Gene Therapy Drug Development for Ultra-Rare Disease: Challenges & Opportunities

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grace science

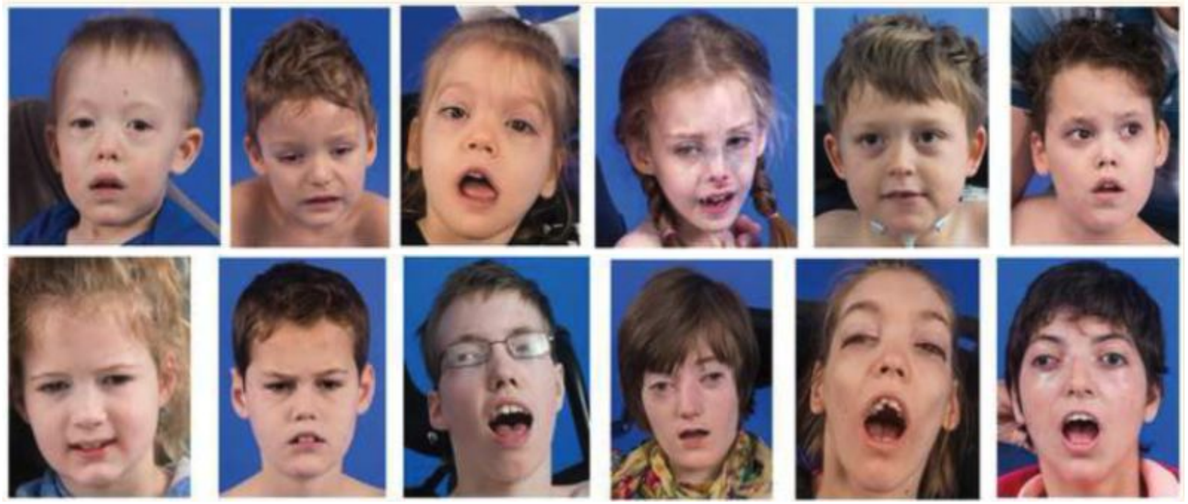
N-glycanase-1 (NGLY1) Deficiency is a Severely Debilitating, Ultra-rare Disease with Limited Life Expectancy and No Approved Therapy

Core phenotypes

1. Global developmental delay and / or intellectual disability
2. A hyperkinetic movement disorder
3. Transient elevation of transaminases
4. (hypo)alacrima
5. Length-dependent sensorimotor neuropathy

Additional phenotypes

- Failure to thrive
- Cerebral atrophy and acquired microcephaly
- Epilepsy
- Ophthalmologic symptoms
- Feeding difficulty with oromotor dysfunction
- Constipation
- Hepatomegaly
- Hypocholesterolemia
- Muscle atrophy
- Joint contractures



Average Age of Diagnosis

6.5 years (GSF database information on 73 patients)

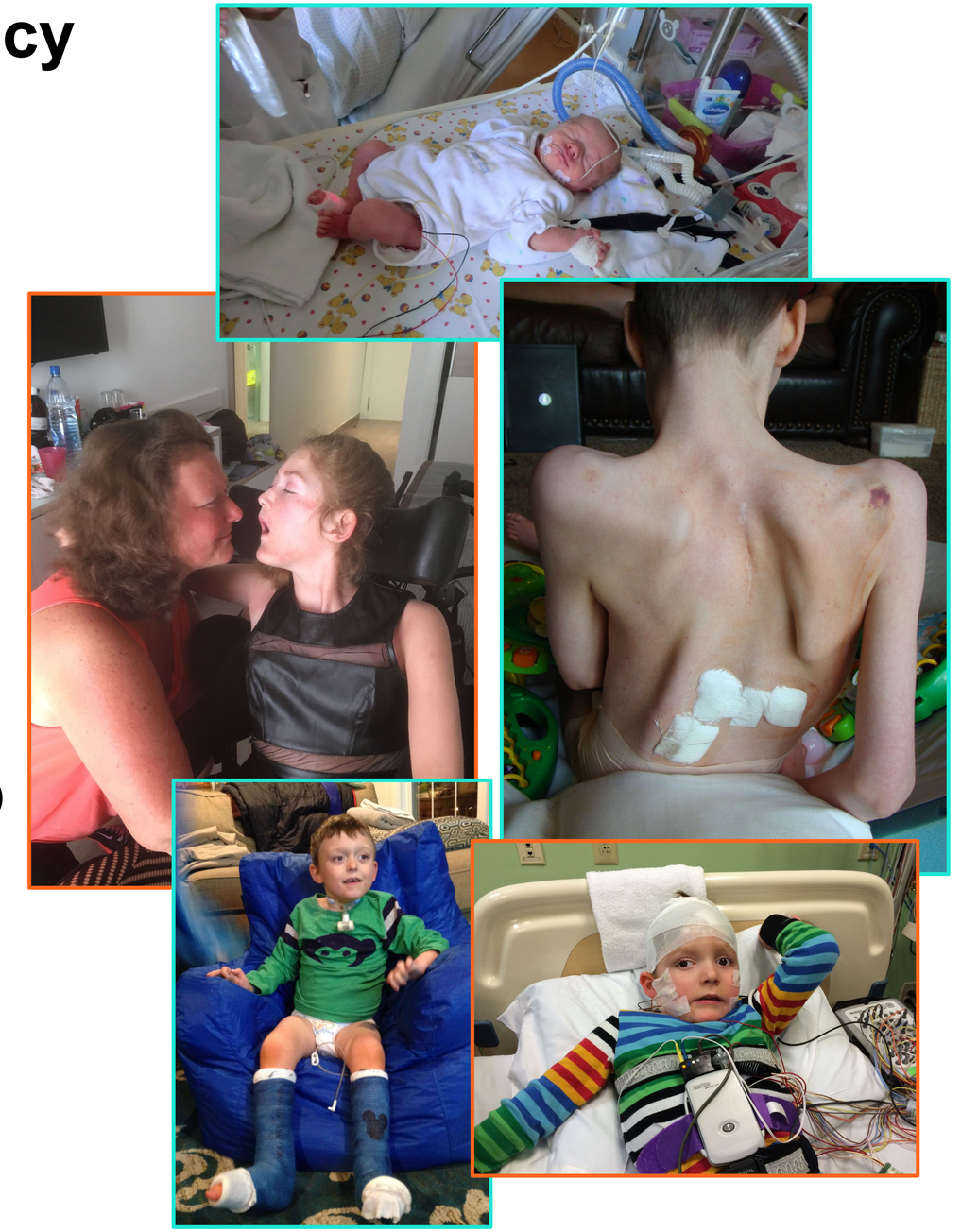
Mean Age of Death for 15 patients in the GSF database

15.9 years (Median: 17 years)

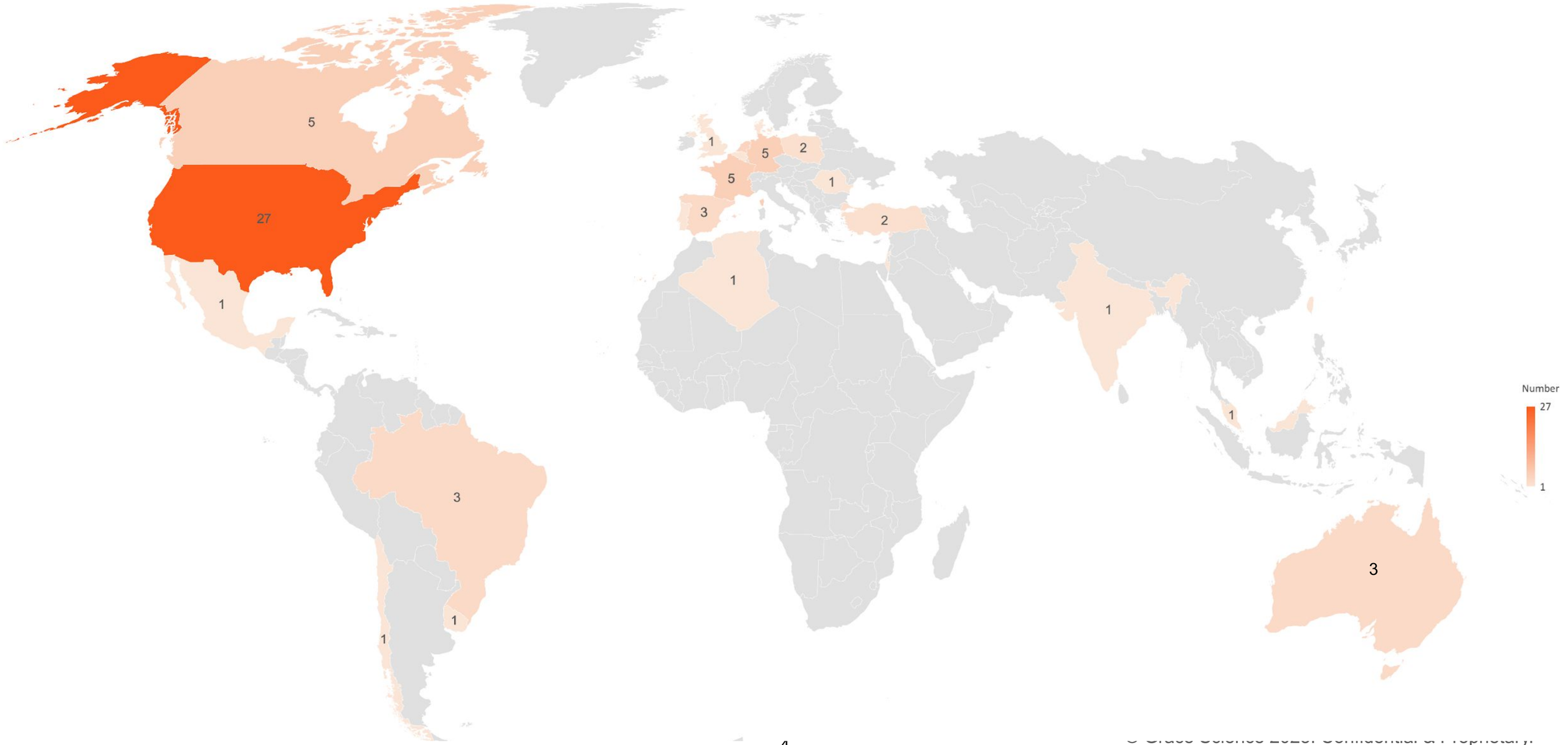
Methods of Diagnosis: Whole Exome Sequencing (WES), Whole Genome Sequencing (WGS), GNA biomarker analysis, symptomology

The Devastating Nature of NGLY1 Deficiency

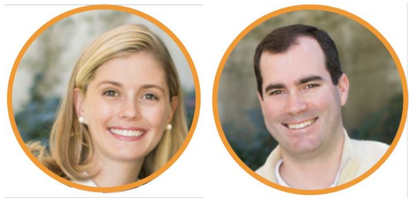
- NGLY1 requires constant daily care for all aspects of living (feeding, bathing, toileting)
- Most patients cannot walk unassisted
- Nearly all patients are non-verbal
- Physical, occupational, and speech therapy are necessary to help with physical issues and lack of speech
- Many patients require a G-tube to maintain adequate nutrition
- NGLY1 patients often present with medical issues that require frequent hospitalization or surgery
- Hospitalization: seizures, infections (pneumonia, urinary tract infections)
- Surgeries: Spinal fusions, inguinal hernias, tracheostomies, hand surgeries, femoral osteotomies, foot surgeries, and G-tubes
- Shortened life expectancy with a median age of death of ~15 years



There are ~100 Known Patients Living with NGLY1 Deficiency Worldwide



Grace Science, LLC Co-founded by Matt Wilsey and Nobel Laureate, Dr. Carolyn Bertozzi, to Develop Treatments based on NGLY1 Biology



Kristen & Matt Wilsey create the Grace Science Foundation (GSF) to find a cure for NGLY1 Deficiency

Matt Wilsey and Nobel Laureate Carolyn Bertozzi co-found Grace Science, LLC to bring treatments for NGLY1 Deficiency & other diseases to the clinic

FDA Orphan drug designation

FDA Rare pediatric disease designation

Nonclinical POC studies related to GS-100

GS-100 IND Enabling Studies

2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023

First GSF conference in Palo Alto, CA USA

GSF brings researchers & patients from around the world & establishes a biobank & patient registry

Natural history study begins at Stanford, following 29 patients for up to 3 years

Pilot movement disorder study begins at Baylor, followed by two-year natural history study

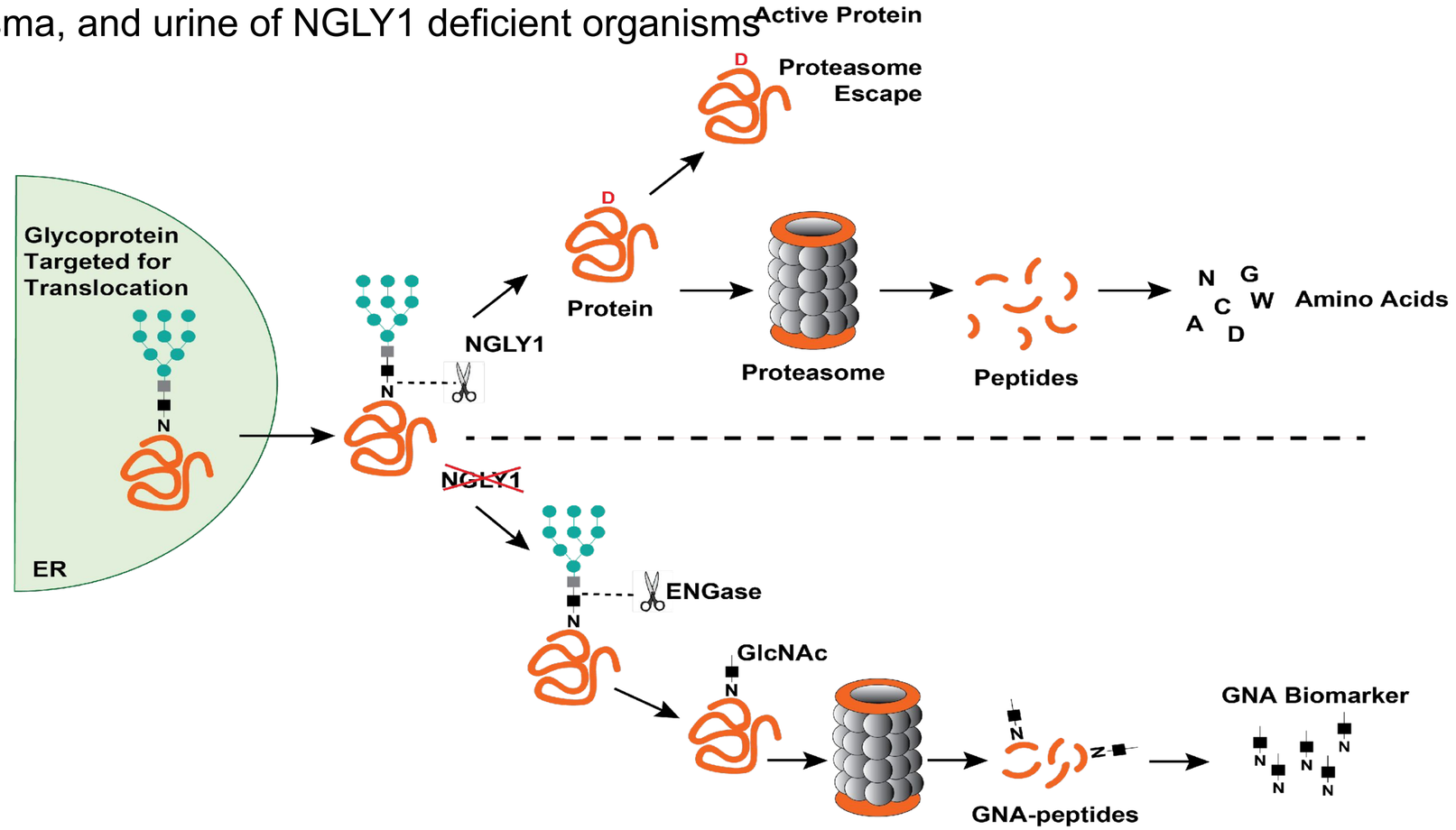
5

The Grace Science story is exemplary of many of the challenges and opportunities ultra-rare disease companies face

https://www.youtube.com/watch?v=EQES3qynVKU

NGLY1 Plays a Pivotal Role in the Degradation of Glycoproteins

- NGLY1 is a cytosolic enzyme that cleaves N-glycans from misfolded glycoproteins facilitating their degradation by the proteasome
- The absence of NGLY1 disturbs proteostasis and results in the accumulation of cytoplasmic ubiquitinated proteins
- The alternate cleavage of N-glycans leads to the accumulation **GNA (N-acetylglucosamine-asparagine; GlcNAc-Asn)** in CSF, plasma, and urine of NGLY1 deficient organisms



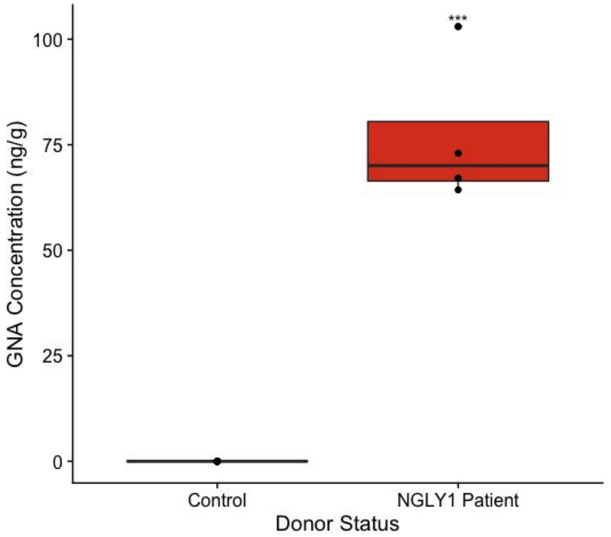
- GNA can be detected in the CSF and plasma of NGLY1 Deficiency patients
- GNA is a primary disease activity biomarker (PDAB)

Mueller et al, 2022 J. BioChem

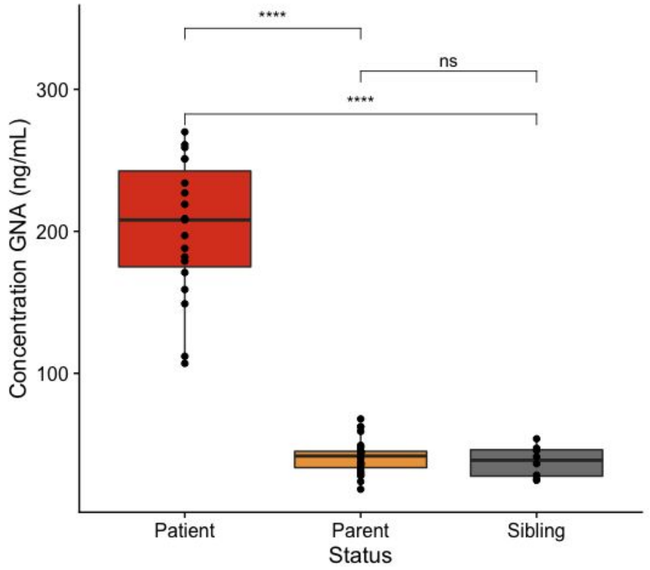
GNA is a Primary Disease Activity Biomarker (PDAB*) that is Increased in the Plasma and CSF of NGLY1 Deficiency Patients and in the Rat Model of Disease

NGLY1 Deficient Patients

CSF

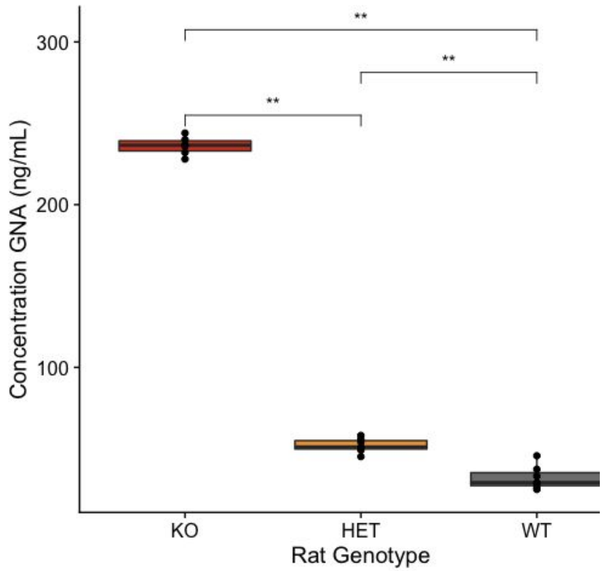


Plasma

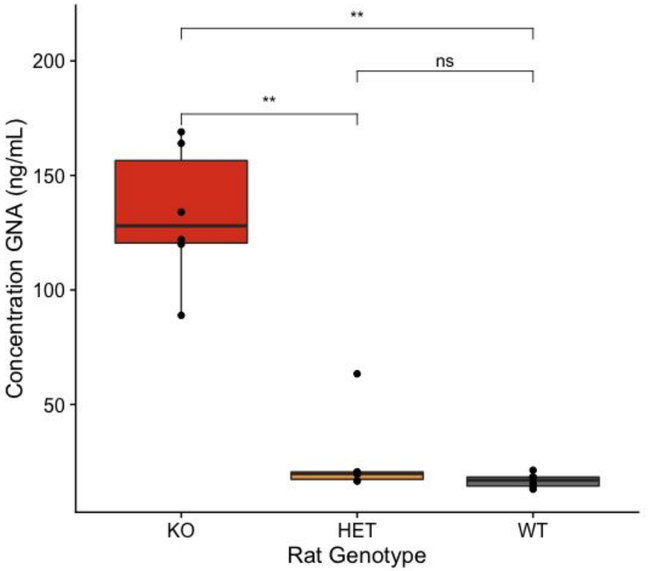


Ngly1 Deficient Rats

CSF



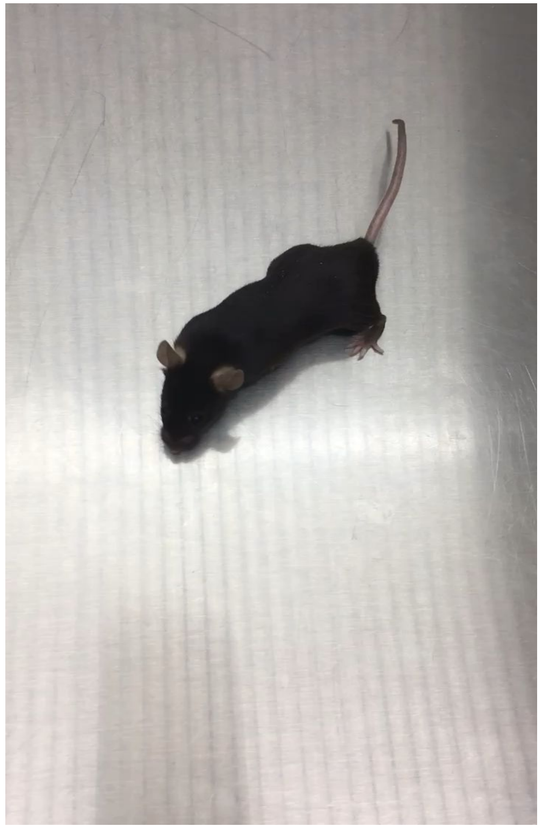
Plasma



*Kakkis et al, 2022 Molecular Genetics & Metabolism
Data from Mueller et al. 2022

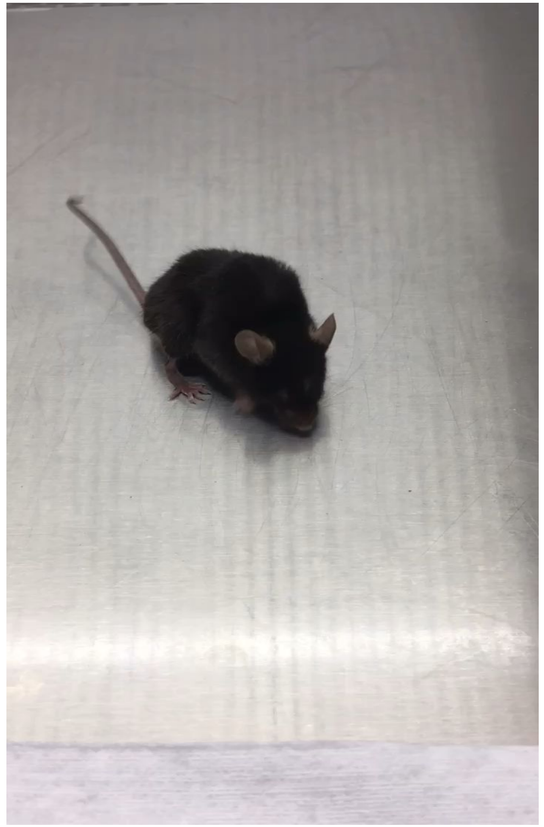
Ngly1 Deficient Rodents Have Severe Neurological Symptoms

Wild-Type



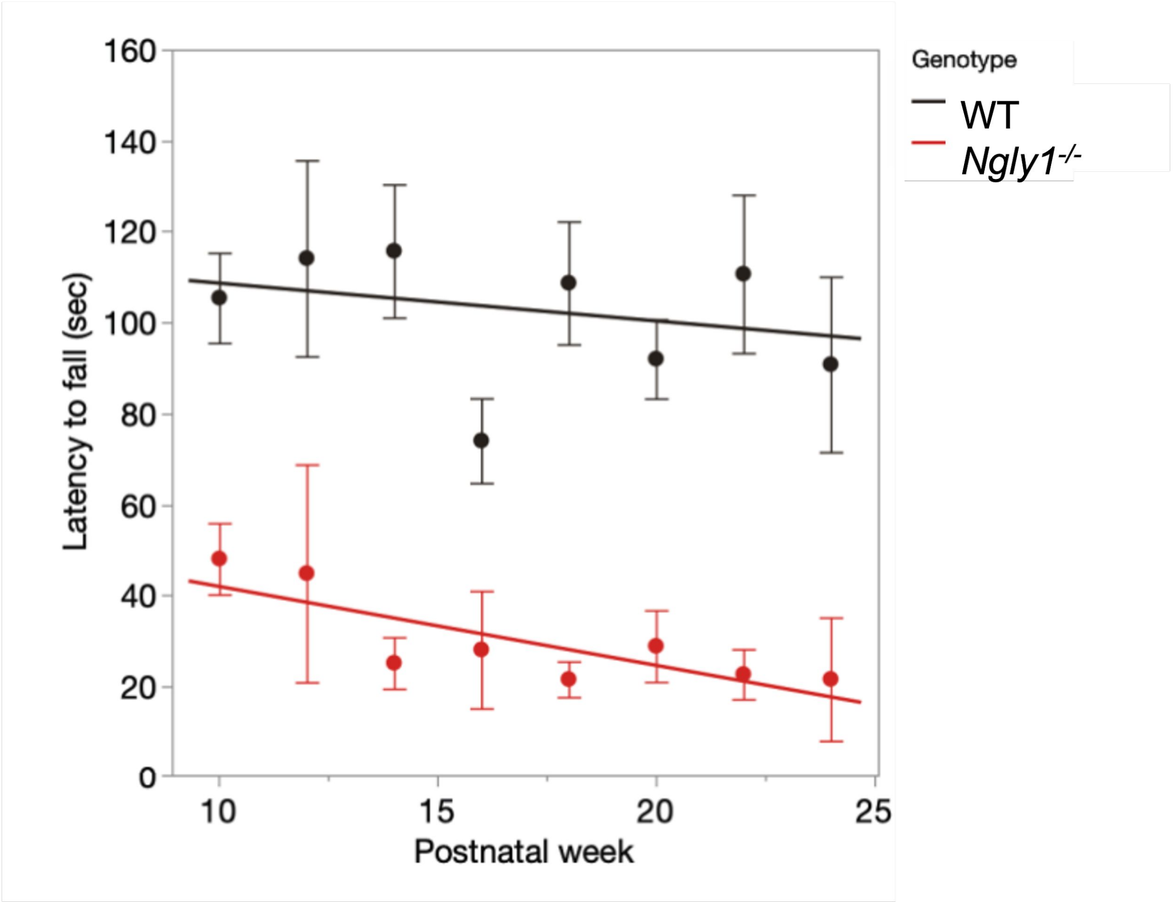
- Fluid movement
- Rapid acceleration
- Exploration

Ngly1 Deficient



- Gait abnormality
- Hind limb drag
- Tremor

Rotarod Performance Impaired in NGLY1 Deficient Rats



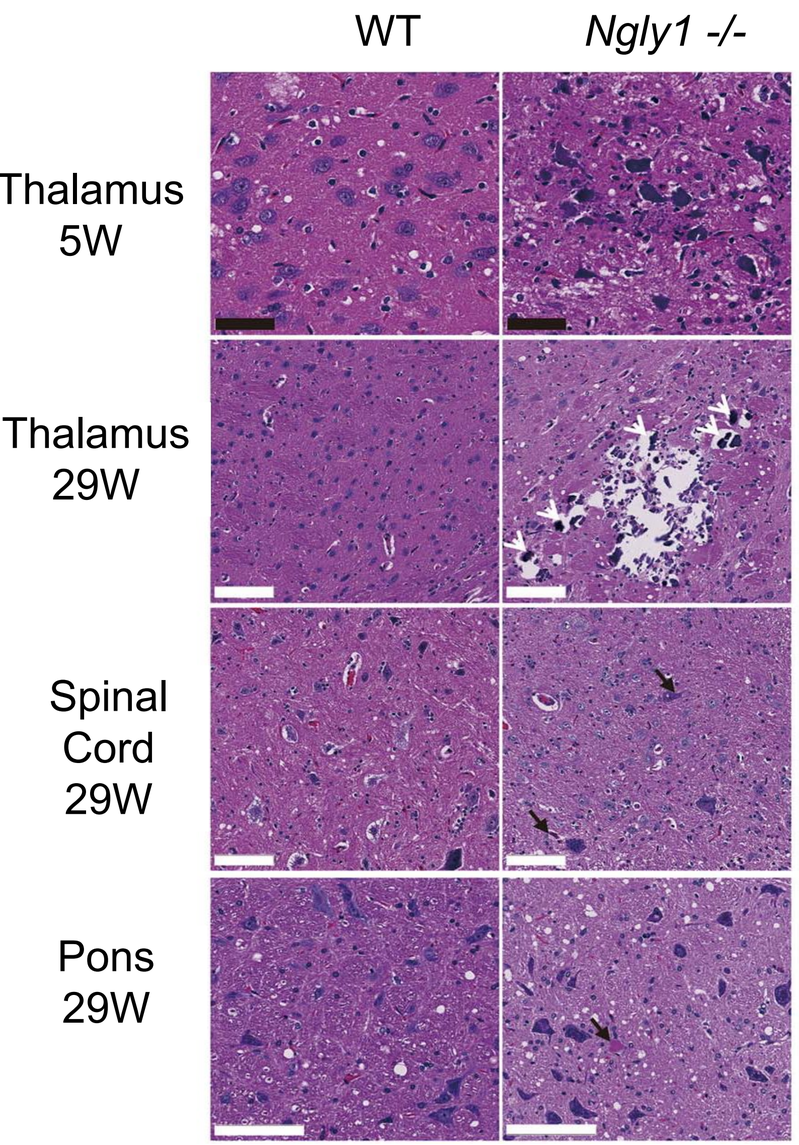
Zhu et al., 2022

Movies provide by Kun Yang at UTSW (GSF Researcher)

NGLY1 Deficient Phenotypes in Humans vs a Rat Model of the Disease

NGLY1 Deficiency Phenotypes in Humans	Ngly1 Deficient Phenotypes in *Ngly1* $^{-/-}$ Rats
Failure to thrive	Reduced survival and fitness
Gait abnormalities Hypotonia Peripheral neuropathy	Gait abnormalities Motor neuron function impairment Axonal degradation in the spinal cord, sciatic nerves, and dorsal root ganglia (DRG)
Hyperkinetic movements Seizures	Neurological impairments Seizures
Intellectual disability Delayed cognitive development Lack of language development	Impaired spatial learning Neurodegeneration
Increased GNA in CSF and plasma	Increased GNA in CSF and plasma

Ngly1 Deficiency Causes Neurodegenerative Pathology



Asahina et al., 2020

Disease-specific pathology in Ngly1 deficient rodents (Zhu et al., 2022)

- Brain: necrotic lesions, mineralization, eosinophilic bodies, astrogliosis, microgliosis, and severe loss of neurons in the thalamus
- Brain regions: thalamus, hippocampus, medulla oblongata, cerebellum, pons
- Sciatic nerves: axonal degeneration
- DRG: necrosis of nerve cell bodies, degeneration of nerve fibers, infiltration of mononuclear cells

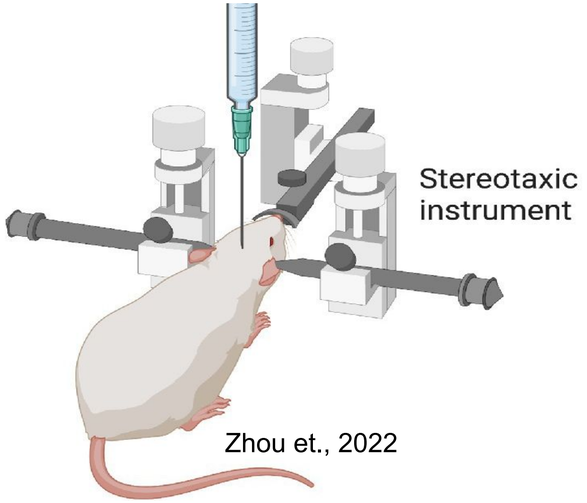
NGLY1 Deficiency patient autopsy pathology (Stuut et al., 2021)

- Eosinophilic cytoplasmic inclusions in thalamus, spinal cord, DRG
- Purkinje cell loss

GS-100 Vector Construct and Route of Delivery

GS-100

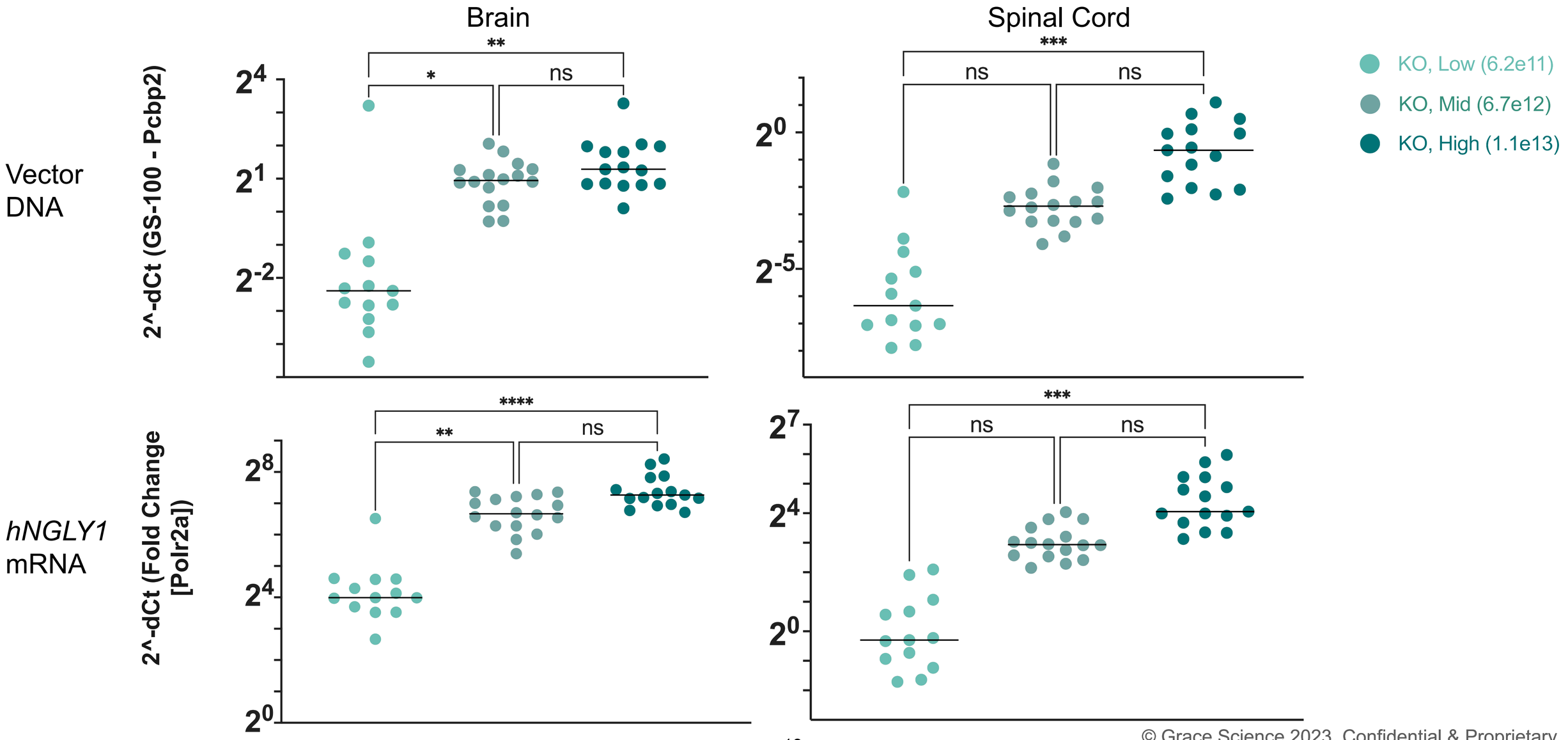
- Recombinant, single-stranded AAV9 vector
- Encodes a codon-optimized full-length version of hNGLY1
- hNGLY1 expression under control of a CAG promoter



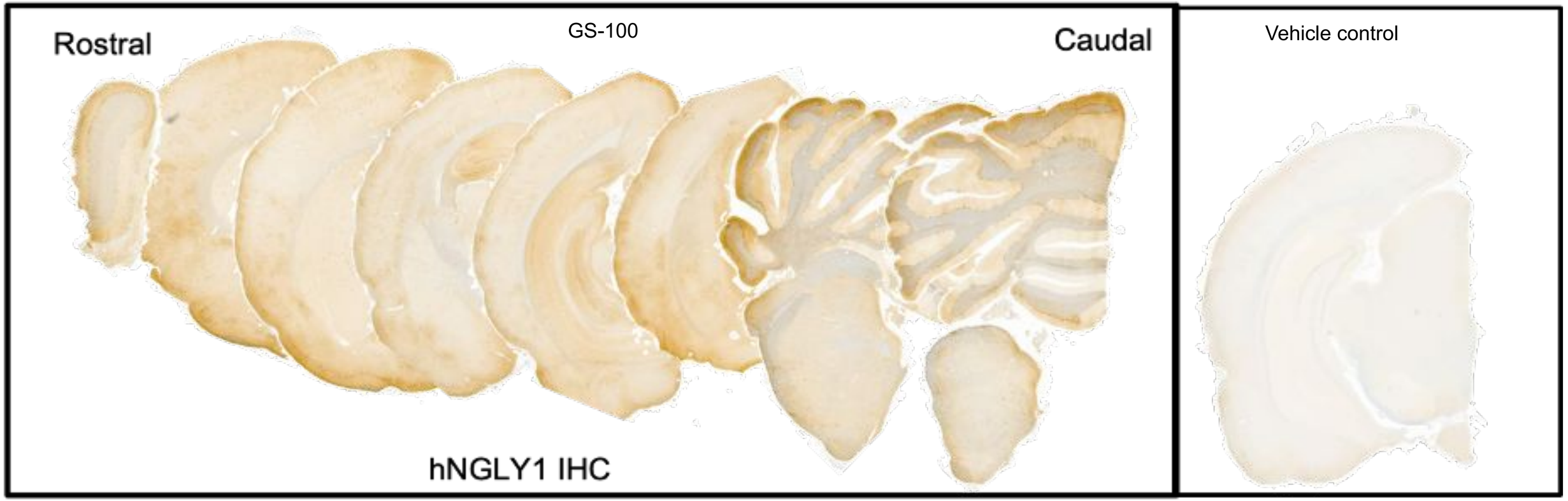
Intracerebroventricular (ICV) Delivery Selected as Route of Administration for GS-100

- NGLY1 Deficiency is primarily a disease of the CNS, with the brain being greatly impacted by neurodegeneration
- In nonclinical studies in the rat, ICV administration resulted in improved motor function and a reduction in GNA biomarker levels in the brain, whereas IV administration did not result in similar improvements
- ICV administration delivers the drug in proximity to the areas of the brain most affected by NGLY1 Deficiency (e.g., thalamus)
- ICV administration is commonly used for delivery of pediatric therapies including ERT, antibiotics, and chemotherapeutics, as well as some gene therapies

Dose-dependent Vector Biodistribution and *hNGLY1* mRNA Expression in Ngly1 Deficient Rats

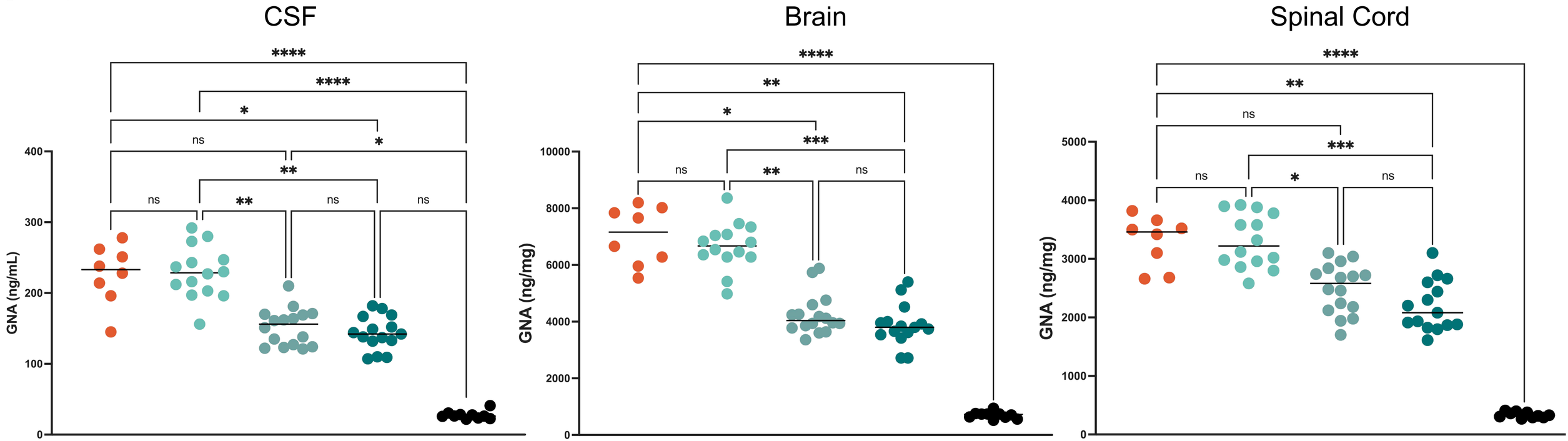


ICV Delivery of GS-100 Results in Widespread hNGLY1 Protein Expression in Ngly1 Deficient Rat Brain

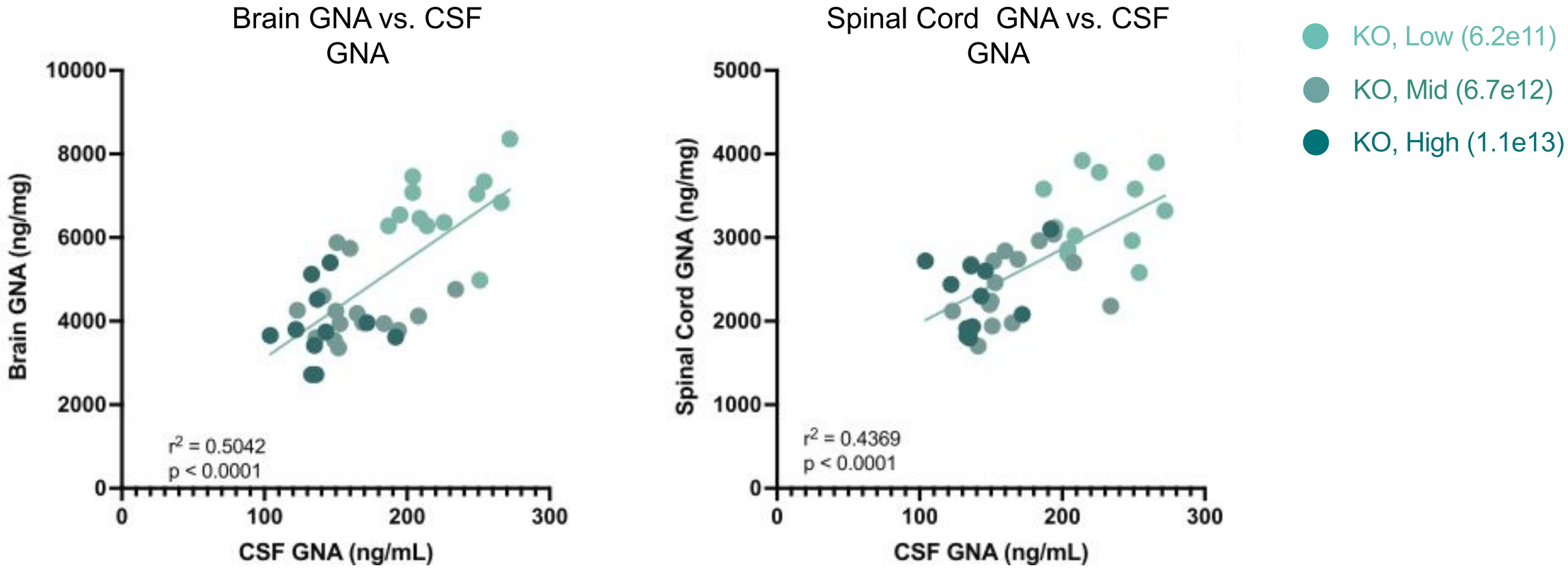


Dose-dependent GNA Biomarker Reduction in Ngly1 Deficient Rats

- KO, Vehicle
- KO, Low (6.2e11)
- KO, Mid (6.7e12)
- KO, High (1.1e13)
- WT, Vehicle

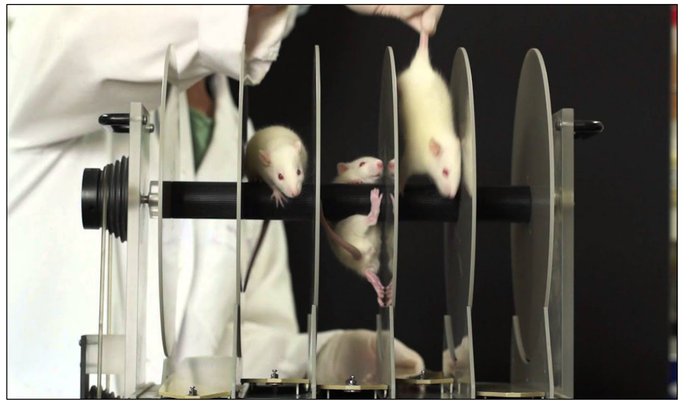


CSF GNA Levels Correlate with GNA Levels in Brain and Spinal Cord Tissue

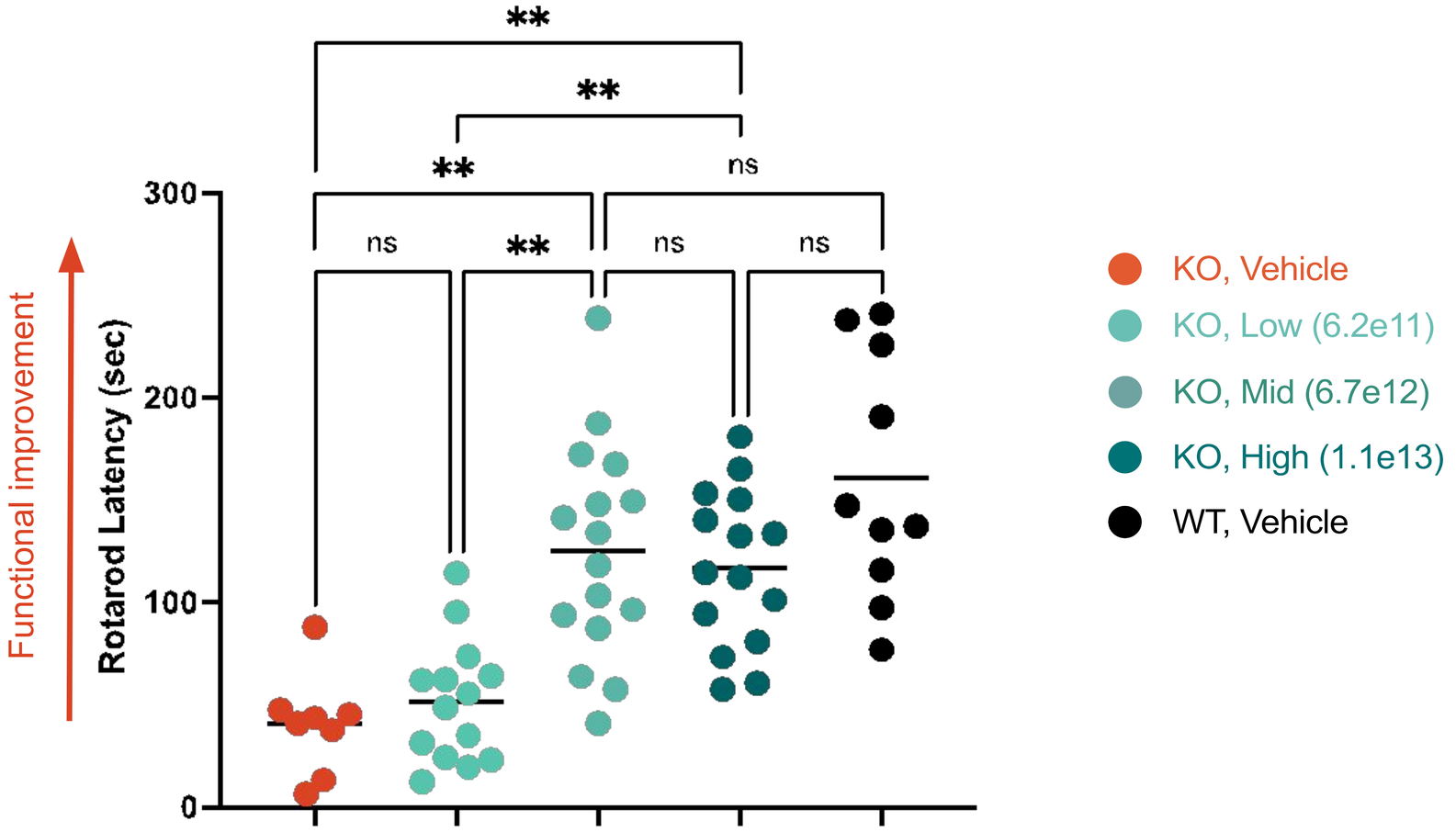


- GNA is a pharmacodynamic substrate biomarker for GS-100 activity and a primary disease activity biomarker (PDAB)
- CSF GNA levels can serve as a surrogate for brain tissue GNA levels in the clinic

ICV Delivery of GS-100 Restores Motor Function in the Rat Model of NGLY1 Deficiency to Near Wild-type Levels

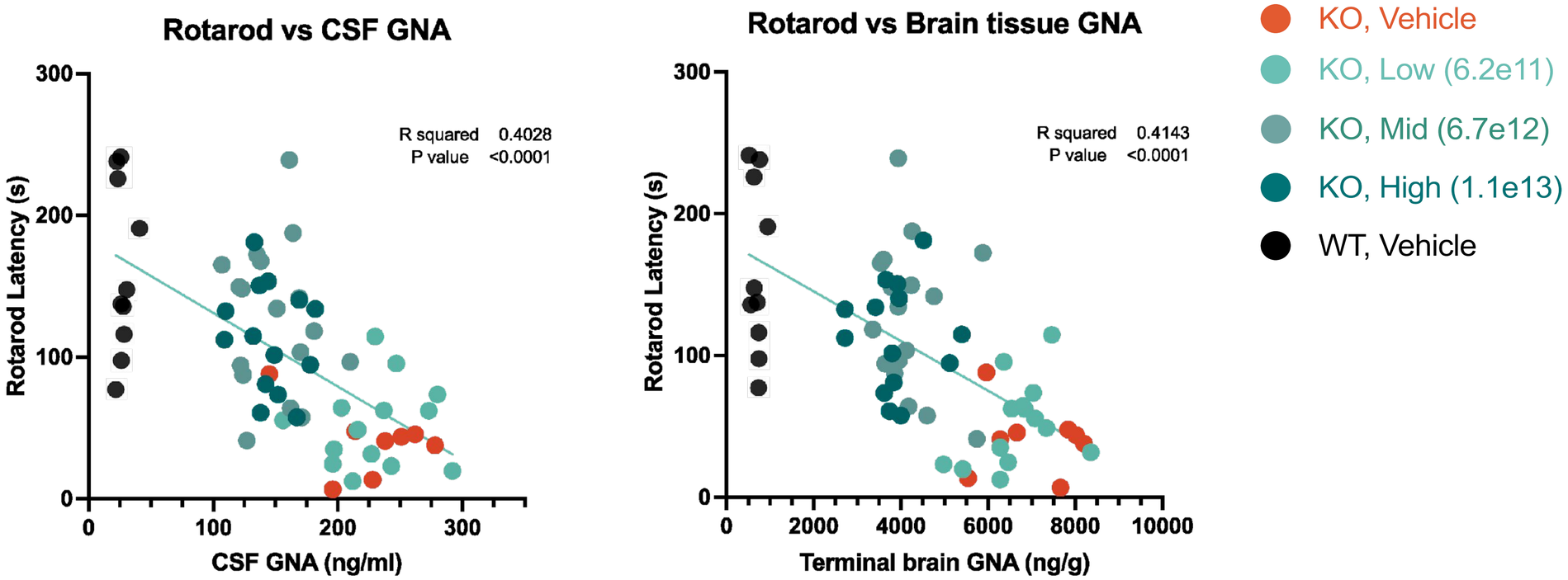


Measuring latency to fall from an accelerating, spinning rotarod



GNA Reduction in the CSF and Brain Correlate with Motor Improvement

- Supports the use of CSF GNA levels as a primary disease activity biomarker (PDAB) that could be used as surrogate for functional endpoint improvement in the clinic



GS-100 Conclusions

- GS-100 treatment resulted in widespread, dose-dependent vector biodistribution, *hNGLY1* mRNA, and hNGLY1 protein expression across the brain and spinal cord that correlated with GNA biomarker reduction and motor function improvement.
- The strong correlation between CSF GNA reduction and functional improvement in the Ngly1 deficient rats suggests GNA will be a useful predictive pharmacodynamic biomarker of clinical benefit in the GS-100 clinical trial.
- GS-100 was well-tolerated with no drug-related systemic toxicities detected in the *Ngly1* deficient rats.
- Dose-dependent, mild to moderate neuronal cell loss was detected in the GS-100 treated rats close to the site of injection, likely due to the spatial constraints of injecting into the 28-day old rat brain.
- These findings were not associated with worsened functional outcomes and are not expected to occur in the clinic when the drug is administered by slow infusion through a catheter placed into the much larger human ventricular space.
- FDA requested a larger animal study to support the first-in-human clinical trial.
- An NHP study was conducted in response. Study completed in June with no drug-related deaths or abnormal clinical chemistry or macroscopic findings. The histopathological analyses are currently ongoing.

Challenges of Ultra-Rare Disease Drug Development

- **Patient availability and recruitment**
  - Enrollment - finding enough patients who fit inclusion and exclusion criteria for a particular trial
  - Accurate diagnosis - patients are often misdiagnosed or not provided a diagnosis for multiple years
  - Patient location - often globally dispersed requiring multi-national trials and challenging travel

- **Designing and evaluating clinical trials**
  - Disease characterization - limited NHS data can lead to poor understanding of disease heterogeneity and progression
  - Statistical analysis - heterogeneous manifestations of disease and phenotypic presentations make analyses challenging
  - Endpoint selection - lack of consensus on clinical outcome measures and poorly defined, unvalidated endpoints
  - Comparator group - placebo control is often impractical, if not impossible, and NHS comparative data is often sparse
  - Patient burden - designing rigorous trials that simultaneously allow enough flexibility to limit patient burden is challenging
  - Multiple regulatory regions - harmonizing trial design across countries is challenging due to differing regulations & standard of care

- **Regulatory approval and payer reimbursement**
  - Collecting and communicating evidence that is convincing and compelling to regulators and payers is challenging due to limited data and inability to run traditional randomized, placebo-controlled clinical trials

AAV Manufacturing Challenges for Ultra-Rare Disease

- **High Cost**
  - Astronomical cost of goods (COGs) – raw materials, extensive testing, FTEs, service costs
  - Limited commercial opportunity due to small number of patients

- **Process Development (PD) and GMP Manufacturing**
  - Available processes are complex and not standardized across the field
  - PD studies are expensive and time-consuming
  - Tendency to stay with sub-optimal status quo because innovation comes with risk and cost
  - Challenging to determine critical process parameters with limited number of batches

- **Analytical Characterization**
  - Challenges with assay development / validation timelines, accuracy, reproducibility
  - No one-size-fits-all standardized testing approach
  - Phase-appropriate challenges for ultra-rare disease when first study may be the pivotal study used for registration
  - Large drug volumes needed for testing reduces amount of drug product available for patients and drives up cost
  - Poor consensus in the field on critical quality attributes (what is truly meaningful to clinical safety and product quality)

- **Comparability**
  - Especially challenging with limited number of batches needed for rare disease clinical trials

Opportunities Created by Rare Disease Drug Development

- The unique challenges require creative problem solving that drive rapid innovation
- Has led to streamlined processes and specialized regulatory pathways for rare disease
  - e.g. Fast Track designation, Priority Review, Accelerated Approval, etc.
- US Orphan Drug Act of 1983 defined rare disease and provided companies with financial incentives to pursue drug development for rare diseases
- Spurred the formation of specialized funding programs and infrastructure
  - e.g. Bespoke Gene Therapy Consortium, NIH Rare Disease program, multiple rare disease grants and funding opportunities, etc.
- Patient advocacy, media coverage, disease severity, etc. drive a passionate call to action and sense of urgency that has created a strong push for new, innovative approaches
- Opportunity to share information freely when there is not a direct conflict of interest

Opportunities for Expediting and Reducing Costs for Ultra-Rare Disease Drug Development by Sharing Information

- Regulatory agencies and companies publish meta-analyses across AAV tox studies
  - Objective: Help the field identify and differentiate between expected AAV class effects, species-specific effects, and drug-specific toxicities that require additional non-clinical investigation

- AAV Manufacturing Collaboration and Information Sharing
  - More open collaboration and information sharing between CDMOs, sponsors, and Regulatory Agencies
  - Publish meta-analyses and white papers that share blinded AAV batch information across programs
  - Objective: Help focus the field on the critical quality attributes that require continued testing, define standard residual and impurity ranges, define expected AAV stability ranges, etc.

Acknowledgements

NGLY1 Deficiency patients & families

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