Stem Cells Network- Complete

Stem cells
Start date: 1 Oct 2014 **Completion date: Jun 2019**
Date of form completion: 4th Nov 2019

Team members funded (full or part-time) by DPUK
Deepak Kumar, Karen Burr and Emma Cope

Team members involved with the project but not funded by DPUK
Richard Wade-Martins, Deepak Kumar, Colin Akerman, Simon Lovestone, **Sally Cowley**, Zameel Cader, John Davis, Julian Knight, Francesca Nicholls, David Owen, **Tom Warner**, Adrian Isaacs, Parmjit Jat, Robin Ketteler, Rickie Patani, Sarah Tabrizi, Selina Wray, **Nicholas Allen**, Yves Bardes, Lesley Jones, Paul Kemp, Emma Kidd, Emyr Lloyd-Evans, Anne Rosser, Julie Williams, Katie Lunnon, John Mill, Andy Randall, Vasanta Subramanian, James Uney, **Siddharthan Chandran**, Ian Deary, Charles ffrench-Constant, Giles Hardingham, David Lyons, Tara Spire-Jones, David Wyllie, **Rick Livesey**, Jenny Gallop, Steve Jackson, **Nigel Hooper**, Stuart Pickering-Brown, Tao Wang, Chris Ward
ECRs: Charmaine Lang, Natalie Connor-Robson, Dayne Beccano-Kelly, Brent Ryan, Tara Caffrey

Objectives
To establish a strategic stem cell network enabling coordinated programmes for:
1. Immortalisation of strategic cell lines
2. High throughput genome editing
3. Standardised protocols for cell differentiation
4. Detailed cell phenotyping based on high throughput high content imaging, physiology and omics.
5. DPUK DSCN Network building

Dependencies to and from other work packages, networks and themes
None

Lessons Learnt (what went well, what did you have to change)
The DPUK Stem Cell Network proved to be a highly effective means to bring together the community of researchers in the UK working to model neurodegenerative disease in human induced pluripotent stem cells. The Network completed its work in June 2019 and the papers cited below provide the record of a highly productive period of work.
The Stem Cell Network is widely acknowledged to be a success story for DPUK and it is of note that many papers shown below have more than one DPUK Stem Cell Network PI as a co-author as evidence of the solid collaborations formed by the network**.**

Were all Milestones completed
YES- all were completed by the conclusion of the DPUK Stem Cell Network in June 2019.

Deliverables	Milestones	Milestone deadline	Work package dependencies	Person(s) responsible
Objective 1:				
D1.1	M1.1.1 Establish automated culture of iPSC lines	M1.1.1 Complete	None	Cowley, Chandran, Wade-Martins
D1.2	M1.2.1 Generation of DPUK iPSC lines	M1.2.1 Complete	None	
Objective 2:				
D2.1	M2.2.1 Establish common approaches for high-content imaging on Opera Phenix platform	M2.2.1 Complete		Hooper, Cowley, Warner, Livesey, Wade-Martins

	D2.2 Establish common approaches for high-content imaging on Opera Phenix platform	M2.2.1 Complete		
Objective 3:				
D3.1	M3.1.1 Standardised protocols for cell differentiation	M3.1.1 Complete	None	Allen
Objective 4:				
D4.1	M4.1.1 Functional analysis of familial and sporadic AD lines to model AD phenotypes	M4.1.1 Complete	None	Livesey, Hooper, Wade- Martins
D4.2	M4.2.1 Functional analysis of familial and sporadic PD iPSC lines to model PD phenotypes	M4.2.1 Complete		
Objective 5:				
D5.1	M5.1.1 DPUK DSCN Network building	M5.1.1 Complete	None	Wade-Martins

Outcomes

The top five outputs from across the network:

1. Lang C,[2]Campbell K, Ryan BJ, Carling P, Attar M, Vowles J, Perestenko OV, Bowden R, Baig F, Kasten M, Hu MT, Cowley SA, Webber C and Wade-Martins R (2019) Single cell sequencing of iPSC-dopamine neurons reconstructs disease progression and identifies HDAC4 as a regulator of Parkinson cell phenotypes. *Cell Stem Cell* 24:93-106.

This paper in Cell Stem Cell undertook the first single cell sequencing study of Parkinson's patient stem cell-derived neurons and identified HDAC4 as a regulator of disease phenotypes in genetic and sporadic Parkinson's. The work went further to show that compounds which modulate HDAC4 activity are able to correct Parkinson's phenotypes in human neurons.

2. Brownjohn PW, Smith J, Solanki R, Lohmann E, Houlden H, Hardy J, Dietmann S, Livesey FJ. (2018) Functional Studies of Missense TREM2 Mutations in Human Stem Cell-Derived Microglia. Stem Cell Reports. 2018 Apr 10;10(4):1294-1307.

This paper describes a robust method to derive microglia from human pluripotent stem calls. These microglia were used to study the consequences of missence mutations of the TREM2 receptor implicated in frontotemporal dementia-like syndrome and Nasu- Hakola disease (NHD). The work demonstrated there is a complex and subtle effect of missense TREM2 mutations on microglial function that could be consistent with the delayed clinical symptoms seen in FTD-like syndrome and NHD.

3. Telezhkin V, Schnell C, Yarova P, Yung S, Cope E, Hughes A, Thompson BA, Sanders P, Geater C, Hancock JM, Joy S, Badder L, Connor-Robson N, Comella A, Straccia M, Bombau G, Brown JT, Canals JM, Randall AD, Allen ND, Kemp PJ. (2016) Forced cell cycle exit and modulation of GABAA, CREB, and GSK3β signaling promote functional maturation of induced pluripotent stem cell-derived neurons. 310(7):C520-41.

This paper describes a simple protocol that employs the sequential addition of supplemented media (Synaptojuice) formulated to separate the two key phases of neural differentiation, the neurogenesis and synaptogenesis, each characterized by different signaling requirements. This new protocol synchronized neurogenesis and enhanced the rate of maturation of pluripotent stem cell-derived neural precursors. Neurons differentiated using this protocol exhibited 1) spontaneous electrical activity; 2) regenerative induced action potential train activity; 3) Na(+) current availability, and 4) synaptic currents. The Synaptojuice method was shared across the network.

4. W Haenseler, SN Sansom, J Buchrieser, SE Newey, CS Moore, FJ Nicholls, S Chintawar, C Schnell, JP Antel, ND Allen, MZ Cader, R Wade-Martins, WS James, SA Cowley, A Highly Efficient Human Pluripotent Stem Cell Microglia Model Displays a Neuronal-Co-culture-Specific Expression Profile and Inflammatory Response, Stem Cell Reports 8(6) (2017) 1727-1742.

In neurodegeneration there has been enormous interest recently in deriving microglia from human induced Pluripotent Stem Cells to model neuroinflammation, particularly since numerous AD-related genes are expressed in microglia. This group has developed methodology to generate microglia in high numbers, with minimal manipulation. As it is a co-culture system with iPSC-neurons, it is physiologically relevant and has become widely adopted; the paper has been cited 65 times in the 24 months since its publication.

5. Magnani D, Borooah S, Burr K, Story D, McCampbell A, Shaw CE, Kind PC, Aitman TJ, Whitelaw CBA, Wilmut I, Smith C, Miles GB, Hardingham GE, Wyllie DJA, Chandran S. (2018) C9ORF72 repeat expansion causes vulnerability of motor neurons to Ca^{2+}-permeable AMPA receptor-mediated excitotoxicity. Nat Commun. 2018 Jan 24;9(1):347.

Mutations in C9ORF72 are the most common cause of familial amyotrophic lateral sclerosis (ALS). This study combined RNA-Seq and electrophysiology of induced pluripotent stem cell (iPSC)-derived motor neurons to show that motor neurons with C9ORF72 mutations have increased expression of GluA1 AMPA receptor (AMPAR) subunit which leads to increased Ca^{2+}-permeable AMPAR expression and enhanced selective MN vulnerability to excitotoxicity.

Other publications from the DPUK Stem Cell Network:

1. Connor-Robson N, Booth H, Martin JG, Gao B, Li K, Doig N, Vowles J, Browne C, Klinger L, Juhasz P, Klein C, Cowley SA, Bolam P, Hirst W and Wade-Martins R (2019) An integrated transcriptomics and proteomics analysis reveals functional endocytic dysregulation caused by mutations in *LRRK2*. *Neurobiology of Disease* 127:512-526.
2. Jarosz-Griffiths HH, Corbett NJ, Rowland HA, Fisher K, Jones AC, Baron J, Howell GJ, Cowley SA, Chintawar S, Cader MZ, Kellett KAB, Hooper NM. (2019) Proteolytic shedding of the prion protein via activation of metallopeptidase ADAM10 reduces cellular binding and toxicity of amyloid-β oligomers. J Biol Chem. 2019 Mar 14. pii: jbc.RA118.005364. doi: 10.1074/jbc.RA118.005364. [Epub ahead of print]
3. Magnani D, Chandran S, Wyllie DJA, Livesey MR.(2019) In Vitro Generation and Electrophysiological Characterization of OPCs and Oligodendrocytes from Human Pluripotent Stem Cells. Methods Mol Biol. 2019;1936:65-77. doi: 10.1007/978-1-4939-9072-6_4.
4. Paonessa F, Evans LD, Solanki R, Larrieu D, Wray S, Hardy J, Jackson SP, Livesey FJ. (2019) Microtubules Deform the Nuclear Membrane and Disrupt Nucleocytoplasmic Transport in Tau-Mediated Frontotemporal Dementia. *Cell Rep.* 2019 Jan 15;26(3):582-593.e5.
5. Zambon F, Cherubini M, Fernandes HJR, Lang C, Ryan BJ, Volpato V, Bengoa-Vergniory N, Attar M, Booth HDE, Haenseler W, Vowles J, Bowden R, Webber C, Cowley SA and Wade-Martins R (2019) Cellular α-synuclein pathology is associated with bioenergetic dysfunction in Parkinson's iPSC-derived dopamine neurons. *Human Molecular Genetics* 28(12):2001-2013.
6. Booth HDE, Wessely F, Connor-Robson N, Rinaldi F, Vowles J, Browne C, Evetts SG, Hu MT, Cowley SA, Webber C, Wade-Martins R. (2019) RNA sequencing reveals MMP2 and TGFB1 downregulation in LRRK2 G2019S Parkinson's iPSC-derived astrocytes. *Neurobiology of Disease* 129:56-66.
7. Evans LD, Wassmer T, Fraser G, Smith J, Perkinton M, Billinton A, Livesey FJ. (2018) Extracellular Monomeric and Aggregated Tau Efficiently Enter Human Neurons through Overlapping but Distinct Pathways. Cell Rep. 2018 Mar 27;22(13):3612-3624.
8. Magnani D, Borooah S, Burr K, Story D, McCampbell A, Shaw CE, Kind PC, Aitman TJ, Whitelaw CBA, Wilmut I, Smith C, Miles GB, Hardingham GE, Wyllie DJA, Chandran S. (2018) C9ORF72 repeat expansion causes vulnerability of motor neurons to Ca^{2+}-permeable AMPA receptor-mediated excitotoxicity. Nat Commun. 2018 Jan 24;9(1):347.
9. Selvaraj BT, Livesey MR, Zhao C, Gregory JM, James OT, Cleary EM, Chouhan AK, Gane AB, Perkins EM, Dando O, Lillico SG, Lee YB, Nishimura AL, Poreci U, Thankamony S, Pray M, Vasistha NA, Rowland HA, Hooper NM, Kellett KAB. (2018) Modelling Sporadic Alzheimer's Disease Using Induced Pluripotent Stem Cells. Neurochem Res. 2018 Dec;43(12):2179-2198.
10. Telezhkin V, Straccia M, Yarova P, Pardo M, Yung S, Vinh NN, Hancock JM, Barriga GG, Brown DA, Rosser AE, Brown JT, Canals JM, Randall AD, Allen ND, Kemp PJ. (2018) Kv7 channels are upregulated during striatal neuron development and promote maturation of human iPSC-derived neurons. Pflugers Arch. 2018 Sep;470(9):1359-1376.
11. Volpato V, Smith J, Sandor C, Ried JS, Baud A, Handel A, Newey SE, Wessely F, Attar M, Whiteley E, Chintawar S, Verheyen A, Barta T, Lako M, Armstrong L, Muschet C, Artati A, Cusulin C, Christensen K, Patsch C, Sharma E, Nicod J, Brownjohn P, Stubbs V, Heywood WE, Gissen P, De Filippis R, Janssen K, Reinhardt P, Adamski J, Royaux I, Peeters PJ, Terstappen GC, Graf M, Livesey FJ, Akerman CJ, Mills K, Bowden R, Nicholson G, Webber C, Cader MZ, Lakics V. (2018) Reproducibility of Molecular Phenotypes after Long-Term Differentiation to Human iPSC-Derived Neurons: A Multi-Site Omics Study. Stem Cell Reports. 2018 Oct 9;11(4):897-911.
12. Watson LM, Wong MMK, Vowles J, Cowley SA, Becker EBE. (2018) A Simplified Method for Generating Purkinje Cells from Human-Induced Pluripotent Stem Cells. Cerebellum. 2018 Aug;17(4):419-427.

13. Haenseler, W, Zambon F, Lee H, Vowles J, Rinaldi F, Duggal G, Houlden H, Gwinn K, Wray S, Luk KC, Wade-Martins R, James WS, and Cowley SA. 2017. Excess α-synuclein compromises phagocytosis in iPSC-derived macrophages. *Scientific Reports*, 7: 9003
14. Sandor C, Robertson P, Lang C, Heger A, Booth H, Vowles J, Witty L, Bowden R, Hu M, Cowley SA, Wade-Martins R, Webber C (2017). Transcriptomic profiling of purified patient-derived dopamine neurons identifies convergent perturbations and therapeutics for Parkinson's disease. *Human Molecular Genetics*, 26(3):552-566.
15. Beevers JE, Lai MC, Collins E, Booth HDE, Zambon F, Parkkinen L, Vowles J, Cowley SA, Wade-Martins R and Caffrey TM (2017) MAPT genetic variation and neuronal maturity alter isoform expression affecting axonal transport in iPSC-derived dopamine neurons. *Stem Cell Reports,* 9: 587-599.
16. Dafinca R, Scaber J, Ababneh N, Lalic T, Weir G, Christian H, Vowles J, Douglas AG, Fletcher-Jones A, Browne C, Nakanishi M, Turner MR, Wade-Martins R, Cowley SA, Talbot K. (2016*)* C9orf72 hexanucleotide expansions are associated with altered ER calcium homeostasis and stress granule formation in iPSC-derived neurons from patients with amyotrophic lateral sclerosis and frontotemporal dementia*. Stem Cells* 34:2063-78.
17. Hall CE, Yao Z, Choi M, Tyzack GE, Serio A, Luisier R, Harley J, Preza E, Arber C, Crisp SJ, Watson PMD, Kullmann DM, Abramov AY, Wray S, Burley R, Loh SHY, Martins LM, Stevens MM, Luscombe NM, Sibley CR, Lakatos A, Ule J, Gandhi S, Patani R (2017) A neuroprotective astrocyte state is induced by neuronal signal EphB1 but fails in ALS models. *Nature Comms* 2017;8:1164.
18. Tyzack GE, Hall CE, Sibley CR, Cymes T, Forostyak S, Carlino G, Meyer IF, Schiavo G, Zhang SC, Gibbons GM, Newcombe J, Patani R, Lakatos A (2017) Progressive Motor Neuron Pathology and role of astrocytes in a human stem cell model of VCP- related ALS. *Cell Reports* 2017;19:1739-1749.
19. Fernandes HJR, Hartfield EM, Christian HC, Emmanoulidou E, Zheng Y, Booth H, Bogetofte H, Lang C, Ryan BJ, Sardi SP, Badger J, Vowles J, Evetts S, Tofaris GK, Vekrellis K, Talbot K, Hu MT, James W, Cowley SA and Wade-Martins R (2016) ER stress and autophagic perturbations lead to elevated extracellular α-synuclein in *GBA-N370S* Parkinson's iPSC-derived dopamine neurons. *Stem Cell Reports* 8;6(3):342-56.

Executive Summary of Project

The DPUK Stem Cell Network was set up to build capacity and provide a focus for the community of researchers in the UK working to model neurodegenerative disease in human induced pluripotent stem cells. The aims of the work were to (i) facilitate the generation of strategically-important iPSC lines; (ii) to help develop new methods of high throughput genome editing, (iii) to ensure best practice and use of standardised protocols for iPSC differentiation into neurons and glia and (iv) to perform detailed cell phenotyping based on high throughput high content imaging, physiology and -omics. The Network proved to be a highly effective means to bring laboratories together to form genuine and lasting collaborations and completed its work in June 2019. The papers cited below provide the record of a highly productive period of work. Further exemplars of the network activity right across the whole UK can be found in the nature of the very successful networking activity shown at our meetings in Edinburgh (2017) and Cardiff (2018).

The Stem Cell Network is widely acknowledged to be a success story for DPUK and it is of note that many papers shown below have more than one DPUK Stem Cell Network PI as a co-author as evidence of the solid collaborations formed by the network**.**

Stem Cell Network End Report.pdf