Next-Generation Therapeutic Targets in Preclinical Mouse Models of Parkinson's Disease

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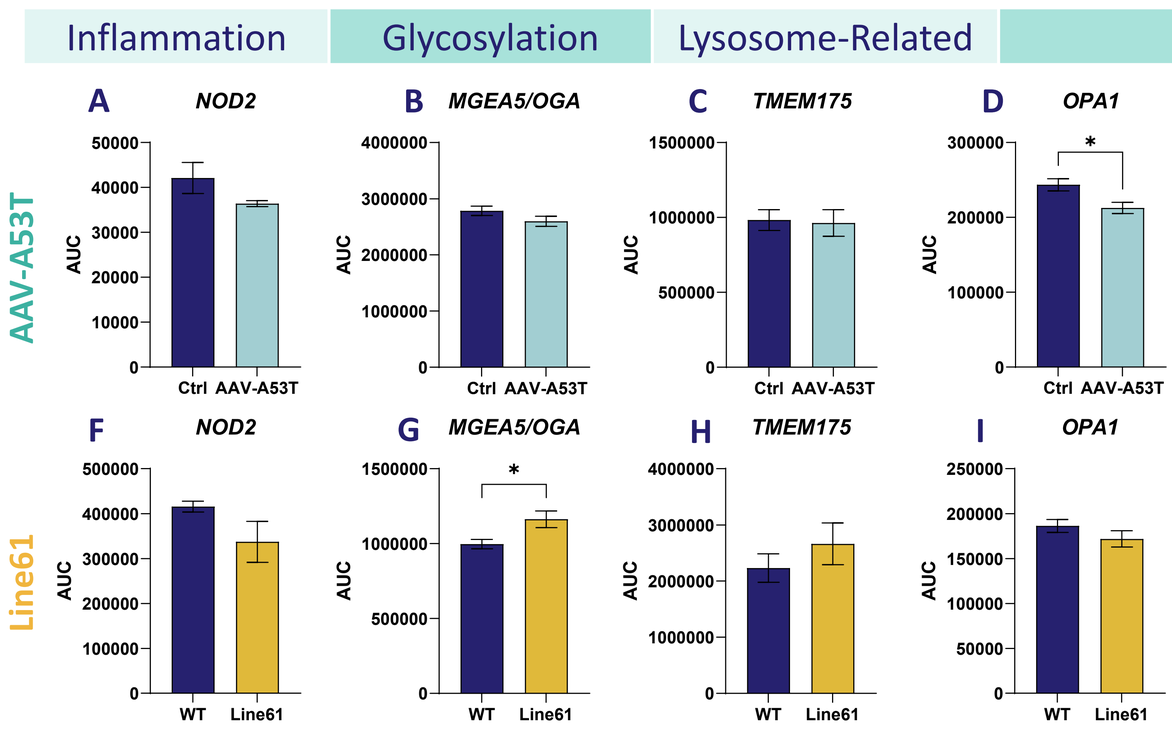
Objectives

Despite ongoing research efforts, disease-modifying therapies for Parkinson's disease (PD) remain elusive. This underscores an urgent need for the development of novel therapeutic strategies that target emerging mechanisms such as mitochondrial dysfunction, inflammation and endo-lysosomal pathway disruptions, aside of the well-known link between GBA1 mutations and PD. To validate the efficacy of these next-generation therapeutic approaches, it is essential to thoroughly characterize the associated targets and affected pathways in appropriate mouse models.

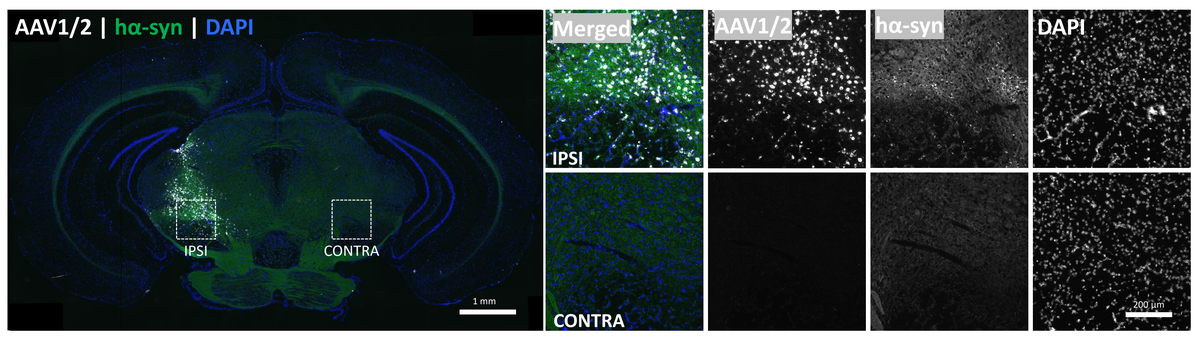
Methods

We characterized expression of several novel therapeutic targets from the Michael J. Fox Foundation's Targets to Therapies (T2T) initiative across four well characterized mouse disease models at a time point when the major hallmarks are developed. Two Parkinson's disease mouse models with α-synuclein pathology were investigated: a line with stable α-synuclein overexpression (Line61) and an AAV-A53T model inducing α-synuclein pathology. Additionally,. two models representing Gaucher disease pathology, including glucocerebrosidase impairment (GBA D409V KI) and combined glucocerebrosidase impairment with reduced saposin levels (4L/PS-NA) were used. Expression levels of a broad set of markers, including NOD2, OGA, TMEM175, TRPML1, ATP13A2 and OPA1, were quantified in hemibrains, using automated Western blotting. For AAV-A53T model also immunohistochemical assessment of pathological markers was performed.

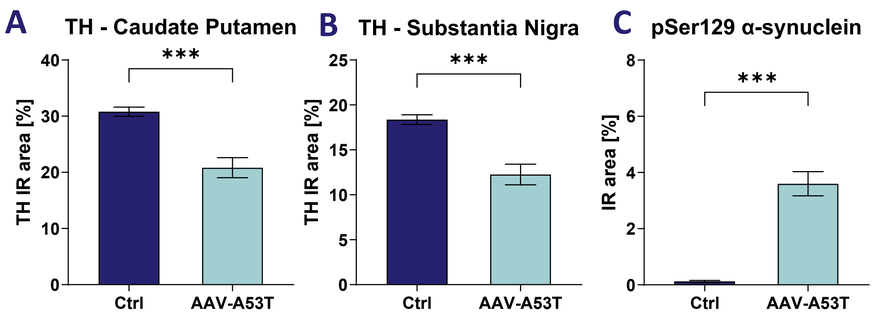
T2T Marker in Parkinson's Disease Models



◄ **Figure 1: Quantification of T2T markers in Parkinson's disease mouse models**. Caudate putamen ipsilaterally AAV2-hA53T-injected (AAV-A53T) and contralaterally AAV2-empty vector control-injected (Ctrl) 9 weeks post injection (**A-E**) or hemibrains of α-synuclein transgenic (Line61) and wild type littermates (WT) (**F-I**) were analyzed for a set of T2T markers via automated Western blotting. While the AAV-A53T samples primarily showed alterations in mitochondrial markers (**D, E**), the Line61 model exhibited a significant increase in MGEA5/OGA levels (**G**). Unpaired T-test; n = 6-8; *p <0.05.

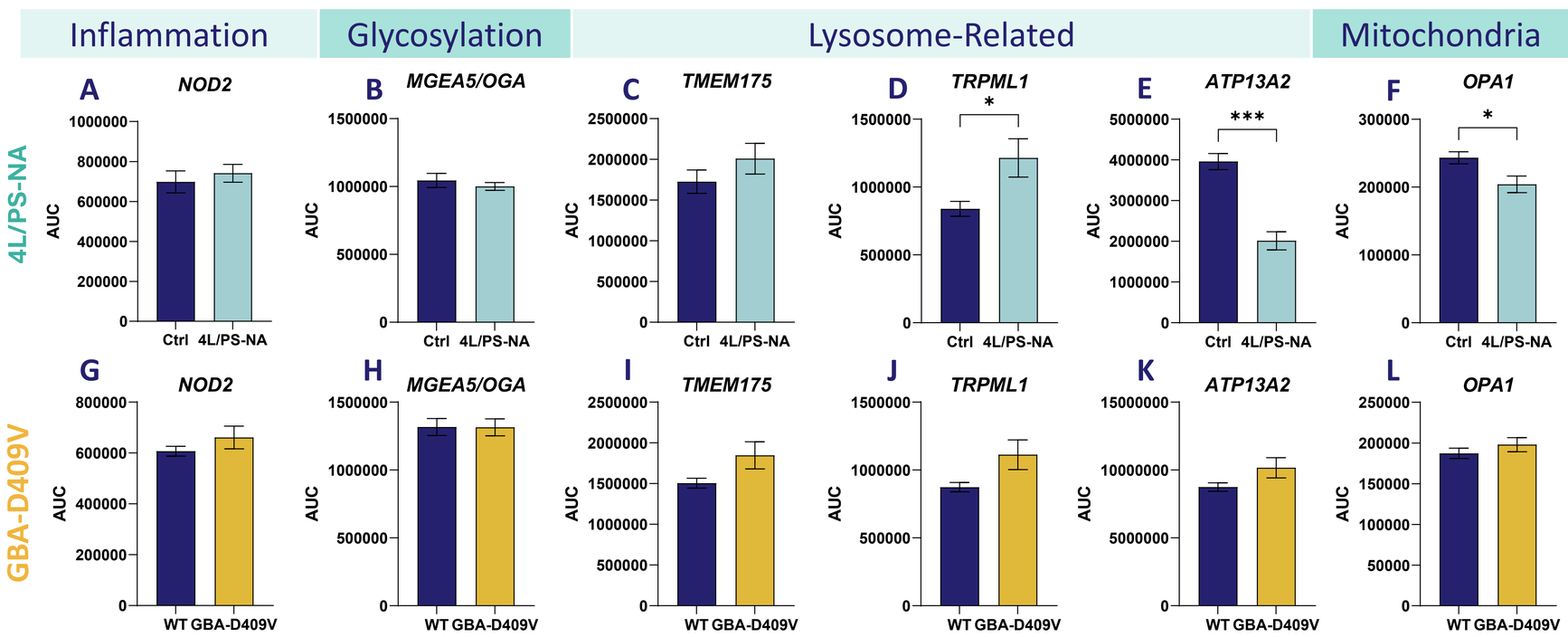


▲ **Figure 2: *In situ* hybridization in combination with immuno-fluorescent labeling in an ipsilaterally AAV2-hA53T-injected and contralaterally AAV2-empty vector control-injected mouse brain**. Fluorescent *in situ* hybridization against the AAV2 serotype (white); Immunofluorescence labeling of hα-syn protein (green). IPSI: ipsilateral hemisphere; CONTRA: contralateral hemisphere. Slides were counterstained with DAPI. Inlets show substantia nigra.



◄ **Figure 3: Quantification of Tyrosine hydroxylase (TH) (A, B) and pSer129 α-synuclein (C) immunofluorescent signal in the caudate putamen or substantia nigra of the hemisphere injected with AAV2-A53T compared to AAV2-empty vector (Ctrl).** Mean ± SEM; 5 brain sections per mouse; n = 8 animals; Paired t-test; ***p <0.001.

T2T Marker in Gaucher Disease Models



◄**Figure 4: Quantification of T2T markers in Gaucher mouse lines.** Hemibrains of 18 weeks old (**A-F**) 4L/PS-NA and control (Ctrl) mice or (**G-L**) D409V homozygous animals and wild type littermates (WT) were analyzed for a set of T2T markers via automated Western blotting. Both models express NOD2 and MGEA5/OGA without detectable changes and show similar but non-significant trends for TMEM175 (**A-C**, **G-I**). The 4L/PS-NA mice, which already exhibit a stronger behavioral phenotype and more pronounced substrate accumulation than the D409V model, also display more alterations in the T2T panel. These include a significant increase in (**D**) TRPML1, a cation channel located on late endosomal and lysosomal membranes, a reduction in (**E**) ATP13A2, a lysosomal ATPase and (**F**) mitochondrial protein OPA1. Unpaired T-test; n = 8; *p <0.05; ***p <0.001.

Conclusion

We found that novel therapeutics for PD and GD are differentially regulated in a number of previously characterized murine disease models. Comprehensive pathway and biomarker profiling supports the selection of the most appropriate models and strengthens the foundation for robust preclinical testing. Assessing the presence and potential alterations of next-generation therapeutic T2T in mouse models that are already well characterized for major pathological hallmarks and widely used in preclinical studies will guide the identification of the most relevant systems for further target evaluation.

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