Progress report: An integrated engineering-computational-biological approach to unravel molecular dysfunction in Parkinson's disease

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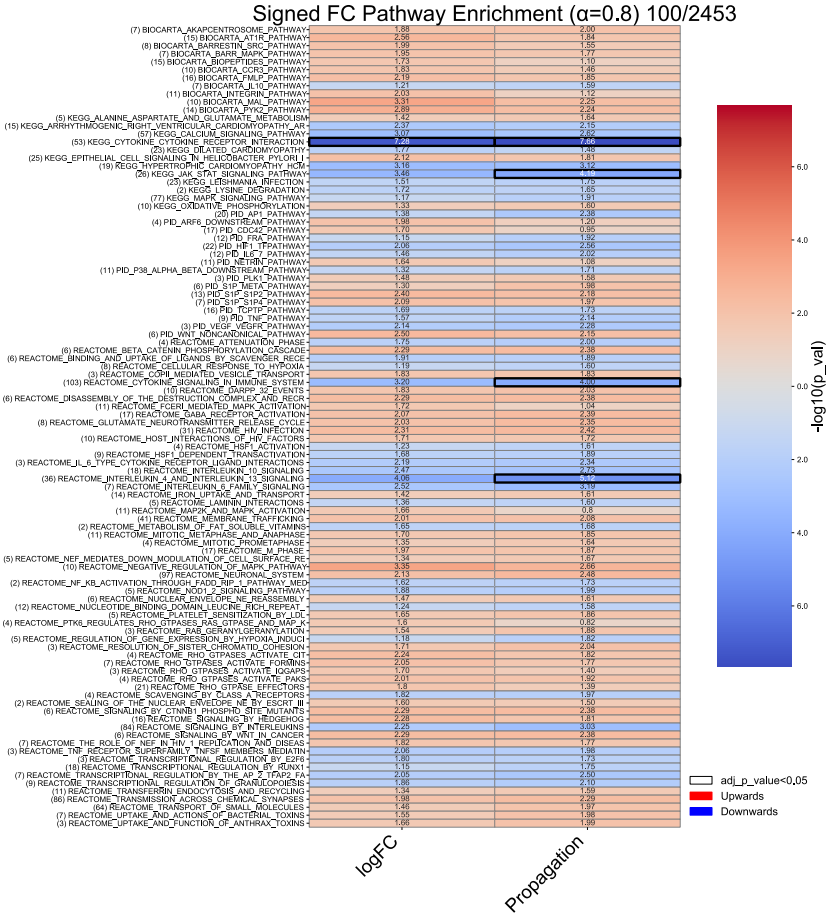
In this cross-campus proposal, we set out to develop a human-relevant *in vitro* platform for studying Parkinson's disease (PD), and to use it for identifying PD-associated abnormal pathways using patient-specific differentiated cells.

Over the first six months, we developed the computational, biological, and engineering infrastructure for tackling this goal, advancing considerably on all fronts.

On the computational side, (Sharan Lab) we developed a tool for pathway enrichment analysis that is based on projecting the raw activity data of disease vs. healthy proteins on a network of protein-protein interactions and then smoothing this information using network propagation techniques. In order to validate and assess the model, we did a massive literature survey to identify specific genes that were identified and published previously (in databases). It is important to note that the next step will be to apply this on the in-house data that will be available from our organoids and iPSC. The data we found was based on the expression data of iPSC-derived dopaminergic neurons from LRRK2-G2019S mutants vs. healthy controls from Carola et al., Nature 2021.

Our initial analysis identified four significantly enriched pathways (circled in bold out of top 100, see **figure below**) known to be associated with PD. As can be also observed, network propagation yields more significant results than the raw fold-change scores, demonstrating the power of analyzing expression data in the context of a network.



In parallel, on the experimental (**biological and engineering**) side, we developed a protocol for growing cortical organoids from PD patients.

We received fibroblasts from a patient with **LRRK2^{G2019S0} mutation, a patient with GBA-1^{N370S}** mutation and healthy control, generated induced pluripotent stem cells (iPSC) (a process which took about 4 months), and were able to expand the culture so that we will have enough cells for future experiments. This step is a major milestone in this work.

Our next step was to grow brain organoids (**Fig. 2**). This step creates a state-of-the-art biological model to model neurodegenerative disease. The brain organoids started to create three-dimensional structures, and are currently at day 6. We will mention that we were able to grow them up to day 30, but due to a power break and failure in the infrastructure, the cells died and we had to restart the organoids.

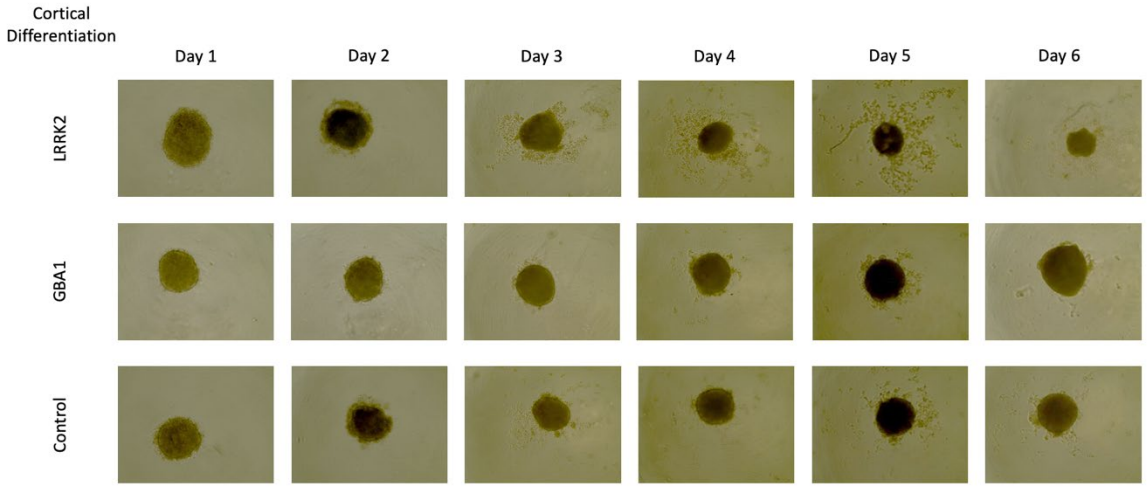


Figure 2 **Derivation of cerebral organoids from induced human pluripotent stem cells** Phase contrast images of days 0-6 EBs subjected to neural induction under combined dual SMAD and WNT inhibition of LRRK2, GBA1 PD and healthy control patients.

Once the organoids grow, we will profile their gene expression and apply the pathway enrichment tool to identify disease-relevant pathways.