Using stem cell models to understand early-onset Alzheimer's Disease and its progression

Peter Bayvel-Zayats

Supervisors: Sam Crawford, Prof. Selina Wray

Wray Lab, Department of Neurodegenerative Diseases, UCL Queen's Square Institute of Neurology

Introduction/Background

Alzheimer's disease (AD) is the most common cause of dementia, affecting around 750,000 people in the UK and 50 million worldwide (Peel, 2025).

AD is characterised by the accumulation of amyloid-β (Aβ) and abnormal forms of the neuronal protein tau, but the early molecular events linking these pathologies remain poorly understood, given it has been considered an ageing disease.

Human induced pluripotent stem cells (iPSCs) provide a powerful way to study these processes. In the lab, patient skin cells can be reprogrammed into stem cells and differentiated into cortical neurons, creating a human cell-based model of disease (Wray et al., 2012).

Project Aims

- This project investigates an exceptionally aggressive form of Alzheimer's disease caused by the PSEN1 L166P mutation, with samples obtained from a patient who developed symptoms in the late twenties.
- We sought to compare L166P mutant cells with a healthy control and a different PSEN mutation R278i to test whether they show increased pTau217, a disease-associated form of tau, phosphorylated at threonine 217 (Kavanagh et al., 2025).
- By first confirming pTau217 antibody specificity and subsequently measuring pTau217 relative to total tau, we aim to determine whether abnormal tau phosphorylation is detectable in both forms of this iPSC model, providing insight into how early PSEN1-driven disease processes progress towards downstream tau pathology.

Methods:

1) iPSC characterization:

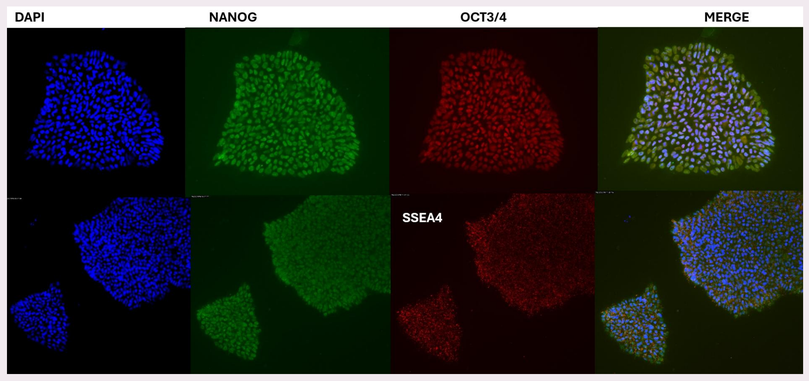

Figure 1: Immunocytochemistry (ICC) staining and imaging of stem cells. DAPI, NANOG, OCT3/4 SSEA4 represent stem cell markers, confirming that iPSC reprogramming was successful.

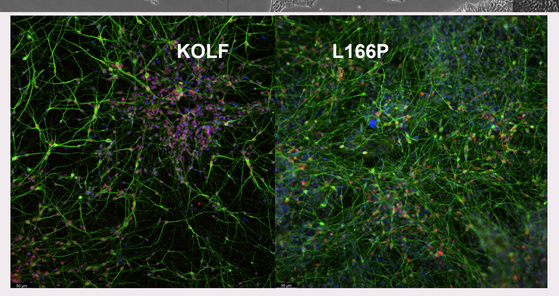

Figure 3: ICC confirming neuronal identity of KOLF and L166P cortical neurones (100DIV)

3) pTau217 Antibody Validation

Dephosphorylation was used successfully to test the phospho-specificity of pTau217 antibodies, alongside familial AD brain lysate as a positive control, before comparison of experimental samples.

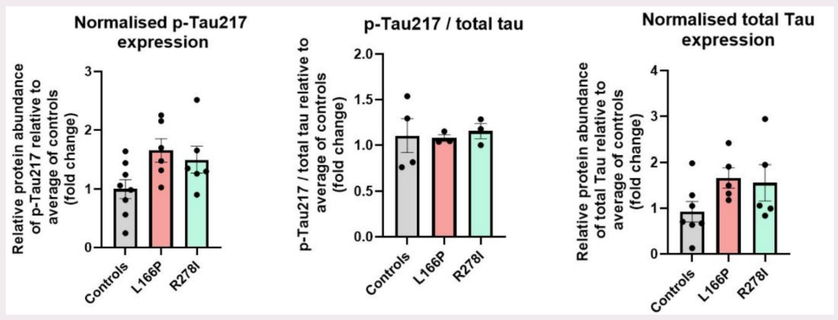

Figure 6: Quantification of Western blot imaging

2) Neuronal differentiation: 2 types of models were used in this project – *cortical neuronal differentiation* involves an 80-day, 3-step process, differentiating iPSCs into cortical neurons (Shi et al., 2012); *i3Neuron induction* is a rapid method which induces NGN2-expressing iPSCs into cortical neurons in 14 days (Fernandopulle et al., 2018). Cortical neurones had been previously generated (samples used were 100 days in vitro), whereas i3 neurones were generated from L166P patient-derived iPSCs **for the first time.**

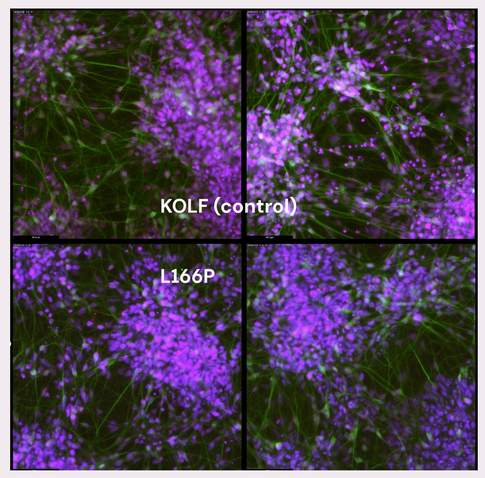

Figure 4: *ICC confirming neuronal identity of KOLF and L166P i3 neurones*

4) pTau217 Expression in Cortical Neurones

Cortical neurones derived from patient and control iPSC lines both express pTau217

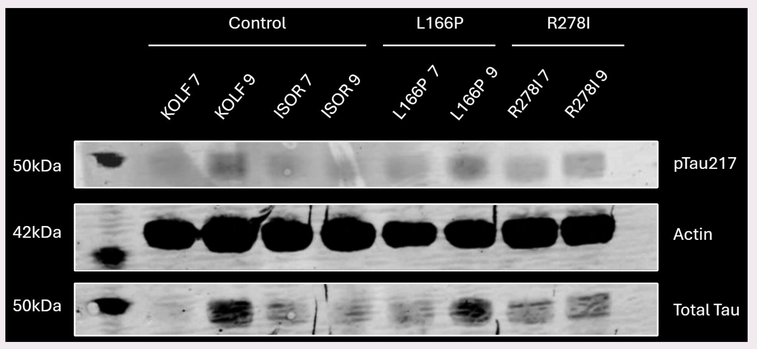

Figure 5: Western blot showing pTau217, Actin and total Tau expression in control and patient-derived cortical neuronal samples

Ptau217 (protein) levels were divided by actin (housekeeper) levels and the average of the controls were then divided by patient values to quantify the signal relative to the controls.

- pTau 217 levels appeared slightly increased; pTau:total Tau does not appear altered; L166P and R278i seem to show slightly elevated total Tau

5) pTau217 Expression in i3 Neurones

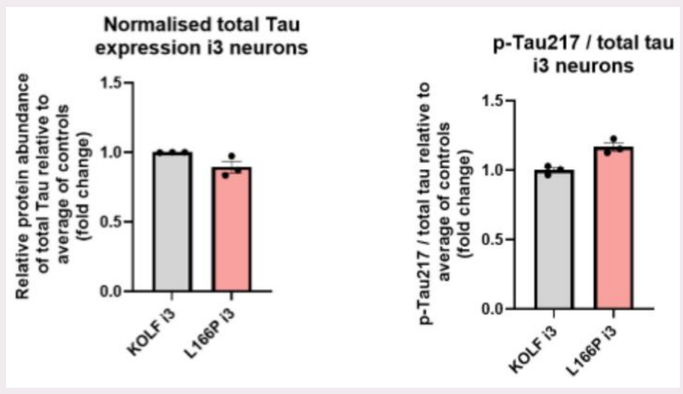

Figure 7: Quantification of i3 Western blot

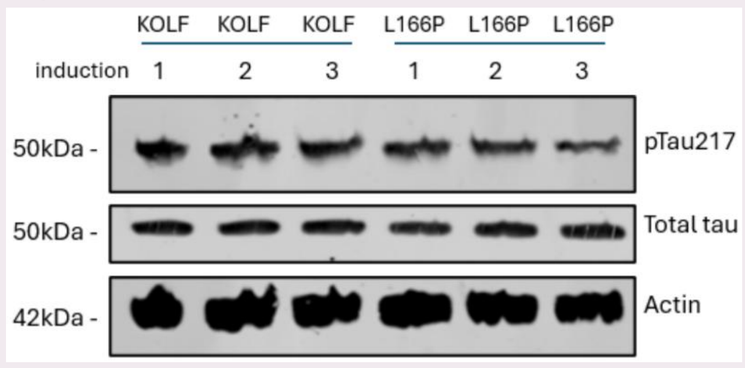

Figure 8: Western blot showing pTau217, Actin and total Tau expression in control and patient-derived i3 neuronal samples

Discussion and Conclusions

- We can use patient-derived iPSCs to generate in vitro models of both cortical and i3 neurons which express pTau217 (first time ever done using i3 neurons!)
- Using both models, we can probe investigate pTau217 expression in patient neurons to understand the contribution of pTau217 to the development of pathology, a valuable step forward in understanding disease progression.
- i3 neurons will allow us to assess this at scale, with further work aim to understand how closely our models mirror the development of pathology in patients.
- Since novel models being used, essential tests had to be performed before lots of functional analysis done. Further replicates are required to confirm significance beyond intrinsic variability and to seek more definitive differences between samples.
- Future work should look in time-course experiments to track changes across ageing i3 lines, which can be paired with imaging/LDH assays to see how tau distribution actually changes between cells and controls and quantify its effect over time.

References

Fernandopulle, M.S., Prestil, R., Grunseich, C., Wang, C., Gan, L. and Ward, M.E. (2018). Transcription Factor-Mediated Differentiation of Human iPSCs into Neurons. *Current Protocols in Cell Biology*, 79(1), p.e51. doi:10.1002/cpcb.51.

Kavanagh, T., Thierry, M., Balcomb, K., Ponce, J., Kanshin, E., Tapia-Sealey, A., Halliday, G., Ueberheide, B., Wisniewski, T. and Drummond, E. (2025). *The interactome of tau phosphorylated at T217 in Alzheimer's disease human brain tissue*. *Acta neuropathologica*, doi:10.1007/s00401-025-02881-8.

Peel, M. (2025). *Proteins in human body reveal warning signs for Alzheimer's and Parkinson's*. [online] @FinancialTimes. Available at: https://www.ft.com/content/f9fa691c-4502-4324-9c4b-b3252abfc40e

Shi, Y., Kirwan, P. and Livesey, F.J. (2012). Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. *Nature Protocols*, 7(10), pp.1836–1846. doi:10.1038/nprot.2012.116.

Wray, S., Self, M., Lewis, P.A., Taanman, J.-W., Ryan, N.S., Mahoney, C.J., Liang, Y., Devine, M.J., Sheerin, U.-M., Houlden, H., Morris, H.R., Healy, D., Marti-Masso, J.-F., Preza, E., Barker, S., Sutherland, M., Corriveau, R.A., D'Andrea, M., Schapira, A.H.V., Uitti, R.J., Guttman, M., Opala, G., Jasinska-Myga, B., Puschmann, A., Nilsson, C., Espay, A.J., Slawek, J., Gutmann, L., Boeve, B.F., Boylan, K., Stoessl, A.J., Ross, O.A., Maragakis, N.J., Van Gerpen, J., Gerstenhaber, M., Gwinn, K., Dawson, T.M., Isacson, O., Marder, K.S., Clark, L.N., Przedborski, S.E., Finkbeiner, S., Rothstein, J.D., Wszolek, Z.K., Rossor, M.N. and Hardy, J. (2012). Creation of an Open-Access, Mutation-Defined Fibroblast Resource for Neurological Disease Research. *PLoS ONE*, 7(8), p.e43099. doi:10.1371/journal.pone.0043099.

LAIDLAW SCHOLARS