NENS Exchange Grant

Targeting *GBA*-related phenotypes in Parkinson's patient iPSC-derived dopaminergic neurons

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Period of Stay: January-June 2022

Parkinson's Disease (PD) is the second most common neurodegenerative disorder, affecting over 6 million people worldwide. The neuropathological hallmarks of this disease are the loss of dopaminergic (DA) neurons in the substantia nigra *pars compacta* (SNpc), leading to striatal dopamine deficiency; and the formation of aggregates of α-synuclein in intracellular inclusions known as Lewy's bodies. The great majority of PD cases are idiopathic, with only 10% attributed to genetic forms. The *GBA* gene encodes the lysosomal enzyme glucocerebrosidase (GCase). Approximately 5-10% of parkinsonian patients present a heterozygous *GBA* mutation, making this the most prevalent genetic risk factor for the development of PD. Specifically, the most common *GBA* risk variant is the *GBA-N370S* mutation.

To date, the mechanisms that underlie the increased risk of developing PD of *GBA* mutation carriers have not been fully elucidated. However, iPSC-derived dopamine neurons from PD patients carrying the *GBA-N370S* mutation have revealed some of the relevant mechanisms by which this mutation may increase cellular susceptibility to disease. These parkinsonian neurons show decreased GCase lysosomal activity, leading to a series of altered subcellular events, including endoplasmic reticulum stress upregulation, autophagy and lysosomal dysfunction and increase release of α-synuclein in the culture media. It is proposed that this combination of disturbances impaired cellular homeostasis in DA neurons contributing to their preferential vulnerability in PD, highlighting the early pathogenic relevance of GCase function.

The present study, performed under the support of the NENS exchange grant, proposes the use of human-patient iPSC-derived DA neurons as powerful tools for high-throughput screening of potential compounds for treatment of PD. Moreover, considering the published data about the

early pathogenic relevance of the *GBA-N370S* mutation, we propose that modulating GCase activity may be beneficial for the treatment of PD. To asses this, we have studied the impact of several candidate compounds from ongoing GSK screening programs on the described altered phenotypes of patient-specific dopaminergic neuronal cultures, differentiated from iPSCs lines derived from PD patients carrying the *GBA-N370S* mutation. Immunocytochemistry and high-content imaging system (Opera Phenix) was used to confirm the successful generation of dopaminergic neurons (e.g. expression of midbrain markers and tyrosine hydroxylase). Compound and control conditions treatment protocol was optimized using HEK293 cells. After the application of the identified compounds, several assays were performed on the dopaminergic neurons to analyze the effect of these compound: GCase enzymatic activity assay and α-synuclein release immunoassay. Alterations at the downstream signaling were also studied using qPCR for genes involved ER stress (BiP, IRE+1a, PDI), endolysosomal pathway and autophagic perturbations (LAMP1, LAMP2A, LC3). Results cannot be disclosed due to confidentiality issues with the partner pharmaceutical company.

The NENS Exchange Program has given me the opportunity to perform my Master thesis project at the University of Oxford, in the Laboratory of Molecular Neurodegeneration, under the supervision of Dr. Mootaz Salman. Here, I have been able to benefit from the extensive knowledge and experience of one of the top labs working in Parkinson's Disease. Under the guidance of my supervisor, I have expanded my theoretical formation in the molecular research of Parkinson's Disease and learn several cutting-edge experimental techniques. This experience has allowed me not only to develop new skills as a scientist, but also at the personal level. The friendly atmosphere in the lab and at the University of Oxford, as well as the internationality surrounding me, has given me a unique opportunity to connect with new people and enjoy different cultures and ways of thinking. I would like to thank NENS for their support in this experience, and I strongly encourage other young scientists like me to apply for the NENS exchange grant to have the opportunity to learn new methodological skills and increase their international experience.