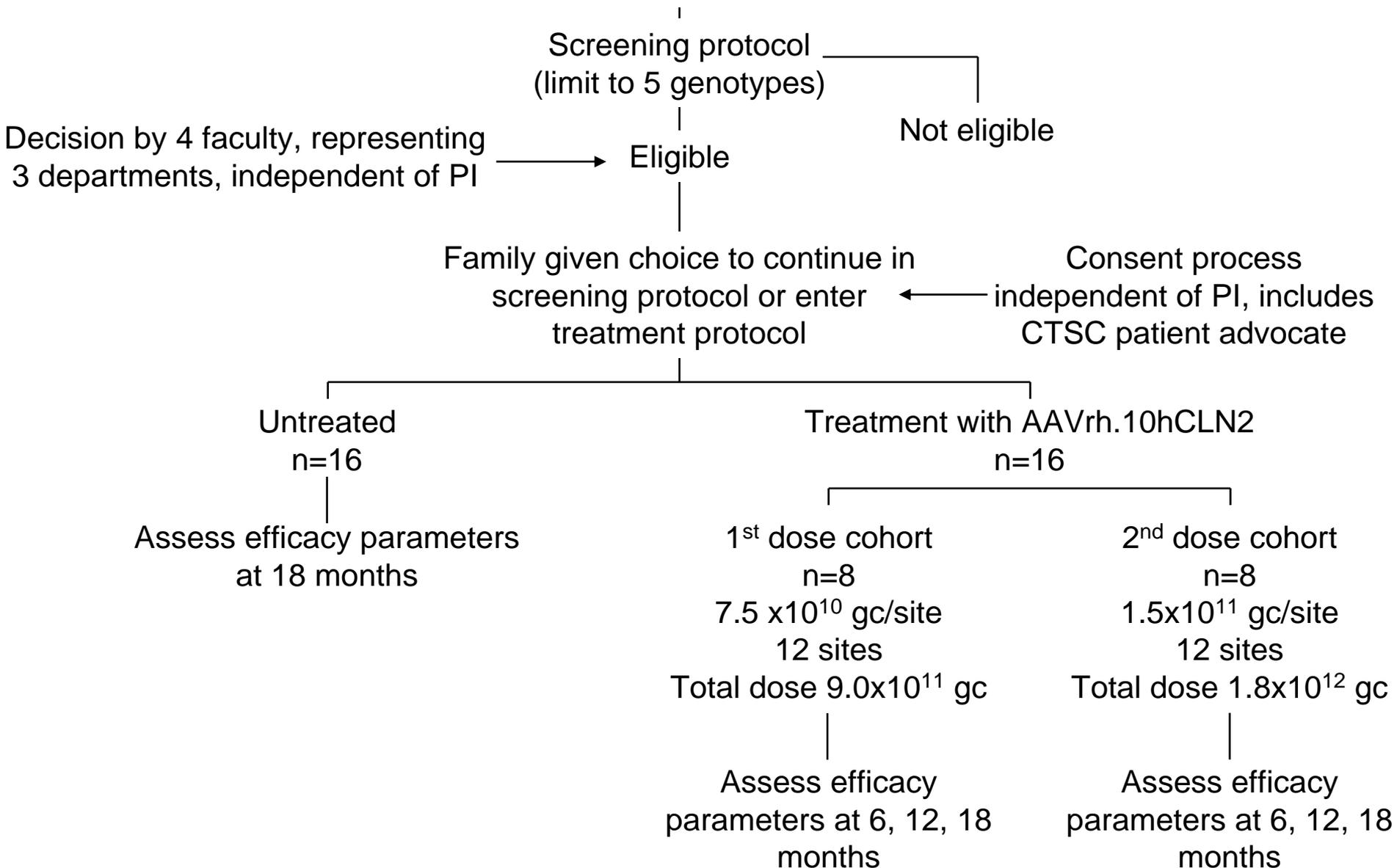
- Cannot do a randomized, placebo controlled, blinded study

## Strategies

- Pre-therapy as the control
  - Once established, disease is rapidly progressive
- Historic controls
- Contemporaneous untreated controls

# Overall Design of the Trial

## Subject with LINCL



# Outcome Measures

## Primary

- Weill-Cornell clinical score<sup>1</sup>
- Unified Batten Disease Rating Scale for JNCL
- CHQ Quality of Life questionnaire
- Mullen score

## Secondary

- Quantitative MRI
  - % Ventricular volume
  - % Grey matter volume
  - Cortical apparent diffusion coefficient

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<sup>1</sup> Feeding 0-3, gait 0-3, motor 0-3, language 0-3; assessed independently by 2 pediatric neurologists; total score = sum of score for the 4 parameters, 12 = normal, 0 = maximum impairment

# Review of J. Bartlett

- Control group?
- Outcome measures?
- Goals for the trial?
- Other delivery strategies?
- Other capsids?
- Concentration of AAVrh.10 vs AAV2?
- Anti-rh.10 immunity as exclusion?
- Exclusion if participated in other experimental studies? Anti-transgene?
- Number of vector lots?
- Why do the treated CLN2-/- mice die? Immunologic?  
Does this argue against therapy of early disease?

# Review of H. Federoff

- Relationship of high and low dose cohorts?
- Are supra-physiologic levels of TPP1 deleterious?
- Observed spongiosis in toxicology studies?
- Peripheral distribution and immune responses?
- Immunity against transgene?
- Expression data non-human primates?
- Why 2 stage depth per burr hole?
- Anesthesia for MRI?
- Serious adverse events; stopping rules?

# Review of J. Flint

- Neuron transduction data *in vitro* and *in vivo*?
- Choice of promoter; why not neuron specific promoter?
- Choice of doses?

# Review of E. Clayton

- Description of risks in protocol vs published paper?
- Description of benefit?