

‡M02

Impact of mitochondrial mutations on the metabolite-dependent epigenetic profile of human induced pluripotent stem cell derived myotubes

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Background: There is accumulating evidence that cell metabolism and the maintenance of cellular identity are intrinsically linked. A number of chromatin modifying enzymes are reliant on intermediate products of metabolism and constitute a mechanistic focal point linking a cells metabolic state with transcriptional profile. Mitochondrial disease mutations might impact the epigenetic profile through alterations in the cellular levels of intermediate metabolites and represent a pathomechanism which contributes to the tissue specific dysfunction of mitochondrial disease patients.

Aims: We set out to investigate the effect that mitochondrial mutations causing Myoclonic Epilepsy with Ragged Red Fibre (MERRF) syndrome and Mitochondrial Encephalopathy Lactic Acidosis (MELAS) syndrome have on the epigenetic and metabolic profiles of disease relevant myogenic cell types.

Methods: Fibroblasts obtained from patients' biopsies harbouring the m.8344A>G MERRF and m.3243A>G MELAS mutations have been reprogrammed using non-integrating delivery methods. Human induced pluripotent stem cell (hiPSC) lines are being differentiated towards myotubes using defined factors which recapitulate the developmental stages of myogenesis. Live confocal imaging will be used to assess mitochondrial function and metabolism in differentiated myotubes and correlated with the histone modification profile obtained through chromatin immunoprecipitation sequencing.

Results: hiPSCs harbouring the m.8344A>G mutation are being successfully differentiated into myotubes alongside isogenic clones. Preliminary results will be presented.

Conclusions: These results will provide new insight into pathomechanisms of mitochondrial disease in muscle. Intermediate metabolites affected by mitochondrial mutations may show promise as a supplement based therapy for mitochondrial disease patients. Genes which show clear epigenetic alterations could also represent new targets for tissue specific treatment.

M03

Development of Cerebral Organoid cultures for the study of the neuronal pathomolecular mechanisms of Mitochondrial NeurogastroIntestinal Encephalomyopathy (MNGIE)

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Background: Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) is a rare metabolic disorder caused by a mutation in the thymidine phosphorylase (TP) gene, leading to secondary aberrations to the mitochondrial genome. The disease is clinically characterised by neurological and gastrointestinal dysfunctions, including neuropathy and leukoencephalopathy.

Aims: The understanding of molecular mechanisms of MNGIE affecting the nervous system is hindered by the lack of a representative model. We are developing *in vitro* 3-D cerebral organoids as a neuronal model for MNGIE.

Methods: Patient-derived somatic cells were reprogrammed to generate *bona fide* induced pluripotent stem cell (iPSC) lines. Pluripotent cells were differentiated into neuroepithelium, embedded in Matrigel and cultured in retinoic acid within bioreactors to generate CNS organoids. TP activity of organoids was monitored by weekly UPLC analysis of spent culture medium and intact organoids were sampled for morphological evaluations and for RNA extraction.

Results: The iPSCs were positive to the immunofluorescence detection of pluripotency markers TRA-1-60 and SOX2. Germ layer differentiation assay indicated that iPSCs were capable of differentiating into Endoderm, Mesoderm and Ectoderm tissues. Karyotyping indicated no chromosomal abnormalities

in generated lines. iPSCs successfully generated cerebral organoids displaying presence of neuromelanin pigments and cystic structures. Levels of thymidine quantified by UPLC, showed no significant changes in concentration for MNGIE whereas in Control organoids, levels of thymidine decreased over weeks, indicating that thymidine phosphorylase is active.

Conclusions: We have established a neuronal organoid model of MNGIE which will be employed in whole genome expression profiling to investigate differential expression between MNGIE and Control and gene editing for the generation of a rescue phenotype.

M04

A feasibility study of bezafibrate in mitochondrial myopathy

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Background: Mitochondrial disorders are genetically determined metabolic diseases arising due to biochemical deficiency of the respiratory chain. Current treatment focuses on alleviating symptoms and there is therefore an unmet need for therapies that modify the biochemical deficit and disease trajectory. Improving deficient OXPHOS pathways through induction of mitochondrial biogenesis is a potential approach.

Peroxisome proliferator activated receptor- γ coactivator-1 α (PGC-1 α) is a pivotal transcriptional co-factor widely considered the 'master regulator' of mitochondrial biogenesis. Whilst several pharmacological agents can influence PGC-1 α expression, bezafibrate (a pan-PPAR agonist) is already licensed for hypertriglyceridaemia, and has shown promise in pre-clinical animal studies of mitochondrial myopathy. We therefore wanted to assess the effects in patients.

Aims: This study aims to ascertain proof of concept data and determine the safety profile of bezafibrate in individuals with mitochondrial myopathy.

Methods: We designed an open-label, non-randomised phase IIb feasibility study open to participants with the m.3243A>G mutation with clinical features of myopathy.

Participants take bezafibrate 200 mg TDS for 6 weeks followed by 400 mg TDS for a further 6 weeks. Participants are reviewed weekly to enable clinical blood testing for safety purposes and adverse event capture.

To assess the effects of bezafibrate on mitochondrial biogenesis, participants undergo muscle biopsy at weeks 0 and 12. In addition, non-invasive measures of mitochondrial function (skeletal muscle ³¹P-MRS; cardiac muscle ³¹P-MRS; cine-gated cardiac MRI, and sub-maximal exercise test) are also performed. Clinical outcome measures (NMDAS, TUG, FIS, NMQ, IPAQ) are undertaken at weeks 0, 6 and 12, alongside objective activity monitoring to control for the known effects of exercise on mitochondrial biogenesis.

Results: Five participants have completed the protocol and 1 participant will complete in January 2017. Preliminary safety results demonstrate participants have tolerated bezafibrate, including at higher doses. There have been no episodes of rhabdomyolysis. 4 participants have experienced hypoglycaemia. Efficacy results are awaited.

Conclusions: Bezafibrate, in standard and higher doses has been tolerated by participants. The analysis of biopsy, MRI and exercise endpoints is expected in 2017.

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