Stem Cell Network

Objective(s):

The aims of the DPUK Stem Cell Network were to establish a network of researchers 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.

Overview Summary:

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 cells.

Executive Summary:

The DPUK Stem Cell Network is widely acknowledged to be a success story for DPUK. It was established 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 Network proved to be a highly effective in bringing laboratories together to form genuine and lasting collaborations, completing its work in June 2019. The papers cited below illustrate the productivity of the group. Close collaboration is illustrated by the fact many publications had more than one DPUK Stem Cell Network PI as a co-author. Further exemplars of network activity right across the whole of the UK was illustrated by the successful hosting of workshops in Edinburgh (2017) and Cardiff (2018).

Summary of Outputs: (as per Researchfish categories)

Publications:

Outcomes

The top five outputs from across the network:

1. Lang C,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. Wallings R, Connor-Robson N, Wade-Martins R. (2019). LRRK2 interacts with the vacuolar-type H+-ATPase pump a1 subunit to regulate lysosomal function. *Human molecular genetics, 28*(16), pp. 2696-2710. doi: 10.1093/hmg/ddz088.
8. Wallings RL, Humble SW, Ward ME, Wade-Martins R. (2019). Lysosomal Dysfunction at the Centre of Parkinson's Disease and Frontotemporal Dementia/Amyotrophic Lateral Sclerosis. *Trends in neurosciences, 42*(12), pp. 899-912. doi: 10.1016/j.tins.2019.10.002.
9. Bogetofte H, Jensen P, Okarmus J, Schmidt SI, Agger M, Ryding M, Nørregaard P, Fenger C, Zeng X, Graakjær J, Ryan BJ, Wade-Martins R, Larsen MR, Meyer M. (2019). Perturbations in RhoA signalling cause altered migration and impaired neuritogenesis in human iPSC-derived neural cells with PARK2 mutation. *Neurobiology of disease, 132*, pp. 104581. doi: 10.1016/j.nbd.2019.104581.
10. Bogetofte H, Jensen P, Ryding M, Schmidt SI, Okarmus J, Ritter L, Worm CS, Hohnholt MC, Azevedo C, Roybon L, Bak LK, Waagepetersen H, Ryan BJ, Wade-Martins R, Larsen MR, Meyer M (2019). Mutation Causes Metabolic Disturbances and Impaired Survival of Human iPSC-

Derived Neurons. Frontiers in cellular neuroscience, 13, pp. 297. doi: 10.3389/fncel.2019.00297.

11. Überbacher C, Obergasteiger J, Volta M, Venezia S, Müller S, Pesce I, Pizzi S, Lamonaca G, Picard A, Cattelan G, Malpeli G, Zoli M, Beccano-Kelly D, Flynn R, Wade-Martins R, Pramstaller PP, Hicks AA, Cowley SA, Corti C (2019). Application of CRISPR/Cas9 editing and digital droplet PCR in human iPSCs to generate novel knock-in reporter lines to visualize dopaminergic neurons. Stem cell research, 41, pp. 101656. doi: 10.1016/j.scr.2019.101656.
12. Lee DS, Luo C, Zhou J, Chandran S, Rivkin A, Bartlett A, Nery JR, Fitzpatrick C, O'Connor C, Dixon JR, Ecker JR. (2019). Simultaneous profiling of 3D genome structure and DNA methylation in single human cells. Nature methods, 16(10), pp. 999-1006. doi: 10.1038/s41592-019-0547-z.
13. Baker E, Sims R, Leonenko G, Frizzati A, Harwood JC, Grozeva D, GERAD/PERADES , CHARGE , ADGC , EADI , IGAP consortia , Morgan K, Passmore P, Holmes C, Powell J, Brayne C, Gill M, Mead S, Bossù P, Spalletta G, Goate AM, Cruchaga C, Maier W, Heun R, Jessen F, Peters O, Dichgans M, FröLich L, Ramirez A, Jones L, Hardy J, Ivanov D, Hill M, Holmans P, Allen ND, Morgan BP, Seshadri S, Schellenberg GD, Amouyel P, Williams J, Escott-Price V. (2019). Gene-based analysis in HRC imputed genome wide association data identifies three novel genes for Alzheimer's disease. PloS one, 14(7), pp. e0218111. doi: 10.1371/journal.pone.0218111.
14. Harwood JC, Kent NA, Allen ND, Harwood AJ. (2019). Nucleosome dynamics of human iPSC during neural differentiation. EMBO reports, 20(6), doi: 10.15252/embr.201846960.
15. Garcia VJ, Rushton DJ, Tom CM, Allen ND, Kemp PJ, Svendsen CN, Mattis VB. (2019). Huntington's Disease Patient-Derived Astrocytes Display Electrophysiological Impairments and Reduced Neuronal Support. Frontiers in neuroscience, 13, pp. 669. doi: 10.3389/fnins.2019.00669.
16. 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 reports, 26(3), pp. 582-593.e5. doi: 10.1016/j.celrep.2018.12.085.
17. 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. *The Journal of biological chemistry, 294*(17), pp. 7085-7097. doi: 10.1074/jbc.RA118.005364.

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19. 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.
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21. 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.
22. 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.
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26. 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.
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30. 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.
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Collaborations & Partnerships

Establishing a network of researchers fully committed to collaborating and sharing underpinned this work. It was highly successful in these aims.

Further Funding

Funding Scheme: MRC Partnership Award (Dementias Platform UK Stem Cell Partnership)
Organisation Name: MRC-UK

Type: Capital/infrastructure (including equipment)
Funding Amount: £1,200,000
Reference Number: MR/N013255/1
Start Month / Year: July 2016
End Month / Year: June 2020

Next Destinations

Deepak Kumar has moved into translational grant portfolio management.

Engagement Activities

MRC Stem Cell Partnership Workshops

The inaugural DPUK Stem Cell Network Spring School was held in April 2017 in Edinburgh. 65 scientists attended and 22 were invited to speak, with an emphasis and priority for Early Career Researchers. There were extensive opportunities for networking, discussions and sharing of expertise and developing new collaborations.

The 2nd annual DPUK Dementia Stem Cell Network workshop in Cardiff in 2018 brought together 93 delegates from all partner universities. The two-day workshop consisted of 21 talks split into seven diverse sessions ranging from genetics to transcriptomics and from iPSC models of neurodegeneration to synaptic activity and the blood brain barrier. Day one culminated in a poster event which showcased work from nine DPUK researchers and provided an opportunity for them to not only communicate their research but to also network and build future collaborations within our partnership.

- 2014. Richard Wade-Martins was a speaker at the launch of UK Dementia Platform representing the DPUK Stem Cell Network.

- 2015. Richard Wade-Martins attended the DPUK Stakeholders Meeting representing the DPUK Stem Cell Network.
- 2019: Rick Livesey presented at the DPUK Five Year event representing the DPUK Stem Cell Network.
- 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.
- YouTube channel for Alzheimer's Research UK Oxford network centre. Includes talks from open days, panel discussions and short Q&A videos.
- Oxford Parkinson's Disease Centre Facebook and Twitter. Used to update on OPDC activity. Focus on outreach events, but also includes news about publications etc.
- 2016. Richard Wade-Martins appeared on BBC One Saturday Breakfast News -to discuss research using stem cell technology to grow and study dopamine neurons from skin cells.
- 2016. "The Funny Side of Parkinson's" on BBC1. Paul Mayhew-Archer Documentary. Richard Wade-Martins talked about OPDC stem cell work.

Influence of policy, practice, patients & the public

None

Research Tools & Methods

The DPUK Stem Cell Network has its own dedicated space on the DPUK web-site (https://www.dementiasplatform.uk/our-impact/stem-cells-network). This includes information on available iPSC lines in AD, PD and normal ageing cohorts; information on team expertise and of the Technology resources available.

All protocols are published and made available by network members. More details are provided here:
https://www.dementiasplatform.uk/our-impact/stem-cells-network/research-programmes/differentiation-2013-brain-cells

Research Databases & Models

Stem cell lines:

See: https://www.dementiasplatform.uk/our-impact/stem-cells-network

All the StemBANCC iPSC lines are fully catalogued and available on: EBiSC https://cells.ebisc.org/ hPSCreg catalog https://hpscreg.eu/ Edinburgh Lothian Birth Cohort lines may be obtained from Siddharthan Chandra
Intellectual property & licencing
None
Medical products, interventions & clinical trials
None
Artistic & creative products
None
Software & technical products
None
Spin outs
None
Awards & recognition
See individual network members laboratory pages
Other outputs & knowledge/future steps
See individual network members laboratory pages
Use of facilities & resources
https://www.dementiasplatform.uk/our-impact/stem-cells-network/state-of-the-art-technologies A variety of resources can be made available to researchers as detailed above.
Most successful outcome and what it means for future dementia research:
The use of patient-derived iPSC models has proved to be transformative for our understanding of neurodegenerative diseases. The DPUK Stem Cell Network was a highly successful endeavour to bring together the leading laboratories in the UK working on generating, characterising and exploiting stem cell models into a collaborative network. The Network was launched at exactly the

right time in the development of the technologies to establish the UK as a major international leader in the work and the 36 papers cited above span across Alzheimer's disease, Parkinson's disease, motor neuron disease and Huntington disease, often representing highly collaborative activities. The work has greatly increased our knowledge of disease mechanisms and the UK is now well-placed to exploit such findings in the next phase of the work, being target and drug discovery for dementias.

Lessons learned:

The DPUK Stem Cell Network is widely acknowledged to be a success story for DPUK. It was established 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 Network proved to be a highly effective in bringing laboratories together to form genuine and lasting collaborations, completing its work in June 2019. The papers cited below illustrate the productivity of the group. Close collaboration is illustrated by the fact many publications had more than one DPUK Stem Cell Network PI as a co-author. Further exemplars of network activity right across the whole of the UK was illustrated by the successful hosting of workshops in Edinburgh (2017) and Cardiff (2018).

Other:

Network members (Lab leaders in bold text)

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.

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

Deepak Kumar, Karen Burr and Emma Cope

ECRs: Charmaine Lang, Natalie Connor-Robson, Dayne Beccano-Kelly, Brent Ryan, Tara Caffrey

Date of Report:

6 April 2020