Exploring the Role of a Common Genetic Variant in Parkinson’s Disease Pathogenesis

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Introduction

• Genetic variation in and around the \textit{LRRK2} gene is associated with both familial and sporadic Parkinson’s disease.

• The present study aims to determine why the single nucleotide polymorphism \(\text{rs76904798}[C/T]\) is highly associated with Parkinson’s disease.
Methods

**Single Nuclei RNA sequencing (snRNAseq)**
- Nuclei isolation from human frontal cortex
- snRNAseq using 10x Genomics platform
- Samples were obtained from the University of Maryland Brain and Tissue Bank

**Differentiation of iPSC to microglia**
- Published protocol from Blurton-Jones lab
- 11-12 days to intermediate hematopoietic stem cell (iHSC) stage
- 28 days from iHSC to mature iMicroglia (iMGL)
- Quantification of LRRK2 expression by western blot and qPCR

![Image courtesy of 10x Genomics](image_url)

Abud et al. Neuron 2017
Results

- snRNAseq of frontal cortex nuclei from 15 donors with known genotype at rs76904798: 5x “CC”, 5x “CT”, 5x “TT”
- 117,379 total nuclei
- Clustering analysis yielded expected brain cell populations
Results

- A statistically significant relationship between genotype at rs76904798 and LRRK2 expression is observed only in microglia population #13 and not in other cell types.
Results

- iPS cells can be reliably differentiated to microglia-like cells that robustly express microglia marker Iba1

- Differentiated iMicroglia respond to LPS, an inflammatory stimulus, by upregulating inflammatory cytokines IL1β, TNFα, and IL6
Results

• “CT” iMicroglia carrying the risk variant “T” at rs76904798 have increased LRRK2 expression at mRNA and protein levels compared to “CC” iMicroglia.

• “CT” iMicroglia have increased phosphorylation of LRRK2 substrate Rab10, with a synergistic effect of genotype and treatment (interaction \( p = 0.009 \)).
Summary

• Nuclei isolated from frozen human frontal cortex yield high quality snRNAseq data that allows measurement of all expected cell types.

• Robust expression of LRRK2 RNA can be measured in human frontal cortex by snRNAseq, with particularly high expression observed in populations of oligodendrocyte precursor cells, excitatory neurons, and microglia.

• Comparing 5 donors per genotype at rs76904798 revealed a cell type-specific eQTL, with increased dosage of PD risk allele ‘T’ corresponding to increased LRRK2 expression in microglia. This relationship was not found in other cell types.

• iPS cell lines can be reliably differentiated to microglia-like cells.

• Increased LRRK2 expression at both the mRNA and protein levels was measured in iMicroglia carrying the PD risk allele ‘T’ at rs76904798, along with increased LRRK2 activity as measured by phosphorylation of physiological substrate Rab10.