

## SUMMARY OF FINAL REPORT

### **Restoration of *Frataxin* gene expression in Friedreich's ataxia - identification and characterisation of novel epigenetic therapies**

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#### **Background and aims:**

Friedreich's ataxia (FRDA), the most common autosomal recessive ataxia, is the result of a GAA repeat expansion in the *frataxin* gene which causes its repression. This repeat induces gene silencing which resembles the archetypal epigenetic phenomenon of Position Effect Variegation, caused by heterochromatin formation. Importantly, this silencing can be modulated *in vivo* by chromatin modifiers providing a potential radical therapeutic approach for this frequently devastating and largely untreatable condition (Saveliev *et al.*, 2003). We have now confirmed that a similar form of silencing occurs in cells from FRDA patients. As a result of our studies we and others have identified histone deacetylase (HDAC) inhibitors which can overcome such silencing and are potential therapeutic agents (Festenstein, 2006; Herman *et al.*, 2006).

The overall aim of the project was to identify pathways whereby we could overcome the aberrant silencing of the *frataxin* gene in patients with a view to developing a radical therapy for this currently incurable condition. We took a candidate approach to screen for modifiers of frataxin expression in EBV transformed cell lines, primary cells and mice.

In a previous Ataxia UK-funded project (entitled 'Epigenetic modifiers as potential disease modifying factors in Friedreich's ataxia') we had shown that the GAA-repeat expansion could induce chromatin modifications consistent with heterochromatinisation *in vivo*. In addition, we identified a novel RNA polymerase II stalling site within the *frataxin* gene locus. The stalled PolII was shown to be more sensitive to proteasomal degradation in cells from people with FRDA than healthy controls. This was a preliminary finding in a limited number of cell lines and has been extended during the course of this grant to examine the effect on primary cells and to investigate the relationship between heterochromatinisation and this effect.

#### **Results:**

**Inhibition of proteasome prevents PolII degradation and upregulates Frataxin expression in FRDA**

RNApolIII was found to be stalled in the *frataxin* gene and was more sensitive to proteasomal degradation in cells from people with FRDA than controls. Inhibiting the proteasome using proteasome inhibitor PS341 restored RNA PolIII levels at the stalling site and this correlated strongly with the ability of PS341 to upregulate frataxin in these cell lines.

Inhibition of the proteasome with proteasome inhibitor MG132 also restored RNAPolIII levels at the stalling site in cells from people with FRDA and this strongly correlated with an increase in frataxin expression.

### **HDAC inhibitors upregulate Frataxin expression**

Our studies have also revealed a classical class III HDAC inhibitor that can upregulate frataxin levels in primary cells from patients. We have shown that other class III HDAC inhibitors can also perform a similar function.

Treatment of mice with the classical HDAC inhibitor upregulated human frataxin in the cerebella of a mouse model of FRDA; frataxin mRNA levels and protein expression were both increased (determined by Q-RT-PCR and Mitosciences dipstick assay respectively).

In parallel we have examined the effect on resting peripheral blood lymphocytes from people with FRDA and shown upregulation of frataxin. In these cells we have monitored the effects of this HDAC inhibitor on the chromatin structure of the frataxin locus using ChIP and the results showed a decrease in heterochromatin histone modifications.

### **Summary**

Taken together our results have identified both class III HDAC inhibitors and proteasome inhibitors as potential therapies for FRDA and seeded an Ataxia UK-funded proof-of-concept study in humans to determine whether a classical ClassIII HDAC inhibitor can upregulate frataxin in people with FRDA (entitled, 'Pharmacodynamic studies of a histone deacetylase inhibitor in Friedreich's ataxia'). A study to treat mice for long periods with class III HDAC inhibitors will also be undertaken to determine their efficacy in a mouse model of FRDA. In addition, a European initiative pulling together basic science and clinical groups has recently been funded by the EU to provide a platform to translate these findings for clinical benefit (EFACTS).

### **Lay summary of the results:**

Friedreich's ataxia (FRDA) is caused by an abnormality in a gene called *frataxin* which inappropriately switches off the gene. We have examined the way this gene is switched off in blood cells obtained from people with FRDA. Our results reveal two new classes of drugs, HDAC inhibitors and proteasome inhibitors, that can restore frataxin expression in patients' cells (switch the gene back on again). This reveals important details about the way the gene is abnormally regulated in people with FRDA and may lead to more therapeutic options aimed at restoring frataxin expression. Since FA is essentially a deficiency disease, switching the *frataxin* gene back on promises to be a radical new approach for this currently incurable condition. Our findings have led to further funding for a proof-of-concept study in humans to determine whether the HDAC inhibitor can increase frataxin levels in people with

FRDA (also funded by Ataxia UK; project entitled, 'Pharmacodynamic studies of a histone deacetylase inhibitor in Friedreich's ataxia'). If this is the case further studies will be needed to test its clinical efficacy.

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

Friedreich's ataxia is the commonest autosomal recessive ataxia causing significant morbidity and premature mortality. There is at present no effective treatment for this disease. If successful our research will provide a new treatment for this disease in the future. Such treatments may also be effective in other diseases caused by inappropriate gene silencing. This project has taken us significantly further in that our findings have led to a proof-of-concept study in humans with FRDA.

**Publications arisen from this project:**

Manuscripts in preparation

**Conferences/ meetings where this research has been presented:**

Epigenome Network of Excellence (FP6) meeting, Madrid - 2008

FARA-Philadelphia – 2009

EFACTS (FP7) –Kick-off meeting – Brussels - 2010

Epigenetics and Disease Meeting, Vienna - 2010

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