Can single-nucleus transcriptomics identify microglial cell states relevant to Alzheimer's Disease

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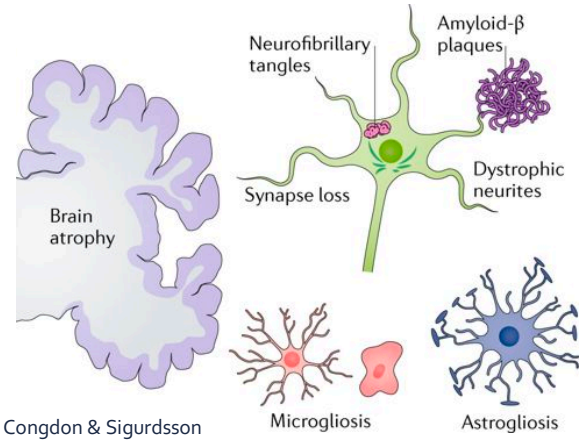
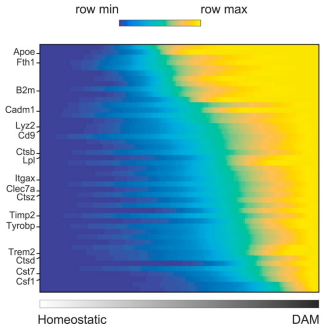
Microglia (the brain's immune cells) contribute to Alzheimer's pathology



Congdon & Sigurdsson (2017), *Nature.*



Keren-Shaul *et al.* (2017), *Cell.*

Disease-associated microglia (DAM) characterised in AD mouse

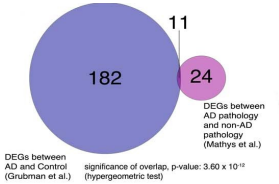
- Show upregulation of genes relating to immune function, lipid metabolism (1)

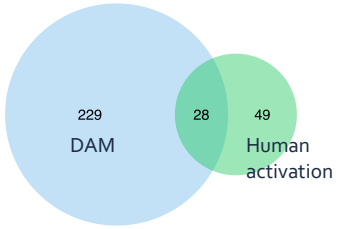
Can we identify a DAM signature in human

- Using postmortem tissue from Alzheimer's patients
- Can't extract cells from frozen tissues
- So we generally extract single nuclei

Very little consensus between human studies (2,3)

Grubman *et al.* (2019), *Nat. Neuroscience.*





Very little overlap with mouse DAM signature (1,2)

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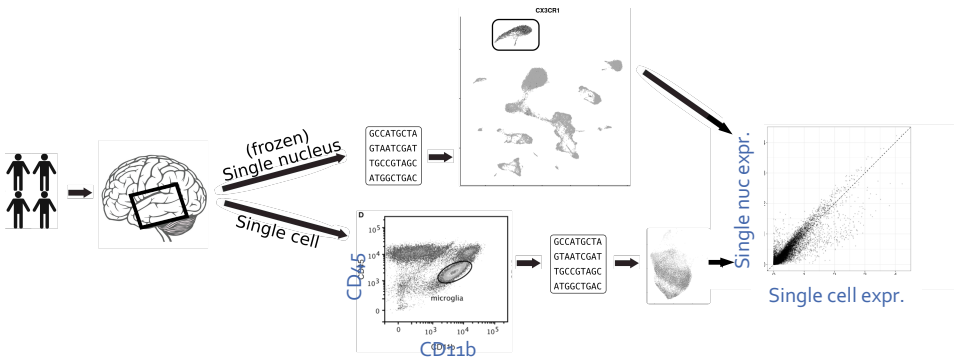
- **So we asked:**

Could this lack of a human activation signature be a result of the single nucleus sequencing technology?

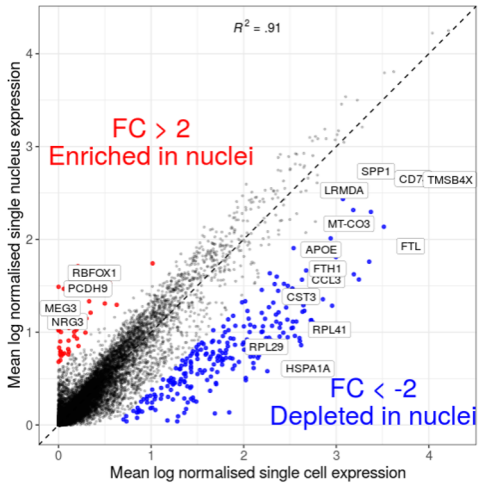


Healthy cortical tissue from 4 neurosurgical patients:

- Single cell sequencing of FACS-sorted microglia
- Single nucleus sequencing of whole tissue, *in silico* extraction of microglial nuclei

Comparison of expression profiles from microglial cells and nuclei

A small population of genes are detected at lower levels in nuclei than in cells:



Differential abundance between single cell and single nucleus expression:

- most genes show similar normalised abundance (r^2=0.91)
- 255 genes with depleted abundance in nuclei (FC < -2, p.adj. < 0.05)

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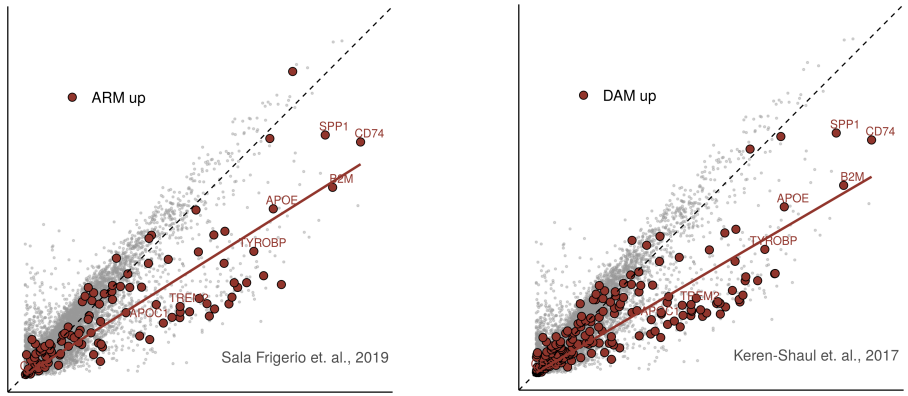
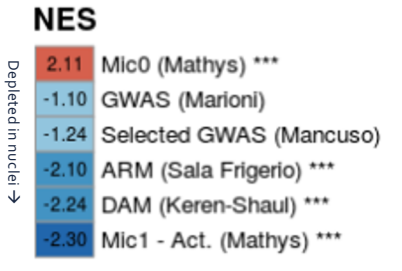
Many genes depleted in nuclei have been implicated in microglial activation in mouse



Microglial genes identified in mouse models of AD are depleted in nuclei

Enrichment analysis of activation genes against fold change confirms depletion of microglial activation genes in single nuclei. * *p.adj < 0.00005*



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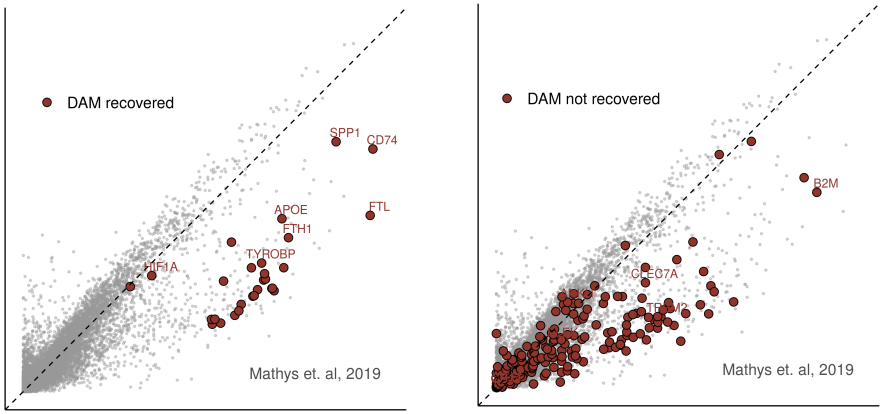
A previous single nucleus study of AD patients identifies only higher-expressing DAM genes as human activation genes



SnRNA-Seq of human postmortem tissue identified a subpopulation of microglia enriched genes in AD patients compared with controls ("Mic 1"). Within this population, only higher-expressing activation genes (identified previously in mouse) were detected (L). Lower-abundance genes were not detected (R).

Mic0: homeostatic microglial genes defined in human cortical tissue (2)
GWAS: genome-wide association study genes implicated in AD (4)
ARM: activation response microglia defined in mouse models of AD (5)
DAM: disease-associated microglia defined in mouse models of AD (1)
Mic1: activation genes defined in human cortical tissue (AD patients) (2)
**** p.adj < 0.0005*

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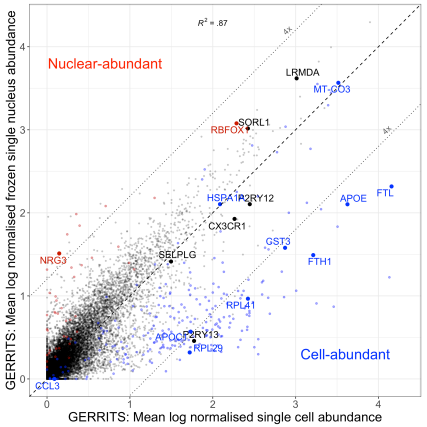
Conclusions

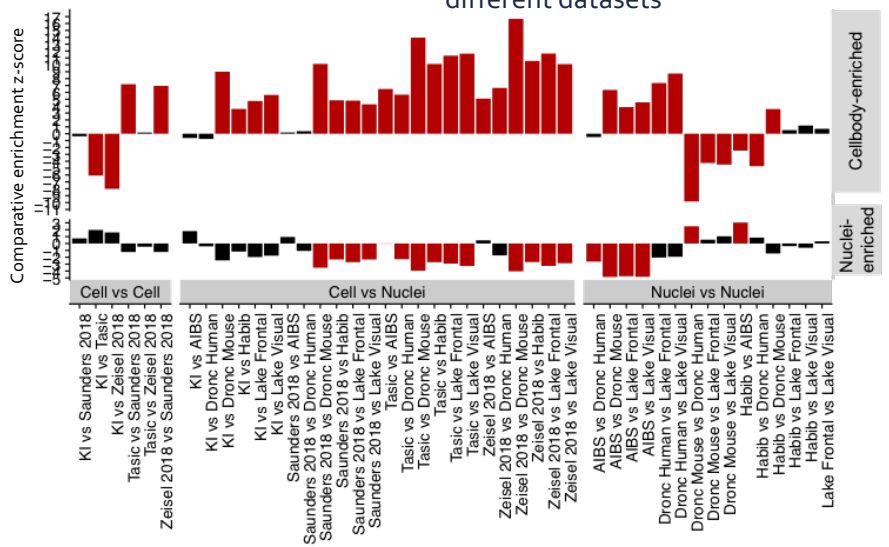
Single nucleus sequencing is not suited to detection of microglial activation genes in human tissue (Thrupp *et al.* , *Cell Reports*, 2020)

- Candidate microglial activation genes show low abundance in nuclei relative to cells
- We see similar results when comparing single nucleus and single cell microglial datasets
- Single nucleus data should be complemented with orthogonal technologies (spatial transcriptomics, iPSC-derived models)
- **Unanswered questions**
- Is this a biological artefact, or a technical artefact?
- Samples with more activation may show different mRNA distributions between nuclei and cellbody
- Deeper sequencing (or large sample sizes) may resolve this issue



Comparison of microglial cells and nuclei from Gerritts *et al.* (6), highlighting in blue our nuclei-depleted genes

Comparison of public single nucleus and single cell microglial data: cell vs. nuclei bars (middle panel) show enrichment of our nuclei-depleted genes in cells in different datasets



References

1. Keren-Shaul *et al.* (2017) Cell.
2. Mathys *et al.* (2019) Nat.
3. Grubman et al. (2019) Nat. Neuroscience.
4. Marioni *et al.* (2018) Transl. Psychiatry.
5. Sala Frigerio *et al.* (2019) Cell Rep.
6. Gerritts *et al.* (2019) Glia.

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