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The presented work by Mgr. Zuzana Matúšová examines the transcriptional profiles of two rare neurodegenerative disorders: Amyotrophic Lateral Sclerosis (ALS) and Alexander Disease (AxD). The study employs single-cell RNA sequencing (scRNA-seq) technology and utilizes the experimental G93A mouse model in ALS and hiPSC-derived cell cultures in AxD.

The research aim is to characterize the involvement of cortical cells in neurodegeneration caused by the G93A mutation in the *SOD1* gene. Based on the author's findings, which reveal only minor changes in glial activation between G93A and control models, she highlights the limitations and questions the suitability of this model for investigating cortical pathology in ALS. For the second aim, focused on AxD, the author and her collaborators employ two novel models of the disease caused by mutations in *GFAP* and describe the mutation's impact on human neurodevelopment.

The dissertation is extensive, well-written, and grounded in the latest scientific literature. It applies cutting-edge methods, demonstrating a strong grasp of the field. I want to highlight Mgr. Matúšová's critical thinking skills and ability to contextualize her findings within current trends, such as the limitations of the *SOD1* G93A model and the broader challenges of using animal models to study neurodegenerative disorders. For her future research, I would appreciate a clear hypothesis statement and the development of specific aims, shifting the focus from methodology to the underlying mechanisms being studied. Additionally, the rationale for combining ALS and AxD in a single study is not well explained and could benefit further clarification.

Throughout her PhD studies, Mgr. Zuzana Matúšová demonstrated significant technical proficiency (e.g., scRNA-seq, library preparation, data analysis, and interpretation), strong scientific writing skills, and the ability to design and execute experiments.

Mgr. Matúšová has summarized her findings as the first author in two original Q1 journal publications: *Scientific Reports* (IF 3.8) and *Glia* (IF 5.4). She also co-authored an article in *Molecular Oncology* and contributed to two reviews. Two additional manuscripts are currently in preparation.

The presented work meets the requirements for a dissertation.

Questions:

1/ Regarding the author's work published in *Scientific Reports*:
The study reports limited transcriptomic changes in glial cells in the G93A model (no significant changes in astrocytes and only minor changes in oligodendrocytes) compared to human tissues. What factors or mechanisms might explain these discrepancies between ALS pathology in humans and the G93A mouse model? Why is the G93A model so distinct from human ALS pathology, and what improvements would you propose?

2/ From the study on AxD, published in *Glia*:
How would you summarize the effects of *GFAP* mutations on neurodevelopment? Which pathways were disrupted in astrocyte-neuron co-cultures and neural organoids derived from hiPSCs?