# Characterization of β-glucuronidase for enzyme replacement therapy in DYT6 Dystonia

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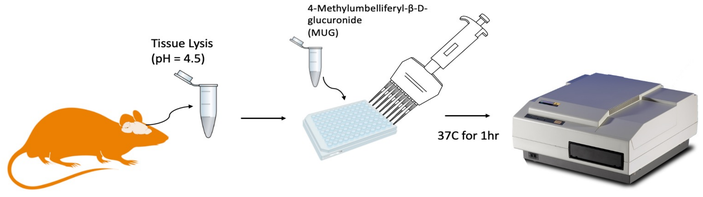
## Introduction

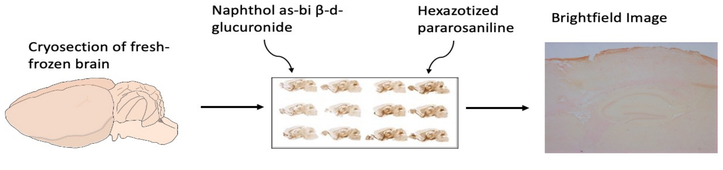
- Dystonia is a debilitating disorder defined by sustained involuntary twisting movements
- Loss of function mutations in the THAP1 transcription factor cause DYT6 (DYT-THAP1) dystonia
- THAP1 deficiency within oligodendrocyte progenitor cells (OPC) directly reduces the lysosomal enzyme β-glucuronidase (GusB) causing the accumulation of glycosaminoglycans (GAGs) that inhibit OPC maturation to myelinating cells. The results are severe dysmyelination and impaired neurodevelopment.
- Genetic overexpression of GusB rescues THAP1 myelination phenotypes suggesting that β-glucuronidase enzyme replacement therapy (ERT) could restore DYT-THAP1 related myelination defects.

## Methods

- We used a Nestin-Cre/Thap1LoxP approach to delete THAP1 from the central nervous system of mice ("THAP1-NCKO") and a GusB transgenic mouse line ("GusB-TG") to overexpress β-glucuronidase.
- We quantified β-glucuronidase activity using a biochemical enzyme activity assay
  - P60 mice were transcaridally perfused with phosphate buffered saline and unfixed brains were harvested
  - Fresh-frozen tissue was lysed and incubated with 4-methylumbelliferyl β-D-glucuronide (MUG)
  - Fluorescence was quantified




- We determined the biodistribution of β-glucuronidase using colorimetric in situ hybridization
  - Cryosections of fresh frozen brains were incubated with naphthol-AS-BI-β-D-glucuronide and stained with hexazotized pararosaniline
  - Enzyme activity produced Insoluble red precipitate




## Results

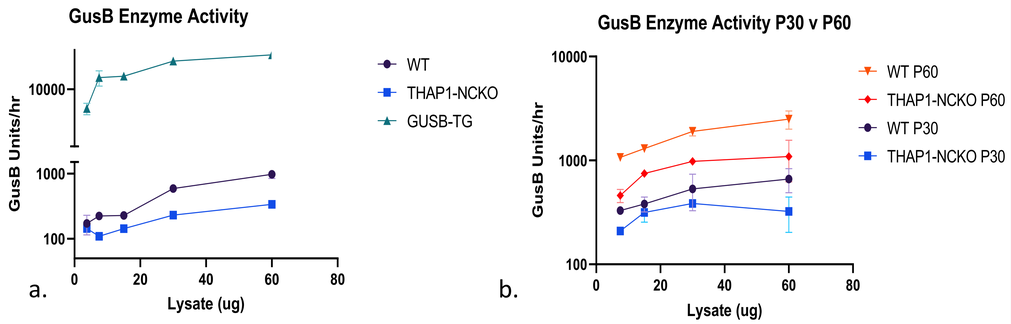



Figure 1. β-glucuronidase biochemical (MUG) assay quantifying enzymatic activity in vitro. **a,** Lysate samples from brain of control, GusB transgenic (TG) overexpressing mice, or THAP1 null (NCKO) mice age P60. **b,** Lysate samples from control or THAP1 null mice ages P30 and P60 show age-related differences.

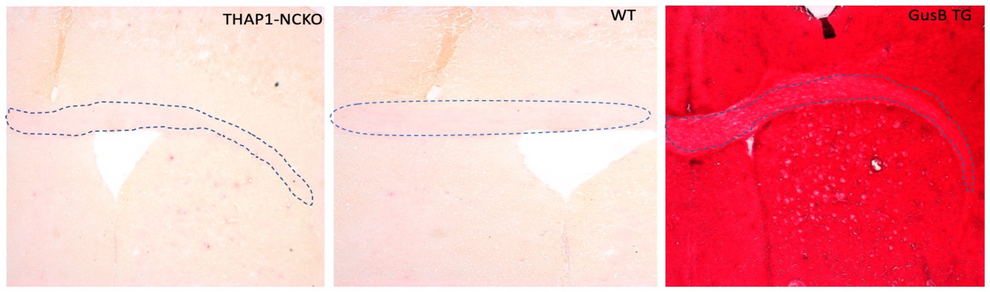



Figure 2. Colorimetric-based in situ assay visualizing β-glucuronidase enzymatic activity. Coronal brain sections from THAP1 null, control, or GusB transgene P30 mice were assayed using naphthol-AS-BI-β-D-glucuronide and stained with hexazotized pararosaniline. Darker color represents more β-glucuronidase activity.

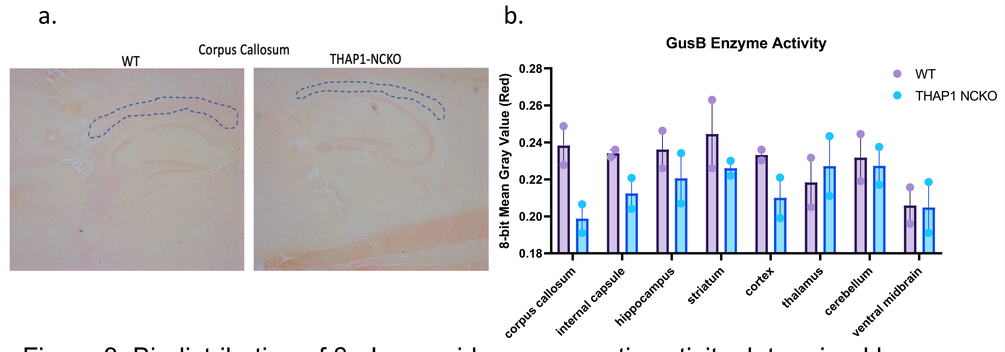



Figure 3. Biodistribution of β-glucuronidase enzymatic activity determined by colorimetric-based in situ assay. **a,** Sagittal section showing the corpus collosum (outlined) from control or THAP null P30 mice. Darker color represents more β-glucuronidase activity. **b,** Differential expression of β-glucuronidase activity in central nervous system brain regions implicated in dystonia neuropathology from control or THAP null P30 mice. White matter tracts in THAP1 null mice exhibit reduction in β-glucuronidase activity compared to other surveyed β-glucuronidase positive areas.

## Conclusions

- We identified that age differentially affects CNS β-glucuronidase (GusB) enzymatic activity in a murine model of DYT6 dystonia as GusB activity increases with increasing age.
- We demonstrated that GusB enzyme exhibits a distinct biodistribution that varies regionally. White matter tracts show the most severe changes to β-glucuronidase defects in THAP1 null mice, providing a region of interest for targeted enzyme delivery.
- Our results provide insights into the specific locations where GusB activity is deficient with THAP1 loss and highlight the importance of a "critical period" in which genetic insults have long lasting neurodevelopmental implications.
- We optimized a robust system of assays to validate whether enzyme replacement will occur in the brain. These findings will aid in the development of a future targeted β-glucuronidase ERT delivery paradigm for novel treatment of DYT-THAP1 dystonia.

## References

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