Development and characterization of a robust *in vitro* disease model to study tauopathies

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1 BACKGROUND & GOAL

Tauopathies, such as frontotemporal dementia (FTD) and Alzheimer's disease (AD), are neurodegenerative diseases characterized by the pathological aggregation of paired helical filaments (PHFs) or neurofibrillary tangles (NFTs) within neurons and glia or extracellular amyloid plaques, leading to neuronal dysfunction [1]. PHFs and NFTs are formed by aggregation of hyperphosphorylated Tau (Figure 1) [1], and amyloid plaques by β-amyloid peptides [2]. The adverse effects of amyloid plaques are dependent on Tau phosphorylation [2]. Mutations in the microtubule-associated protein Tau (MAPT) gene are the cause of genetic tauopathies [3]. Here, we aimed to develop and characterize a physiologically relevant and robust human *in vitro* tauopathy model, to aid the future development of FTD and/or AD disease therapeutics.

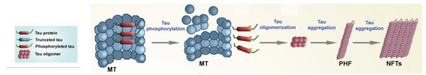

Figure 1 | Diagram showing Tau pathology
Adapted from Simic et al, Biomolecules, 2016

Using CRISPR-Cas9 gene editing technology, familial mutations MAPT P301S or N279K both underlying FTD/AD [3], were engineered into an iPSC line that carries the opti-ox™ technology and can rapidly be reprogrammed into glutamatergic neurons[4]. By means of immunocytochemistry we characterized induction of Tau hyperphosphorylation by β-amyloid oligomer treatment, and then assessed Tau hyperphosphorylation in neurons derived from the lines with a distinct MAPT mutation. Tau hyperphosphorylation was quantified using a newly developed image algorithm.

Goal:
To assess the effect of β-amyloid oligomers and MAPT P301S or N279K homozygous or heterozygous mutations on FTD/AD phenotypes such as increased Tau phosphorylation.

2 METHODS

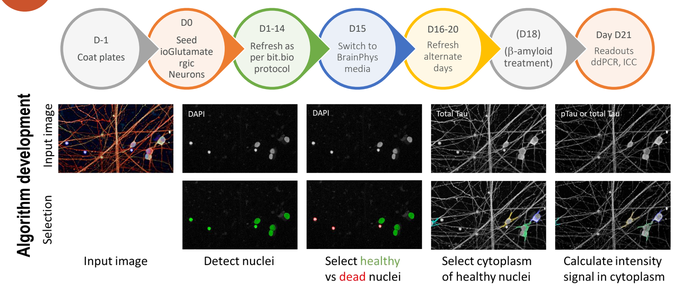

Figure 2 | Experimental protocol using bit.bio generated ioGlutamatergic Neurons containing MAPT mutations
In total four bit.bio-generated cell lines (3 clones with MAPT mutation and 1 isogenic wild type (WT) lines were seeded onto PDL/Geltrex coated plates and refreshed as per bit.bio protocol. On day 15 post seeding cells were switched to BrainPhys media and cells were refreshed on alternate days. On day 18, if required, cells were stimulated with β-amyloid oligomers. On day 21 cells were fixed for immunocytochemistry. Immunocytochemistry was performed for DAPI, total Tau and phospho-Tau. High content imaging was performed using the Yokogawa CV8000 and PE Columbus 2.9.1 was used to create an algorithm to analyse total Tau and pTau intensity in the cytoplasm of healthy neurons (image).

4 CONCLUSION

The elevated pTau to total Tau ratio measured by the immunocytochemistry assay indicates the potential of at least two cell lines as possible disease models to aid future research into developing FTD disease therapeutics.

- pTau 217 and pTau 202/205 antibodies show a β-amyloid-induced dose dependent increase in intensity, whereas pTau404 is increased, but not in a concentration dependent manner. Overall, this suggests that β-amyloid oligomer treatment results in Tau hyperphosphorylation in bit.bio WT ioGlutamatergic Neurons.
- bit.bio generated ioGlutamatergic Neurons with a homozygous or heterozygous MAPT P301S or heterozygous N279K mutation in an isogenic WT line[4], making it possible to directly compare the effect of the mutation on FTD-associated phenotypes.
- All cell lines give rise to mature glutamatergic neurons as they express the classical vGlut1, vGlut2, TUBB3 and MAP2 marker genes, along with minimal cell debris, indicating healthy cell cultures.
- The homozygous P301S cell line showed a clear increase in pTau (pTau202/205, pTau217 and pTau404) to total Tau ratio compared to the wild type (WT), whereas the heterozygous line did not show this increase.
- The heterozygous N279K cell line showed an increase in two out of three tested pTau antibodies.
- Combined, this suggests that a homozygous P301S or heterozygous N279K mutation results in Tau hyperphosphorylation.

References:
1. *Silva MC, eLife, 2019*
2. *Seward ME, et al, 2013. Journal Cell Sci 126(5):1278-1286*
3. *Hutton M, et al, 1998, Nature 393:702-705*
4. *Pawlowski M, et al, 2017. Stem cell reports 8(4), 803-812*

3 RESULTS

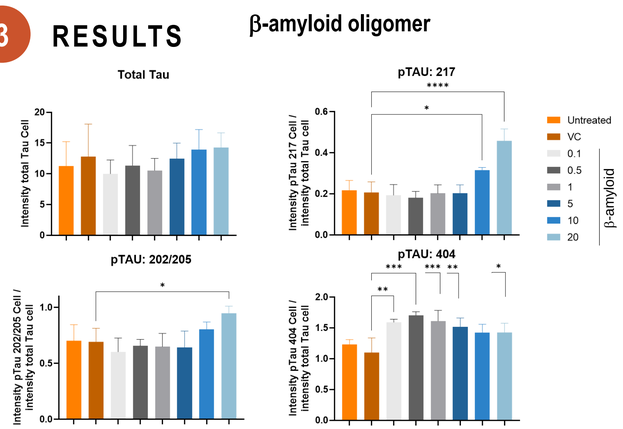

Figure 3 | Tau phosphorylation induced by β amyloid oligomers in WT bit.bio cells
ioGlutamatergic Neurons WT were treated with indicated concentrations of β-amyloid (1-42) oligomers (μM), vehicle control (VC) or were left untreated for 72 hours before harvest and stained with the Tau antibodies indicated in the graphs. Bars, mean; error bars, SD. Significance was calculated by one way ANOVA compared to VC on ≥3 cellular replicates in the same plate.

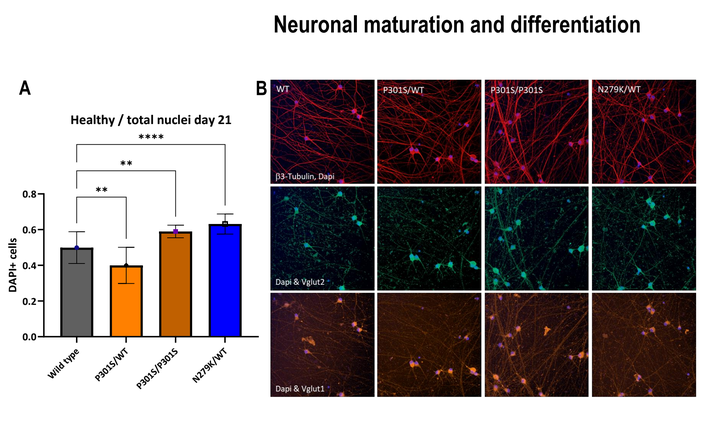

Figure 4 | ioGlutamatergic Neurons (with MAPT mutation) are mature and healthy
A A high content analysis algorithm was developed using PE Columbus 2.9.1 to detect healthy DAPI+ cells based on size and intensity. The graph displays healthy / total DAPI+ nuclei on day 21. Statistical analysis on 15 cellular replicates in the same plate. Bars, mean; error bars, SD. **B** Images taken from ICC analysis to assess neuronal maturation and differentiation to the glutamatergic lineage. Cells were stained for DAPI, β3-tubulin, Vglut2 and Vglut1.

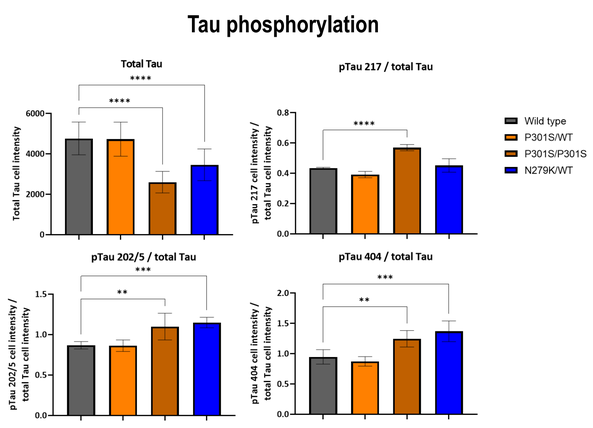

Figure 5 | MAPT P301S/P301S and MAPT N279K/WT show hyperphosphorylation when compared to isogenic WT line
Bar graphs showing total Tau, pTau 217/ total Tau, pTau 202/5 / total Tau or pTau404 / total Tau ICC analysis in the cell body. Statistical analysis (one way ANOVA) are performed on 5 cellular replicates in the same plate. Bars, mean; error bars, SD.