Multidimensional phenotyping of human stem cell-derived midbrain dopaminergic neurons from a LRRK2 mutation carrier for drug screening applications

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Introduction

Parkinson's disease (PD) is a heterogeneous movement disorder caused by environmental and genetic risk factors or mutations in specific genes. Pathological characteristics of PD include the progressive loss of midbrain dopaminergic neurons (mDANs) and often the appearance of Lewy bodies, cytoplasmic inclusions containing aggregated α-synuclein (**αSyn**) protein. The Glycine to Serine substitution at position 2019 (G2019S) in the leucine-rich repeat kinase 2 gene (**LRRK2**) has been associated with PD. One hypothesis is that **LRRK2 G2019S** causes defects in mitochondrial biology, additionally to induce mDAN loss by increasing the levels of phosphorylated αSyn, leading to its aggregation.

The goal of this study was therefore to develop a robust **machine learning** (ML) methodology sensitive enough to detect phenotypic variations based on genetic, but also to identify chemical compound inducing phenotypic changes. Our work outlines a novel strategy to use iPSC-derived mDANs from LRRK2 mutation carriers for imaging-based disease modelling by computationally combining multiple disease relevant phenotypes.

LRRK2 kinase overactivity triggers Parkinson's disease-related mechanisms

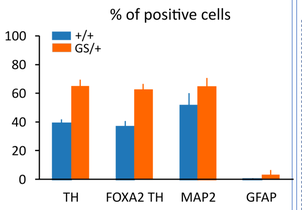
- Leucine-rich repeat kinase 2 (LRRK2) mutations account for roughly 1% of sporadic and 4% of familial PD cases.
- LRRK2 kinase domain G2019S mutation leads to kinase overactivity
- **GS/+**: LRRK2 G2019S-patient iPSCs differentiated into midbrain dopaminergic neurons (mDANs)
- **+/+**: Mutation-corrected isogenic iPSCs differentiated into mDANs

Facilitates misfolded protein buildup — *Increased 4E-BP & 40S phosphorylation*

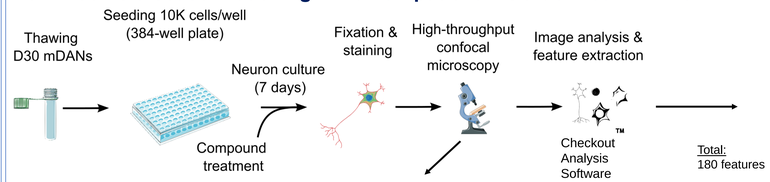
Compromises vesicular sorting — *Increased RAB phosphorylation & inactivation*

Decreases microtubule stability & neurite outgrowth — *Reduced α-tubulin acetylation*

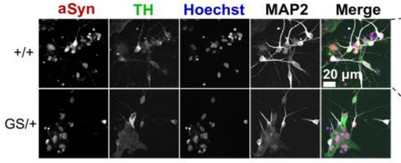
Impairs mitochondrial quality control — *Altered mitophagy*

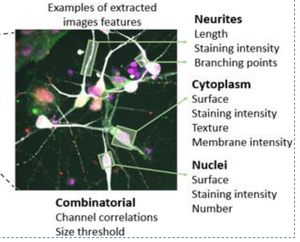


Parkinson's disease modelling in human patient iPSC-derived mDANs

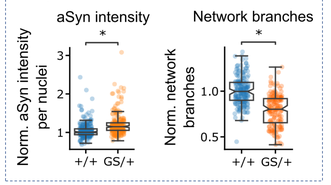


Staining panel in D37 isogenic mDAN pair

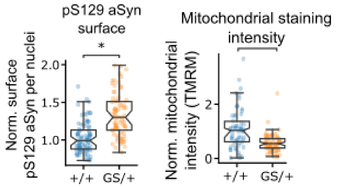




Example neuronal features

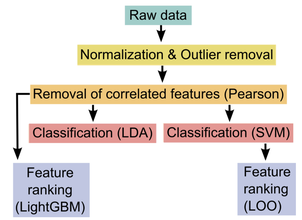


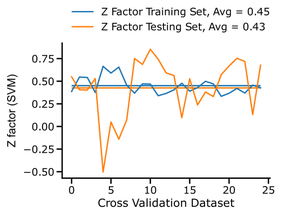
Additional hallmarks of Parkinson's disease in D37 isogenic mDAN pair



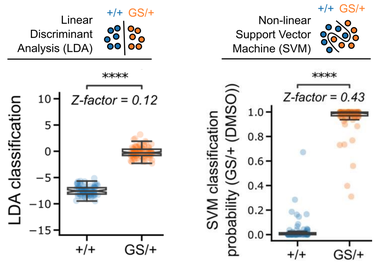
Machine Learning (ML) strategy to classify neurons based on multiple image-derived cellular features

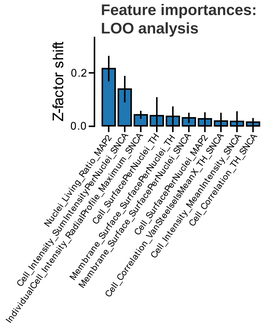
"Unseen" conditions such as novel cell lines or tested chemical compounds can be mapped relative to the two reference classes.





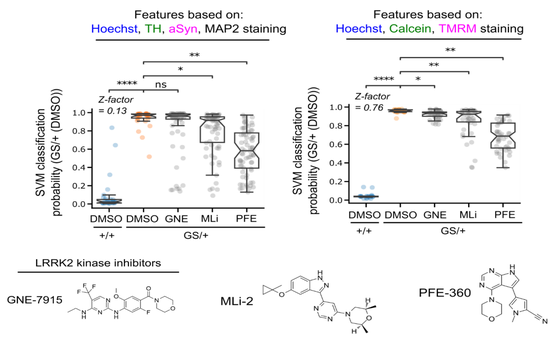
Machine learning classification of mDA neuronal cell lines using Hoechst, TH, aSyn, MAP2 staining





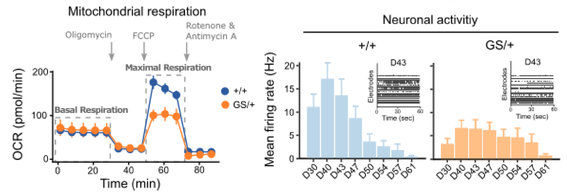
- Machine Learning is used to highlight discriminating features between the two populations
- ML allows to identify relevant Parkinson Disease vs. healthy phenotype

Multi-feature detection of LRRK2 kinase inhibition effects



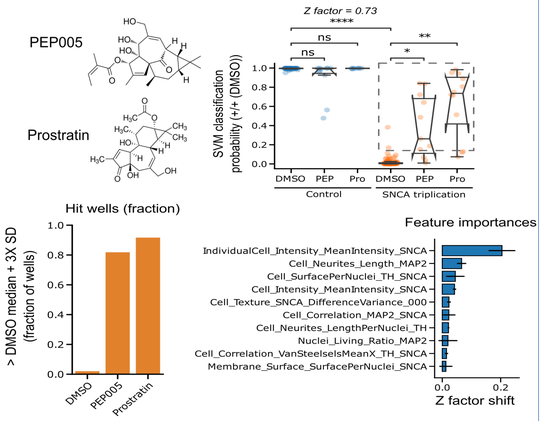
- LRRK2 kinase inhibitor PFE-360 partially rescues a mutant mDAN phenotypic profile using two different sets of cellular stainings.

Additional assays on Parkinson's disease isogenic pair



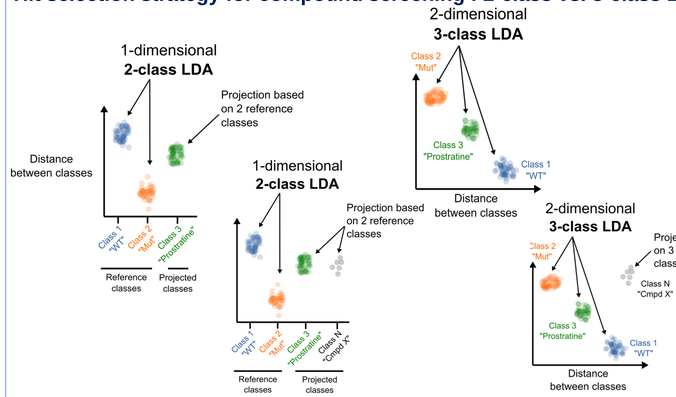
- Seahorse analysis shows a decreased of the GS/+ maximal respiration compared to the control cell line.
- Multi-Electrode Array (MEA) study shows a neuronal activity deficiency of the GS/+ cell line.

Multi-feature detection of aSyn reduction effects



- Prostratin shows the most efficient change toward healthy profile
- ML identified SNCA features as significant discrimination features

Hit selection strategy for compound screening : 2-class vs. 3-class LDA



- Compound X (Cmpd X) and Prostratin are at the same distance of Class 1 and 2
- But is there really no difference between Class 3 and Class N?
- Cmpd X is not similar to Prostratin although the equal distance to Class 1/2
- 3-class LDA allows to quantify distances of a tested compound with all controls

Conclusion

In our study, we demonstrate that image-derived phenotypes in human iPSC-derived mDANs can be used for **cell line characterization** and the **identification of chemical compound** treated neurons by **Machine learning** classification approaches. First, we used isogenic controls to reduce sources of inter-donor genetic variability, and we developed a compact and automated experimental protocol in 384-well plate format to reduce intervention steps and technical variability. Secondly, we developed a reliable **Machine learning** neuronal genotypes classification based on image-extracted features. We were therefore able to identify Parkinson's disease phenotypes in two midbrain neuronal models, together with highlighting compounds partially rescuing the healthy phenotype. We are currently setting up a small molecular screening protocol using 3-class LDA analysis for hit identification.