Development of robust iPSC-based α-Synuclein, Tau and TDP-43 aggregation models for drug discovery

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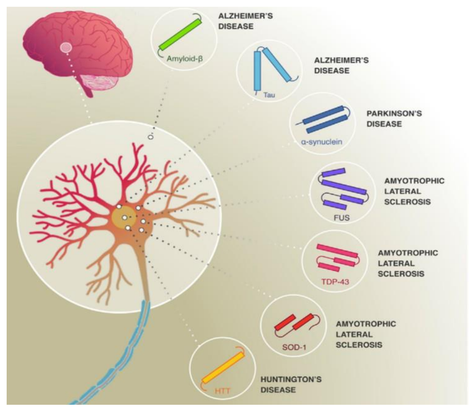
Ncardia Services BV Leiden, The Netherlands - No conflicts of interest

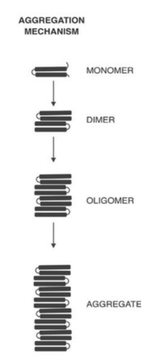
Background

Proteinopathies are disorders driven by protein misfolding and aggregation, resulting in impaired neuronal function and progressive neurodegeneration. Notable examples include Parkinson's disease (PD), Alzheimer's disease (AD), and Amyotrophic Lateral Sclerosis (ALS). These conditions are highly complex and remain challenging to model accurately in both in vitro and in vivo systems. Consequently, many therapies that show promise in animal models fail in clinical trials, highlighting a significant translational gap in drug discovery.

Developing physiologically relevant human disease models is therefore critical to more reliably evaluate therapeutic candidates and improve clinical success rates. Human induced pluripotent stem cells (hiPSCs) offer a powerful solution, as they can differentiate into diverse cell types, preserve patient-specific genetic backgrounds, and recapitulate human pathophysiology while responding predictively to candidate drugs. These features make hiPSC-based systems well suited for drug discovery applications.

In this study, Ncardia established three hiPSC-derived neuronal in vitro assays to model the aggregation of α-synuclein, Tau, and TDP-43—key pathological hallmarks of PD, AD, and ALS.



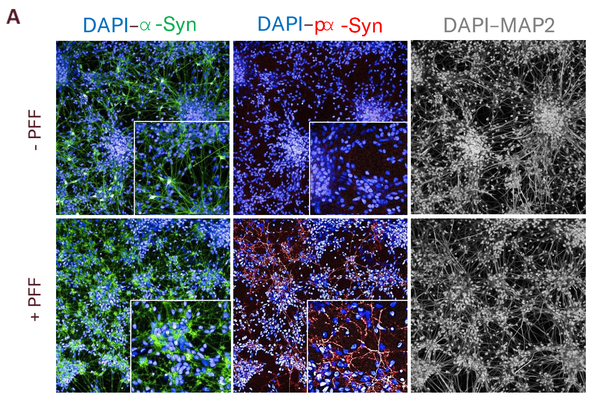


Postulated model for protein aggregation mechanisms in neurodegenerative diseases

- Misfolding of specific characteristic disease related proteins is suggested to be linked to disease progression, resulting in aggregation and fibril formation of these proteins
- Once disease protein aggregates and loses its function or additionally shows a toxic gain of function.

Bahareh Eftekharzadeh, Bradley T. Hyman, Susanne Wegmann. Structural studies on the mechanism of protein a regation in age related neurodegenerative diseases, Mechanisms of Ageing and Development, Volume 156, 2016, Pages 1-13, ISSN 0047-6374, https://doi.org/10.1016/j.mad.2016.03.001.

Aggregation and phosphorylation of α-synuclein



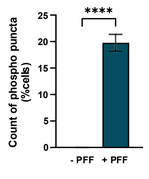
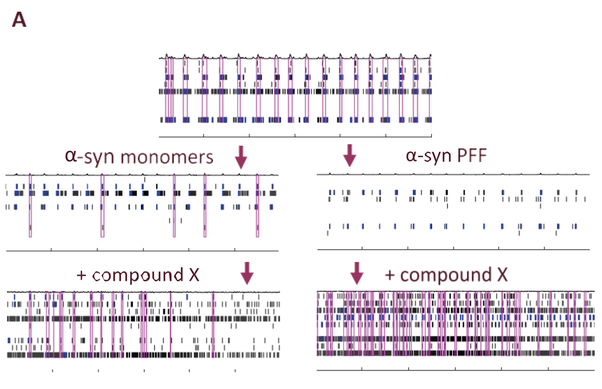


Figure B Quantification of phosphorylated puncta counts of cells without PFFs (purple) compared to cells treated with PFFs (turquoise), expressed as %.

Figure A Fluorescent images of cells, acquired with a 20x objective and stained for DAPI (blue), α-syn (green), MAP2 (grey) and phospho α-syn S129 (red).

Treatment with α-syn pre-formed fibrils (PFF) results in p-α-syn aggregates

Electrophysiological dysfunction in a model of PD



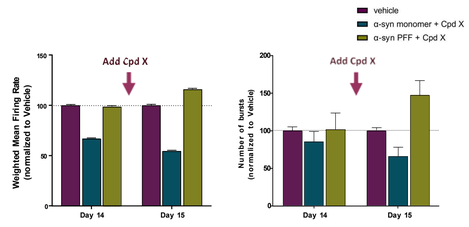


Figure B Quantification of the normalized parameters (weighted mean firing and number of bursts) of cells treated with α- sun monomers or PFFs in combination with compound X , expressed as % vehicle condition set to 100%.

Figure A Representative raster plots of MEA (action potential activity) after treatment with α-syn monomers or PFF for 7 days and compound rescuing neurotoxic phenotype (y-axis: electrodes, x-axis: 5 min recording time).

Treatment of Ncyte CNS with α-syn monomers or PFF disturbed Ncyte CNS electrical activity

Quantification of pTAU, soluble and insoluable Tau

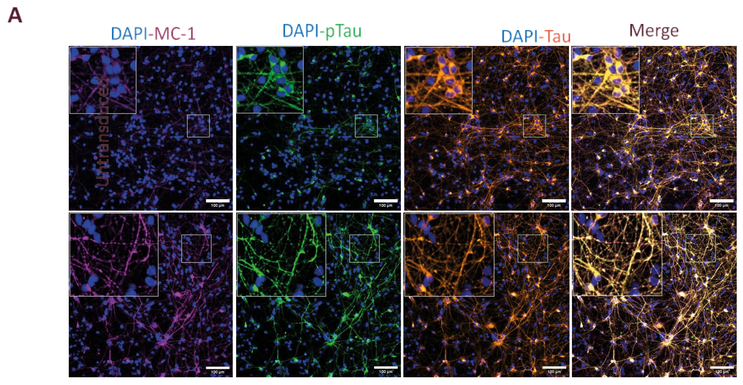


Figure A Tau overexpression by transduction in combination with chronic treatment with Tau PFFs (condition transduced +PFF) showed a notable increase in misfolded Tau (MC-1 in purple) and pTau (green) signals, resembling Tau pathology observed in Alzheimer's disease (magnification 20x)

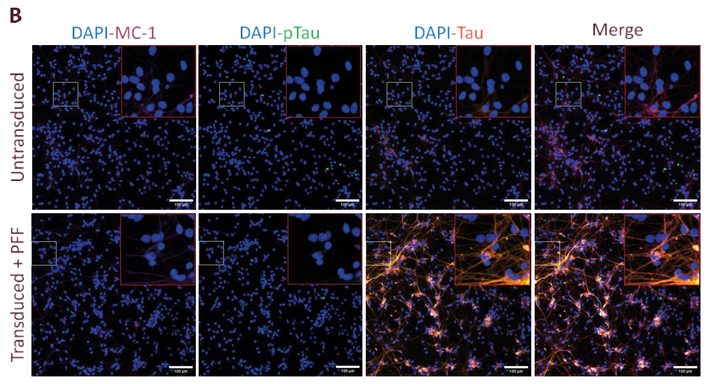


Figure B Fixation of neurons with methanol, removes the phosphorylated Tau (green) and MC-1 positive Tau (purple) but in condition transduced +PFFs, Tau signal (orange) is still present, indicating the formation of insoluble tau a regates (magnification 20x).

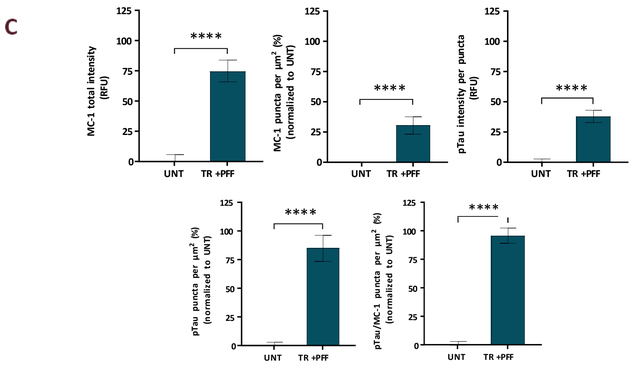


Figure C Quantification of the normalized parameters (intensity and puncta counts of MC-1 and pTau) of untransduced cells (UNT in purple) compared to cells transduced and treated with PFFs (TR+ PFFs in turquoise), expressed as % to condition UNT set to 0% (Plotted values are averages ±SD. Statistical significance calculated with unpaired t-test * p≤0.05; ** p≤0.01; *** p≤0.001; **** p≤0000.1).

Overexpression of TAU combined with chronic exposure to PFFs induces an Alzheimer's disease phenotype

Mis-localization and aggregation of TDP-43 and downregulation STMN2

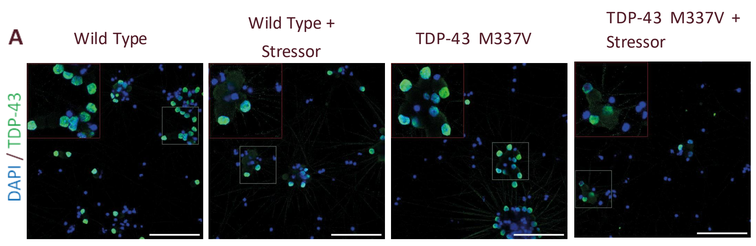
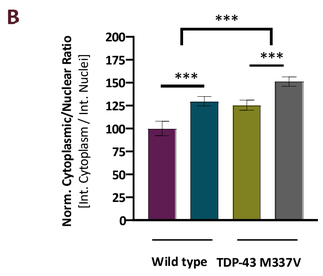


Figure A Fluorescent images of motor neurons expressing wild type and mutated TDP-43 (M337V mutation), treated with vehicle or stressor and stained for TD43. Cytoplasmic staining of TDP-43 can be observed in the mutant neurons as well as in all neurons treated with the stressor (magnification 40x, scale bar 100 µm).



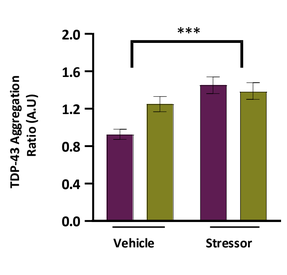


Figure B: Quantification of the normalized parameter (ratio of cytoplasmic/nuclear TDP43) of wild type and mutated TDP-43 (M337V mutation) motor neurons, compared to wild type neurons (in purple) and expressed as % to condition set to 100%.

Figure C Quantification of the normalised parameter (TDP-43 agggretion) by HTRF of wild type and mutated TDP-43 (M337V mutation) motor neurons, compared to wild type neurons (in purple) and expressed as % to condition set to 100%. The mutant motor neurons show a s u estive increase in TDP-43 a regation but the addition of a stressor, consistently and robustly increases TDP-43 aggregation.

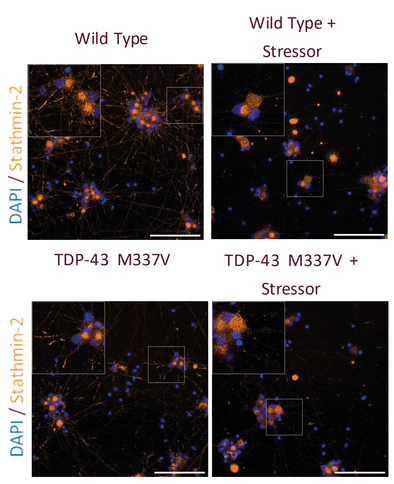


Figure D Fluorescent images of motor neurons expressing wild type and mutated TDP-43 (M337V mutation), treated with vehicle or stressor and stained for Stathmin2 (yellow) and DAPI (blue). Reduction of neurite staining of Sthatmin2 can be observed in motor neurons treated with the stressor (magnification 40x).

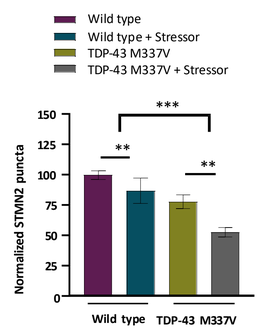


Figure E Quantification of the normalized parameter Stathmin-2 puncta in neurites) of wild type and mutated TDP- 43 (M337V mutation) motor neurons, compared to wild type neurons (in purple) and expressed as % to condition set to 100%. (Plotted values are averages ±SD. Statistical significance calculated with two-way ANOVA * p≤0.05; ** p≤0.01; *** p≤0.001; **** p≤0000.1.

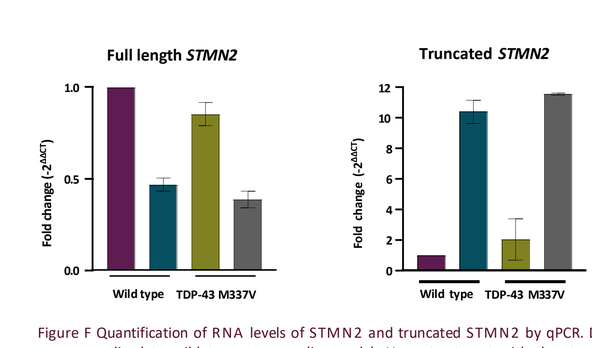


Figure F Quantification of RNA levels of STMN2 and truncated STMN2 by qPCR. Data was normalized to wild type neurons (in purple). Upon treatment with the stressor, STMN2 levels significantly decreased while there was a significant increase in truncated STMN2 levels.

Motor neurons chronically treated with a stressor show mis-localization of TDP-43 to the cytoplasm, a regation of TDP-43, reduction of STMN2 and appearance of the truncated variant

Conclusions

- Neuronal co-cultures were used to quantify disease relevant phenotypes for α-synuclein or TAU aggregation as well as the formation intermediate phosphorylated species after treatment with α-synuclein and Tau recombinant preformed fibrils (PFFs).
- Stressor-treated mutant and wild type iPSC-derived motor neurons (hiPSC-MN) showed disease-specific mis-localization of TDP-43 to the cytoplasm, aggregation of TDP-43, reduction of STMN2 and appearance of the truncated variant.
- We have established a suite of robust, clinically relevant in vitro assays (Z-factor >0.5). These assays are fully automated and performed in a scalable format to support drug discovery at any stage .
- We successfully modelled and evaluated disease-linked phenotypes relevant to AD, PD and ALS, among other neurodegenerative disorders, using complimentary assays. Altogether, offering the opportunity to gain a holistic understanding of the efficacy of therapies targeting aggregation

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