Generation of an authentic inducible Krabbe disease model to develop innovative clinical therapies

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Abstract:
Krabbe disease, is a devastating genetic disorder of the brain, spinal cord and peripheral nerves, principally affecting infants and young children. Extensive demyelination in Krabbe disease has lethal effects: with seizures, blindness, deafness, spasticity and regression of developmental faculties before the age of two years. We seek to develop innovative combinatorial therapy based on successful gene transfer, regenerative cell and enzymatic augmentation now reported in cognate neurological diseases.

Deficiency of galactocerebrosidase (GALC) in Krabbe disease causes loss of oligodendroglia and neurones due to toxic accumulation of the diffusible bioactive sphingoid base, psychosine - with irreversible loss of myelin and dementia. In a natural mutant model, the twitcher mouse, lacking GALC, which authentically recapitulates human Krabbe disease, haematopoietic stem-cell transplantation and gene therapy targeted to the brain and peripheral nervous system extend survival. The host laboratory developed the first eukaryotic system to produce active stable GALC for enzyme replacement therapy and also made possible solution of its 3-D crystal structure; moreover we are undertaking stem cell (mesenchymal-lineage) gene augmentation therapy in the living model. Most importantly we have also shown that, unique to severe Gaucher and Krabbe diseases, neural cell loss is due to necroptosis regulated by the kinases RIP1 and RIP3 and critical for programmed cell-death. There is thus a strong case for exploring the adjunctive role of pharmaceutical inhibitors RIP kinase inhibitors and these studies are planned – particularly since with existing interventions, limited and temporary curative success only has been obtained, and although symptom onset is delayed, disease is not prevented.

Myelination is incomplete at birth and human maturation occurs over at least a decade; but in the mouse, myelination peaks at 20 days after birth and continues throughout the lifetime. The twitcher mouse appears indistinguishable from its littermates at birth; abnormal twitching/tremor first becomes apparent at age 15 days, and a fulminant disease ensues with death by 40 days. Compression of the pathological course is a major stumbling block for developing new treatments and unravelling the exact mechanisms of disease.

We will develop a living Krabbe model in which disease can be induced and reversed at will in a tissue-specific manner at any time during the life of the animal by addition of dietary...
doxycycline. Technical advances in recent years have made the creation of such invaluable models possible, and we have recently generated a highly informative inducible transgenic mouse of Tay-Sachs disease, a counterpart of Krabbe disease with devastating neuronal effects. Our programme of work on Tay-Sachs and related disease has recently attracted substantive funding from the public sources (NIHR and Medical Research Council) to conduct a clinical trial of gene therapy in human patients.

Building on this initiative, we propose to create an inducible knock-in mouse model of Krabbe disease to: (1) increase the window of opportunity for evaluating powerful therapies – including RIP 3 kinase inhibitors and gene transfer – not fully studied; (2) in model human early (infantile) and late onset (juvenile and adult) forms of disease; and (3) study how the disease progresses using sophisticated means for molecular and chemical analysis at our disposal.

Specifically, the project will provide 3-3.5y training in gene therapy, enzymatic/protein complementation as well as pharmaceutical drug development, with a strong view to clinical application and meeting the key requirement to use molecular cell biology definitively to identify the mechanisms of cell death and demyelination in Krabbe disease.

References


**Related Grants**


MRC Stratified Medicine Consortium award Predictive measurements to stratify clinical outcomes in children and adults with Gaucher disease and responses to specific therapies (GAUCHERITE consortium: TM Cox Lead applicant . £3,740,000. 1/10/2013-30/9/2017