Interrogating the gene regulatory network in human stem cell models of Alzheimer's Disease

Dementia, and particularly, Alzheimer's Disease (AD), is the major health care challenge of the 21st century. A major roadblock to developing novel therapeutic tools is our paucity of knowledge about the underlying mechanisms driving neurodegeneration. Much evidence suggests that these mechanisms are highly complex suggesting that we need to adopt a systems approach to properly understand the pathogenic process. One such approach is to examine the interactions among all the genes that respond to a known aetiopathological insult to establish a 'gene regulatory network' (GRN), in order to identify common underlying biological pathways. More formally, we need to examine how the topology and state of the GRN determines the contribution of any particular gene to the network output. This systems approach requires close collaboration among experimental and computational scientists. This project sits on the experimental side of this collaboration and comprises a multifaceted approach encompassing stem cell biology, genomics, transcriptomics, epigenomics and genome editing.

We have shown that Clusterin, one of the few novel genes to be associated with late onset AD, lies on the ß-amyloid cascade through a complex pathway involving p53/ Dkk1/Wnt signaling [1]. As such, stem cells, bearing genetic alterations in clusterin present an ideal model to interrogate the underlying gene regulatory network that links clusterin to neurodegeneration. Further, this focus on clusterin allows us to see how the impact of variation in a single gene is not simply a function of the gene in isolation but is determined by gene interactions across the network i.e. the network topology. We expect this study to provide a general methodological and conceptual framework to understand how underlying genetic architecture determines neuronal susceptibility to neurodegeneration.

The essence of this project is to distil gene regulatory networks from time series transcriptome data harvested from human stem cells differentiated toward neurons. By interrogating the transcriptome at multiple time-points during neuronal differentiation, we can infer causal relationships and build a network where the nodes represent genes and edges represent regulatory connections among the genes. Collectively, these gene interactions constitute a GRN, the topology of which allows us to understand the contribution of individual genes and biological pathways to the overall network output. Our challenge is to determine if applying network inference to data derived from human stem cell derived neurons lends insight into AD mechanism and further, allows identification of novel therapeutic targets that may delay or abrogate neurodegeneration.

The key objectives will be to:
- Harvest transcriptome data from human ESCs bearing wild-type and genetically altered clusterin as they differentiate from neural progenitors to differentiated neurons.
- Work in collaboration with a team of computational biologists and bioinformaticians (although you will not be expected to undertake the computational analyses, you will be expected to be an active member of this cross-disciplinary team) to identify key genes and pathways involved in clusterin-mediated neurodegeneration.
- Develop cellular assays of neuronal phenotypes to assess network output
- Test candidate pathways to attempt to rescue or prevent neurodegeneration using RNAi and genome-editing to engineer modifications to multiple genes and/or pathways.

Location and supervision: This project allows you to carry out a PhD program of research that investigates basic molecular mechanisms underlying Alzheimer's Disease using stem cell biology, gene network discovery, next-generation sequencing, target identification and the opportunity to work within a multidisciplinary team comprising basic and translational neuroscientists, stem cell biologists, bioinformaticians, and to develop collaborations with computational biologists. While your

focus will be on the molecular and cell biology, we fully expect you to become part of this multidisciplinary team and to take advantage of the training opportunities offered by this unique studentship. The proposed studentship would have dual supervision from Professors Buckley[2] and Lovestone[1].

1   Killick, R. et al. Clusterin regulates beta-amyloid toxicity via Dickkopf-1-driven induction of the wnt-PCP-JNK pathway. *Mol Psychiatry* 19, 88-98, doi:10.1038/mp.2012.163 (2014).

2   Raposo ASF, et al. Ascl1 coordinately regulates gene expression and the chromatin landscape during neurogenesis. Manuscript in press *Cell Reports* (2015).

Deadline for applications: Friday 29th May 2015.
Start date: October 2015.
Anticipated interview date: 10th/ 11th June 2015.
Funding: A tax free stipend of £18,000 pa for 3 years is provided by the Department of Psychiatry.
Entry requirements: A minimum of an upper second class undergraduate degree in a relevant subject. Applicants whose first language is not English will be required to provide evidence of proficiency as required by the University of Oxford.