

SUMMARY OF FINAL REPORT

Epigenetic modifiers as potential disease modifying factors in Friedreich's Ataxia.

Principal researcher/s: Professor Richard Festenstein, Imperial College London

October 2004 – August 2008

Background and aims:

Friedreich's ataxia (FA) is caused by an abnormality in a gene called frataxin that inappropriately switches off the gene. The purpose of this project was to investigate the way in which the gene is switched off in blood cells taken from people with FA.

Our previous work has shown that the abnormal gene might be switched off in a way that is very similar to a gene-switching defect that has been extensively studied in fruit flies. This project, which formed a PhD studentship, investigated whether the type of switching defect seen in fruit flies also occurs in people with FA and whether knowledge from fruit fly studies can be used to turn the gene back on again in people with FA.

Results:

In FA, the *frataxin* gene contains an abnormal expansion of a GAA-repeat which results in low levels of *frataxin* mRNA.

We have used chromatin immunoprecipitation (ChIP) to demonstrate the presence of an RNA Pol II transcriptional pausing site in the *frataxin* gene. This pausing site is found in people with FA as well as unaffected individuals and may be a rate-limiting step for frataxin regulation. Consequently, it may provide a way to modify gene expression in FRDA.

One type of compound that is known to alleviate repression of the mutated *frataxin* gene is HDAC inhibitors. We treated normal cells with a class III HDAC inhibitor and investigated the effects of this on negative elongation factor, NELF-E, which is known to mediate transcriptional pausing. We found that in normal cells treated with the class III HDAC inhibitor, NELF-E levels were increased. There was also a significant increase in H4K16ac, an established mark for open ('readable') chromatin, in the treated cells.

In cell lines established from people with FA, increased NELF-E binding was seen before as well as after treatment with the HDAC inhibitor, compared to controls. Therefore, NELF-E-mediated RNA Pol II pausing might contribute to the repression

of the mutated *frataxin* gene, not only by blocking transcription but also by inducing pausing of RNA Pol II in the presence of an abnormal GAA-repeat expansion. This new finding could explain the relatively minor effects of different drug approaches to up-regulate this gene, as these approaches may not address the increased pausing in *frataxin* gene transcription.

DNA methylation (ie the number of methyl groups attached to the DNA) can alter gene expression and this may be mediated by other proteins, for example CCCTC-binding factor (CTCF). CTCF is a protein that binds to specific unmethylated regions of DNA and our investigations showed that this protein is present at the RNA Pol II pausing site on the *frataxin* gene. Another protein that is found at this site is the histone demethylase LSD1, which removes methyl groups from DNA. Our results indicate that LSD1 removes methyl groups from DNA at the pausing site and also from the abnormal GAA repeat expansions that are seen in people with FA. Therefore, LSD1 may be involved in maintaining DNA in a more open, 'readable' form in genes that need to be expressed. Finally, we used a technique called primary transcript RNA-FISH, which allows the visualisation and measurement of RNA transcription in cells, to confirm that there is a dramatic reduction in RNA Pol II-mediated transcription of the *frataxin* gene in the presence of long GAA repeats.

In summary, this project has investigated the mechanisms underlying decreased gene expression of the *frataxin* gene in FA. We identified and characterised a transcriptional pausing site in the *frataxin* gene. This pausing site is likely to repress *frataxin* expression and promote the progression of FA in the case of over stimulation and GAA repeat expansion.

Lay summary of the results:

We have found that the *frataxin* gene is abnormally switched off in FA. The purpose of this project was to further investigate the nature of this 'switch' and this was done using cells taken from people with FA.

Normally, special cellular machinery 'reads' the gene and turns it into a message to the cell to make frataxin protein. We have found that this machinery is abnormally paused on the mutated *frataxin* gene and have made advances towards understanding the nature of this pausing. We have also identified compounds that could potentially switch the gene back on again.

This has opened up the possibility of potential new therapeutic avenues and has triggered related research internationally. Our in vivo studies have also led to further studies which are now underway to determine the whether these gene switching factors are able to work in the cells that are affected in Friedreich's ataxia.

Benefits to people with ataxia arisen/likely to arise from this research:

Work is currently ongoing in a number of labs to look at the ability of HDAC class III inhibitors and other drugs to upregulate frataxin in cells and mice. We hope that some of these compounds will be eventually developed into treatments for FA. During the period of this project we have been actively involved in a European wide initiative to perform controlled trials in this disease.

Publications arisen from this project:

GAA-repeats modulate epigenotype in Friedreich's ataxia (working title) Chapman-Rothe N Mayan Santos MD; Torres Torres R; Yandim C; Pombo A and Festenstein R (MS under preparation, *Nature Genetics*, 2009)

Conferences/ meetings where this research has been presented:

London Chromatin Club Meeting, 2007

FASI – International Conference on Friedreich's Ataxia Dublin, 2008

Epigenome Network of Excellence Meeting, Madrid, 2008

HGBS Symposium on Epigenetics and Disease, Helsinki, 2009

If the grant awarded funded a PhD studentship, has the student obtained their PhD?
If not please give details of current status.

For more support or information please contact: Ataxia UK, Lincoln House, Kennington Park, 1 – 3 Brixton Road. London SW9 6DE

Website: www.ataxia.org.uk.

Helpline: 0845 644 0606 **Tel:** +44 (0)20 7582 1444 **Fax:** +44 (0)20 7582 9444

Email: helpline@ataxia.org.uk