# Failure of major proteostatic mechanisms and lysosomal malfunction in p.A53T-αSyn PD patient iPSC-derived astrocytes

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The accumulation of aggregated alpha-Synuclein (αSyn) is typical in Parkinson's disease (PD)-patients' brains and the prion-like spreading hypothesis is gaining ground. However, the astrocytic contribution in PD pathology is understudied, despite PD-related mechanisms including neuroinflammation and aggregate resolution pathways may involve non-cell autonomous components. Dysregulation of neuronal autophagy is known to cause accumulation of aggregated αSyn. Here, we aimed to investigate how the p.A53T-αSyn mutation affects proteostasis in astrocytes, using our previously established induced pluripotent stem cell (iPSC) model derived from PD patients harboring the p.A53T-αSyn mutation. Ventral midbrain astrocytes differentiated from PD iPSCs (PDa) displayed accumulation of protein aggregates, including the pathological phosphorylated form of αSyn. Proteome profiling of PDa versus Ha revealed endocytosis and protein catabolic processes, including autophagy, among the most affected pathways. Upon investigation of the endocytic capacity of Ha and PDa treated with neuronal conditioned medium, we observed that unlike Ha, PDa have diminished capacity to uptake neuronal αSyn. Moreover, proteasome activity was reduced in PDa and autophagy was disturbed, as revealed by increased LC3II levels and reduced autophagosome to autolysosome transition. Additionally, PDa exhibited decreased LAMP1 levels, reduced lysosomal acidity and enzymatic activity, alongside with increased number of lysosomes and altered lysosomal positioning, essential for proper autophagosome-lysosome fusion. These observations couple dysregulated autophagy with impaired lysosomal integrity in PDa. Overall, our data demonstrate that the p.A53T-αSyn mutation causes intrinsic malfunctions in astrocytes, related to proteostasis and clearance mechanisms that may have a critical contribution in PD pathology. **Funding:** HFRI Project 1019-DiseasePhenoTarget; GSRI Project TAA TAEDR-0535850 - Brain Precision.