Evaluation Report of Zuzana's Matúšová PhD Thesis

The submitted PhD thesis by Zuzana Matúšová, titled "*Gene expression profiling of glial cells in neurodegenerative diseases*" explores a diverse group of disorders affecting the nervous system, often with unknown causes and limited treatment options. The research focuses on the role of glial cells in disease onset and progression, emphasizing their reactive states as potential therapeutic targets. Using single-cell RNA sequencing, Zuzana analysed transcriptional profiles in models of amyotrophic lateral sclerosis (ALS) and Alexander disease (AxD), revealing distinct pathologies. The study identified limited cortical pathology in the SOD1(G93A) mouse model of ALS, while the human iPSC-derived models of AxD uncovered a GFAP mutation-driven differentiation phenotype, offering novel insights into disease mechanisms and pathology. The work has been published in two distinguished articles: one in *Scientific Reports* (Filipi & Matusova et al., 2023) and another in *Glia* (Matusova & Dykstra et al. 2024), both of which Zuzana co-first authored. Furthermore, during her studies, Zuzana contributed as a co-author to an additional original research paper and two review articles. With two manuscripts currently in preparation, this highlights her active and valuable role as a member of the research team.

The introductory section is well-written and provides a thorough background relevant to the work. It covers the general topics of ALS, AxD, and the heterogeneity of glia cells in health and disease. It also effectively introduces the animal and *in vitro* model of 2D and 3D human neuronal cultures, as well as the RNA transcriptomics methods used in the study. Overall, the author addresses all relevant topics, and the literature is generally cited appropriately. The discussion is clear and well-presented, systematically addressed step by step in light of the obtained results. It demonstrates that the author has a strong understanding of the topic and, based on the findings, is capable of formulating hypotheses for further testing. The thesis stands out for its logical structure and coherence. The research described in this work will be of interest to a broad range of scientists working in the fields of neurodegenerative diseases and single-cell RNA sequencing.

In summary, I wholeheartedly congratulate Zuzana on her work. It was a pleasure to read this thesis, and I fully support its acceptance by the thesis committee and the Faculty of Science, Charles University, for defense and the awarding of the PhD degree.

However, before proceeding, I have a few questions that I would like to ask the student, which are as follows:

1) Why is there so much heterogeneity in neurodegeneration and disease onset, even when caused by mutations in a single gene? Which environmental factors might contribute to this variability, and could this explain why many mouse models of neurodegenerative diseases, maintained in more sterile conditions, fail to fully recapitulate human pathology? Furthermore, can RNA sequencing data provide any insights into this issue or help identify factors contributing to this heterogeneity?

2) Could you please comment on the differences between your protocols and findings (Filipi & Matusova et al., 2023) and those reported in the study by Burg et al. (2020)? Is it possible that the sorting conditions, particularly the use of specific markers for glial cells, could result in inefficient

binding to certain populations, such as disease-associated cells, where marker expression might be partially affected? Additionally, how does scRNA-seq account for variations in gene expression across different cell cycle phases, as well as differences in cell differentiation and maturation levels?

3) If I understood correctly, in your human iPSC-derived models, you do not observe overexpression of GFAP or protein aggregations, making it challenging to explain the impact on neurodevelopment through altered mechanical properties. Is it known, whether both hiPSC lines (GFAP-mutated and corrected) differentiate similarly into muscle or epithelial cells, where GFAP is not typically expressed? Based on your scRNA-seq data, could you propose potential druggable targets that might benefit patients, and how would you test their efficacy? Additionally, do you predict that the same molecular mechanisms are involved in the onset of neurodegeneration in humans, and if so, why?

4) With all the knowledge and experience you have gained during your PhD studies; how would you design a new project to identify the mechanisms of disease pathology in patients with various homozygous mutations in a single gene associated with neurodegeneration? Specifically, which models would you use, how many samples would be required, and what methods, preparations, and transcriptomics tools would you implement if you had sufficient funding and access to the necessary resources and technologies?

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