Roles and mechanisms of synuclein and ataxin-3 spreading in Parkinson and Machado-Joseph diseases – *SynSpread*

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Parkinson's disease (PD) and Machado Joseph disease (MJD) represent two prototypical neurodegenerative disorders associated with protein aggregation and progressive spread of disease.

The SynSpread project hypothesised that PD and MJD share a common mechanism intersecting autophagy and exosome secretion, ultimately promoting accumulation and spreading of pathogenic proteins. This project aimed at investigating how such common mechanism may contribute to PD and MJD using neurons and astrocytes derived from PD and MJD patients' inducible pluripotent stem cells (iPSCs) and *in vivo* models. Ultimately, the whole- brain data on protein spreading were planned to be plugged into a novel computational model of protein propagation along with an experimentally derived reconstruction of known mouse brain connectivity to predict disease progression and identify biochemical pathways underlying progression.

The Synspread was organized into four work packages and was initiated in January 2015. A large amount of work was performed and contributed to disclose important mechanisms related to alpha-synuclein and ataxin-3 spreading in the context of PD and MJD, respectively. Through the establishment of co-culture systems with neuroepithelial stem cells (NESCs) derived from PD and MJD patients, we were able to perform studies of alpha-synuclein and ataxin-3 spreading/transport. Currently, induced pluripotent stem cells (iPSCs) derived from patients' fibroblasts are widely used for *in vitro* disease modelling and also for experimental cell replacement approaches. However, in the particular case of protein spreading studies in PD, further investigations indicated that the increased alpha-synuclein levels failed to induce spreading or aggregation in the mouse brain. Therefore, there was the need to move towards the cultivation of PD patient specific neurons derived from mutant LRRK2 iPSCs and optimize it in 3D microfluidics. This approach supported the use of advanced *in vitro* models for future patient stratification and personalized drug development.

Pathology from neurodegenerative disorders affects neuronal populations that are anatomically connected although the contribution of neuronal connectivity remains to be quantitatively explored. The contribution of the connectome alone to the spreading of arbitrary aggregates using a computational model of temporal spread within an abstract representation of the mouse mesoscale connectome was simulated. It was found that neuronal connectivity appears to be compatible with the spreading pattern of alpha-synuclein pathology however, it may be *per se* insufficient to determine the anatomical pattern of protein spreading observed in experimental animals, suggesting a role of selective vulnerability of neuronal pathways to alpha-synuclein diffusion, accumulation and pathology. Furthermore, it was concluded that neuronal connectivity is necessary but not sufficient to computationally predict the anatomical pattern of induced alpha-synuclein pathology observed in rats. If one assumes that mouse brainstem connectivity is a fair representation of human brainstem connectivity, then these results suggest that the connectome is necessary, but not alone sufficient, to predict the histopathological patterns of Lewy pathology observed in PD. Overall, the SynSpread project contributed to the comprehension of synuclein and ataxin-3 transport between cells and prediction of toxic proteins' spreading patterns, using 3D and computational models.

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