# Framework for Defining Evidentiary Standards for Biomarker Qualification:

# Alexander Disease

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BIOMARKERS CONSORTIUM
IMPROVING HEALTH THROUGH MEANINGFUL MEASUREMENTS

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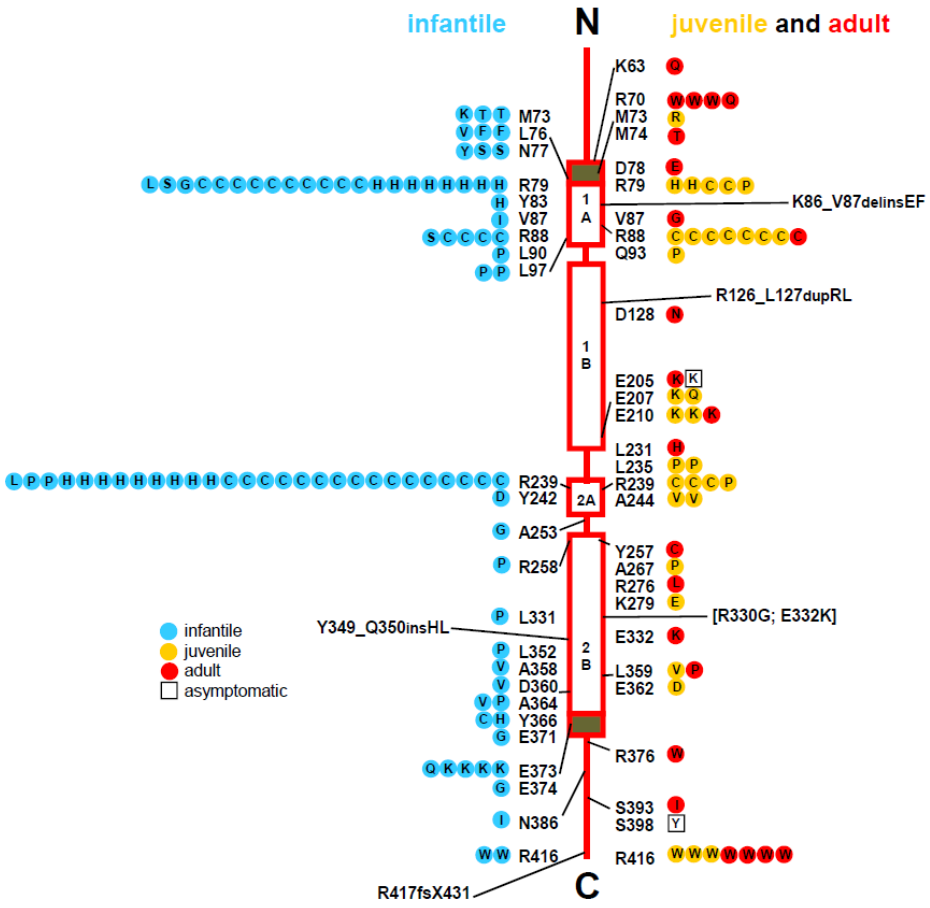
Foundation for the National Institutes of Health

# Alexander Disease

A genetic condition caused by heterozygous, most often sporadic, mutations in *GFAP* (Glial Fibrillary Acidic Protein)

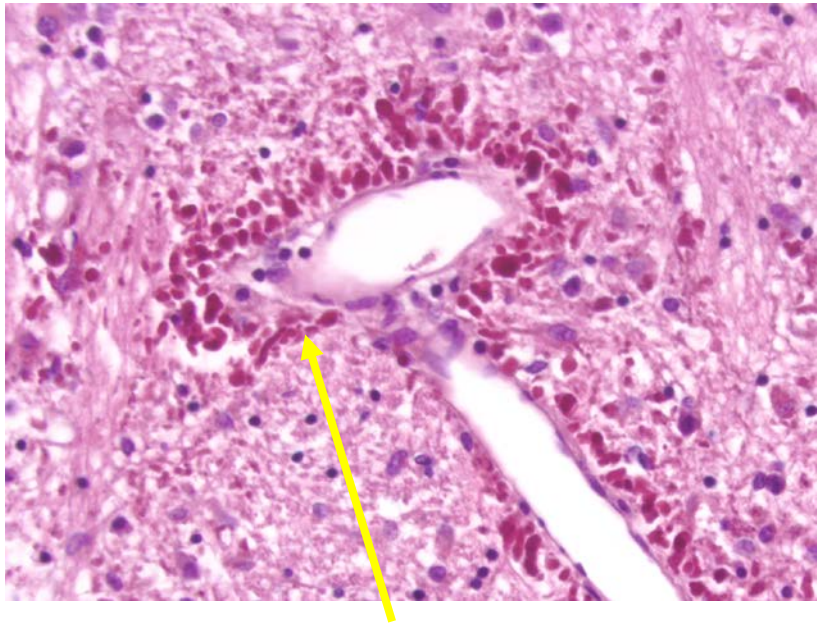



Flint D, Brenner M. Alexander disease. In Raymond G, Eichler F, Fatemi A, Naidu S, ed. *Leukodystrophies*. London: MacKeith Press; 2011: 106-129.

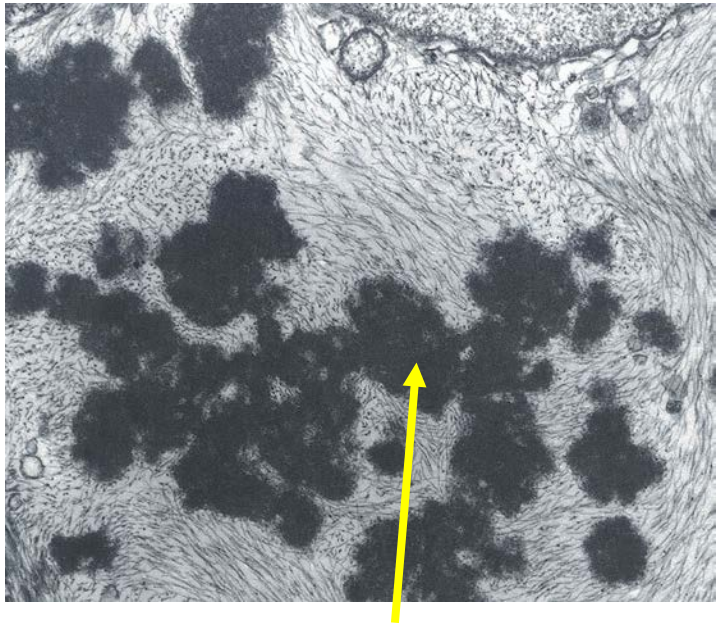
# Alexander Disease

Alexander Disease was originally defined by the pathologic findings prior to genetic identification

Primarily affects astrocytes (which produce GFAP), resulting in accumulation of aggregates of proteins called Rosenthal fibers, a pathologic hallmark of the disease



Human brain tissue (Light microscopy): H&E staining demonstrates Rosenthal fibers



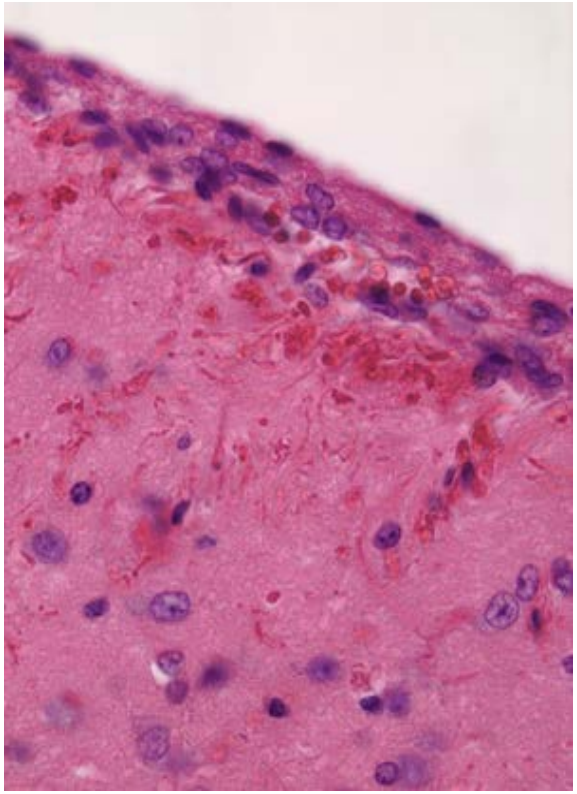
Human brain tissue (Electron microscopy): Rosenthal fibers contain GFAP, ubiquitinated stress protein inclusions

# Alexander Disease

- Results in symptoms, typically with onset in toddlers, including macrocephaly, spasticity, delayed and loss of motor milestones, abnormal speech patterns and seizures
- Progresses over 10+ years to complete loss of motor, speech and vital functions and death.
- However, disorder also encompasses less frequent phenotypes:
  - Neonatal with severe early onset encephalopathy, hydrocephalus and seizures
  - Older child or adolescent with more slowly progressive abnormalities including anorexia, recurrent vomiting, scoliosis, seizures and slow deterioration of motor skills
  - Adult onset presentation with autonomic dysfunction, gait disturbance, obstructive sleep apnea

# Alexander Disease

- A transgenic mouse model engineered to produce excess wild-type GFAP develops Rosenthal fibers similar to the human disease
- Mouse model replicates biochemical and pathologic features of disease

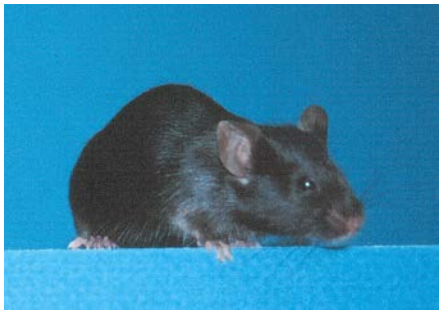


Morphologically and biochemically identical to human Rosenthal fibers

- Too much GFAP is WORSE than deficiency – even normal protein can be lethal

GFAP transgenic
(over-expression of wt protein)

Messing et al. *Am J Pathol* 152:391, 1998



GFAP-null

McCall et al. *PNAS* 93:6361, 1996

# Biomarker Evidentiary Framework

**Statement of need**
- Clinical features may develop over many years and may be variable
- Clinical outcomes are not specific to AxD (gait and language abnormalities)
- No clinical outcome assessments (COA) validated for AxD
- Lack of COA would prevent adequately testing dosing and response
- Novel ASO based therapies tested in rodent models
- Urgent need for clinical trial readiness

**Benefits of the marker**
- The patients would benefit because direct brain measurement of GFAP is not accessible. *CSF testing less invasive*
- The current COA relies on manifesting severe signs of disease, generally irreversible and developing over years. *Would allow more rapid development of therapies with real-time assessment of impact of therapies*
- This biomarker will allow *quantitative testing in a population*.

**Risks of the marker**
- GFAP levels may not be directly related to phenotype. *Preliminary data suggests that GFAP correlates to disease severity. [risk not likely and low magnitude]*

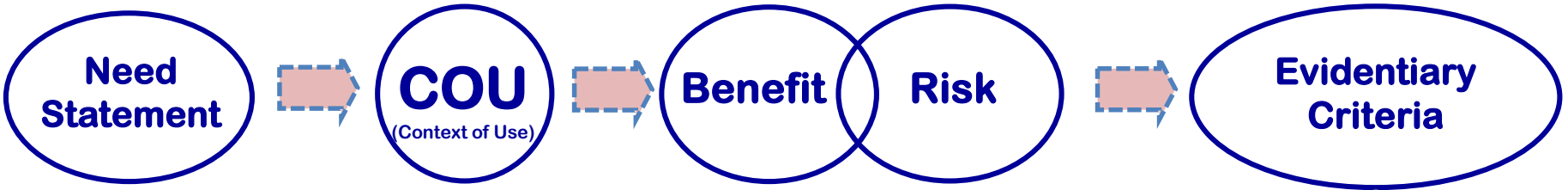
**What is the acceptable level of uncertainty**?
- *The patient population is motivated to take on more risk to help achieve beneficial therapies.*

**In Drug Development**

**Factor likelihood and magnitude**

Informs Required Stringency of EC

**What is the acceptable level of uncertainty?**




The surrogate endpoint will be used to measure response in individuals with AxD by assessing GFAP in CSF or plasma before and some time after treatment to predict clinical outcomes at a later date

**Surrogate Endpoint Evidentiary Issues**
- Biological plausibility
  - Genetics
  - Animal model data
- Causality-Genetics
- Universality
- Proportionality-GFAP levels proportional to age of onset
- Specificity-GFAP aggregates are the hallmark of the disease

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# Statement of Need

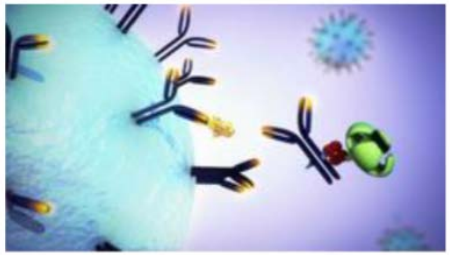
- Clinical features may develop over many years. This would prevent adequately testing dosing and response.
- Clinical outcomes are not specific to AxD (gait and language abnormalities)
- No clinical outcome assessments (COA) validated for AxD
- Novel ASO based therapies recently tested in rodent models and offer hope of therapeutic effect
- Therefore, urgent need for clinical trial readiness to enable more efficient, safer and potentially faster drug development

# Description of the Biomarker

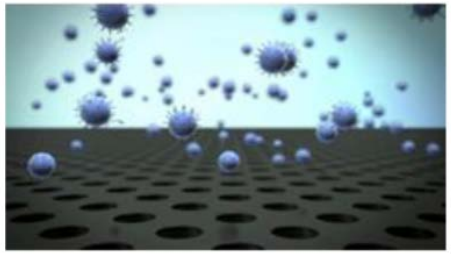
- The biomarker to be measured is GFAP (glial fibrillary acidic protein)
- This protein can be measured in CSF (and blood)
- Processes for sampling well established
- Methodology commercially available
- Correlation between levels in body fluids and brain
- Quantitative validation ongoing
- GFAP is the target of therapeutic intervention

# GFAP assay-Methodology

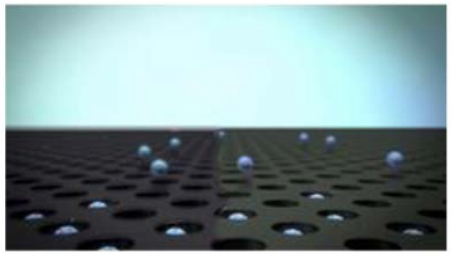
- Simoa: Single Molecule Array (Quanterix)
- Loading, sealing, and imaging of single paramagnetic beads (ø2.7µm) in arrays of femtoliter-sized wells.



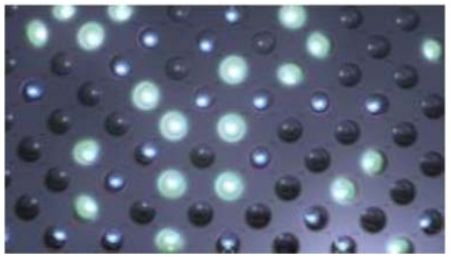
Beads, a fraction of which are associated with captured and enzyme-labeled protein molecules, are introduced into the array.



Beads settle by gravity onto the surface of the array, and a fraction of them fall into microwells. The remainder lie on the surface.



Oil is introduced into the channel to displace the aqueous medium and excess beads and seal the wells.



Sealed wells are imaged. Fluorescent signals are generated in sealed wells that contain beads associated with captured and enzyme-labeled protein molecules.

Source: Images from Quanterix

# Context of Use

- The surrogate endpoint will be used to measure response in individuals with **Type I AxD** by assessing GFAP in CSF (or plasma) before and some time after treatment

- The surrogate endpoint will be used to measure response in individuals with **Type II AxD** by assessing GFAP in CSF (or plasma) before and some time after treatment

# Benefit Assessment

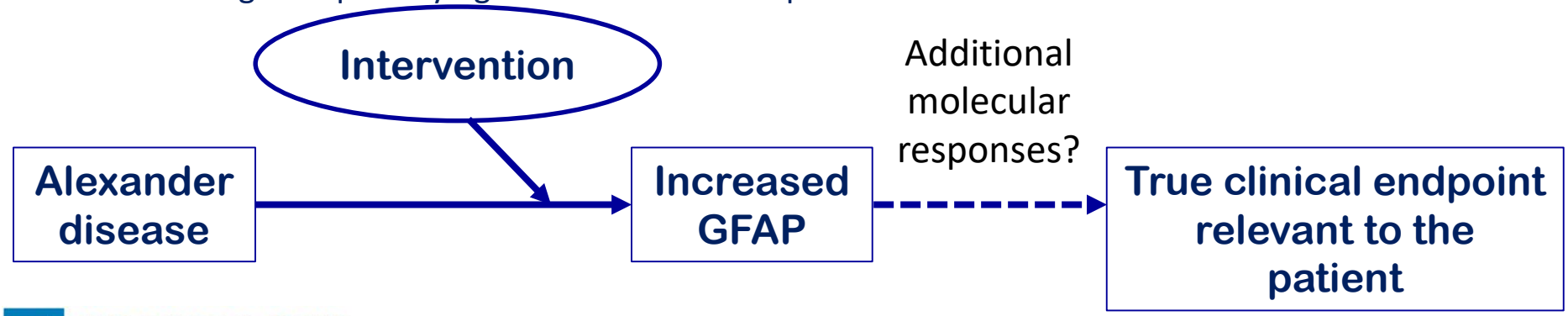
- The patients would benefit because direct brain measurement of GFAP is not accessible. *CSF testing less invasive*
- The current COA relies on manifesting severe signs of disease, generally irreversible and developing over years. A biomarker would allow more rapid development of therapies for these patients and assessment of efficacy earlier in treatment regimes.
- Society would benefit because the biomarker would provide benefit at a critical time in drug development, facilitating study of dosing and therapeutic effect. *A validated biomarker would allow more rapid development of therapies with real-time assessment of impact of therapies.*
- AxD disease is severe with high morbidity, health care costs and mortality
- This biomarker will allow *quantitative testing in a population*.

# Risk Assessment

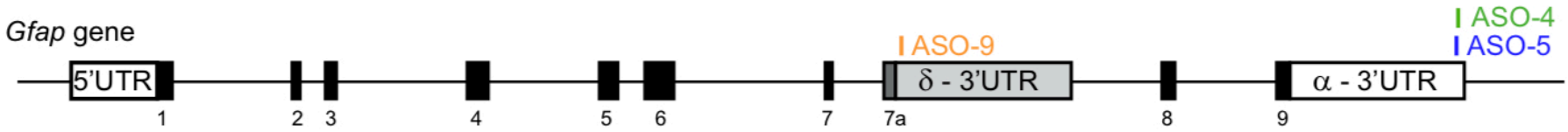
- GFAP levels may not be directly related to phenotype. Although *GFAP* is the causal mutation, a change in GFAP expression may not be proportional to the Rosenthal fibers in brain tissue or astrocyte dysfunction.
  - Human and animal data suggests that GFAP levels correlate with disease severity.
  - Animal data suggests that CSF fraction corresponds to tissue levels
- The variability of symptoms in AxD and the small number of patients make correlation of GFAP levels to a clinically meaningful outcome measure more challenging.
- CSF GFAP measures are an invasive test. This disease has no current therapeutic approaches so the patient population is motivated to take on more risk to help achieve beneficial therapies.
- Mitigation strategy is to rely on current slow and imprecise standard testing of Clinical Outcome Assessments, which will delay therapy development.

# Evidentiary characteristics that support GFAP as a potential surrogate endpoint

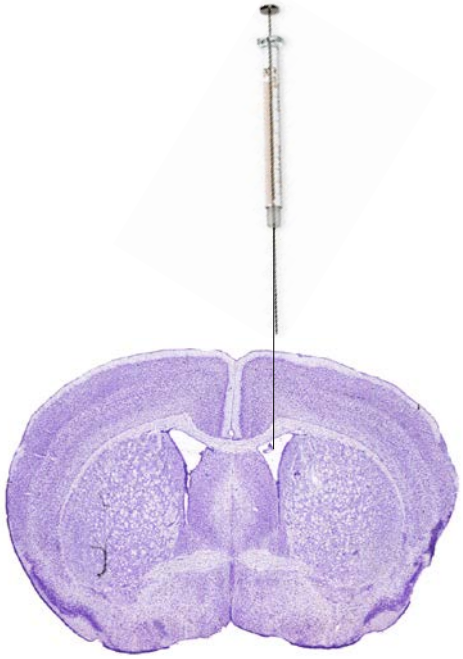
- Universality
  - Unproven as only ASO treatment is being tested and it is unclear if multiple treatment effects correlate with clinical function
- Plausibility
  - Biologic effects of reversal on fiber formation based on ASO in animal models
- Causality
  - Genetic mutation in *GFAP*
- Specificity and potential for off target effects
  - GFAP alone is not specific, but in combination with mutation data is clear
- Proportionality
  - The extent to which the surrogate explains the disease or the change in disease has not yet been defined (next slides)
- Challenge:
  - Defining and quantifying the true clinical endpoint



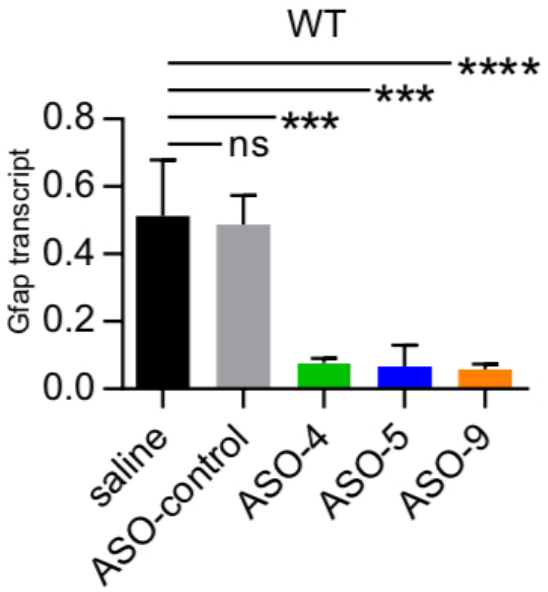
# Brain GFAP is responsive to ASO treatment in mice




- ASO=Antisense Oligonucleotide in wild-type mice
- GFAP was measured in response to intracerebroventricular ASO treatment
- GFAP is increased in AxD and RF are the diagnostic hallmark
- AxD toxicity may be related not to RF but to earlier steps in GFAP assembly



Single ICV bolus injection



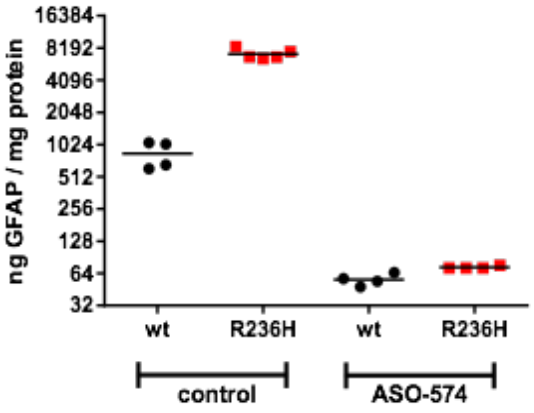

ICV at 12 weeks; analysis 2 weeks later

Plausibility

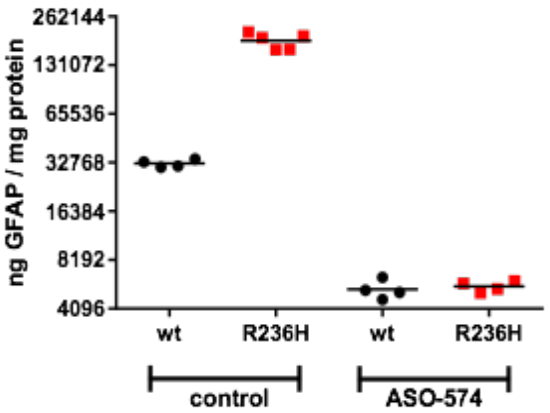
Hagemann TL et al., *Ann Neurol,* 2018

# Reduction of GFAP using ASO in mice

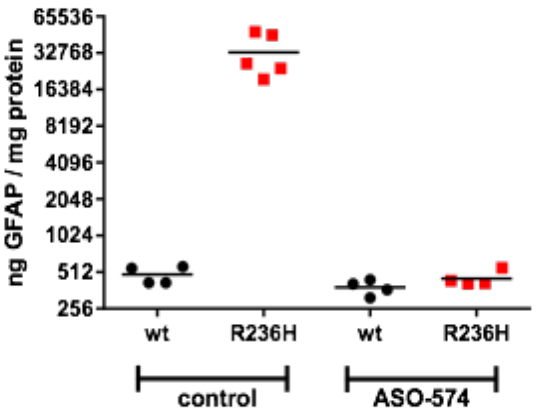
Soluble

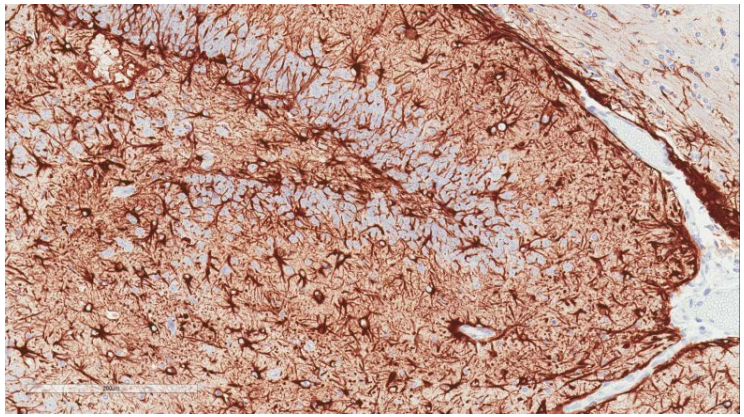


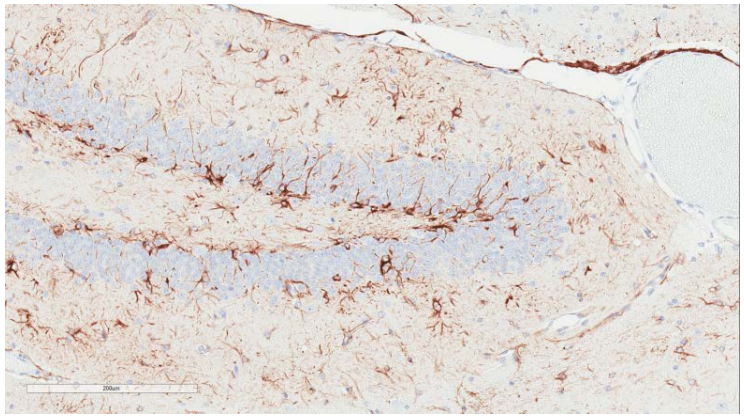

Insoluble




RF enriched





Plausibility

Saline-treated R236H

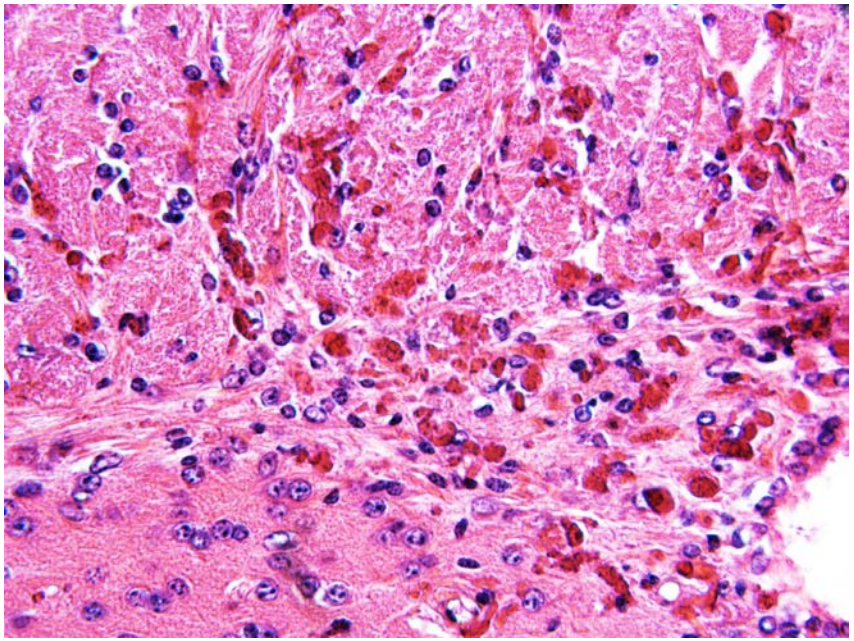
ASO-treated R236H

Hagemann TL et al., *Ann Neurol,* 2018

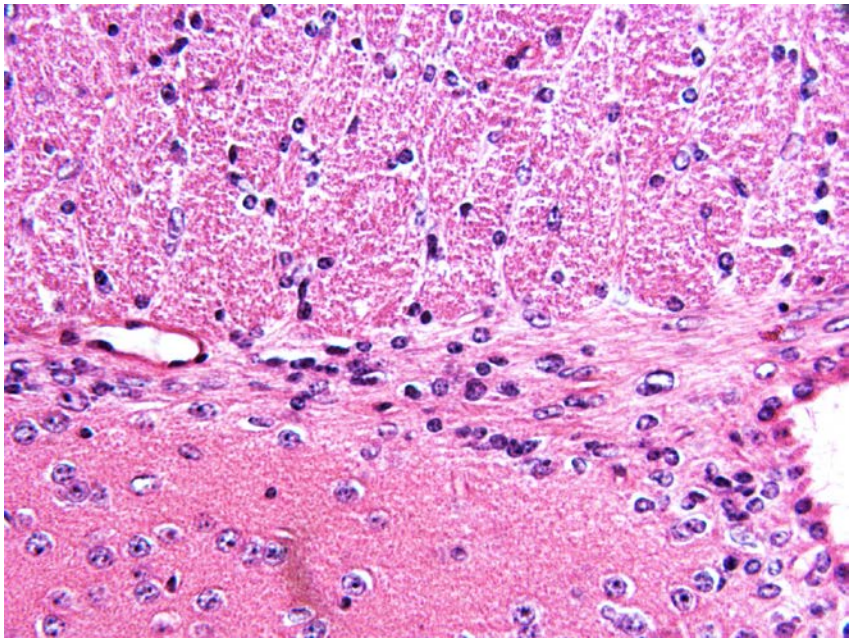
# Reduction of GFAP using ASO reduces Rosenthal Fibers in mice

- Reversal of Rosenthal fibers 8 weeks post-treatment

Saline-treated



ASO-5 treated



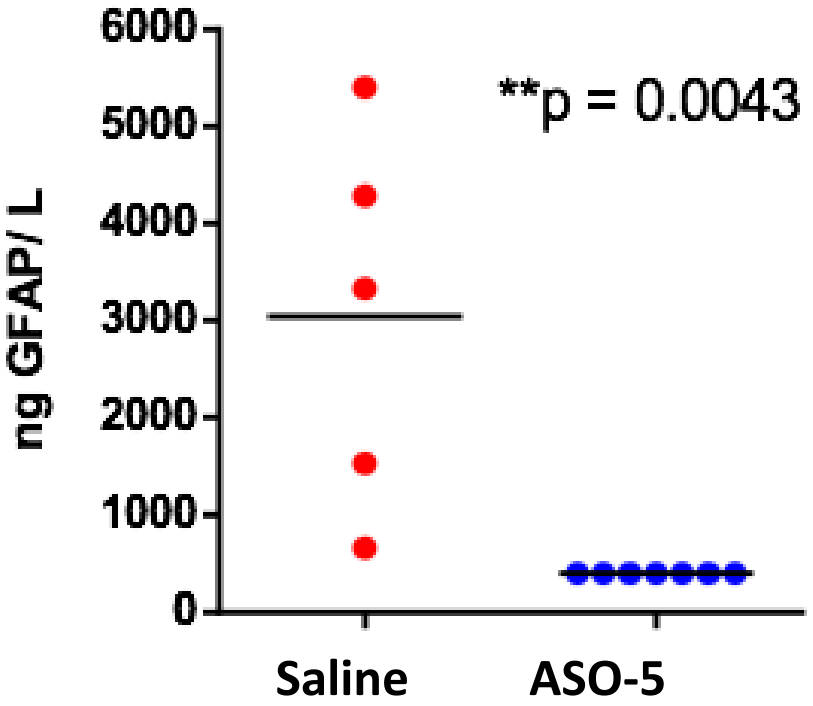
Plausibility

Hagemann TL et al., *Ann Neurol* 2018

# Reduction of CSF GFAP using ASO in mice

- GFAP in murine CSF can be decreased by ASO treatment

CSF GFAP levels
8 weeks post treatment

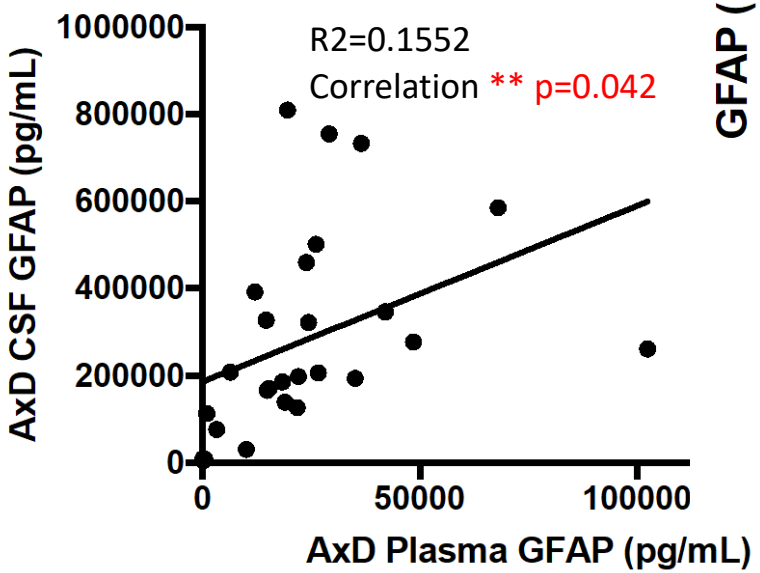


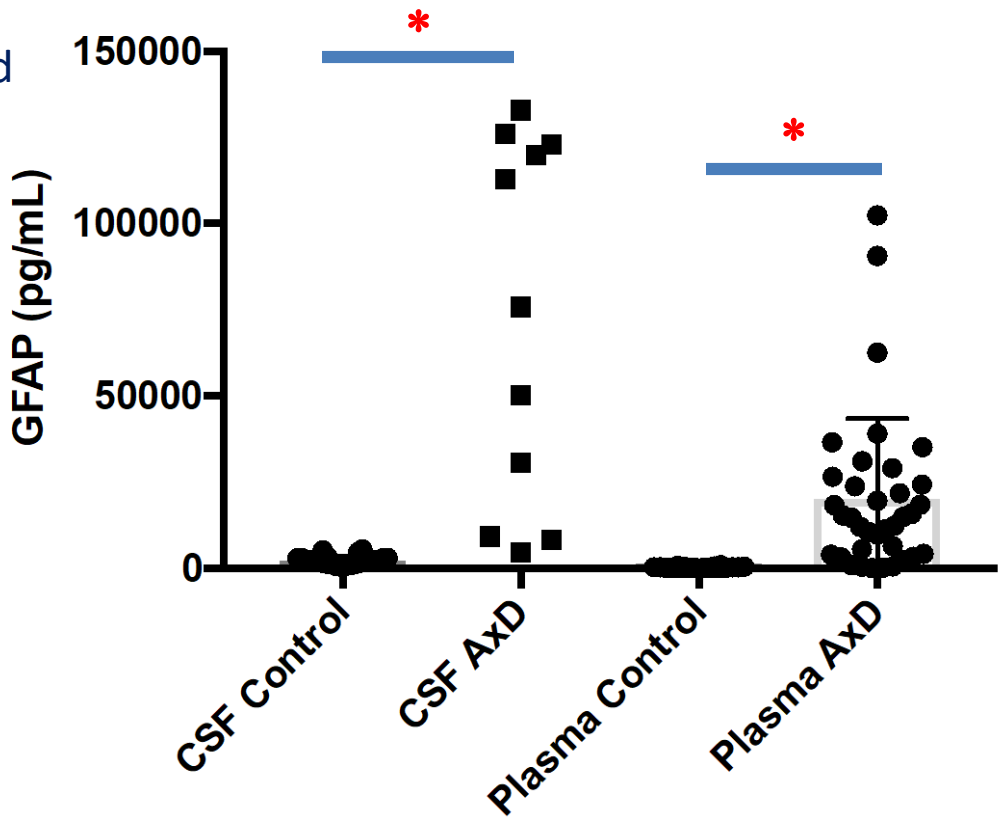

Plausibility

Hagemann TL et al., *Ann Neurol* , 2018

# GFAP is elevated in CSF and Plasma of Alexander disease

- GFAP is elevated in plasma and CSF compared to controls
  - CSF controls: oncologic or other neurologic conditions, suspected infections
  - Plasma controls: other leukodystrophies



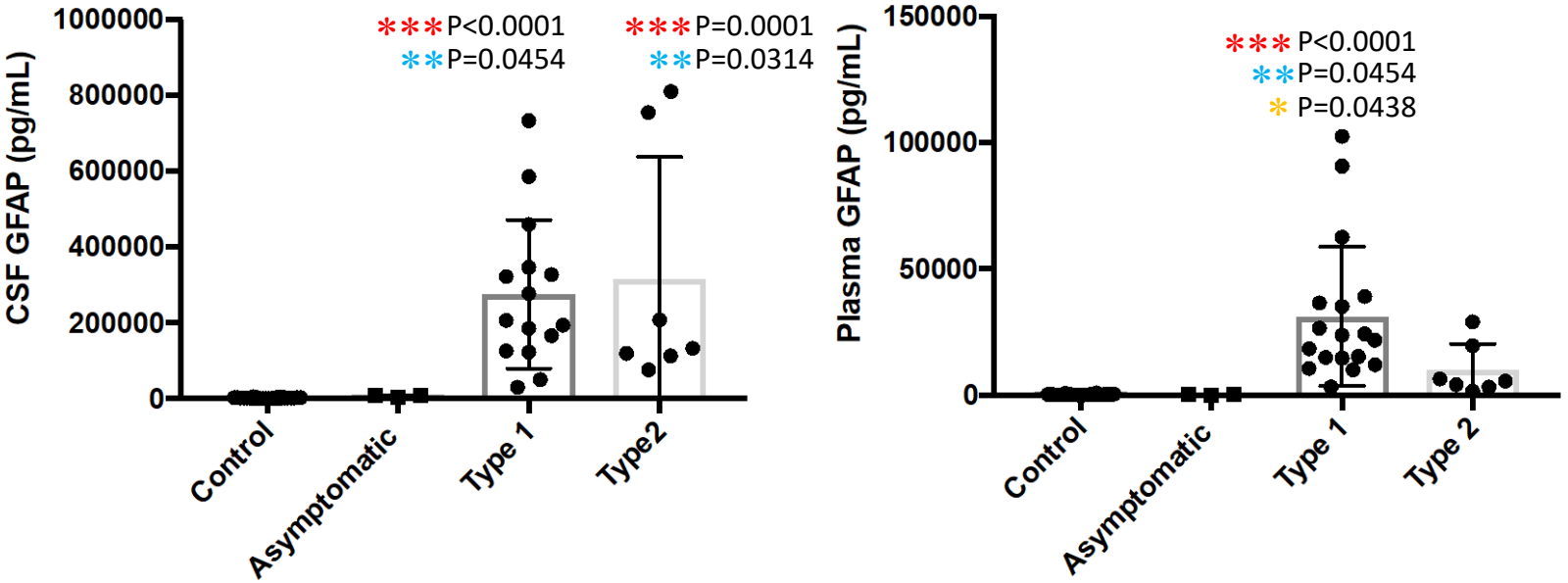




Mann-Whitney test, *p<0.0001 vs. controls

Causality and Plausibility

# GFAP is elevated in CSF and plasma of Alexander disease, and not in individuals with non-pathogenic variants

- GFAP was tested in 3 individuals with genetic variants but no evidence of clinical disease
  - 2 mosaic parents (variants R79C, I84M) that are clinically and radiologically unaffected
  - 1 adult with a GFAP variant (R136Q) but without the clinical or imaging features of AxD




One-Way ANOVA multiple comparisons
(*** vs. controls, ** vs. Asymptomatic, * Type 1 vs. Type 2)

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## Causality and Plausibility

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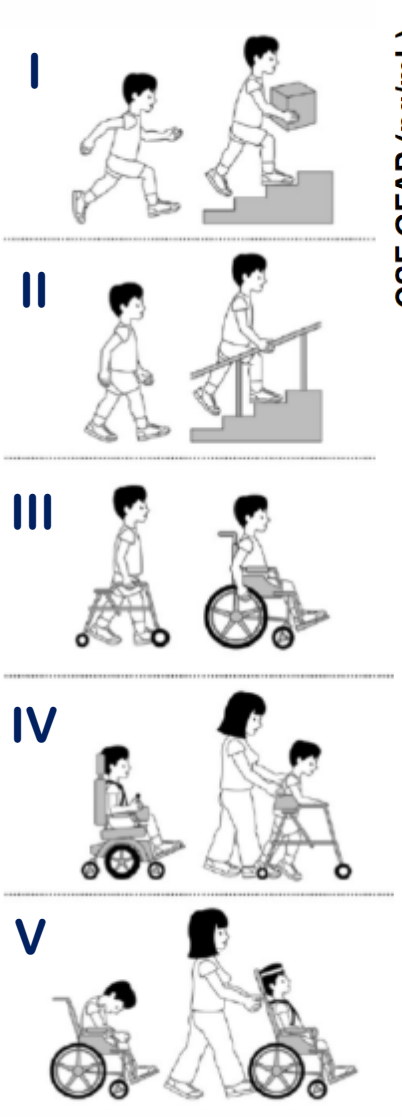
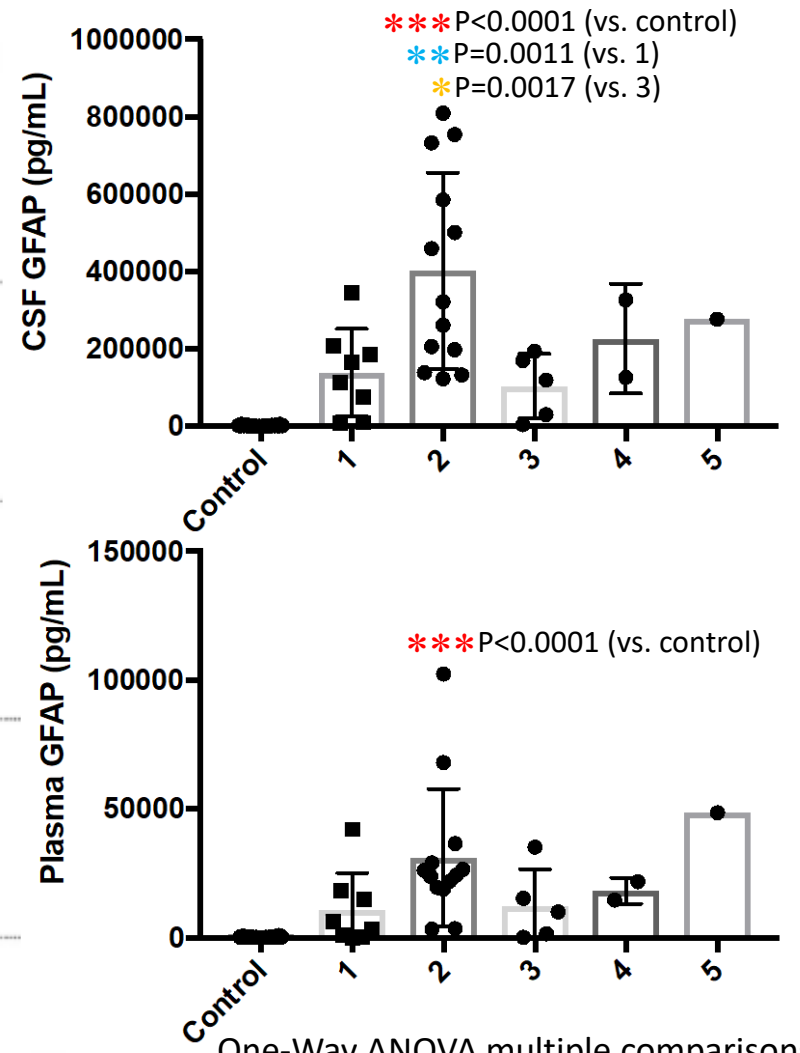
# Clinical endpoint: gross motor

- **Gross Motor Function Measure-88**
  - Designed for longitudinal testing in cerebral palsy
  - Activities:
    - A) Lying and rolling
    - B) Sitting
    - C) Crawling and kneeling
    - D) Standing
    - E) Walking, running, jumping
- **Gross Motor Function Classification System**
  - Descriptive tool
  - Designed for longitudinal testing in cerebral palsy

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# Question and Answer Session

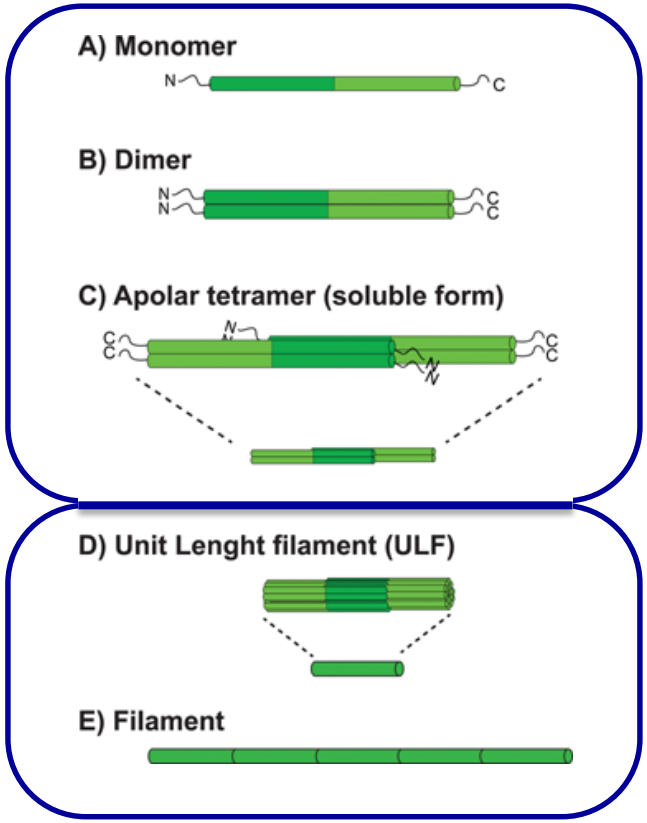
- How much weight can you give to animal models? What validation does that provide?
  - How different in a monogenic rare disease?
  - How to use markers as a surrogate endpoint? Clinical relevance?
  - Do different models help? Mouse, rat, fly?
- How about human IPS cells or other human cellular models? Advantages and disadvantages.
- How heterogeneous is the population in progression and presentation? How feasible/easy will it be to assess clinical assessments alone?
- Can surrogacy be an ultimate goal with useful milestones of progression or response along the way? Go/NoGo, dose selection, many other possibilities.
- What level of evidence is needed for this surrogate end-point in this rare disease? Does correlation between GFAP and functional outcomes need to be demonstrated?

# Additional figures

# GFAP pools and predictions about decline

soluble

insoluble

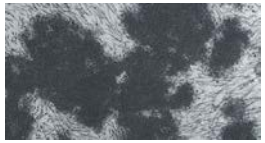



**Triton X 0.5%**

**Urea 6M**

**SDS 2%**

**Rosenthal fibers**



- ~95% of normal GFAP is in the ULF or filament form
- half-life (from Eng 1985) estimated at 9 weeks in mouse spinal cord
- Goldman studies show proteasome dysfunction

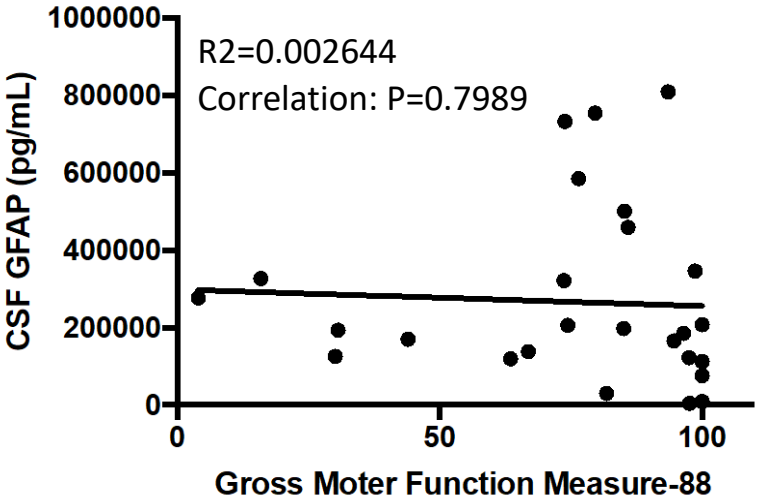
- predictions:

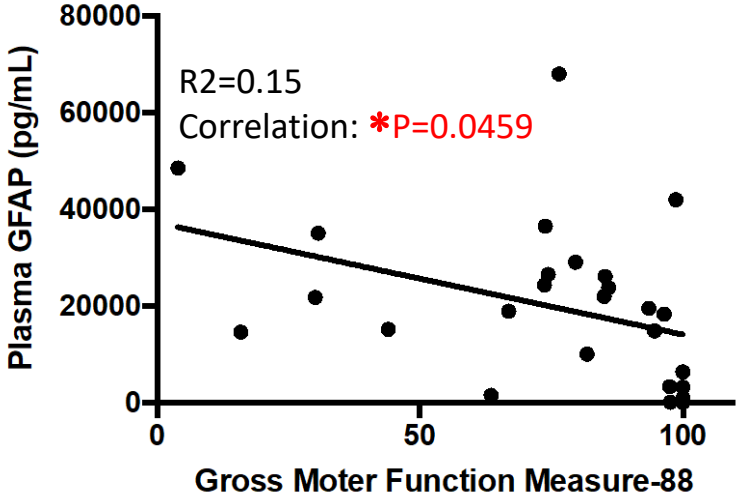
1. soluble pool drops first
2. insoluble pool drops second
3. RF fraction may persist a long time

Robert A et al. *Bioessays* 38:232-243 (2016)

# primary endpoint

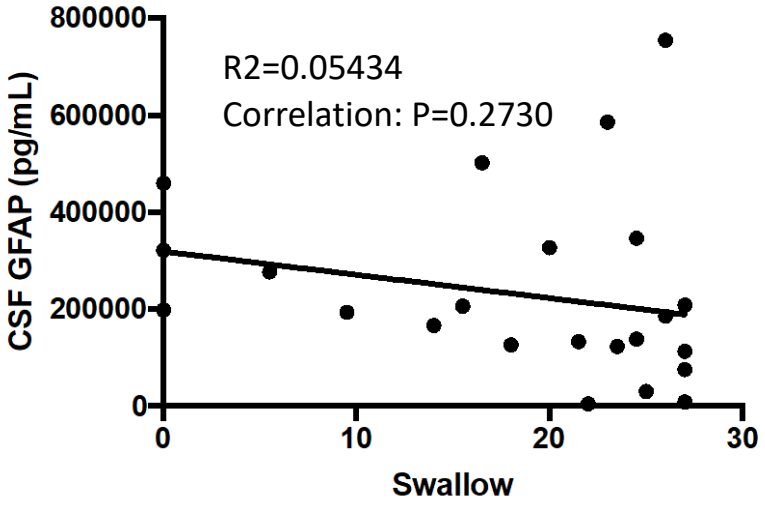
– Candidate outcome measure: Gross Motor Function Measure-88

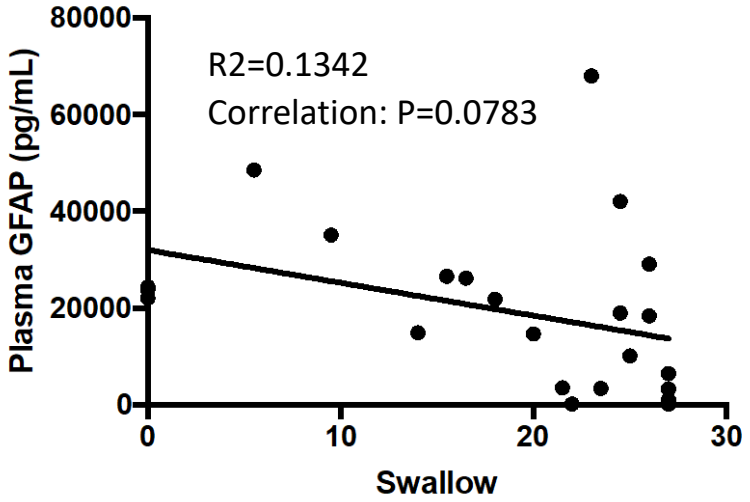


# Clinical endpoint: Swallow

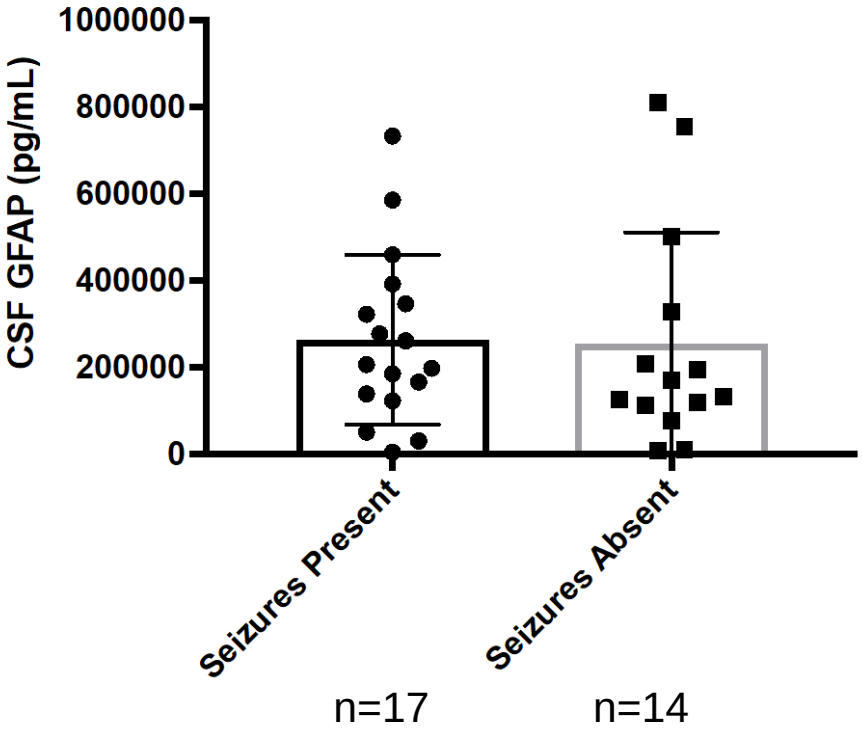
- Swallowing function
  - Custom scale designed by Chrissy Minkoff, SLP, to evaluate safety and efficacy of the oral, pharyngeal and esophageal phases of swallowing function using thin liquids, purees, and solids
- Scoring
  - Individual items range 0 – 3
  - Total score = 27 (normal swallowing function)
  - Not tested in those who are exclusively G-tube fed (safety)



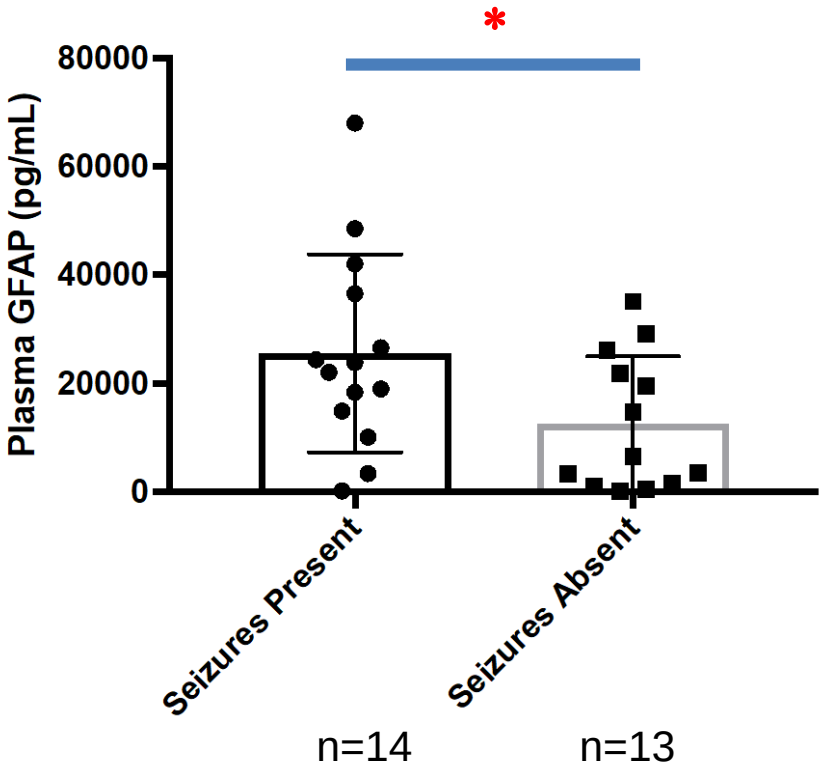



# Clinical endpoint, seizures

## Seizures: GFAP



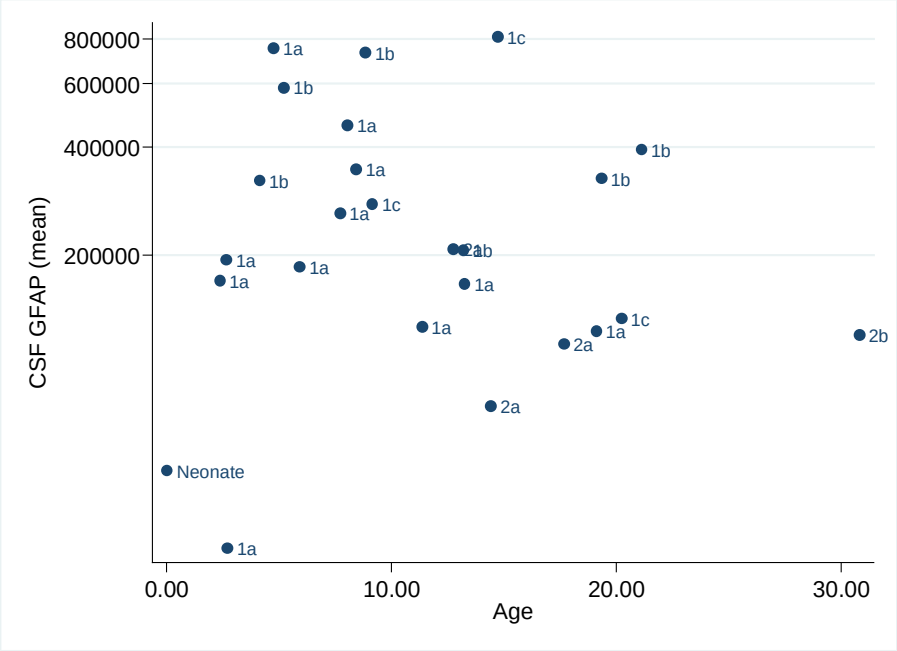

Unpaired t-test P=0.9028



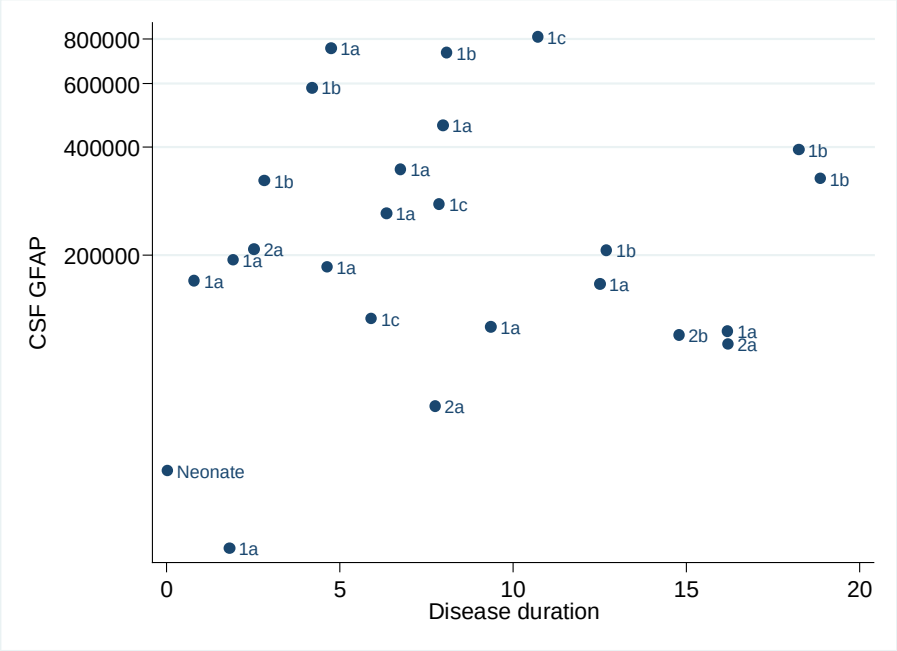

Unpaired t-test *P=0.0414

# Demographics

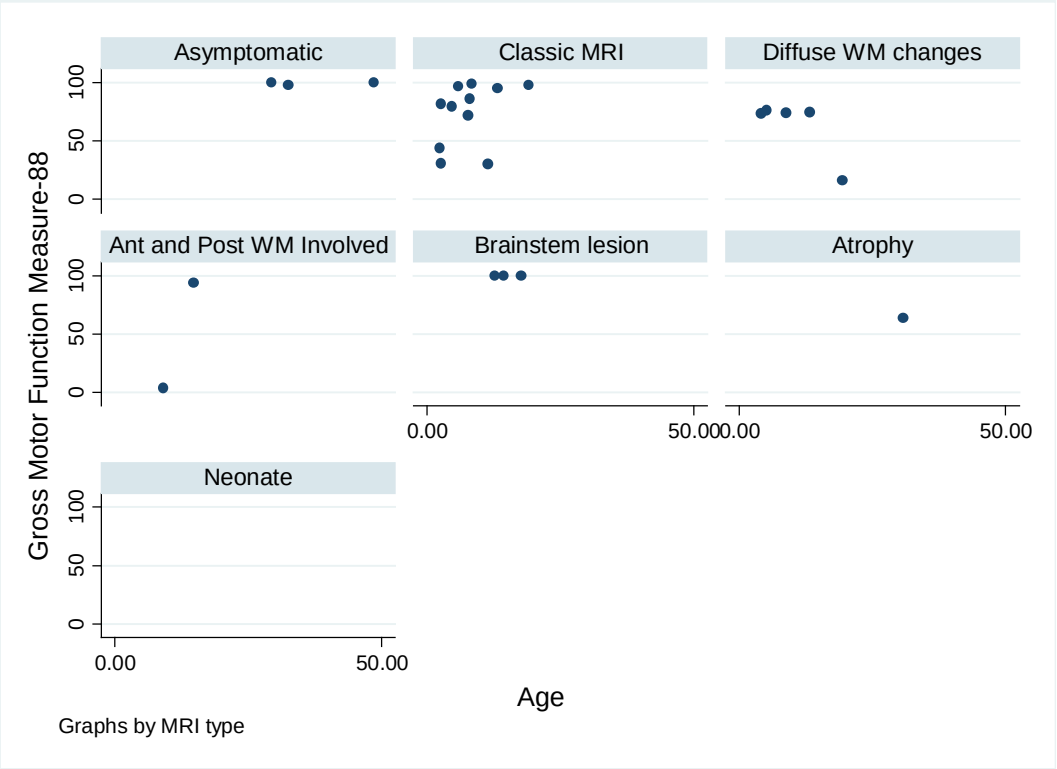
GFAP levels by age



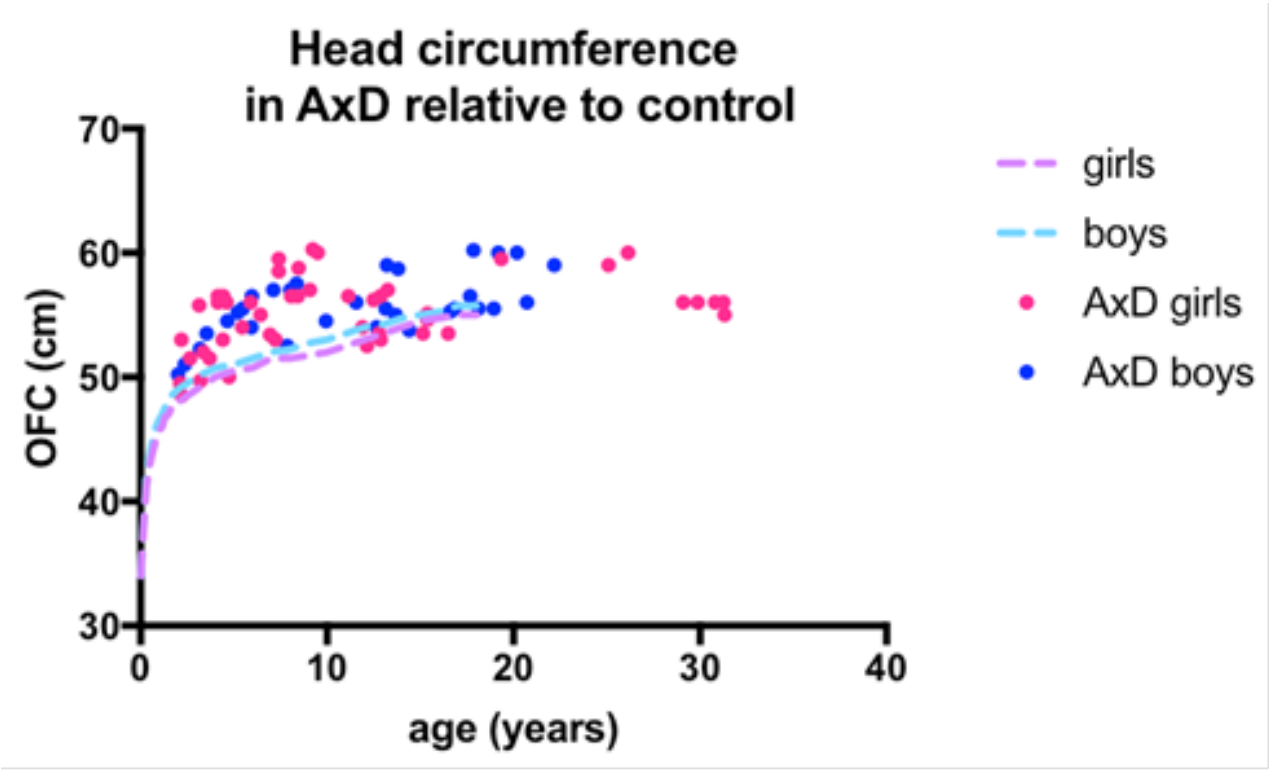

GFAP levels by disease duration



# GMFM-88 by age, MRI

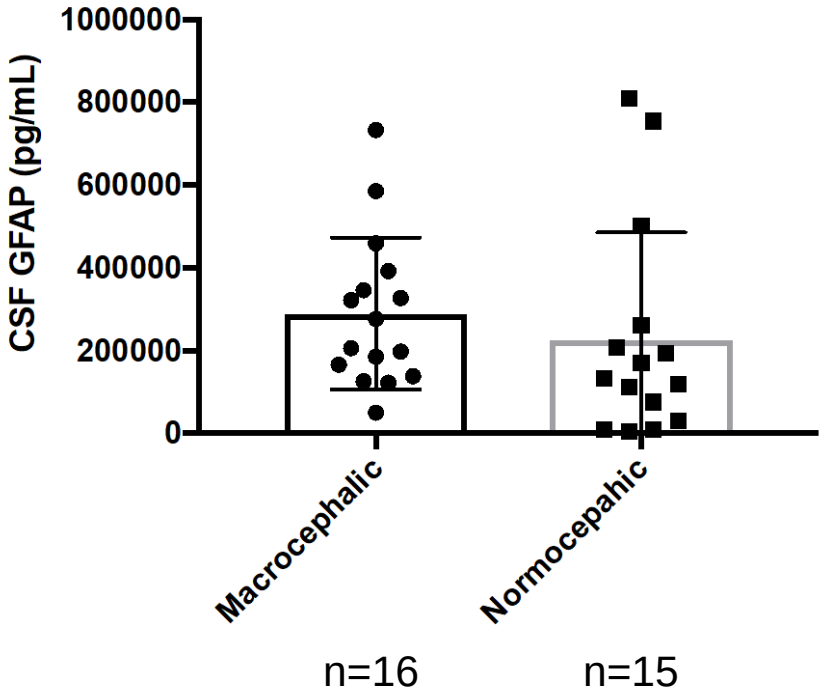


# Alexander disease, macrocephaly



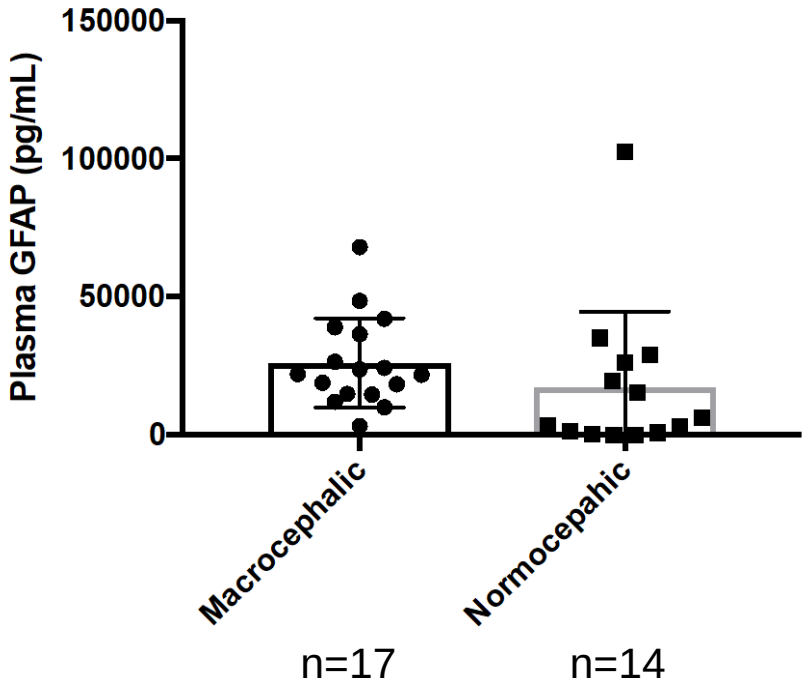
# Macrocephaly

## Macrocephaly: GFAP




n=16 n=15

Unpaired t-test P=0.4340




n=17 n=14

Unpaired t-test P=0.2767

# Alexander Disease

## Variable clinical classification systems and disease subtypes are a challenge

### Age-based

- Neonatal (1%) – Seizures, hydrocephalus, severe motor and intellectual disability
- Infantile (42%) – Progressive psychomotor retardation with loss of milestones, megalencephaly, frontal bossing, seizures
- Juvenile (22%) – Bulbar/pseudobulbar signs, ataxia, gradual loss of intellectual function, seizures, breathing difficulty
- Adult (33%) – Bulbar/pseudobulbar signs (palatal myoclonus, dysphagia, dysphonia, dysarthria), spasticity, ataxia, sleep apnea, gait disturbance, cerebellar signs

### Phenotype-based

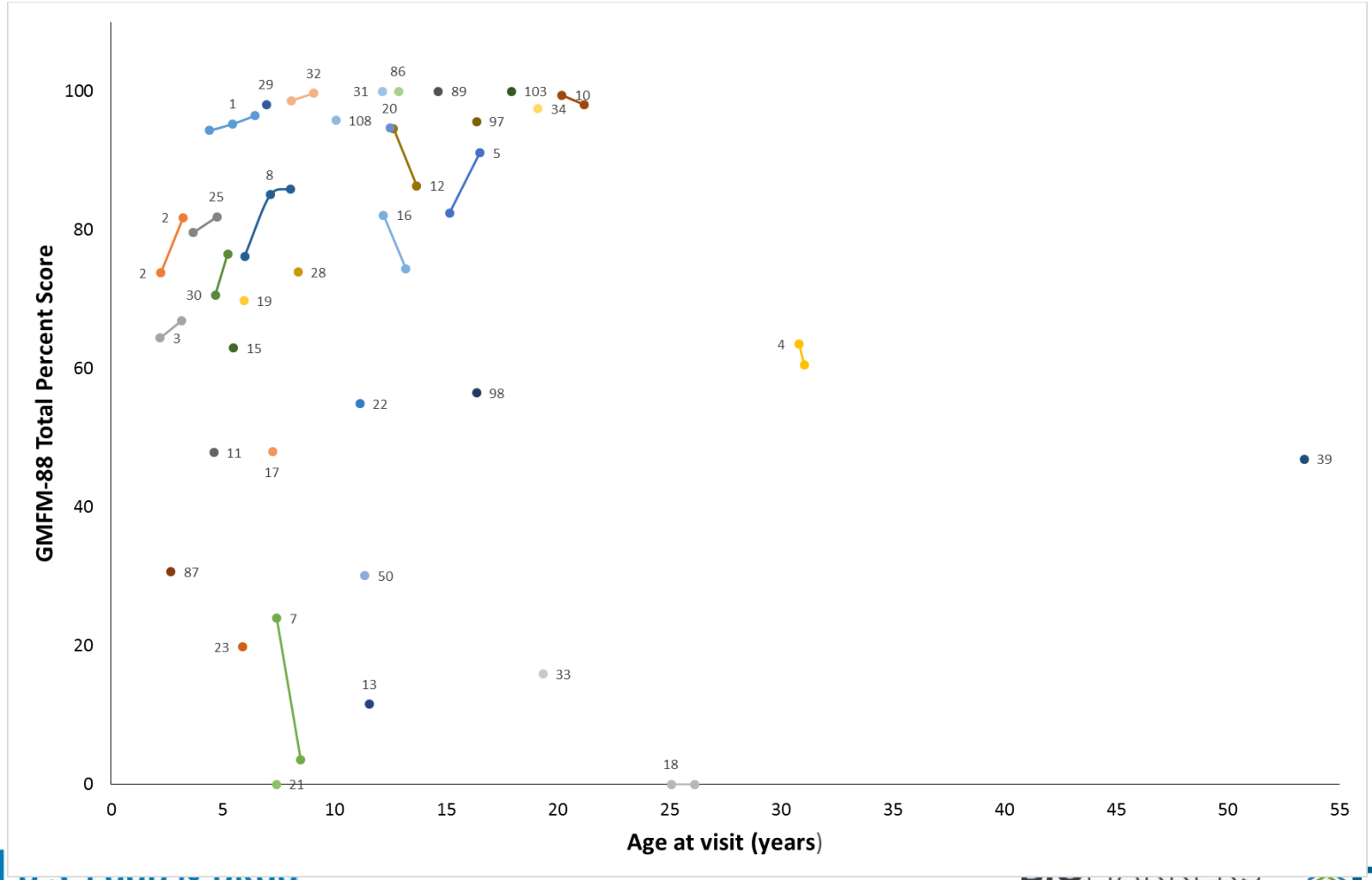
| Type I AxD | Type II AxD |
|---|---|
| Early age at onset (often before 4 years) | Manifests across the lifespan |
| Seizures | Autonomic dysfunction |
| Macrocephaly | Bulbar symptoms |
| Encephalopathy | Ocular movement abnormalities |
| Paroxysmal deterioration | Palatal myoclonus, |
| Failure to thrive | Often negative for neurocognitive or developmental deficits |
| Developmental delay | Atypical radiologic features |
| Classic radiologic features[10] | |


1. Srivastava S, Naidu S. Alexander Disease. 2002 Nov 15 [Updated 2015 Jan 8]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1172/
2. Prust M, Wang J, Morizono H, et al. GFAP mutations, age at onset, and clinical subtypes in Alexander disease. Neurology 2011;77:1287.

# AlexandER DISEASE

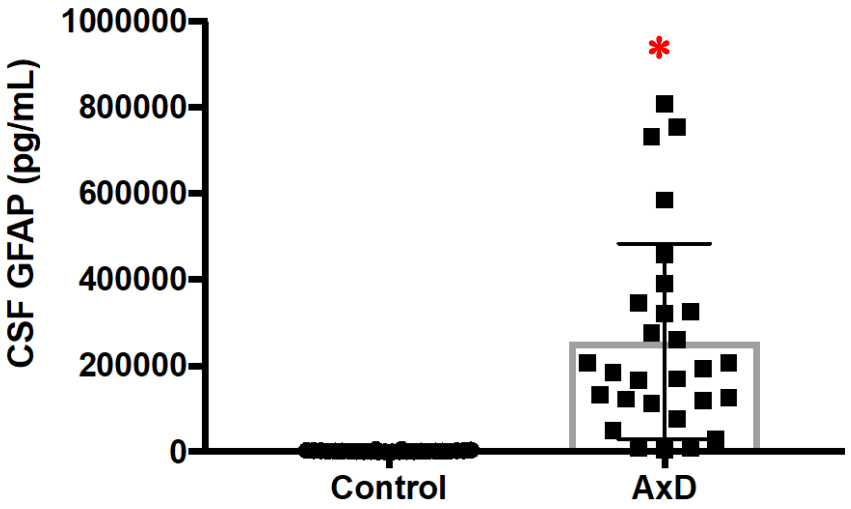
| Classification | Testing |
|---|---|
| Gross motor | • Gross Motor Function Measure (GMFM-88)<br>• Peabody Developmental Motor Scales (PDMS-2)<br>• Bruininks-Oseretsky Test of Motor Proficiency (BOT™-2)<br>• Berg Balance scale<br>• 6-minute walk |
| Fine motor | • Peabody Developmental Motor Scales (PDMS-2)<br>• Bruininks-Oseretsky Test of Motor Proficiency (BOT™-2)<br>• Functional Dexterity Test<br>• 9-hole Peg Test |
| Speech and language | • Rosetti Infant-Toddler Language Scale<br>• Preschool Language Scale 5<br>• Clinical Evaluation of Language Fundamentals® (CELF®-5)<br>• Peabody Picture Vocabulary Test<br>• Goldman Fristoe Test of Articulation-3<br>• Khan-Lewis Phonological Analysis<br>• CHOP speech classification |
| Swallowing | Custom evaluation that assesses safety and efficiency with thin liquids, purees, and solids |
| Other | Pediatric Evaluation of Disability Inventory Computer Adaptive Test (PEDI-CAT) |

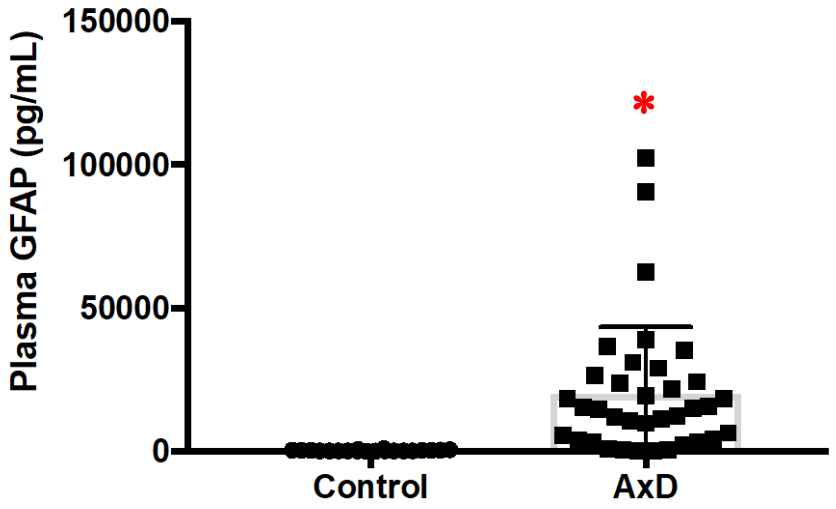
# ALEXANDER DISEASE



# Simoa GFAP assay results

## CSF vs Plasma




n=28 n=26

n=36 n=20

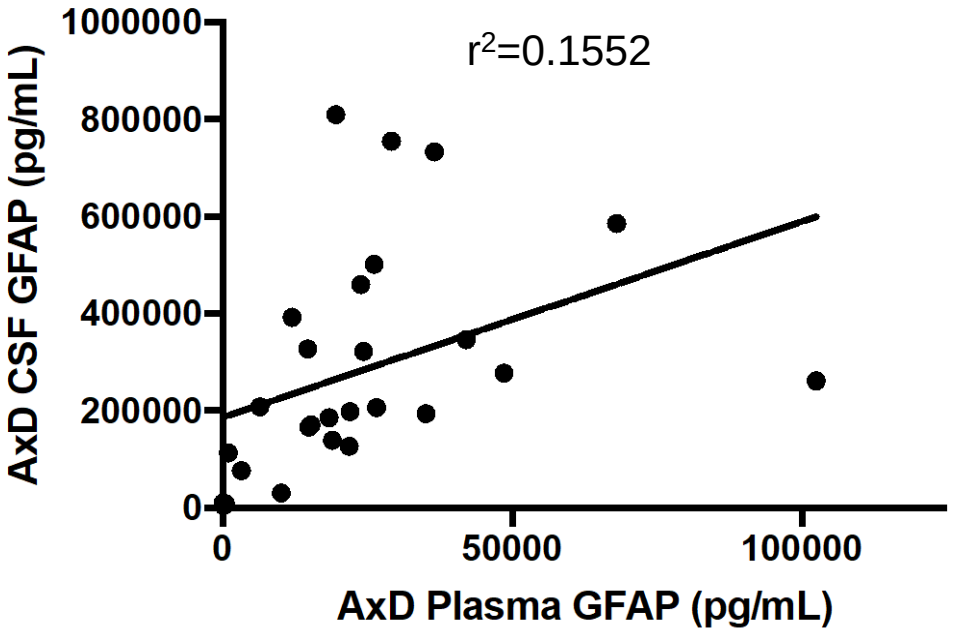
P<0.0001

P<0.0001

*t*-tests, Mann-Whitney test

# Simoa GFAP assay results

## CSF vs Plasma

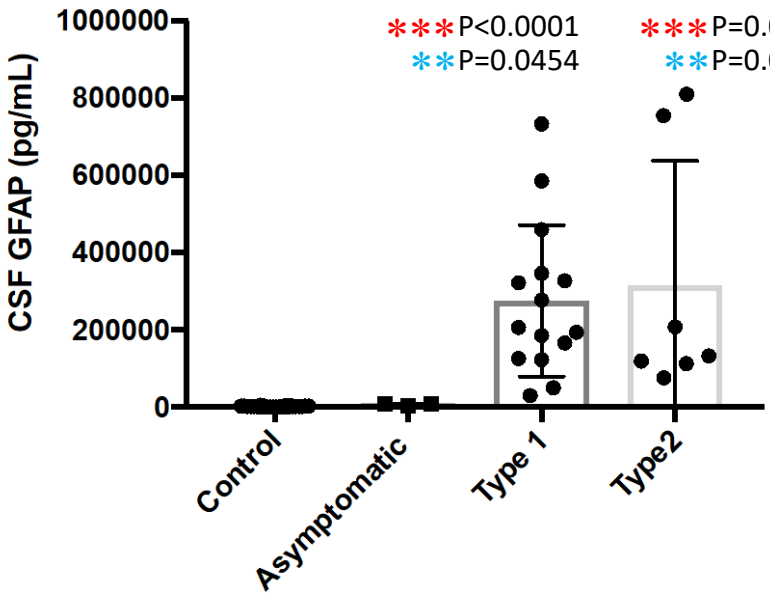


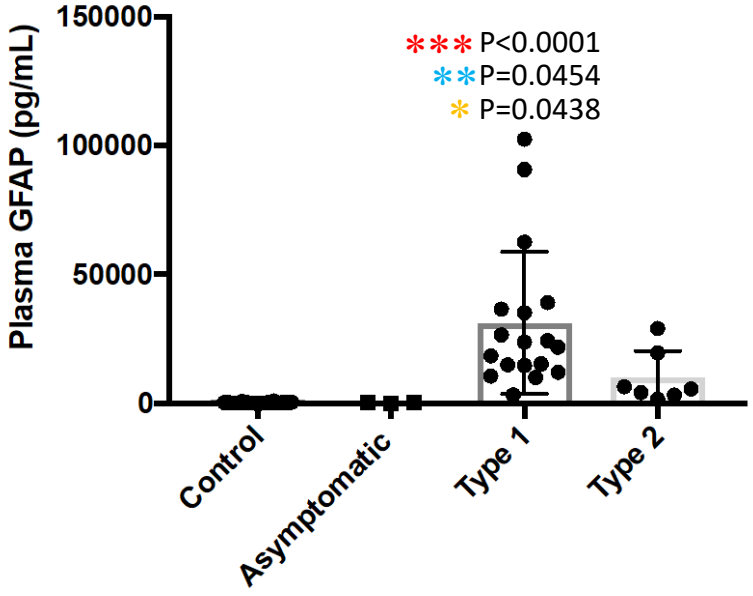

n=27

Correlation *P=0.0420

# Simoa GFAP assay results

## By AxD Clinical Type



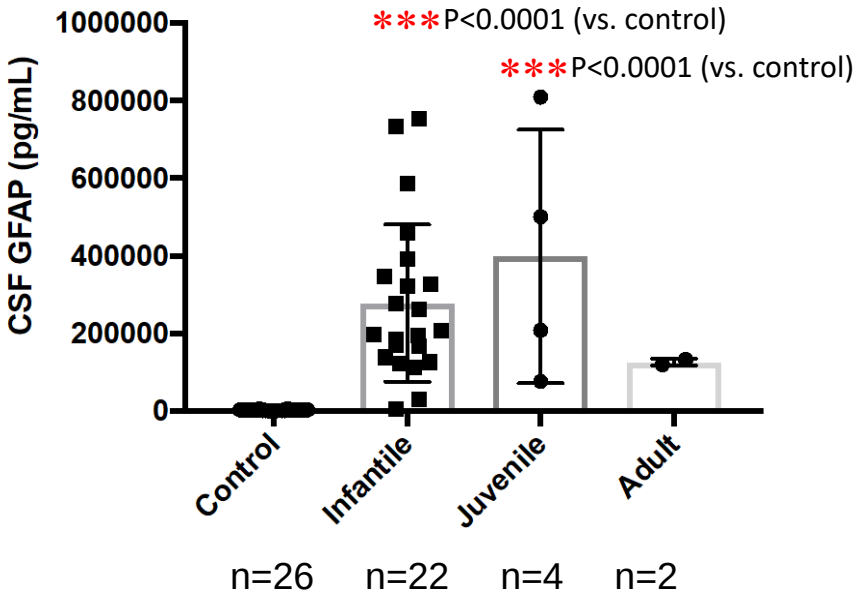

n=26 n=3 n=15 n=7

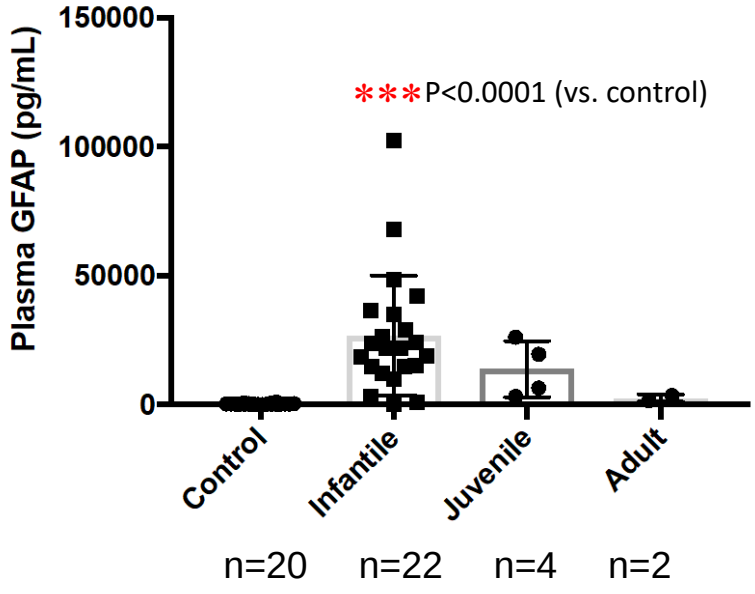
n=20 n=3 n=18 n=7

One-Way ANOVA multiple comparisons
(*** vs. controls, ** vs. Asymptomatic, * Type 1 vs. Type 2)

# Simoa GFAP assay results

## Age of Onset




One-Way ANOVA multiple comparisons