Dissecting the role of microglia in *C9ORF72* ALS using cerebral organoids

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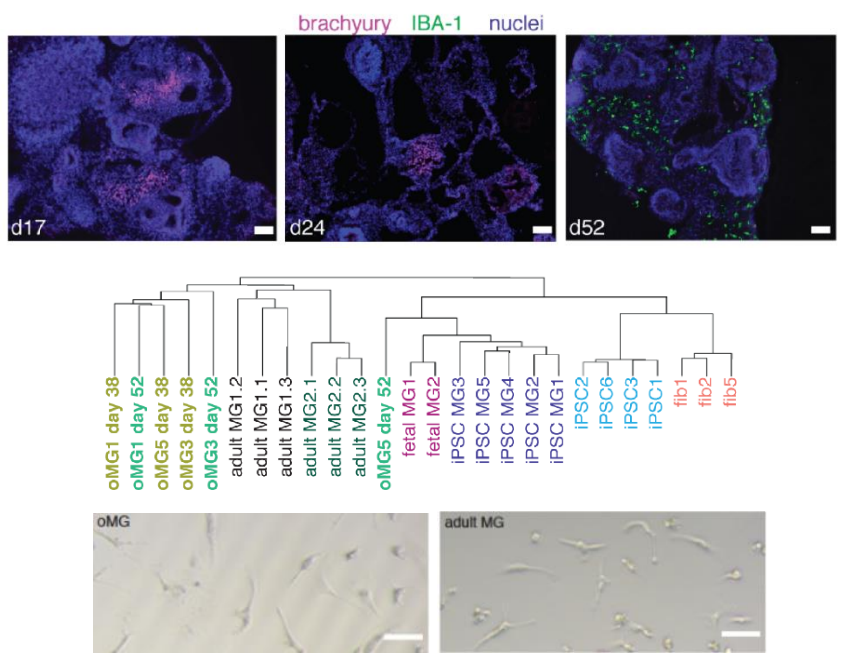
Background and Aim

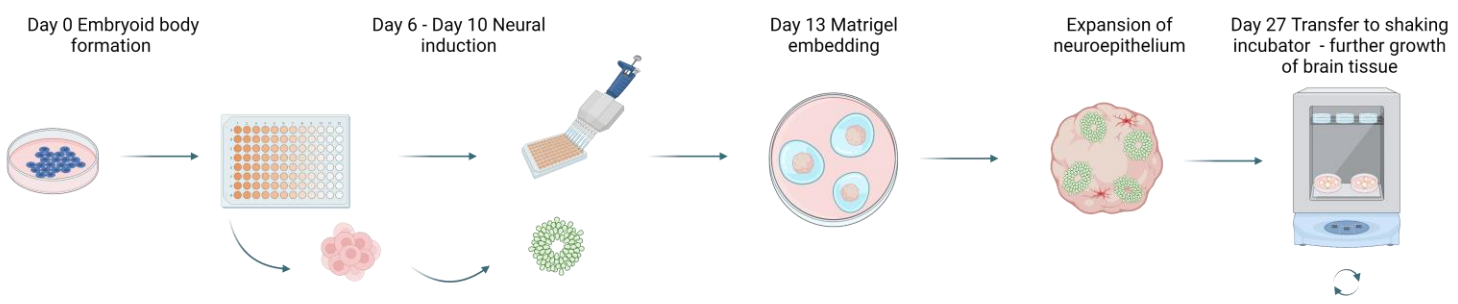
- Activated microglia are a hallmark of ALS pathology.
- We have previously developed a model of 3D brain organoids where microglia develop innately.
- Organoid grown microglia (oMGs) cluster together with adult microglia based on their transcriptomic profile.

- We aim to answer the question: **What is the contribution of *C9ORF72* to an altered immunity in ALS?**
- To this end we grow *C9ORF72* - ALS organoids and organoids from healthy age matched controls.
- Here we characterize *C9ORF72* - oMGs by assessing their **transcriptome signature, morphology, phagocytic activity** and **inflammatory response.**



Experimental set up

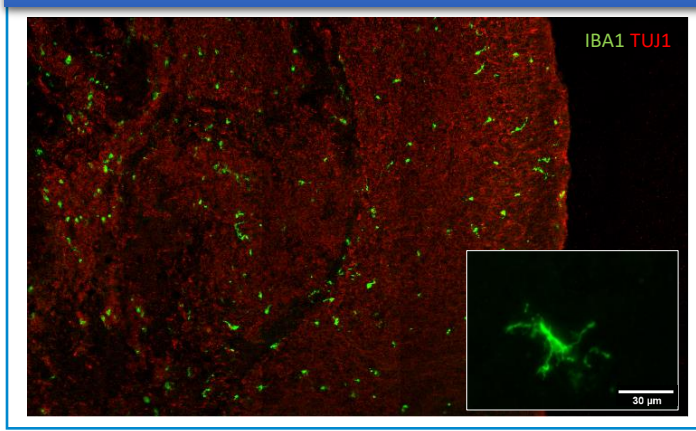
Generation of cerebral organoids that innately develop microglia

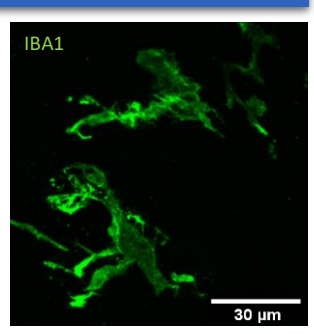


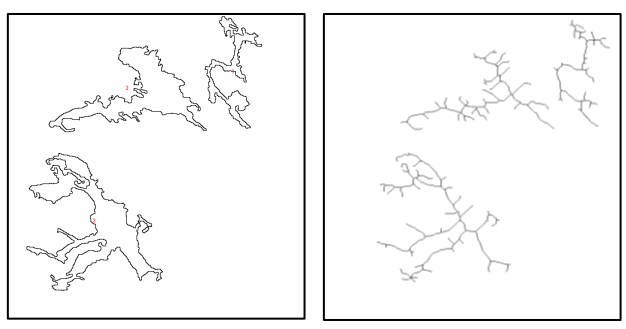
iPSC lines used:

- 3 C9ORF72 –ALS lines
- 3 age matched healthy controls lines

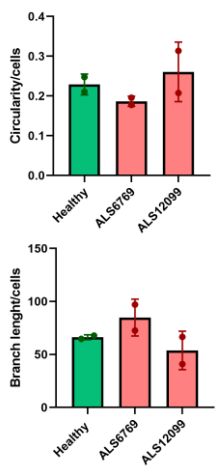
Morphology analysis of C9ORF72 oMGs



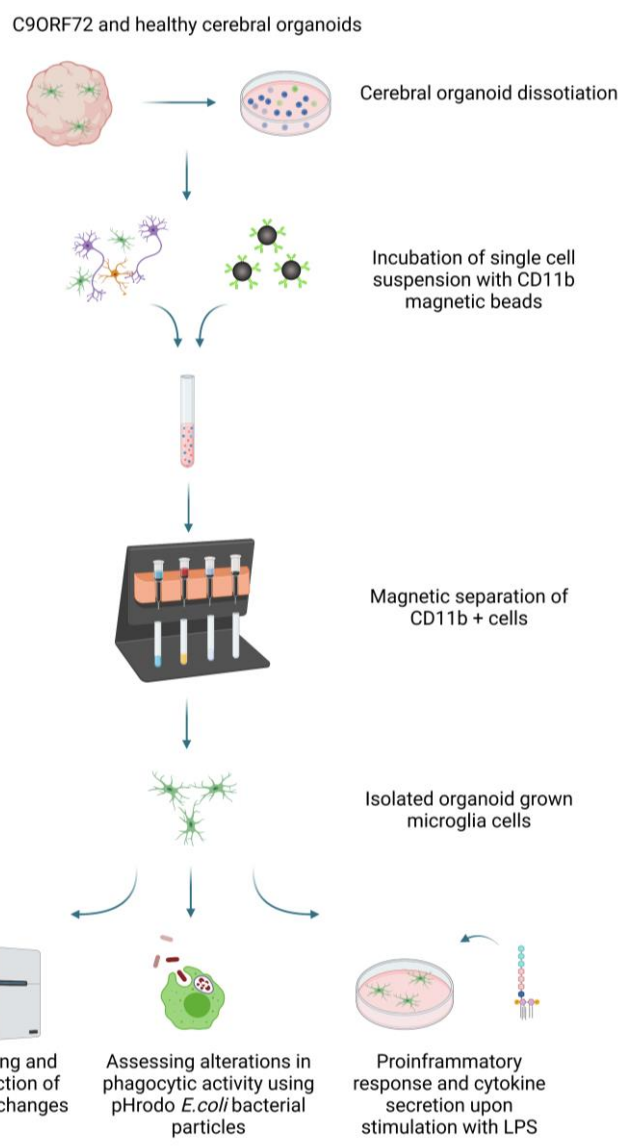




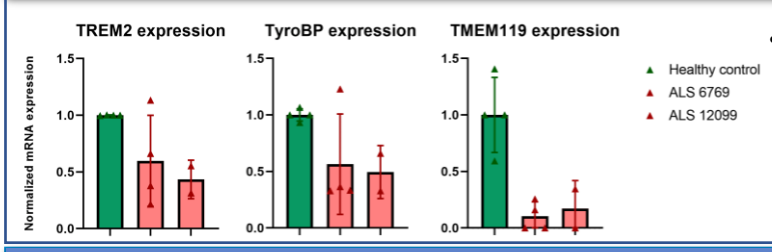
Each dot in the graph represents 50 cells from a single batch



Magnetic isolation of oMGs for detection of functional and transcriptomic changes

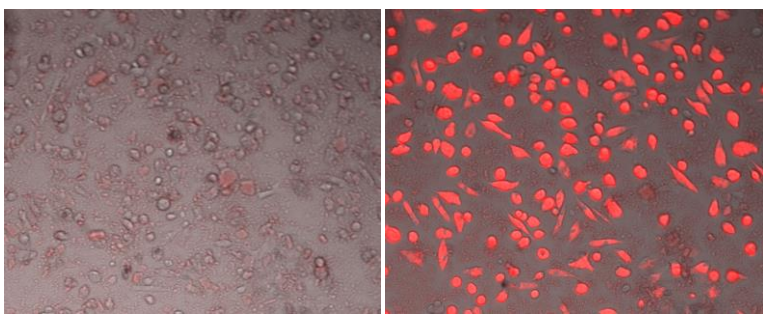


I. Dysregulation of homeostatic microglia genes



- qPCR of ALS-oMGs show downregulation of some homeostatic microglia genes like TREM2, TyroBP and TMEM119.

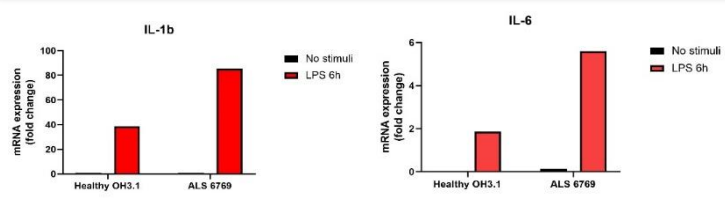
II. Phagocytosis – Assessing phagocytic activity with Live imaging



t=0 t=120min

- pH - sensitive red *E.coli* bacterial particles (pHrodo) were added to previously isolated oMGs.
- Images were taken every 10 min for up to 6h.
- We intend to measure the change in florescence over time between healthy and ALS microglia.

III. LPS stimulation – Higher expression of proinflammatory markers in C9ORF72 microglia



- qPCR of LPS treated oMGs shows elevated reactivity of ALS microglia

Conclusions and future plans

- Initial analysis of C9ORF72 organoid grown - microglia shows dysregulation at the transcriptomic and functional level.
- RNA sequencing will further elucidate molecular pathways that are dysregulated

References: Ormel *et al*. Nat. Comm. 2018

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