**Title:**

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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)
    - General introduction to monocytes and macrophages (Jung, 2018).
    - (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. Though others later refuted this, claiming that the additional monocyte subtype was actually a misclassified group of NK cells (Kapellos et al., 2019).
  + *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)
      * Monocle3 (Cao et al., 2019; Trapnell et al., 2014)
      * 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 findVariableGenes 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**
* **To Do**
  + **Update PD genes list in enrichment**

**Abstract**

**Introduction**

**Methods & Materials**

**Results**

**Discussion**

**Conclusions**

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**Figures**

**References**

Cao, J., Spielmann, M., Qiu, X., Huang, X., Ibrahim, D. M., Hill, A. J., … Shendure, J. (2019). The single-cell transcriptional landscape of mammalian organogenesis. *Nature*, *566*(7745), 496–502. https://doi.org/10.1038/s41586-019-0969-x

Chang, D., Nalls, M. A., Hallgrímsdottir, I. B., Hunkapiller, J., Van Der Brug, M., Cai, F., … Graham, R. R. (2017). A meta-analysis of genome-wide association studies identifies 17 new Parkinson’s disease risk loci. *Nature Genetics*, *49*(10), 1511–1516. https://doi.org/10.1038/ng.3955

Grozdanov, V., Bliederhaeuser, C., Ruf, W. P., Roth, V., Fundel-Clemens, K., Zondler, L., … Danzer, K. M. (2014). Inflammatory dysregulation of blood monocytes in Parkinson’s disease patients. *Acta Neuropathologica*, *128*(5), 651–663. https://doi.org/10.1007/s00401-014-1345-4

Hirsch, E. C., & Hunot, S. (2009). Neuroinflammation in Parkinson’s disease: a target for neuroprotection? *The Lancet Neurology*, *8*(4), 382–397. https://doi.org/10.1016/S1474-4422(09)70062-6

Hofmann, M. A., Drury, S., Fu, C., Qu, W., Taguchi, A., Lu, Y., … Schmidt, A. M. (1999). RAGE Mediates a Novel Proinflammatory Axis: A Central Cell Surface Receptor for S100/Calgranulin Polypeptides. *Cell*, *97*(7), 889–901. https://doi.org/10.1016/s0092-8674(00)80801-6

Hujoel, M. L. A., Gazal, S., Loh, P., Patterson, N., & Alkes, L. (2019). Combining case-control status and family history of disease increases association power, 1–20.

Jung, S. (2018). Macrophages and monocytes: Of tortoises and hares. *Nature Reviews Immunology*, *18*(2), 85–86. https://doi.org/10.1038/nri.2017.158

Kang, H. M., Subramaniam, M., Targ, S., Nguyen, M., Maliskova, L., McCarthy, E., … Ye, C. J. (2018). Multiplexed droplet single-cell RNA-sequencing using natural genetic variation. *Nature Biotechnology*, *36*(1), 89–94. https://doi.org/10.1038/nbt.4042

Kapellos, T. S., Bonaguro, L., Gemünd, I., Reusch, N., Saglam, A., Hinkley, E. R., & Schultze, J. L. (2019). Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases, *10*(August), 1–13. https://doi.org/10.3389/fimmu.2019.02035

Li, Y. I., Wong, G., Humphrey, J., & Raj, T. (2019). Prioritizing Parkinson’s disease genes using population-scale transcriptomic data. *Nature Communications*, *10*(1), 1–10. https://doi.org/10.1038/s41467-019-08912-9

Marshak, D. R., Pesce, S. A., Stanley, L. C., & Griffin, W. S. T. (1992). Increased S100β neurotrophic activity in Alzheimer’s disease temporal lobe. *Neurobiology of Aging*, *13*(1), 1–7. https://doi.org/10.1016/0197-4580(92)90002-F

Mukherjee, R., Kanti Barman, P., Kumar Thatoi, P., Tripathy, R., Kumar Das, B., & Ravindran, B. (2015). Non-Classical monocytes display inflammatory features: Validation in Sepsis and Systemic Lupus Erythematous. *Scientific Reports*, *5*(May), 1–14. https://doi.org/10.1038/srep13886

Nalls, M. (2018). Parkinson’s disease genetics: identifying novel risk loci, providing causal insights and improving estimates of heritable risk. *Bioarxiv*. https://doi.org/http://dx.doi.org/10.1101/388165

Nalls, M. A., Blauwendraat, C., Vallerga, C. L., Heilbron, K., Bandres-Ciga, S., Chang, D., … Andrew B. Singleton for the International Parkinson’s Disease Genomics Consortium. (2018). Expanding Parkinson’s disease genetics: novel risk loci, genomic context, causal insights and heritable risk. *BioaRxiv*.

Nalls, M. A., Pankratz, N., Lill, C. M., Do, C. B., Hernandez, D. G., Saad, M., … Singleton, A. B. (2014). Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson’s disease. *Nature Genetics*, *46*(9), 989–993. https://doi.org/10.1038/ng.3043

Nissen, S. K., Shrivastava, K., Schulte, C., Otzen, D. E., Goldeck, D., Berg, D., … Romero‐Ramos, M. (2019). Alterations in Blood Monocyte Functions in Parkinson’s Disease. *Movement Disorders*, (June), mds.27815. https://doi.org/10.1002/mds.27815

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., … Abstract. (2018). Parkinson disease. *Nature Reviews*, *3*, 17013. https://doi.org/10.1016/B978-0-444-63916-5.00011-2

Raj, T., Rothamel, K., Mostafavi, S., Ye, C., Lee, M. N., Replogle, J. M., … Jager, P. L. De. (2014). Polarization of the effects of autoimmune and neurodegenerative risk alleles in leukocytes. *Science*, *344*(May), 519–524.

Ramdhani, S., Navarro, E., Udine, E., Schilder, B. M., Parks, M., & Raj, T. (2018). Tensor Decomposition of Stimulated Monocyte and Macrophage Gene Expression Profiles Identifies Neurodegenerative Disease-specific Trans-eQTLs. *BioRxiv*, 499509. https://doi.org/10.1101/499509

Raudvere, U., Kolberg, L., Kuzmin, I., Arak, T., Adler, P., Peterson, H., & Vilo, J. (2019). g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Research*, *47*(W1), W191–W198. https://doi.org/10.1093/nar/gkz369

Stoeckius, M., Zheng, S., Houck-Loomis, B., Hao, S., Yeung, B. Z., Smibert, P., & Satija, R. (2018). Cell “hashing” with barcoded antibodies enables multiplexing and doublet detection for single cell genomics. *Genome Biology*, 237693. https://doi.org/10.1101/237693

Trapnell, C., Cacchiarelli, D., Grimsby, J., Pokharel, P., Li, S., Morse, M., … Rinn, J. L. (2014). The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nature Biotechnology*, *32*(4), 381–386. https://doi.org/10.1038/nbt.2859

Villani, A. C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., … Jardine, L. (2017). Single-cell RNA-Seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science*, *101*(9), 1955–1956. https://doi.org/10.1097/TP.0000000000001890

Xia, C., Braunstein, Z., Toomey, A. C., Zhong, J., & Rao, X. (2018). S100 proteins as an important regulator of macrophage inflammation. *Frontiers in Immunology*, *8*(JAN), 1–11. https://doi.org/10.3389/fimmu.2017.01908