Characterization, comparison, and cross-validation of in vivo alpha-synuclein models of parkinsonism

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INTRODUCTION

As part of its aggressive strategy to accelerate efforts to find a cure for Parkinson’s disease (PD) and to improve therapies for PD patients, the Michael J. Fox Foundation for Parkinson’s research (MJFF) endeavors to generate and rigorously characterize preclinical tools and animal models and provide them to the PD research community with minimal barriers. Here we highlight MJFF-generated alpha-synuclein preclinical tools and animal models and describe in vivo characterization and validation efforts with the objective of informing the PD research community of the utility of these tools for potential use in studies aimed at understanding alpha-synuclein pathology or to test potential therapeutics targeting alpha-synuclein.

AAV Viral Vectors Expressing Human Alpha-Synuclein Transduce Nigral DA Neurons in vivo

AAV2 and AAV5 viral vectors expressing human WT alpha-synuclein or eGFP were constructed, produced, and titrated by the UNC Vector Core. In vivo validation in rats was performed by the laboratories of Dr. D. Kirik (BRAINS Unit, Lund University) & Dr. R. Mandel, (The McKnight Brain Inst., University of Florida).

Study Design for In vivo Validation of Alpha-synuclein Expressing AAV Vectors in Rats

- AAV2
- AAV5

Figure 1. TH Staining in SNC Following Stereotaxic Injection of AAV Vectors in Rats

A. AAV2
B. AAV5

Figure 2. Analysis of TH+ Nigral Neurons, TH striatal density, and Striatal DA Content

A. AAV2
B. AAV5

Figure 3. Loss of TH in Neurons Expressing GFP Does Not Correlate with Cell Death

A. AAV2
B. AAV5

Comparative Study of Alpha-synuclein Transgenic Mouse Models

Numerous transgenic alpha-synuclein mouse models have been developed over the years. However, a lack of standardization and reproducibility of phenotype make it difficult to select appropriate preclinical mouse models to use in efficacy studies for potential alpha-synuclein therapeutics. Thus, MJFF endeavored to independently compare and cross-validate several alpha-synuclein mouse models using standardized outcome measures.

Table 1. Transgenic Mouse Models in the Alpha-synuclein Comparison Study

<table>
<thead>
<tr>
<th>Model</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>Human A53T</td>
<td>Reduced striatal dopamine content, reduced TH expression, decreased nigrostriatal survival</td>
</tr>
<tr>
<td>Human E46K</td>
<td>Reduced striatal dopamine content, decreased TH expression, decreased nigrostriatal survival</td>
</tr>
<tr>
<td>Human A53T expression</td>
<td>Reduced striatal dopamine content, decreased TH expression, decreased nigrostriatal survival</td>
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</tr>
</tbody>
</table>

Figure 4. Human Alpha-Synuclein Protein Expression in Transgenic Mouse Models

A. Human A53T
B. Human E46K
C. Human A53T expression

Figure 5. Behavior Analyses in Alpha-Synuclein Transgenic Mouse Models

A. Relaxed (Latency to drop)
B. Forelimb Grip

Figure 6. Striatal Neurochemistry for Neurontin in Tg Mouse Brain

A. Dopamine (DARPP
B. Serotonin
C. Noradrenaline

Figure 7. Immunohistochemistry: Human Alpha-Synuclein In Tg Mouse Brain Tissue

A. TH
B. DARPP
C. Serotonin

Figure 8. Dopamine Neuron Counts in SNC in Tg Mouse Brain

A. TH
B. Serotonin

Replication of Alpha-syn Fibrils Propagation Study

Numerous studies including the Braak hypothesis of alpha-synuclein pathology[14] and transfer of alpha-synuclein in cellular and animal models[16] support a hypothesis of neurotoxic transfer of alpha-synuclein protein. Moreover, single intracerebral injection of alpha-synuclein pre-formed fibrils (PFF) is reported to induce Lewy-like pathology in cells – that can spread from affected to unaffected regions – along with concomitant neurodegeneration and motor dysfunction[29]. To independently replicate these seminal findings, the MJFF partnered with PsychoGenics in collaboration with Drs. Kelvin Luk and Virginia Lee.

Figure 9. Synthetic PFF Inoculation Significantly Reduces Striatal DA Content and Increases Phospho-S129 Alpha-Synuclein Immunoreactivity

A. Real Time PCR
B. CEF
C. S129

Figure 10. No Significant Effects on Behavior Following α-syn PFF Inoculation

A. CEF
B. S129

Figure 11. Significant Decrease in Striatal DAT Following α-syn PFF Inoculation

A. CEF
B. S129

SUMMARY

Taken together, the data presented here can help inform the PD research community of the utility and reproducibility of various in vivo rodent models of alpha-synucleinopathy, thereby potentially informing selection of appropriate models in which to test prospective therapeutics targeting alpha-synuclein. More information on other available MJFF tools and resources (as well as new projects in development) can be found at the MJFF website.


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