EM 2 Project report

Integration of clinical and cellular phenotypes in the DPUK Deep and Frequent Phenotype Cohort

Start date: 1 Jan 2017 **Completion date: PhD student will work on project until October 2021 . DPUK end date agreed as 30 April 2020**

Team members funded (full or part-time) by DPUK

None: consumables costs only

Team members involved with the project but not funded by DPUK

Anne Hedegaard, Helen Rowland, Simon Lovestone, Liting Wei, Zameel Cader, Bryan Ng, Richard Wade-Martins

ECRs: Bryan Ng, Liting Wei, Helen Rowland

Location(s):

Oxford University

Objectives	**Dependencies to and from other work packages, networks and themes**
1) Generation of iPSC lines from 24 individuals in the DPUK Deep and Frequent Phenotype study 2) Analysis and characterisation of patient samples 3) SomaLogic profiling and assay development/validation	Stem cell network

Updates on delivery against milestones since last report

All remaining milestones are underway:

D2.3 Define the pathological interaction between extracellular Abeta and intraceullar Tau using cell viability, biochemistry, electrophysiology and RNA-Seq

D 3.1- Comparison of the levels of 3000 proteins in patient blood, CSF with that in conditioned medium harvested from cultures differentiated from patient iPSCs

Deliverables	**Milestones**	**Milestone deadline**	**Work package dependencies**	**Person(s) responsible**
Objective 1: Generation of iPSCs lines from 24 individuals in the DPUK Deep and Frequent Phenotype study				
D1.1 -Lines from each of the 24 individuals will be generated and quality-controlled	M1.1.1	M1.1.1 Completed	None	ZC
D1.2- iPSC lines from a reprogrammed ApoE allelic series from StemBANCC will be generated and differentiated into cortical neurons and brain endothelial cells using established methods	M1.2.1	M1.2.1 Complete	None	ZC
Objective 2: Analysis and characterisation of patient samples				
D2.1- Protocols and assay details finalised for cortical differentiation methodology in formats required for, biochemistry, cell viability, neuronal morphology, electrophysiology and RNA-Seq.	M2.1.1	M2.1.1 Complete	None	RWM
D2.2- Measurement of neuronal morphology, neurite outgrowth and spine density using the Perkin Elmer Opera Phenix platform	M2.2.1	M2.2 Complete	None	RWM
D2.3 Define the pathological interaction between extracellular Abeta and intraceullar Tau using cell viability, biochemistry, electrophysiology and RNA-Seq	M2.3.1	M2.3 Dec 2020	None	RWM, ZC, SL
Objective 3: SomaLogic profiling and assay development /validation				
D 3.1- Comparison of the levels of 3000 proteins in patient blood, CSF with that in conditioned medium harvested from cultures differentiated from patient iPSCs	M3.3.1	M3.3.1 Jun 2020 (was Dec 2018)	None	SL
D3.4-Comparison of patient autophagy levels in peripheral PBMc with CNS levels in iPSC-derived cortical neurons	M3.3.4	M3.2.1 Dec 2020	PBMCs required	SL, RWM

EM 2 Project report

Summary of plan to deliver on outstanding work

- We have **now completed the optimisation of high-throughput differentiation of all 14 iPSC lines in parallel across the three collaborating laboratories** (data below).
- We have defined a common stock of large volume across the collaborating laboratories of Abeta extracted from AD brains as required and established standard dilution conditions for its use. (data below). Healthy brain extracts and Abeta immune-depleted AD brain extract have been selected as control conditions.
- Distribution of distinct phenotyping tasks across the laboratories has been finalised:

Wade-Martins: High content imaging of neuroanatomy and synapse formation, neurite extension, axonal morphology and complexity at 80 days

Lovestone: Somalogic on medium and cell pellet; Phospho tau and total tau by westerns and IHC; Cell viability by LDH, caspase 3 or live/dead assay

Cader: MEA and transcriptomics at 80 days

- We are now ready to undertake the full experiment to quantify the effect of Abeta extracts to all 14 iPSC-derived neuronal cultures.

Risks	**Mitigation**
1) n/a	1) n/a

Please tell us the most successful outcome and what it means to dementia research

The most successful outcome has been the development of efficient methodologies for the differentiation and characterisation of many Alzheimer's patient stem cell-derived neurons in parallel. This will allow us to directly compare in parallel in patients the cellular response to an A-beta insult of an iPSC neuron in the laboratory, with cognitive decline in the clinic.

Outcomes

PUBLICATIONS

None yet, experimental work ongoing.

ENGAGEMENT ACTIVITIES

- 2014. Richard Wade-Martins was a speaker at the launch of UK Dementia Platform.
- 2015, 2016, 2017, 2018 & 2019 Dementia Awareness Day. The Oxford ARUK Network Centre organise this event to discuss current dementia research taking place within the network centre, which includes the University of Oxford, Oxford Brookes University and University of Reading.
- November 2019. Richard Wade-Martins gave an ARUK Target Drug Discovery talk.
- 2015. Richard Wade-Martins attended the DPUK Stakeholders Meeting.
- 2016. Richard Wade-Martins gave an interview for the Civil Service Fast Stream blog about his research into dementia at which Richard discussed his DPUK work.
- 2016. Participation of the Pint of Science Public Engagement Talks, Oxford.
- 2019. Bryan Ng attended and supported the ARUK Oxford Network Research Day and gave a presentation. Bryan Ng helped out at a dementia awareness event at the John Radcliffe Hospital. This was a morning of talks about research into dementia in and around Oxford and a chance for the public to meet researchers and find out about support services available for people living with dementia.
- 2019. Bryan Ng attended the Alzheimer's Association International Conference and presented a poster.

FURTHER FUNDING

- Funding Scheme: Travel grant for conference for Bryan Ng
  Organisation Name: Alzheimer's Research UK
  Type: Travel/small personal
  Funding Amount: £600

NEXT DESTINATION

- AH: moved to a post-doctoral research position at the University of Oxford

Lessons Learnt

We have encountered several challenges throughout the project to do with generating and characterising many iPSC lines in parallel. Progressing cell phenotyping assays to a miniature form in 96 and 384 well plates has been essential to developing the work, as has been the use of DPUK-funded equipment purchased as part of the Dementia Stem Cell Network. The network of the three laboratories (Wade-Martins, Lovestone and Cader) has worked together extremely well helped by a strong sense of collaboration and regular update meetings.

Project narrative

Update: Lovestone Laboratory

Since the last reporting period the group has now made the major advance of successfully differentiating the 14 Ngn2-transduced DFP iPSC lines from patients to day 40 neurons **in parallel**. As demonstrated in Figure 1, all the cell lines show positive staining for the upper (Figure 1A) and lower (Figure 1B) layer cortical markers Cux2 and CTIP2 respectively. Likewise, the neuronal populations of each line show relatively low expression of contaminant astrocytic or non-neuronal cells (Figure 1C, 1D). In differentiating these lines, an optimised seeding cell density for further experiments was also been established. Thirteen of the 14 lines were characterised at that point and are shown here, whereas the remaining line has subsequently been successfully differentiated.

The lab has also further optimised the output measures to subsequently be assessed in the 14 lines. This has been carried out in 3 of the 14 lines (BPC-939, BPC-940, and BPC-943). Initial data represented in Figure 2 suggests that day 40 neurons treated with 20 µM of Aβ25-35 for 48 hours show detectable changes in morphology (Figure 2A), cell death (Figure 2B) and neurite length (Figure 2D). Several measures of cell death were assessed including the live/dead assay using calcein AM and ethidium homodimer, LDH levels and a CytoTox assay fluorescently measuring the amount of dead cells over the total cell number. This last assay (Figure 2B) proved to be the most reliable and demonstrates a trend (but not significant) towards a decrease in survival after Aβ treatment. Different measures of neurite length were also assessed (Figure 2C). Neurons treated for 48 hours with Aβ showed decreases in segment length, length to cell, and neurite branch number.

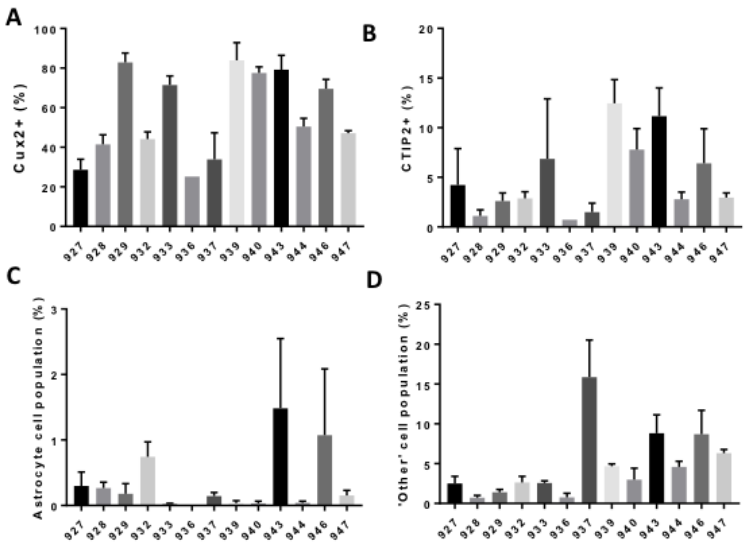

Figure 1: Expression of cortical and glial markers in 13 of the 14 Ngn2-transduced iPSC lines from patients differentiated to day 40 neurons. (A) Percentage of Cux2 positive cells in each cell lines. (B) Percentage of CTIP2 positive cells in each cell lines. (C) Percentage of s100β positive cells in each cell lines representing the proportion of cells that are astrocytes. (D) Percentage of cells with nuclear size above a threshold representing non-neuronal cells.

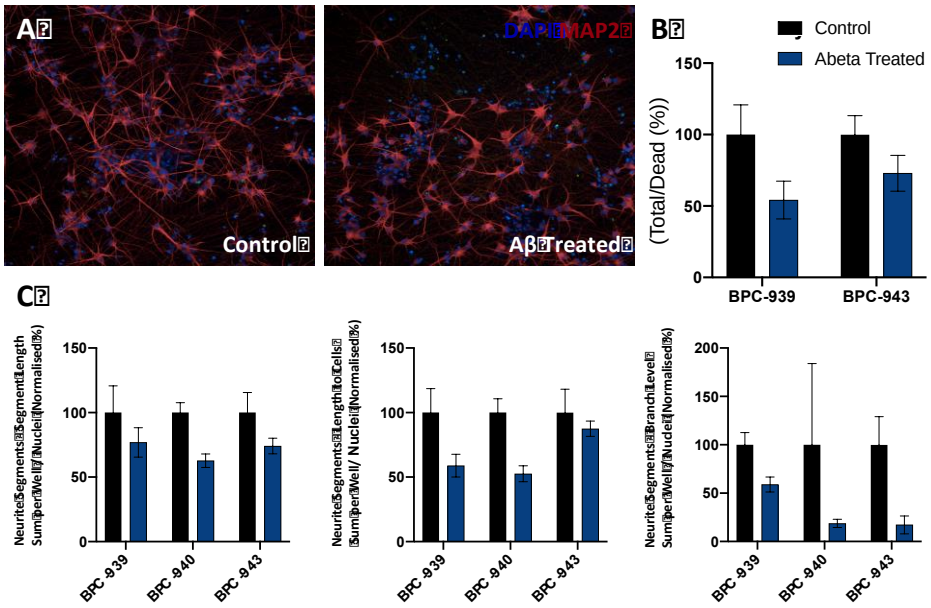

Figure 2: Assessment of cell death and neurite length of day 40 neurons after treatment with Aβ25-35. (A) Representative images of morphology changes in neurons treated with Aβ. Neurons stained with MAP2. (B) Cell death appears increased in response to Aβ as measured by CytoTox Assay. (C) Neurite length measures also demonstrate a decrease in length, branching number and segment length to cell in response to Aβ treatment.

Update: Wade-Martins Laboratory

Importantly, since the last reporting period, we have decided on the six treatment conditions for the fourteen individual patient lines of iPSC-derived cortical neurons.

- Artificial CSF (vehicle)
- Brain homogenate from a healthy subject
- Brain homogenate from an AD patient
- Brain homogenate from the same AD patient with Aβ immunodepleted
- $A\beta_{25-35}$ oligomers
- $A\beta_{35-25}$ (reversed peptide as control) oligomers

The Wade-Martins Laboratory has focussed on preparation and characterisation of the AD patient brain extracts. A range of experiments has been proposed to quantify the impact of treatment conditions on the fourteen neuronal cultures, such as synaptic imaging, neurite measurement, tau phosphorylation quantification, electrophysiology, transcriptomics, proteomics and metabolomics. In particular, the proteomics and metabolomics experiments will require a large amount of brain homogenate, hence it is necessary to prepare brain tissue from a second AD patient. To minimise variability between the brain homogenates from two individual AD patients, we intend to pool the brain homogenates 1:1 for the neuronal culture treatments. All brain homogenates were derived from post-mortem frozen frontal cortical tissues.

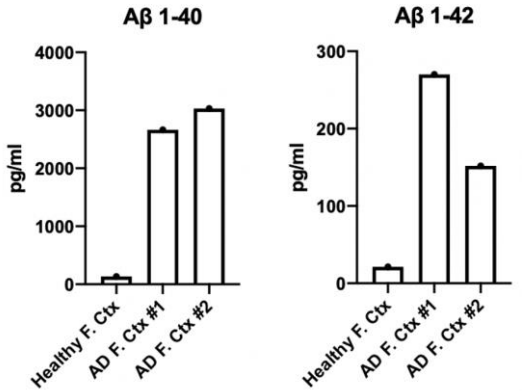

Figure 3: MSD immunoassay quantification of soluble $A\beta_{1-40}$ and $A\beta_{1-42}$ in brain homogenates from frontal cortex material from healthy and Alzheimer's patients.

The MSD immunoassay (Figure 3) indicates that AD brain homogenates from each AD patient contained higher levels of soluble Aβ than that from healthy individual. As we had not worked with pooled brain extract before, we wished to confirm that the pooled extract from AD patients 1 and 2 had the same effect on cultured neurons as each extract on its own. In a small pilot experiment, day 70 iPSC-derived cortical neurons from three DFP lines seeded on rat astrocytes were then treated with AD brain homogenates at 25% for three days before being fixed and stained for synaptic markers (Figure 4). We did indeed observe that the response of synapse outgrowth to the individual brain homogenates and the pooled brain homogenates appeared to be similar in terms of their ability to cause the loss of synapses. Interestingly, however, each line responded differently to the AD brain extracts, which we will now investigate in the full experiment to begin shortly.

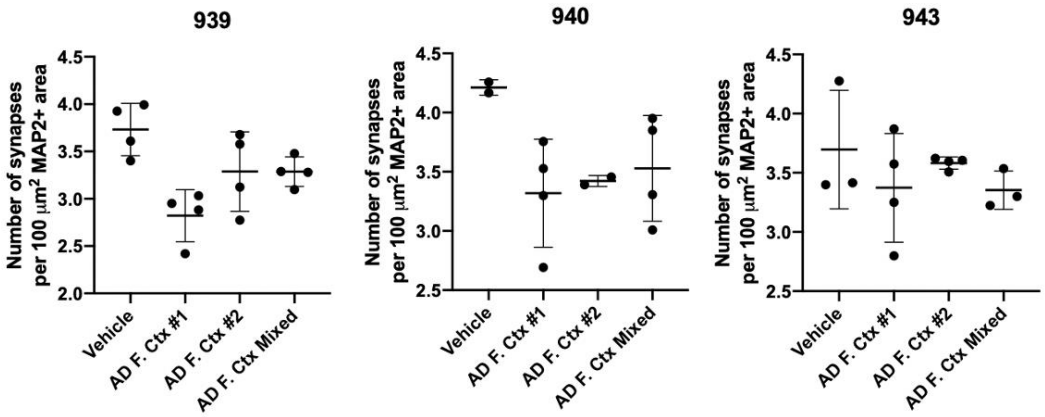

Figure 4: Synapse quantification on Day 70 iPSC-derived cortical neurons from three sporadic AD patients after treatment with brain homogenate at 25 % for three days. Mean ± SD and n = average of 15 images per well of neurons imaged. From one differentiation.

The Day 70 neurons were also treated with $A\beta_{1-42}$ oligomers for one day before going through the same experimental procedure for synapse quantification (Figure 5). It was shown that the oligomers could effectively cause downregulation of synapses and the responses from each line broadly correspond to the dataset from Figure 4 (i.e. 943 line was the least responsive to extrinsic toxic insult).

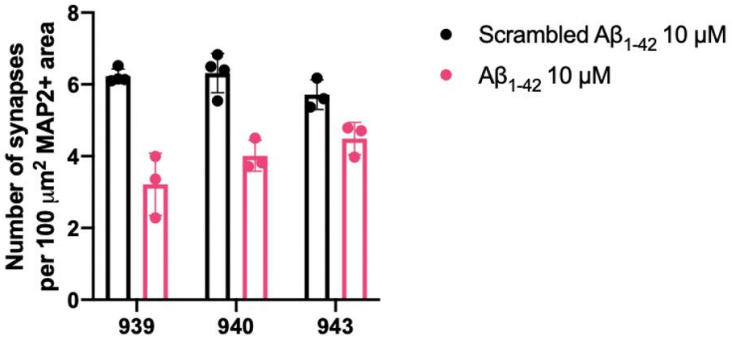

Figure 5: Synapse quantification on Day 70 iPSC-derived cortical neurons from three sporadic AD patients after treatment with $A\beta_{1-42}$ oligomers for one day. Mean ± SD and n = average of 15 images per well of neurons imaged. From one differentiation.

Cader Laboratory update

The Cader Laboratory is focussed on analysis of iPSC-derived cortical neurons by multi-electrode array (MEA). Further statistical analysis has been carried out the recordings collected from DIV 0 - DIV85 of the three DFP iPSC lines differentiated and matured on MEA plates as part of the pilot work (BPC939, BPC943, BPC940). A summary graph of the mean firing rate (+/-SEM) of each line is shown below (Fig. 6). From DIV 59, activity on the plate starts to become significantly different to the start for all 3 cell lines

due to neuronal maturation (one-way ANOVA p<0.05). The line BPC940 overall activity is significantly less than the other 2 lines. Analysis of cell morphology confirmed that this line is slower growing (2-way ANOVA at D85).

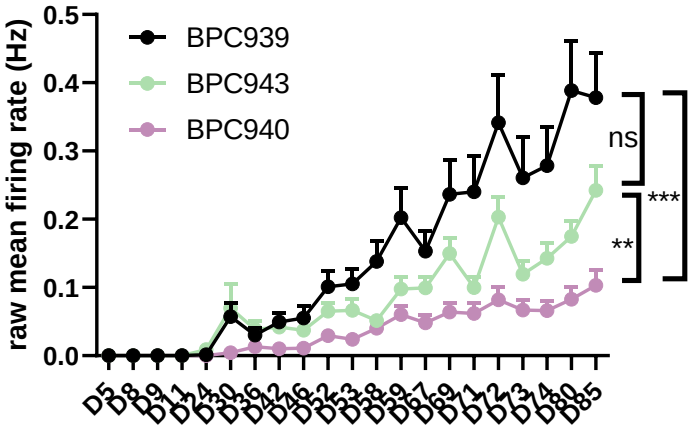

Figure 6: Activity on MEA changes from DIV5 to DIV85 for the 3 cell lines. (statistics: graph includes all electrodes, no filtering performed. mean+/-SEM; 2-way ANOVA)

<u>Effect of treatments on MEA activity</u>

Four treatments were used on the 48 well MEA plate at >DIV 85 with four repeated wells per treatment per cell line used and applying a 1:4 dilution of AD brain extract.

- ACSF (1:4)
- AD extract in ACSF (1:4)
- Ab peptide 25-35 (20 uM)
- Healthy Brain extract in ACSF (1:4)

Recordings were performed before the treatments were added on the cells (Baseline); 1 hour post treatment (1h); 1 day post treatment (1d); 2 day post treatment (2d); 3 day post treatment (3d); 4 day post treatment (4d); 5 day post treatment (5d); 6 day post treatment (6d).

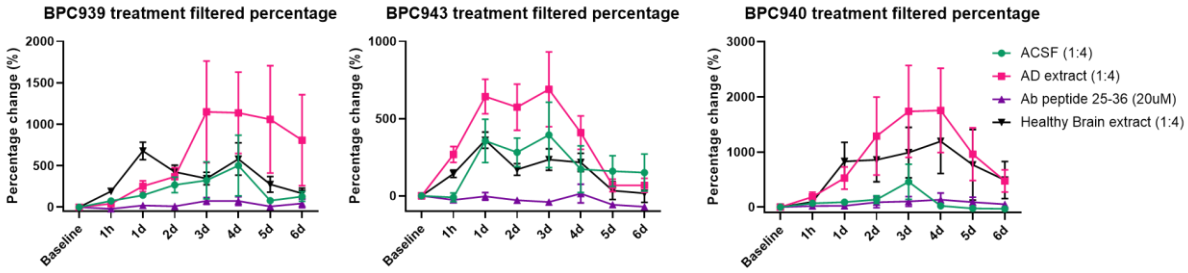

Figure 7: Percentage change in activity for the 3 cell lines with each treatment conditions. Statistic: electrodes that weren't recording any activity at the baseline is filtered out from all analysis. The percentage change is calculated per electrode. Graph shows mean +/- SEM.

We note that in all cases AD Brain extract (1:4) treatment consistently leads to the highest increase in activity in all three lines. However, the difference between AD extract treatment and controls (ACSF and healthy brain extract) is not significant with 2-way ANOVA test. The variability of the data is high. To reduce this, we will improve three factors for the next round of testing: 1) pre-screen activity in MEA plate before treatment to filter out silent wells; 2) check the Osmolarity of ACSF; 3) check Ca2+ concentrations in all testing conditions.