**THESIS REVIEW**

**Ewelina Latoszek**

**Characteristics of generated human iPSC-derived models of Huntington's disease, identification of calcium signaling dysregulation and dendritic spine dysfunction**

The PhD thesis of Ewelina Latoszek explores the molecular and cellular mechanisms underlying Huntington's disease (HD), a progressive neurodegenerative disorder caused by expanded CAG repeats in the HTT gene. The research focuses on calcium signaling abnormalities and morphological impairments in neuronal cells derived from human induced pluripotent stem cells (hiPSCs) of HD patients. Key findings indicate that disruptions in [redacted]. The study also highlights the role of Siah-1-interacting protein (SIP) in modulating mutant huntingtin aggregation. The thesis findings are relevant as they enhance our understanding of HD progression and demonstrate the potential of hiPSC-derived models for investigating neurodegenerative mechanisms.

The thesis is based on 4 published papers in Stem Cell Research, Frontiers in Cell and Developmental Biology, Cell & Bioscience. Ewelina is first author in 3 and she also has two additional research papers published.

**Aims of the study are the following:**
The main aim of this PhD thesis was to generate new hiPSC-derived 2D cellular models and 3D human striatal organoids from adult and juvenile onset HD patients and relevant control individuals to investigate HD pathophysiology.

6 objectives were defined including: The study focuses on exploring the role of Siah-1-interacting protein (SIP) in mutant huntingtin (mHTT) aggregation, characterizing hiPSC-derived neural cell lines, examining calcium signaling disturbances, and analyzing gene expression changes linked to HD progression.

**Appearance of the Thesis**
The thesis is well-written, easy to follow, balanced, and well-structured. It spans 190 pages, with the following distribution: 32 pages for the Introduction and Literature Review, 1 page for Aims and Objectives, 26 pages for Materials and Methods, 63 pages for Results, 31 pages for Discussion, and 3 pages for Conclusion and Limitations.

The 396 references are more than adequate—though perhaps slightly overwhelming, as a typical range of 150 to 200 references is usually sufficient for a thorough review. The thesis

includes a well-written and precise list of abbreviations at the beginning, which helps guide the reader. Figures are generally clear, with adequate legends, except for a few where the font size is too small. The thesis contains 48 main figures and 19 tables. The deductions are moderate and well-reasoned, without being excessive, and the limitations are carefully raised and discussed throughout the text.

**Methods**

The Methods section is thorough, well-written, clear, and precise, ensuring reproducibility. The use of tables is particularly effective and enhances clarity.

**Minor comments:**

Mind to use a **,** after thousand for numbers (like page 17: 2,494 patients, or in the methods)
With longer, misspelled page 17
Brightfield images where unfortunately very dark
Fig. 5.4 GAPDH is quite weak- I would have increased the intensity for clarity
Fig 5.7. way too small. Figures would have benefited if split to 2 and be more visible as 5.8
5.10 Figure is more a table and is probably unnecessary
Page 90: HD is not just an age-related but also a neurodevelopmental disease
On some cases figure legends (y and x axis is quite small and hard to read: 5.15, 16, 25 etc)

**Questions & Comments (for the oral defense)**

1. Factors Affecting Disease Onset – Besides age and CAG repeat length, what other factors influence disease onset in HD? [redacted] Could you elaborate on its role?

2. Future Directions – What future experiments would you suggest to further understand the molecular regulation and mechanisms of HD? How could you age your 3D models? What is epigenetic aging of organoids?

3. HD Staging – The Huntington's Disease Integrated Staging System (HD-ISS) defines four stages of HD based on biological, clinical, and functional markers. You only mention three—how does your classification differ?

4. Data Presentation – Individual data points on graphs were missing. Please consider showcasing them in future work for greater clarity.

5. What are the most common drug used in HD treatment?

[redacted]

7. Aging Organoids & Direct Reprogramming – What are your thoughts on aging organoids? Are there existing methods for studying aged human neurons? Direct reprogramming was not discussed in the thesis—any reason why?

8. iPSC Modeling of Neurodegenerative Diseases – Table 1.2 states that iPSCs are a good reflection of AD, PD, and HD. Is this entirely accurate? What percentage of AD and PD cases can be effectively modeled using iPSCs? Also, what is the representative CAG repeat length for HD in iPSC models? How many 2D and 3D iPSC studies use the most commonly observed repeat lengths in patients highlighted in your table?

9. Key Novel Findings – A clearer emphasis on novel and original findings would have strengthened the thesis.

10. While juvenile-onset HD (JHD) is typically associated with more than 60 CAG repeats, there are cases where individuals with repeat lengths in the low-to-mid 50s develop symptoms before age 21. Therefore, classification depends on the age of onset:

Before age 21 → Considered juvenile-onset HD
After age 21 (e.g., 30s or 40s) → Classified as adult-onset HD
With 54 repeats, the case falls at the upper limit of adult-onset HD but slightly below the typical JHD range. Given this, why was 54 repeats chosen for the juvenile group?

Additionally, was separating the HD groups into 2-2 individuals beneficial, or would combining them have provided stronger insights? I found the classification somewhat puzzling, and in many cases, combining data, tables, and graphs from both HD donors might have improved clarity and comparability.

11. Lentivirus Titration – No titration was performed for lentiviral vectors (LVs)—why? What would be the advantages of including this?

12. CAG Repeat Sequencing – Did you perform CAG repeat sequencing? If so, what method did you use?

13. Triton X-100 Concentration – You used 0.3% Triton X-100, which is a bit high. Was this applied to all experiments or only HEK cells?

14. Automated Microscopy – Did you consider using automated microscopy? How many neurons were analyzed per condition—100, 500, or 1000 (e.g., Fig. 22)?

15. Statistical Testing – Did you perform a normality test on your data? If so, which one?

16. Western Blot for Aggregates – What are the key technical challenges when performing WB for protein aggregates and very large proteins?

17. Cell Passage Number – Why were cells passaged until P6?

18. Flow Cytometry for Cell Composition – Did you use flow cytometry to determine the exact cellular composition of cultures (e.g., Fig. 5.19)? If not, what is your estimated ratio?

19. Data Visualization – It would be helpful to show graphs rather than tables for datasets like Table 5.2 (with fold-change and p-values). Did you conduct a GO analysis for the bulk data in Fig. 5.26?

20. HEK Model Limitations – The HEK model is not an HD model, but rather an HD aggregation model. Please clarify this distinction.

21. SIP & Autophagy – Did you check for autophagy involvement in the SIP-HEK experiments?

22. Structural Validation of SIP – Did you use AlphaFold to validate SIP results?

23. Somatic Expansion – The discussion lacked mentioning somatic expansion.

24. Age of Animal Models – In Table 6.1, including the age of the animals at the time of observation would improve clarity.

**The thesis and papers include the following novel and original findings**
SIP's dual role in mHTT aggregation, identifying a potential therapeutic target.
[redacted]
Validation of hSOs as an effective intermediate model for HD research.

**Final Conclusion**
The candidate demonstrates substantial theoretical knowledge in the field of HD and modeling. The thesis clearly showcases her ability to conduct independent scientific work. It also specifies the contributions made by the doctoral student in the dissertation, distinguishing them from the collective work.

I, the undersigned, hereby state that the doctoral dissertation of Ewelina Latoszek meets the requirements specified in Article 187 of the Act of July 20, 2018 – Law on Higher Education and Science (c.t., Journal of Laws of 2024, item 1571, as amended). I hereby recommend to the Doctoral Committee of the International Institute of Molecular and Cell Biology in Warsaw to admit Ewelina Latoszek to the subsequent stages of the procedure for the conferment of the doctoral degree in the field of natural sciences, in the discipline of biological sciences.

Budapest, 6th March 2025

Karolina Pircs

Digitally signed by Karolina Pircs
Date: 2025.03.06 23:16:38 +01'00'

Karolina Pircs, PhD