Evaluating TMEM175 Mutation Phenotypes in Human iPSC-derived Dopaminergic Neuron Models for Parkinson's Disease

Scott Schachtele[1], Rebecca Fiene[1], David Majewski[1], Stella Donato[2], Teresa Sorbo[2], Fernanda Ricci[2], Anna Mondini[2], Simon Hilcove[1], Coby Carlson[1], Jean-Francois Rolland[2], and Eugenia Jones[1]
[1]FUJIFILM Cellular Dynamics, Madison, WI ; [2]Axxam S.p.A. OpenZone, Bresso (Milan), Italy

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Abstract

Genome-wide association studies have associated Parkinson's Disease (PD) risk with mutations in TMEM175, a cation channel important for maintaining lysosomal pH. Specifically, the TMEM175 heterozygous M393T mutant has reduced pH and is associated increased protein aggregates in PD. The role of TMEM175 on lysosomal function has increased its potential as a druggable target for modulating protein degradation in both PD and across neurodegenerative diseases hallmarked by protein aggregates. While TMEM175 overexpressing cell lines were important for initial channel characterization, studies in human-relevant dopaminergic neurons are needed to link channel physiology with cellular biology and PD disease pathology. This study establishes and characterizes a panel of human induced pluripotent stem cell (iPSC)-derived midbrain dopaminergic neurons containing PD-relevant TMEM175 mutations. To this end, dopaminergic neurons (iCell DopaNeurons) were differentiated from isogenic iPSC donor lines containing various TMEM175 mutations, including the PD-relevant heterozygous M393T variant, an apparently healthy normal M393M control, a loss-of-function T393T mutation, or the Q65P gain-of-function mutation. The reproducibility of the differentiation protocol, licensed from Memorial Sloan Kettering Cancer Center, is demonstrated by the robust and consistent expression of characteristic midbrain dopaminergic neuron markers (FoxA2, TH, and BIII-tubulin), with levels comparable across mutations indicating neurons of similar purity and quality. iPSC-derived dopaminergic neurons should develop spontaneous and synchronized network bursts in culture. Indeed, using microelectrode array (MEA), all mutants achieved synchronized bursting with preliminary data suggesting similar firing patterns. Lysosomal patch-clamp experiments were performed to evaluate the effects of TMEM175 mutations lysosome function. Compared to M393M neurons, the M393T mutation showed a slight reduction in conductance, while conductance was significantly reduced in the T393T loss-of-function mutant. Conversely, conductance in the Q65P mutant was significantly increased. These changes align with results reported in previous literature using TMEM175 overexpressing cell lines. Using cell-based assays to evaluate lysosomal and mitochondrial function, our preliminary data also showed phenotype variation across mutations. This study shows that iPSC-derived dopaminergic neurons containing TMEM175 mutations exhibit expected channel physiology and offer unique phenotypic differences, making them a useful addition for PD drug discovery research.

Background and Methods

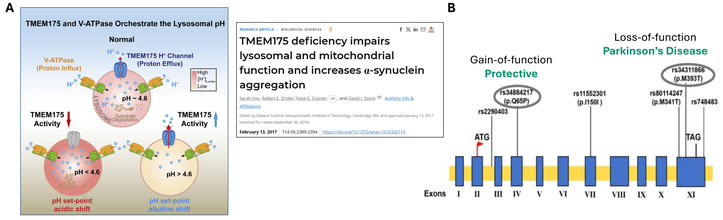
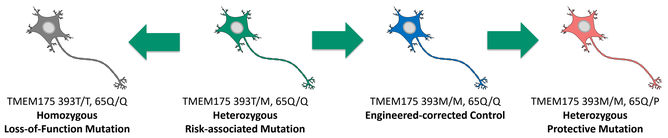


Figure 1. TMEM175 Background and Association with Parkinson's Disease. A) Schematic of TMEM175 role in maintaining lysosomal pH (*Hu, et al., (2002) Cell. 185:13*). Degreased channel function is associated with lysosomal dysfunction and altered protein degradation and aggregation (*Jinn et al., (2017) PNAS. 114:9*). **B)** Single nucleotide polymorphisms in TMEM175 are associated with pathogenesis of Parkinson's Disease. 393M/T heterozygous mutation is a loss of function mutation reduces channel permeability and is an increased risk factor for PD. 65Q/P heterozygous mutation is a gain of function, increasing channel permeability and is suggested to be protective variant (*Palomba et al. (2023) Mol. Neurobiology. 60:2150*).



Product Name	Donor	Cat #	393 Genotype	65 Genotype	Description
iCell DopaNeurons	01279	R1032	M393T HZ	Normal	PD Risk-associated
iCell DopaNeurons TMEM175 393M/M	01279	R1264	M393M HO	Normal	Mutation-corrected control
iCell DopaNeurons TMEM175 393M/M, 65Q/P	01279	R1266	M393M HO	Q65P HZ	Protective
iCell DopaNeurons TMEM175 393T/T	01279	N/A	T393T HO	Normal	Loss-of-function mutation

Figure 2. TMEM175 iCell DopaNeurons Engineering. Schematic of engineering to generate a panel of TMEM175 iCell DopaNeurons featuring risk-associated loss-of-function mutants, protective gain-of-function mutant, and corrected controls. Using this panel a thorough examination of TMEM175 in human iPSC-derived dopaminergic neurons can run in various cell-based assays for drug discovery and basic Parkinson's disease research.

Characterization of TMEM175 iCell DopaNeurons

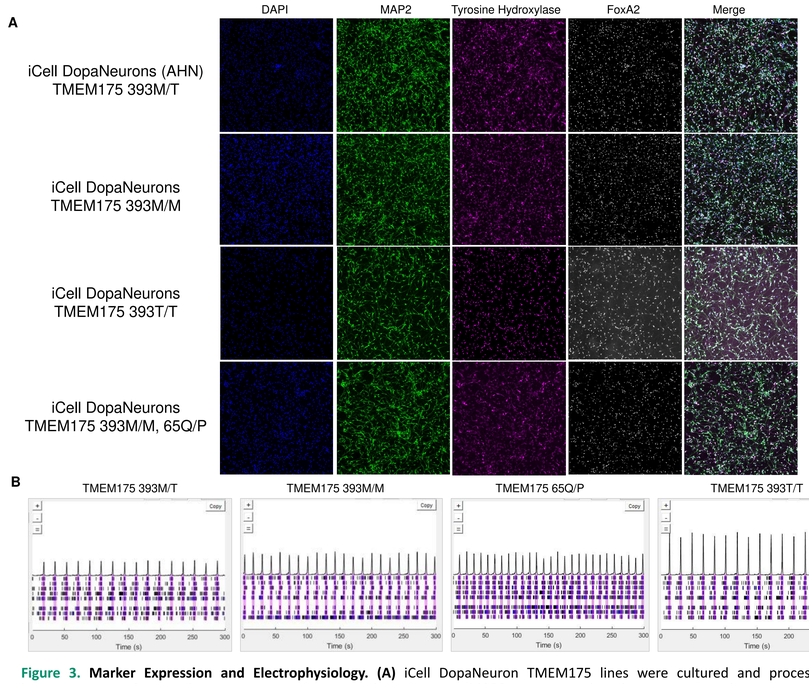


Figure 3. Marker Expression and Electrophysiology. (A) iCell DopaNeuron TMEM175 lines were cultured and processed for immunocytochemistry at 4 days post-thaw for expression of neural (MAP2) and dopaminergic markers (tyrosine hydroxylase and FOXA2). Staining shows ubiquitous and similar expression across all TMEM175 lines of iCell DopaNeurons, indicating a consistent differentiation and consistent starting cell population across lines. **(B)** iCell DopaNeuron TMEM175 mutants will form active neural networks (synchronized bursting) when cultured with iCell Astrocytes 2.0 and recorded on microelectrode array (MEA) plates, demonstrating that these cells are highly functional iPSC-derived dopaminergic neurons.

Lysosomal Electrophysiology in TMEM175 iCell DopaNeurons

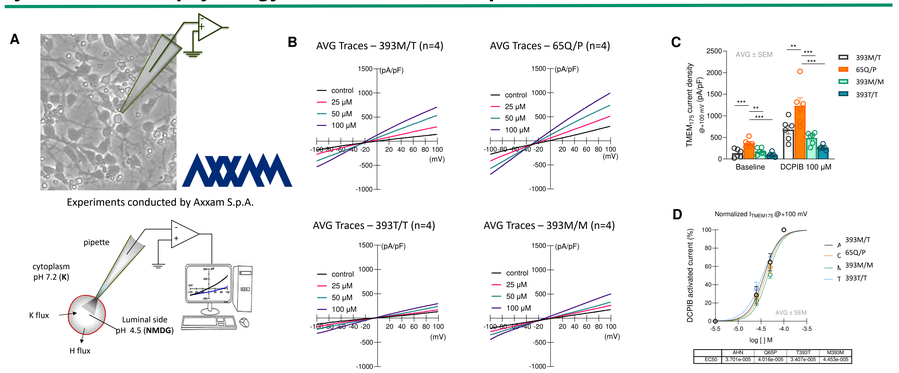


Figure 4. I-V Currents Observed Across TMEM175 Genotypes. iCell DopaNeurons TMEM175 lines were cultured for 14 days and then treated with vacuolin-1 for 24 h to induce lysosome enlargement. **(A)** Manual patch clamp of enlarged vesicles was performed to characterize baseline electrophysiological properties and responses to DCPIB, a TMEM175 channel agonist. **(B)** Average current-voltage (I-V) plots for each cell line exposed to (0, 25, 50, or 100 µM of DCPIB. **(C)** In agreement with literature data, measuring TMEM175 current density revealed higher baseline conductance from the 65Q/P mutation and equal or reduced conductance for 393M/T and 393T/T, respectively. **(D)** The mutation did not affect responsiveness to DCPIB as shown by similar EC_{50} curves between the mutants.

Evaluating Parkinson's Disease MEA Phenotypes in TMEM175 iCell DopaNeurons

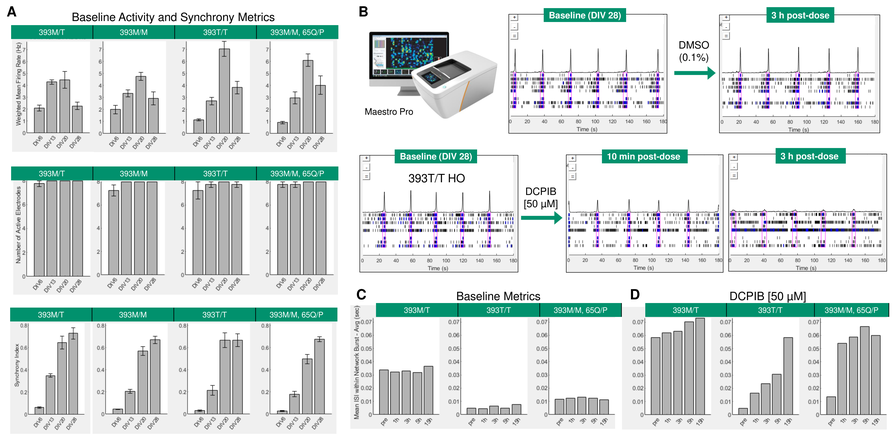
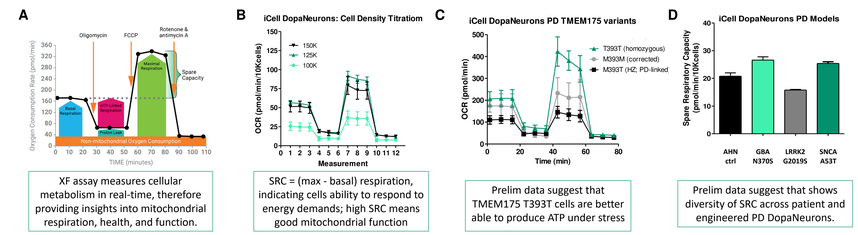


Figure 5. MEA Network Activity and Compound Testing. TMEM175 iCell DopaNeurons were co-cultured with iCell Astrocytes, 01434 and neuronal activity was recorded on the Maestro Pro (Axion) at multiple days in culture. **(A)** Weighted MFR, Active Electrodes, and Synchrony Index parameters show consistent activity development across the panel of DopaNeurons, indicating healthy and robust neural populations. On DIV 28, cells were dosed with either DMSO vehicle control or DCPIB (a known TMEM175 channel activator) and spike activity was monitored every hour over night. As illustrated by the **(B)** raster plots, **(C)** baseline metrics, and **(D)** DCPIB post-dose metrics, it was evident that DCPIB had an effect as early as 10 min post-dose and continued to change over time, reducing network burst strength and synchrony. Kinetic analysis further highlighted the apparent differences between mutations. DCPIB treatment increased the "Mean ISI within Network Burst", which measures the time between spikes and smaller values mean more intense network bursts. Importantly, the Mean ISI metric changed significantly in the 65Q/P cells within 1 h (5X greater), whereas the same value was not reached until 15 h for the 393T/T mutant.

Bioenergetic Profiling of TMEM175 iCell DopaNeurons



XF assay measures cellular metabolism in real-time, therefore providing insights into mitochondrial respiration, health, and function.

SRC = (max - basal) respiration, indicating cells ability to respond to energy demands; high SRC means good mitochondrial function

Prelim data suggest that TMEM175 T393T cells are better able to produce ATP under stress

Prelim data suggest that shows diversity of SRC across patient and engineered PD DopaNeurons.

Figure 6: Seahorse XF Assay with TMEM175 and other PD iCell DopaNeurons. (A) Schematic for the Seahorse XF Assay and compound treatment. **(B)** Cell density titration of AHN iCell DopaNeurons showed changes in OCR assay signal on Day 21 on the Seahorse XF Pro Analyzer. Recommended cell density is 125,000 cells/well. **(C)** Panel of TMEM175 iCell DopaNeurons were then assayed with 1 µM Oligomycin, 2 µM FCCP and 0.5 µM Rot/Ant A on Day 14. Data suggest that TMEM175 393T/T cells are better able to produce ATP under stress. **(D)** Separately, other PD iCell DopaNeurons (LRRK2 G2019S, GBA1 N370S, and SCNA A53T) were analyzed for spare respiratory capacity. Together, these lines along with the TMEM175 lines can produce a powerful panel for drug discovery and basic research.

Conclusions

These data demonstrate the utility of a panel of iCell DopaNeurons featuring TMEM175 mutations associated with Parkinson's Disease for use in research and drug discovery programs.

iCell DopaNeurons TMEM175 Mutants:

- Consistent expression of dopaminergic markers (TH, FoxA2) across lines supporting a robust differentiation protocol and similar health and cell quality.
- Develop spontaneous and synchronous network activity across lines, indicating healthy neural populations and function.
- Expected lysosomal patch clamp conductance for 393M/T, 393T/T, and 65Q/P PD-relevant mutations, corroborating previous research and substantiating functional effects of mutation.
- Respond to compounds via MEA and patch clamp suggesting utility in drug discovery programs.

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