

Kathryn Wright, PhD student researcher at the Wolfson Centre for Inherited Neuromuscular Disease reported on her work.

McArdle Disease (Glycogen Storage Disease V) is a rare inherited metabolic muscle disease caused by mutations in the Muscle Glycogen Phosphorylase (MGP) gene. MGP (myophosphorylase) is a muscle-specific enzyme needed for glycogen metabolism.

The most common mutations in Caucasian patients are the early stop codon R50X (incidence around 80%) and missense G205S (incidence around 10%) mutations. Existing animal (cow and sheep) models do not have the same mutations as humans and there are few existing cell culture models of McArdle's Disease. Patient biopsies have limited availability and can only be grown for a short period of time. Muscle biopsies must be innervated (be incubated with a nerve dissected out of a freshly sacrificed rat) to induce expression of MGP or have multiple additional copies of the MGP gene added to the muscle cultures using an adenovirus.

In order to study therapeutic approaches such as correction or read-through of these mutations, we have made cell culture models of McArdle Disease using in-vitro site-directed mutagenesis to introduce the R50X and G205S mutations into full length wildtype mouse MGP cDNA in the mammalian expression vector pCIneo. Green fluorescent protein (GFP)-R50X and GFP-G205S mutant MGP constructs were also made to assess the stability of the mRNA and production of protein. Stable cell lines were created by transfection into CHO-K1 cells, which does not express endogenous MGP.

Expression of the MGP protein was detected immunologically in cells with wildtype MGP but not in cells with R50X or G205S mutant MGP or GFP R50X or G205S mutant MGP fusion constructs

RT-PCR has shown that MGP mRNA is being transcribed by cells transfected with mutant R50X and G205S, and with GFP-R50X and GFP-G205S MGP. Work is in progress to quantify levels of mRNA expression. mRNA and MGP protein expression from transfected clones are currently being characterized and investigated to establish a baseline prior to investigating treatments to stabilize mRNA and increase protein expression.

Although sheep with naturally-occurring McArdle disease do not have the same mutations as found in humans, it results in an absence of MGP in the muscle cells. We plan to investigate the use of McArdle sheep muscle cells as another model system to examine mRNA and protein expression since they may be easier to differentiate than human cells. We also hope to be involved in analysing some of the results of a drug trial currently underway in the McArdle sheep to increase expression of an alternative isoform (the brain isoform) of Glycogen Phosphorylase to replace the absent MGP in muscle cells.

In summary, these cell models may be useful in better understanding the cellular mechanisms of McArdle disease and in identifying drugs or other treatments that might enable functional MGP production from the mutant forms of the gene.