Parkinson's disease (PD) is the second most age related neurodegenerative disease affecting approximately 1% of the world population above 60 years. The disease is commonly associated with bradykinesia, impaired ability of voluntary movements and resting tremors. Pharmacological strategies for PD revolve around increasing the levels of dopamine in brain and carry the risks of side-effects like psychotic side-effects in PD. The classical immunopathological feature of PD patient's brain is occurrence of Lewy bodies, majorly composed of α-synuclein & ubiquitin. Aberrant level of α-synuclein is cited in familial as well as idiopathic PD subjects (McCormack et al. 2016).

Mutations in Leucine Rich Repeat Kinase 2 (LRRK2) contribute significantly to large number of familial as well as sporadic cases of PD. LRRK2 is a large 2527 amino acid protein consisting of several functional domains including a Ras-like small GTPase domain (ROC), a carboxy-terminal of Roc (COR) domain, and a kinase domain3. The various mutations in LRRK2 involved in PD, are R1441C, R1441G, I1371V in the Roc domain, Y1699C in the COR domain, and G2019S, I2020T in the kinase domain. Research on PD related LRRK2 mutations have been focused majorly on the mutations located in the kinase domain, particularly on the G2019S mutation, which increases the kinase activity. G2019S mutation is predominant in the Caucasian PD patients. The prevalence of this mutation in the Indian population is very rare with less than 0.1% harboring this mutation. On the other hand clinical studies for LRRK2 mutation in Indian population for PD have shown in the I1371V allele, specifically in the East Indian population.

The I1371V SNP is located in the Ras of complex proteins (ROC) domain that may act as a GTPase to regulate its protein kinase activity. The ROC domain has a dimeric fold; the I1371V SNP is located at the interface of two monomers . This mutation partially disrupts the tertiary structure of the protein at the dimer interface, which results in the decrease of the GTP hydrolysis and therefore prolongs GTP-mediated activation of the kinase. Impaired GTPase activity leads to more accumulation of the GTP bound form of LRRK2.

Mutations in the GTPase domain are not explored as extensively as the kinase domain counterparts. There are yet no PD patient-specific iPSCs reported with I1371V allele mutation for LRRK2. *In vitro* studies have shown that LRRK2 mutation (G2019S allele) is associated with α-synuclein aggregation and phosphorylation and its release. For the GTPase domain mutations of R1441C, R1441G, R1441H allele has been shown to lead to impaired phosphorylation of LRRK2 but there are yet no studies on its association with ontogeny of dopaminergic neurons and α-synuclein phosphorylation machinery.

While post-mortem samples provide valuable information on end-stage of PD, an understanding of the early stage molecular mechanisms holds the key in developing therapeutics to prevent or halt PD progression. Cell line models such as PC12, SH-SY5Y, HEK or transgenic and primary rodent neuronal cultures fail to recapitulate the required DA neuron subtype, while animal models fail to replicate human ethnicity variations. It is well established that humans and mice have considerable developmental, genetic and physiological differences (Mouse genome sequencing consortium et al., 2002), and that genetic-mutations for human PD do not replicate the disease-phenotype in mice. Pluripotent stem cells are thus attractive candidates to study the progression of PD *in vitro* via directed differentiation into neuronal progenitors and subsequent neuronal types such as DA neurons. iPSCs greatly help in circumventing the ethical issues associated with the use of embryonic stem cells. Additionally, iPSC-derived neuronal cells provide a patient-specific perspective on PD, of particular relevance considering that majority of the PD cases are idiopathic. The rapidly growing knowledge of efficient iPSC generation and various differentiation protocols for diverse cell types has brought the promise of personalized medicine from the into reality. It is increasingly known that in complex neurodegenerative diseases, the clinical heterogeneity with respect to the severity of the disease, the stage, drug responsiveness and gene environment interaction are prevalent. IPSCs offer a unique opportunity to study these

complex disease-associated differences varying from patient to patient. Woodard et al in 2014, demonstrated the effect of a single glucocerebrosidase N370S mutation in iPSC-derived mDA neurons of twins clinically discordant for PD. Nguyen et al in 2011, showed the effect of G2019S mutation in the LRRK2 gene in multiple clones of a single iPSC line derived DA neurons which lead to increased susceptibility under 6OHDA stress. The effect of synuclein locus triplication to increased susceptibility of neurons to oxidative stress and synuclein accumulation was revealed by Byers et al in 2011, using iPSCs derived from a 48 year old male PD patient in comparison to iPSC derived from the unaffected sister. The varying responses of patients to the L-Dopa treatment coupled with the complex heterogeneity of PD cases indicate that differences in the biochemical pathways play major role in the drug responsiveness. Patient specific genetic differences largely influence the metabolic pathways governing the drug uptake and activity. (Stoddard-Bennett et al, 2019)

Hence in this PhD work, we are studying the LRRK2 I1371V allele mutation on cellular pathogenesis of Parkinson's disease along with a comparative analysis in sporadic PD case using patient-specific induced pluripotent stem cells. In the present study are generating midbrain floor plate cells (FPCs) from iPSC lines harboring the I1371V mutation , a sporadic PD line along with iPSC derived from age-matched healthy control. The FPCs are characterized for their commitment to mature dopaminergic neurons. The intracellular $Ca_{2+}$ and vesicular dopamine release from these neurons under physiological stimuli, detected through real time fluorescence imaging, FACS and ELISA – show significant differences between the PD and healthy lines. Neurite analysis, axon degeneration index, along with synaptic vesicle release proteins, synapsin, VMAT2 and RAB protein expression and phosphorylation will be determined. Along with the cellular and biophysical differences, the differential ontogeny of the FPC population into substantia nigra pars compacta (SNPc) and ventral tegmental area (VTA) type neurons is also checked since the SNPc dopamine neurons exhibit increased degeneration compared to VTA type neurons with PD progression . Enzyme expression and activity and phosphorylation of LRRK2, PLK2, CK2 and GRK5 (involved in α-synuclein phosphorylation), phosphatase (PP1 and PP2A) will be assessed. The susceptibility to 6-OHDA stress of these neurons will be assessed through ROS generation and apoptosis.

Indrani Datta

INDRANI DATTA. Ph.D.
Associate Professor
Department of Biophysics
National Institute of Mental
Health & Neurosciences
Bengaluru - 560 029.