Single-cell RNA sequencing of ALS mouse model with attenuated reactive astrogliosis

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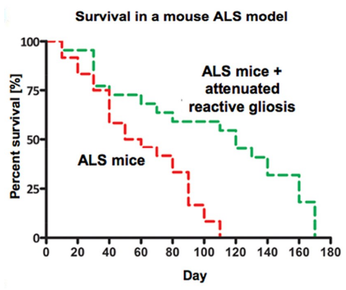
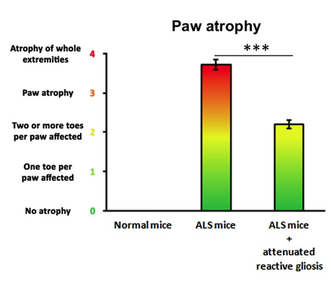
Background

Progress of amyotrophic lateral sclerosis (**ALS**), neurodegenerative disease **affecting** lower motor **neurons** in spinal cord and brain stem, **is accompanied by** astrocyte activation and **reactive astrogliosis** involving **upregulation of** intermediate filaments (**IFs**). Complete **absence of IFs** proteins, glial fibrillary acidic protein (Gfap) and vimentin (Vim), leads to inhibition of glial scar formation, improvement of regeneration after CNS injury and to **attenuation of reactive astrogliosis**[1]. We crossbred *Gfap-/-Vim-/-* mouse with **wobbler mouse** (recessive point mutation in *Vps54* causing ALS–like pathology). Resulting mice showed **slower disease progression**, improved survival and decreased neuronal loss in cervical spinal cord.

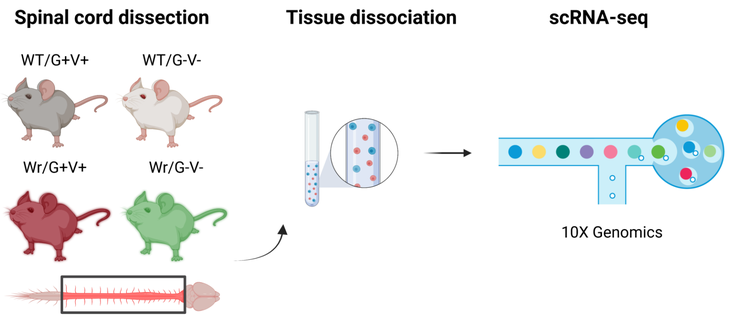




Here, we used **single-cell RNA sequencing** of cervical spinal cord samples of 30 days old wobbler mice (**Wr/G+V+**), wobbler mice carrying *Gfap-/-Vim-/-* (**Wr/G-V-**), control wild type mice (**WT/G+V+**), and wild type mice carrying *Gfap-/-Vim-/-* (**WT/G-V-**) to characterize cell type specific transcriptomic changes leading to improvement of ALS survival.

[1] Li et al., Protective role of reactive astrocytes in brain ischemia. J Cereb Blood Flow Metab. 2008, Mar;28(3):468-81. doi: 10.1038/sj.jcbfm.9600546. Epub 2007 Aug 29. PMID: 17726492.

Experimental design



Conclusions

- ALS–like pathology caused activation of astrocytes and decrease of homeostatic and neuroprotective populations.
- The pathological changes are mitigated by lack of reactive astrogliosis causing shifts in composition of astrocytic populations to control–like state.
- The protective effect is likely mediated by genes involved in lipoprotein particle assembly and lipid metabolism.
- Microglia showed signs of early activation in ALS-like pathology which was reduced by lack of reactive astrogliosis, indicating interaction of astrocytes and microglia.
- The subpopulations of astrocytes and microglia have active communication network that is changed by presence or lack of reactive astrogliosis.

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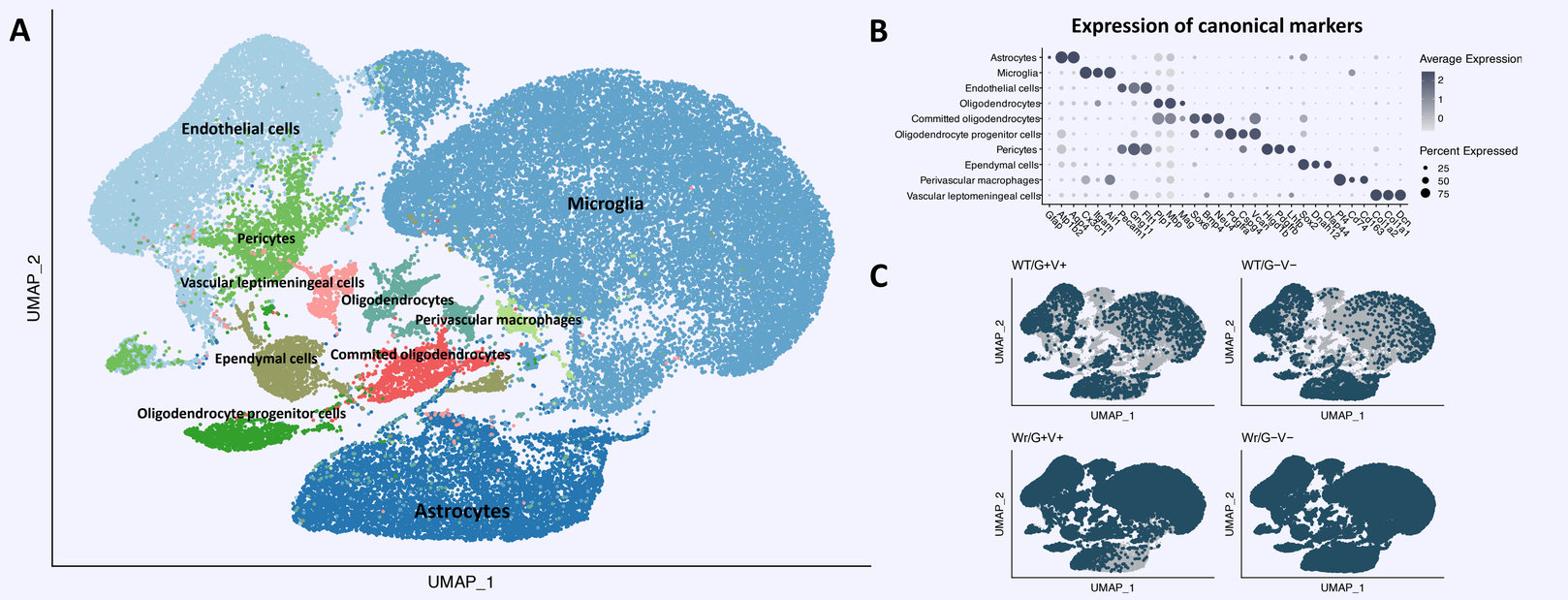
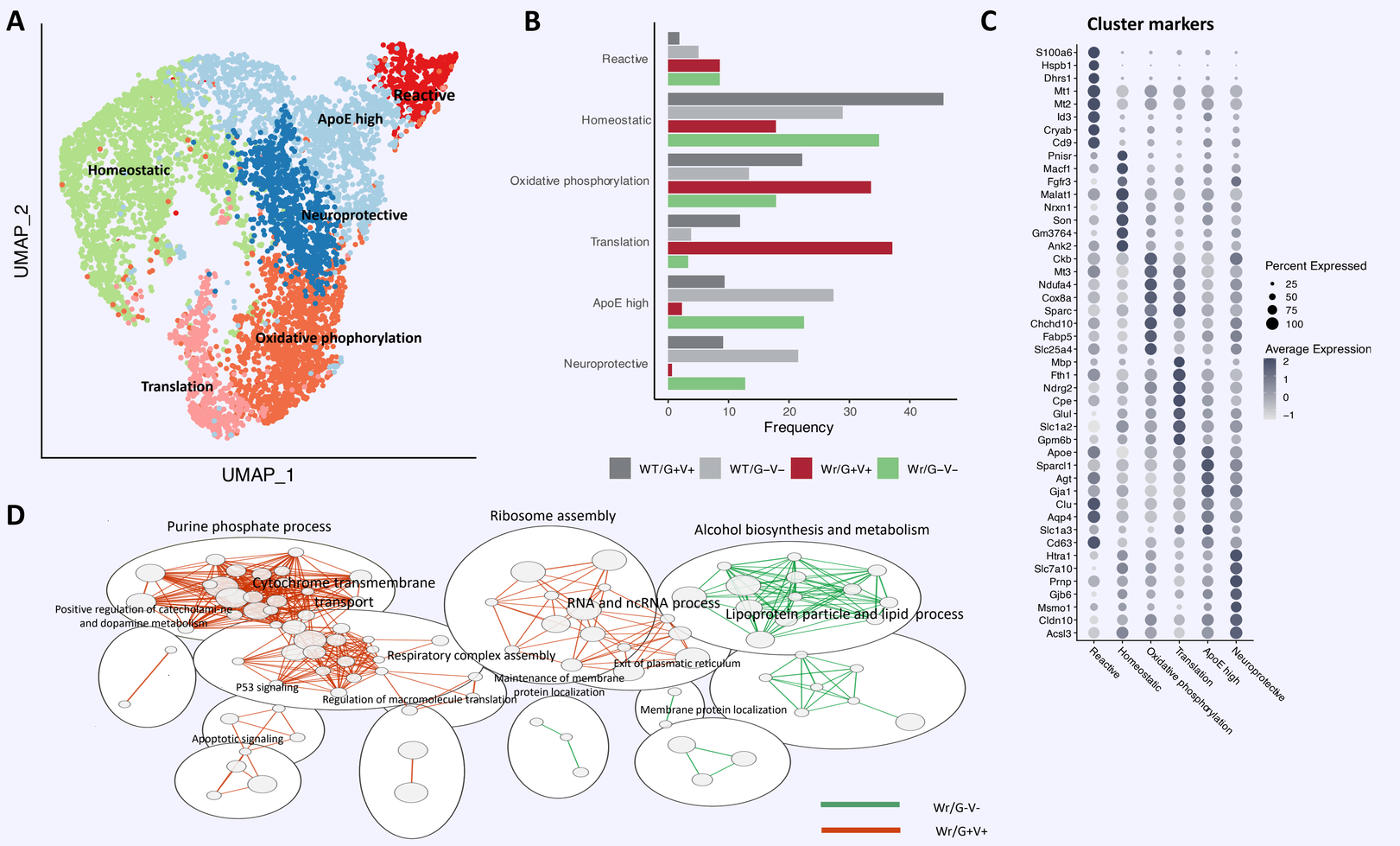
scRNA-seq captured main glial cell populations



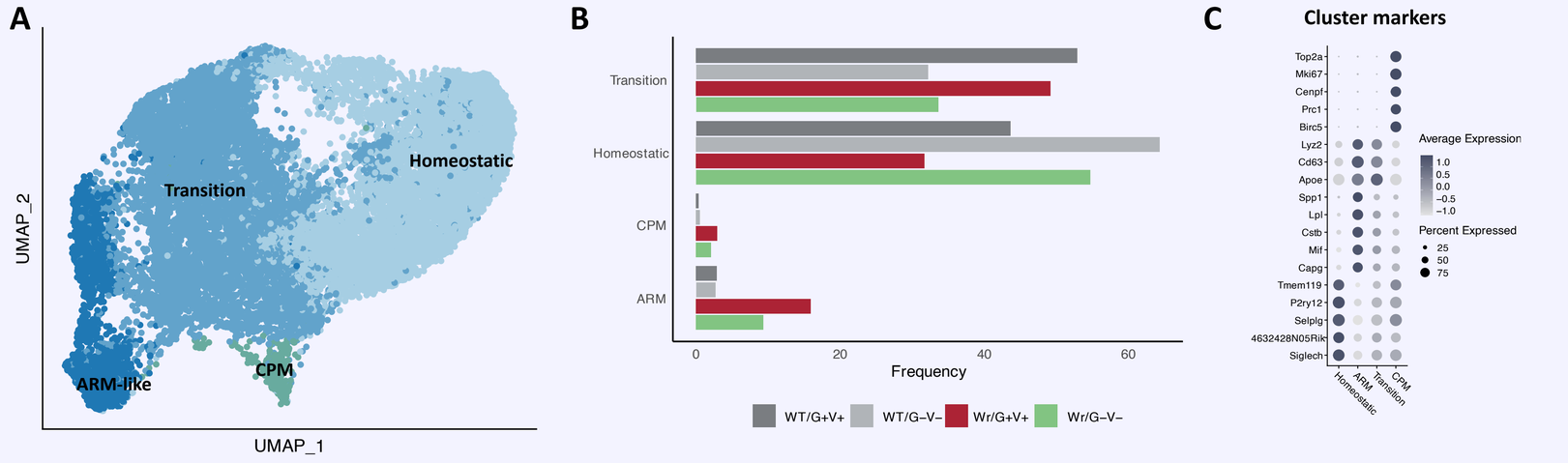
A, UMAP visualization of all manually identified cell types within integrated dataset obtained by combining all four conditions using Seurat 4 package. **B,** Dotplot with scaled expression of canonical cell type markers in identified cell type populations. **C**, UMAP visualization of cells in each condition.

Astrocytes showed diverse transcriptional profiles influenced by pathology and presence of reactive astrogliosis



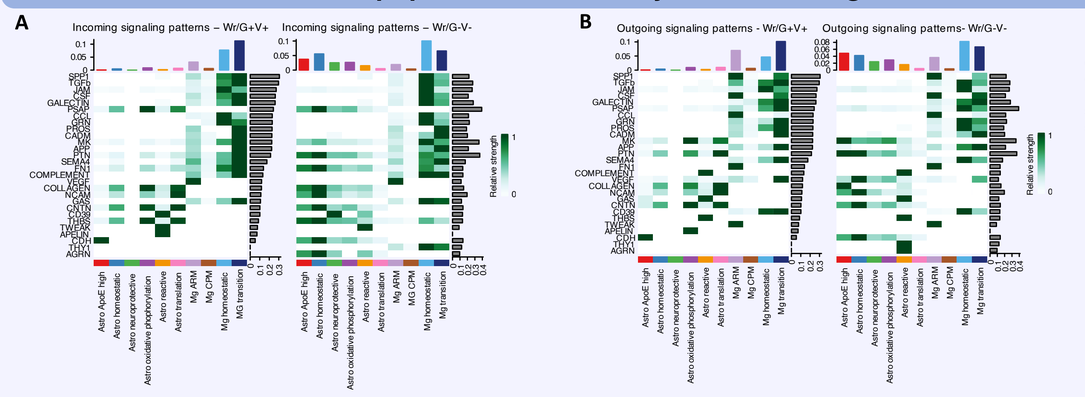
A, UMAP visualization of astrocyte clusters calculated (using Seurat 4 package) on all conditions. Names of the clusters were derived manually according to function of marker genes. **B,** Frequency was calculated as a ratio of number of cells in each astrocyte cluster to total number of astrocytes in each condition. **C,** Marker genes calculated using Wilcoxon rank sum test. **D,** Displayed parent terms were derived from gene ontologies of biological processes significantly enriched (using over-representation analysis, q-value < 0.05) in genes differentially expressed (padj < 0.05) between Wr/G+V+ and Wr/G-V- astrocytes.

Microglia are activated by ALS–like pathology and subtly affected by the lack of IFs



A, UMAP visualization of microglia clusters calculated (using Seurat 4 package) on all conditions (**ARM-like**; activated response microglia, **CPM**; cycling and proliferating microglia). Names of the clusters were derived manually according to the functions of marker genes. **B**, Frequency was calculated as a ratio of number of cells in each microglia cluster to total number of microglia in each condition. **C**, Marker genes calculated using Wilcoxon rank sum test.

Cell-cell communication analysis revealed changes in signaling (pathways) between populations of astrocytes and microglia



Heatmaps of relative level of signalization in each cell type for expressed signaling pahways. **A,** incoming signaling, **B,** outgoing signaling.

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