Phenotyping of Human iPSC-derived Dopaminergic Neurons Containing the Engineered A53T α-Synuclein Mutation

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Abstract

Parkinson's disease (PD) affects ~1% of people over the age of 65 and is the second most common neurodegenerative brain disorder after Alzheimer's. The physiological decline associated with PD is generally thought to be caused by a marked pathological deterioration of dopaminergic neurons located in the substantia nigra. Mutations in several different genes have been clearly linked to PD, including *SNCA* that encodes the alpha-synuclein (α-syn) protein, which is predominantly expressed in the brain at presynaptic terminals. The mutation in α-syn at A53T renders the protein more susceptible to aggregation and accumulation, which are hallmark indicators of PD pathology. Despite its low occurrence, A53T is one of the most highly penetrant and widely studied mutations.

The combination of cutting-edge genome-editing and induced pluripotent stem cell (iPSC) technologies offers the opportunity to study patient-specific risk factors or disease-specific mutations (such as the A53T mutation in α-syn) in a physiologically-relevant cell type (dopaminergic neurons) and compare the function and phenotype in a series of assays to cells derived from healthy control iPSC lines. This approach is revolutionary for disease modeling and drug discovery.

In this poster, we show data comparing healthy (WT) and A53T dopaminergic neurons that demonstrate alterations at the synapse, both functionally (electrophysiological MEA readout) and anatomically (neurite outgrowth and branching). The observed differences between healthy and A53T suggest early physiological changes tilted towards producing a more connected and highly-active neuronal network. In correlation with the known disease pathology, these "aging" cultures show synaptic deterioration and dendritic atrophy.

Current studies are underway to further determine if additional hallmarks of PD patho-physiology, including α-syn aggregation or mitochondrial dysfunction, can be measured in these human cell models.

Genome-Engineered Disease Modeling

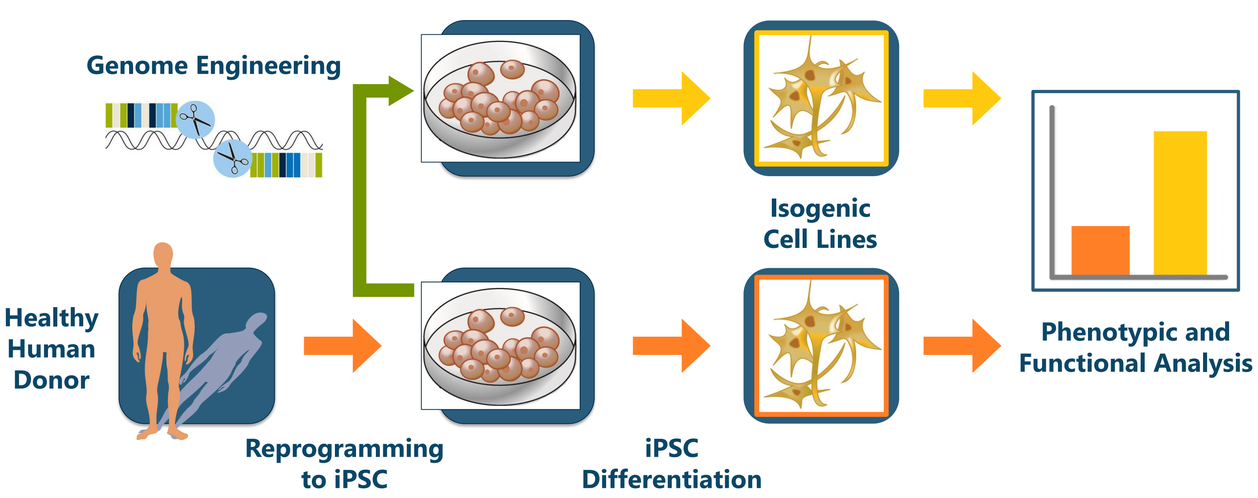


Figure 1. Creating the iPSC lines. Nuclease-mediated SNP alteration of a healthy control iPSC line was performed to introduce a site-specific mutation into the gene for alpha-synuclein (*SNCA*) in order to generate the A53T allelic variant. This genome-engineered iPSC, as well as the isogenic control, was then differentiated into human midbrain floorplate dopaminergic neurons (MyCell® and iCell® DopaNeurons) was then differentiated into human midbrain floorplate dopaminergic neurons according to protocols adapted from Memorial Sloan Kettering and industrialized at CDI.

What is a Dopaminergic Neuron?

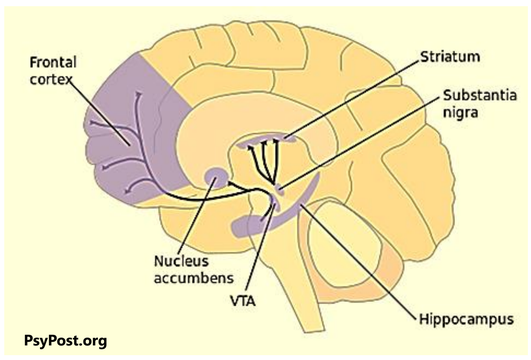
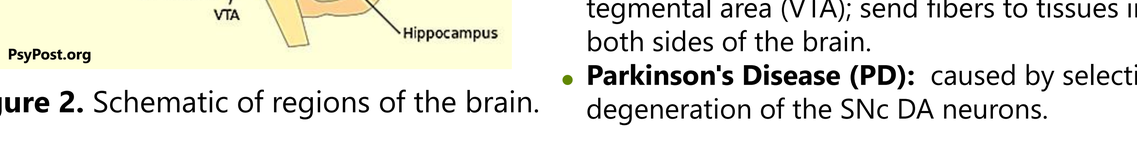


Figure 2. Schematic of regions of the brain.

- **Dopaminergic (DA) neurons:** producers of dopamine; found in different regions in the CNS with the largest concentration in the midbrain.
- **Dopamine:** role in **voluntary movement** and a broad array of **behavioral processes** such as mood, reward, addiction, and stress.
- **Midbrain DA neurons:** located in the substantia nigra compacta (SNc) and the ventral tegmental area (VTA); send fibers to tissues in both sides of the brain.
- **Parkinson's Disease (PD):** caused by selective degeneration of the SNc DA neurons.

Cell Type Characterization Data

Figure 3. Expression of relevant midbrain dopaminergic markers. Analysis of iCell DopaNeurons by high content imaging and flow cytometry at Day 14 post-thaw indicates that these cells are a highly pure neuronal cell type by MAP2 staining (>95% MAP2-positive; nestin-negative). Cells are positive for prototypical markers FoxA2 (94%) and LMX1 (96%), and they are co-positive for FoxA2/LMX1 (>90% in overlay). Finally, these human iPSC-derived dopaminergic neurons express high levels of TH (>80%).



Identifying Parkinson's Disease Phenotypes in a Dish

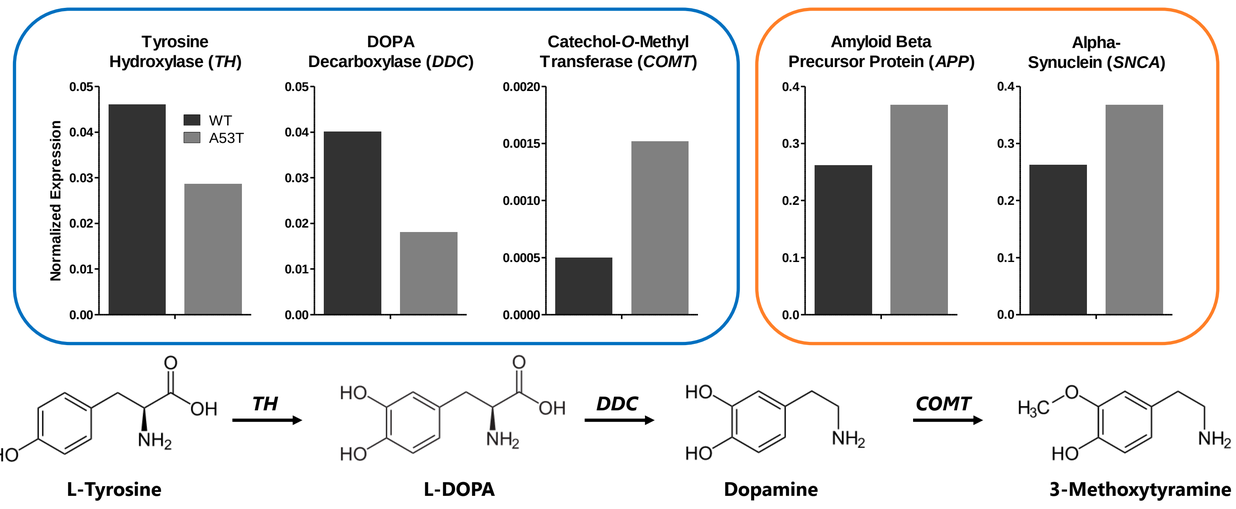


Figure 4. Differential expression of key genes between WT and A53T dopaminergic neurons. Isogenic iCell and MyCell DopaNeurons were cultured until DIV 21 in standard maintenance medium (MM) or in BrainPhys neuronal medium. Cells were then processed for qRT-PCR analysis of gene expression using a dopamine and serotonin pathway array (Bio-Rad; SAB target list). Interestingly, key enzymes (circled in **BLUE**) involved in the biosynthesis (*TH* and *DDC*) and degradation (*COMT*) of dopamine were dysregulated toward the lower production of or the increased catabolism of **dopamine** in cells with the A53T mutation (in both media). Circled in **ORANGE**, it was shown that expression of *APP* and *SNCA* were elevated above WT levels. These two proteins have been reported to target and accumulate in the mitochondria, impacting the pathophysiology in PD.

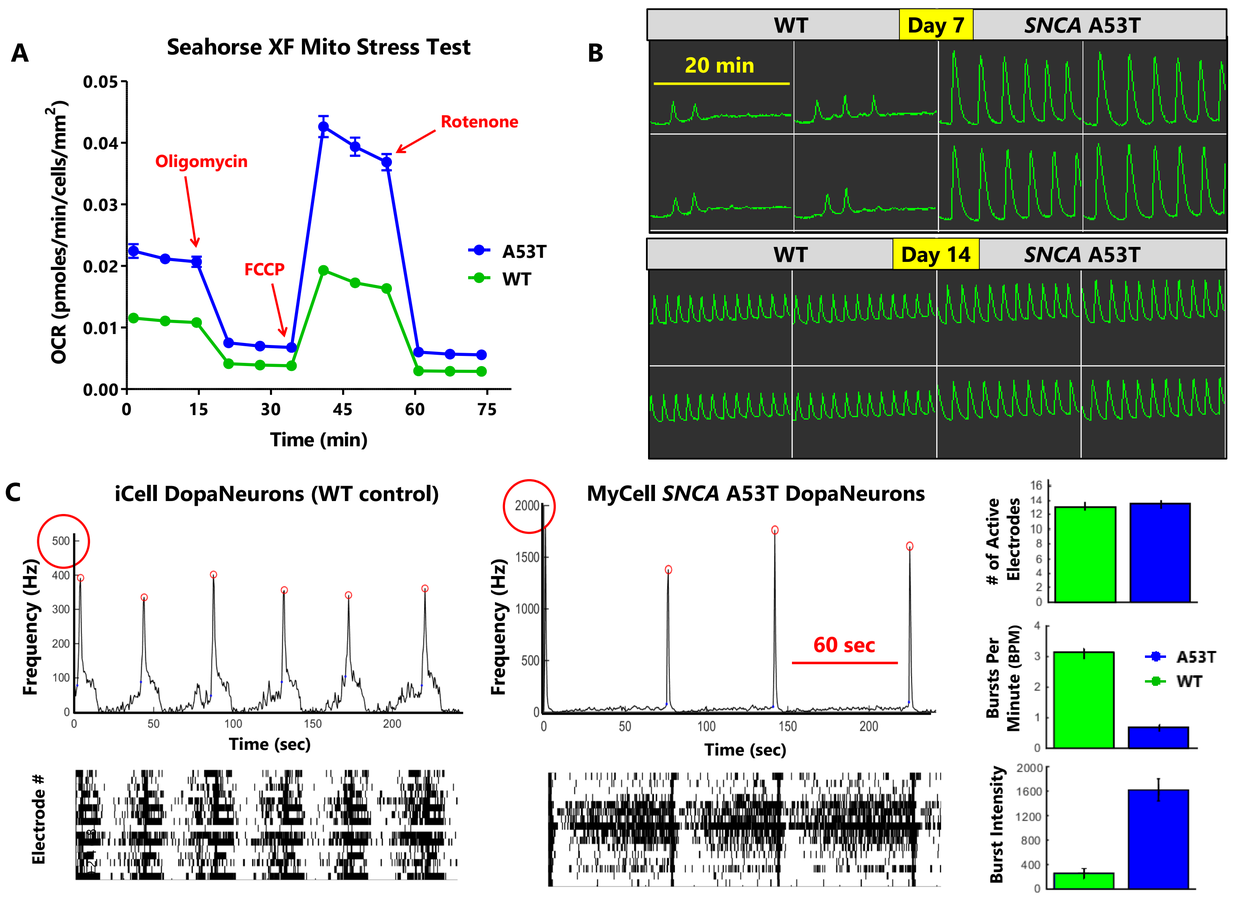
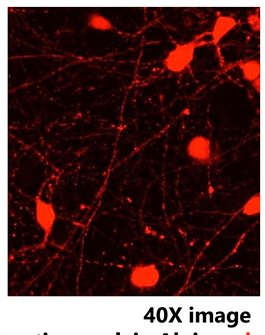


Figure 5. Identification of phenotypic differences observed between WT and A53T dopaminergic neurons. Isogenic iCell and MyCell DopaNeurons were cultured in BrainPhys media and compared in various functional readouts. (A) *Seahorse XF Analyzer* mitochondrial assay for metabolism revealed >2-fold greater spare respiratory capacity for the A53T line. (B) *FDSS µCell* calcium assay demonstrated spontaneous oscillations earlier in culture for A53T with amplitudes and peak-to-peak timing significantly different than the WT control. (C) *Axion Maestro* multi-electrode array (MEA) data showed >3-fold decreased burst frequency (BPM) and ~4-fold increased burst intensity for the A53T line with an equal number of active electrodes vs WT control.

Additional iPSC Lines – What's Next?

CDI is actively collaborating with organizations, such as the Michael J. Fox Foundation and Rare Science, to acquire donor samples for the sole purpose of making more PD-related iPSC lines available to the broader scientific community. In the near future, lines from 20 donors who are part of the Parkinson's Progression Markers Initiative (PPMI) will be distributed through the MJFF website. Additionally, Rare Science has partnered with ADCY5.org to bring a collection of samples from patients with ADCY5-related movement disorders to the California Institute of Regenerative Medicine (CIRM). CDI will generate the iPSC lines and add them to the ever-growing CIRM iPSC bank.

MyCell *SNCA* A53T DopaNeurons



40X image
MJFR1 anti-synuclein Ab in red

Summary

- Human iPSC technology holds great promise in the field of regenerative medicine and cell therapy, but the more immediate impact is in the area of *disease modeling*
- Using genome-editing tools, a cell model for *Parkinson's Disease* was created first by engineering of an iPSC line to generate the *SNCA* A53T mutation and then by further differentiating the iPSC to dopaminergic neurons
- The presence of a perfectly matched isogenic control iPSC and an industrialized manufacturing process reduces variation between disease state (A53T) and wild-type condition
- MyCell *SNCA* A53T DopaNeurons differentially express key genes related to PD and display unique cellular phenotypes in that can be measured in both electrophysiological and metabolic functional assays