TITLE: Modeling Parkinson's disease neurodegeneration mechanisms and therapeutic strategies using induced pluripotent stem cells.

ABSTRACT:

BACKGROUND

Parkinson's disease (PD) is the second-most common neurodegenerative disorder that affects 2–3% of the population ≥65 years of age (1). The main neuropathological finding is alpha-synuclein-containing Lewy bodies and loss of dopaminergic neurons in the substantia nigra, manifesting as reduced facilitation of voluntary movements. Although the majority of PD cases are sporadic in nature, there are a growing number of monogenic mutations identified to cause PD in a highly penetrant manner. Mutations in the gene encoding beta-glucocerebrosidase (GBA) are now known to be the largest risk factor for development of PD, conferring a 5- to 7-fold increased risk (2). However, to date, the molecular mechanisms which lead to this increased risk are not fully elucidated. Furthermore, it remains obscure the reason why subjects with the same GBA mutation may or not develop PD.

OBJECTIVES AND METHODOLOGY

An intriguing hypothesis to explain the differences in GBA mutation penetrance is that other lysosomal gene mutations may interact with GBA dysfunction causing the accumulation of alpha-synuclein and potentially other toxic substrates, increasing PD susceptibility (3). With the advent of reprogramming technology it is now possible to capture lysosomal gene mutations into induced pluripotent stem cells (iPSCs) to establish models of PD in a dish. Using a protocol for the efficient differentiation of patient-derived iPSCs into dopaminergic neurons (4), the selected candidate will correlate their functionality to key pathological pathways including alpha-synuclein aggregation, lysosomal and endoplasmic reticulum stress. The study will be performed both at single cell and network level by a combination of advanced imaging (1- and 2-photons, super-resolution STED) and electrophysiological techniques (patch-clamp and multi-electrode arrays). Since the human brain is an extremely complex environment, whose structural and functional properties can only be partially recapitulated by 2D cultures, the project also aim to study 3D midbrain organoids (5) (https://youtu.be/cHyhWv37g74). Finally, therapeutic potential of novel antibodies which prevent alpha-synuclein aggregation will be explored in the designed models.

REFERENCES:

1. Nat Rev Dis Primers. 2017 Mar 23;3:17013
2. Asselta R et al. Parkinsonism Relat Disord. 2014 Nov;20(11):1215-20.
3. Wong YC, Krainc D. Mov Disord. 2016 Nov;31(11):1610-1618.
4. Okano H, Yamanaka S. Mol Brain. 2014; 7: 22.
5. Schwamborn JC, Stem Cells Dev. 2018 Feb 7.

PARTICIPANTS (PI and co-PIs):

UNIPD-PNC members: Mario Bortolozzi (PI, Dept. of Physics and Astronomy and Venetian Institute of Molecular Medicine), Angelo Antonini (Dept. of Neurosciences), Luigi Bubacco (Dept. of Biology), Stefano Vassanelli (Dept. of Biomedical Sciences).

External partners: Alessio Di Fonzo (Policlinico di Milano), Stefano Duga (Humanitas University, Milano), Massimo Aureli (University of Milan), Jens Schwamborn e Reiko Krueger (Luxebourg Centre for Systems Biomedicine, Université du Luxembourg).

EXPERIMENTAL DATA:

To be acquired	X
Already acquired (ready to be used)	

The selected candidate will work at the Venetian Institute of Molecular Medicine (VIMM, Padova), where state-of-the-art biological and biophysical facilities are available. In particular, electrophysiology and imaging infrastructures include the 2P/STED microscope of the PNC coordinated by prof. Bortolozzi. Additional equipment (multi-electrode arrays, MEAs) is available in the laboratory of prof. Vassanelli at the Biomedical Sciences department. The multidisciplinary nature of this project appears evident from the broad range of expertise involved: imaging and electrophysiology (prof. Bortolozzi and prof. Vassanelli), iPSC models (prof. Bortolozzi, dr. Di Fonzo and prof. Schwamborn (5)), biochemistry (prof. Bubacco and dr. Aureli), genetics and medicine (prof. Antonini and prof. Duga). Preliminary experiments in midbrain organoids were already performed in collaboration with dr. Di Fonzo and profs. Antonini and Bubacco (https://youtu.be/wekvd8F7eSU).
Thanks to the large database of GBA-PD patients of prof. Antonini, we plan to recruit in this project 3 healthy subjects and at least 3 GBA-PD patients, in particular a homozygous L444P, a heterozygous L444P and a double heterozygous L444P/N350S. The first year will be devoted to generation, characterization and differentiation of iPSC lines, while the next two years to biochemical, imaging and electrophysiological experiments in dopaminergic neurons derived from iPSCs. In order to corroborate and improve the group's expertise with organoids, we plan a 4 month period to be spent by the PhD student in the Luxembourg Centre for Systems Biomedicine. This period could also give him/her the opportunity to obtain the Doctor Europaeus title.

ETHICS COMMITTEE:

Obtained	
Conditioned submission*	Expected time response (in months): 3
Not required	

* request will be submitted only if a PhD student will be associated to the project