**Title:**

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**Authors**:

Brian M. Schilder1,2,3, Katia Lopes1,2,3, Elisa Navarro1,2,3, Towfique Raj1,2,3

1. Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY 10035
2. Department of Genetics & Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10035
3. Ronald M. Loeb Center for Alzheimer’s Disease, Icahn School of Medicine at Mount Sinai, New York, NY 10035

**Corresponding Autho**r:

Brian M. Schilder

[brian.schilder@mssm.edu](mailto:brian.schilder@mssm.edu)

19 E 98th St, Room E6-6C

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Parkinson’s Disease, monocytes, single-cell, networks

**Outline**

* **Introduction**
  + *Importance of PD research and outstanding questions*
    - General review of PD prevalence, our current state of knowledge, and the lack of treatments (Poewe et al., 2018).
  + *Relationship between PD and the immune system (specifically monocytes):*
    - PD research has implicated a causal role of the immune system (Hirsch & Hunot, 2009).
      * PD GWAS gene hits support this (Chang et al., 2017; M. A. Nalls et al., 2018, 2014)
    - (Grozdanov et al., 2014)
      * PD monocytes show excessive and sustained response.
      * Distinguished between classical vs. non-classical.
    - (Nissen et al., 2019):
      * “﻿PD patients’ [﻿CD14+/CD163+/HLA-DR+ monocytes] were less responsive to stimulation, as shown by the lack of changes in CD163 and CD14 expression, and by the absence of significant upregulation of anti-inflammatory cytokines in culture”.
      * Increased CD14+ monocytes in PD culture after 24h.
      * Also demonstrated sex differences in PD (but not control) monocytes.
      * Hypothesis: PD monocytes are upregulated due to an impaired ability to response to disease.
    - (Ramdhani et al., 2018):
      * PD and AD-related trans-QTLs in monocyte/macrophages.
    - (Li, Wong, Humphrey, & Raj, 2019):
      * LRRK2 eQTLs observed in monocytes (but not bulk brain tissue).
    - (Raj et al., 2014):
      * Large proportion (~2/3) of eQTLs were monocyte markers. AD was about the same, if not slightly more.
  + *History of monocyte subtypes:*
    - (Mukherjee et al., 2015):
      * Classical vs. non-classical vs. intermediate monocytes (in Lupus)
    - (Villani et al., 2017):
      * scRNA-seq of ~2,400 PMBCs. Proposed new classification of 6 DC subtypes and 4 monocytes subtypes.
  + *Purpose of this study:*
    - Investigate the molecular mechanisms of PD within subtypes of monocytes.
    - To do this, we conducted scRNA-seq on CD14+ monocytes in 10 patients (3 controls and 7 PD)
    - In order to assess whether these monocyte subtype specific effect could be detected within a larger cohort of individuals at the bulk-level, we also compared gene co-expression network derived from this data to those derived from bulk RNA-seq of purified monocytes.
* **Methods & Materials**
  + scRNA-seq
    - Samples
      * 10 donors: 3 controls, 7 PD (2 GBA + 1 LRRK2 + 4 sporadic PD)
    - Data Collection
      * CITE-seq, highlight Cell Hashing (Stoeckius et al., 2018)
    - Data Analysis
      * Doublet identification w/ demuxlet (Kang et al., 2018)
      * Preprocessing
        + Cell filtering: ~27k 🡺 ~22k cells
        + Gene filtering ~25k 🡺 ~22k genes
        + Protein-coding only: ~22k 🡺 ~14k genes
        + Normalization: counts ~ nUMI + %mitochondrial genes

Did not regress out individual ID because would remove any disease signal with such a small individual-level sample size.

* + - * Clustering (DR, Louvain)
      * Cell type identification
        + Garnett (w/ provided PBMC dataset, and Villani dataset)
      * Cell type distribution
        + Test # of PD vs. controls cells within each cluster.
      * DGE
        + Method1: Monocle3 DGE w/ quasi-Poisson distribution.
        + Method2: Effie’s robust iterative pipeline.
        + Across Clusters

PD vs. Controls

PD vs. GBA

* + - * + Between Clusters

Cluster 1 (canonical) vs. Cluster 2 (intermediate)

* + - * + Within Clusters

PD vs. Controls

PD vs. GBA

* + - * Enrichment of DGE
        + GO terms (gprofiler2) (Raudvere et al., 2019)
        + PD GWAS gene list (Chang et al., 2017; Hujoel, Gazal, Loh, Patterson, & Alkes, 2019; M. Nalls, 2018)
        + AD GWAS gene list
      * Networks
        + UMAP + Louvain w/ 1000 most variable genes (using Seurat fineVariableGenes function)
        + WGCNA?
        + PINSplus/consensus clustering?
  + bulk RNA-seq
    - Samples: 237 donors (101 Controls, 136 PD)
    - Preprocessing:
      * 11,473 genes after QC and protein-coding filtering.
    - WGCNA networks
    - Data Collection (brief summary w/ reference to prior publications)
  + bulk vs. sc modules
    - module-module overlap/enrichment (gene-level, term-level)
  + Scripts and data availability
* **Results**
  + scRNA-seq
    - Good individual-level mixing in all clusters
    - Cell cluster cell type identification:
      * Key markers, Garnett (w/ provided PBMC dataset, and Villani dataset)
    - DGE
      * Across Clusters
        + PD vs. Controls
        + PD vs. GBA
      * Between Clusters
        + Cluster 1 (canonical) vs. Cluster 2 (intermediate)
        + 1619/12929 genes (12.5%) were DE at Bonferroni-corrected p-value ≤ 0.05.
        + Enriched GO terms (enrichR):

Inflammatory processes

Immune response

Chrohn’s disease

Blood-brain barrier

Recruitment of leukocytes

Promotion of cytokine/

Chemokine production

* + - * + Huge percentage DGEs bind to RAGE receptor (S100A[x]) (Hofmann et al., 1999; Marshak, Pesce, Stanley, & Griffin, 1992; Xia, Braunstein, Toomey, Zhong, & Rao, 2018).

RAGE gene (AGER) itself is DE at p=0.003 LogDC, 0.3), but not significant.

RAGE receptor binding appears in enrichR results.

* + - * Within Clusters
        + PD vs. Controls
        + PD vs. GBA
    - Enrichment of DGE
      * GO terms (gprofiler2) (Raudvere et al., 2019)
      * PD GWAS gene list
      * AD GWAS gene list
    - Networks
      * Summary stats: Number of modules, genes/module,
  + bulk vs. sc modules
    - Overall:
      * 25 / 1122 module-module comparisons show significant overlap (FDR ≤ 0.05) (Table X).
      * ~23% bulk.modules showed enrichment for some sc.module.
      * ~82% of sc.modules showed enrichment for some bulk.module.
    - Top 2 most-similar module-module pairs:
      * ~53% of the sc.module2 genes were contained in the green bulk.module (Mitochondria, Translation, Ribosome)
      * ~31% of the sc.module8 genes were contained in the pink bulk.module (Cell proliferation, Apoptosis, Cytokine response)
    - sc.modules 4,5 & 10 are over-expressed in intermediate monocytes. (Fig. X)
    - sc.modules 8, 13 & 17 are over-expressed in canonical monocytes. (Fig. X)
    - Conclusions:
      * The bulk monocyte modules contain signatures that can be found in 82% of the sc.modules.
      * The green bulk.module corresponds to mitochondrial function and is upregulated in both canonical and intermediate monocytes (and possibly CD14+ neutrophils).
      * The pink bulk.module corresponds to a proliferative cytokine response that is upregulated in canonical monocytes (but not intermediate monocytes). This module is also significantly underexpressed in PD monocytes, possibly suggesting an impaired ability to mount a defensive immune response in the disease.
* **Discussion**
* **Conclusions**
* **Supplementary Materials**

**Abstract**

**Introduction**

**Methods & Materials**

**Results**

**Discussion**

**Conclusions**

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**Figures**

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