

Research into Friedreich's Ataxia

EXPANDING OUR KNOWLEDGE

Since the discovery of the genetic defect that causes Friedreich's ataxia (**FRDA** or **FA**) in 1996, much research has been focused on how this manifests as a neurological disorder.

The disorder results from an abnormal expansion of the FA gene (Campuzano V et al 1996). Normally, the gene may be expanded up to ~33 repeats (GAA trinucleotide repeats), but when the sequence is longer than 59 repeats it appears to alter the architecture of the DNA sequence by causing the usual double-helix structure to fold back on itself into a triplex formation ('sticky DNA') (Sakamoto et al 1999). This interferes with **transcription** of the gene, resulting in a deficiency of the protein encoded in that gene, in this case named **frataxin** (Bidichandani SI, et al 1998). Increasing length of the repeat expansion correlates with greater reduction in levels of frataxin and increased severity of the disorder (Campuzano et al 1997).

Research looking at equivalents of frataxin in other organisms has shown that a decrease in frataxin is associated with a reduction in energy production in the **mitochondria**, an accumulation of free iron, and an increase in susceptibility to oxidative stress from **free radicals** (Wong A et al 1999).

We know that whatever it's precise function is in the body, frataxin protein is essential for survival. Cosseé et al demonstrated that when mouse models were generated by completely deleting the frataxin gene and therefore preventing any frataxin protein being produced, the mice died whilst in the embryonic stages (Cosseé M et al 2000). A paper published in 2007 showed that removing frataxin from a *C.Elegans* (roundworm) model of FA reduces oxygen consumption and respiration in the worm and shortened lifespan (Zarse et al 2007).

In the last decade, researchers have delved further into the function of human frataxin, suggesting lots of possible roles for the protein, and increasing our knowledge of the protein itself; for instance we now know that it is a 210-**amino acid** protein (Musco et al 2000), which is found in the mitochondria, the energy producing units or 'batteries' inside cells. Highest levels of this protein are found in the heart, liver, skeletal muscle, pancreas, brown fat (present in newborn babies and very rich in mitochondria) and in the nervous system predominately in the dorsal root ganglia (Campuzano et al, 1996), structures containing groups of cell bodies of the sensory nerves that relay information along the spinal cord to the brain.

The main theories at present are that frataxin acts as a chaperone for iron, delivering it for the assembly of enzymes containing iron-sulphur clusters, and for heme synthesis, while also having an excess iron detoxification function which helps to protect cells from oxidative stress (Stehling et al 2004; Gakh et al 2005).

The creation of **animal models** of FA helps scientists to learn about the effects of frataxin and is crucial for testing potential treatments. A number of animal models of FA exist, each having specific uses. Simple models such as yeast and the *C.Elegans* worm have provided much valuable information about how genes work and because of their relative simplicity and short life cycles enable scientists to study disease processes much more quickly than would be possible in humans. Now there are also more advanced mouse models. The first mouse models created have one specific organ completely without frataxin, for example the heart, producing only the cardiac symptoms associated with FA or in other models only the neurological problems (Puccio 2001). A more 'realistic' mouse model which produces mice with low levels of frataxin throughout the body and symptoms more similar to that seen in the human condition has now been produced by Dr Mark Pook and his team at Brunel University, with funding from Ataxia UK (Al-Mahdawi et al 2006).

RESEARCH INTO TREATMENTS

ANTIOXIDANTS

Oxidative stress is thought to be central to the disease mechanism in FA and thus ways to reduce this stress are being investigated. FA cells are more susceptible to oxidative damage due to several reasons:

- the abnormal use of iron leads to changes in metabolism and increased production of harmful free radicals (via the Fenton reaction), (Radisky et al 1999; Koenig and Mandel 1997)
- because cells which are deficient in frataxin appear to be less efficient at generating natural antioxidant defences (Chantrel-Groussard et al 2001)

Antioxidants are molecules which help 'mop up' free radicals and prevent other molecules undergoing damaging oxidative reactions. Antioxidants come in many different types (e.g. Glutathione, vitamin C, vitamin E, beta-carotene, Coenzyme Q10) and may be important in many diseases, as well as being used in many dietary supplements and anti-ageing beauty treatments.

Preliminary results from a trial of **vitamin E** and **Coenzyme Q10 (CoQ10)** show some stabilisation of the progression of ataxic symptoms when the therapy was taken over 47 months (Hart et al 2005). In this trial, funded by Ataxia UK, 10 adults received high doses of vitamin E and CoQ10 (2,100 IU/day, 400mg/day respectively). At the end of the study period testing showed that energy production in heart and skeletal muscle had improved and in 8 out of the 10 patients their clinical symptoms had remained stable or even improved. The researchers planned to go on to conduct a larger trial with 50 patients and results of this are eagerly awaited.

Idebenone is a variant of CoQ10 and is a powerful synthetic antioxidant. The first major studies on idebenone for FA were carried out by Pierre Rustin's research team (Rustin P et al 1999) and showed that when given to patients with FA and hypertrophic cardiomyopathy, idebenone could decrease the abnormalities in the heart muscle. In 2004 a group of researchers demonstrated the effects of administering idebenone to mice which had been engineered to be deficient in frataxin in their heart and muscle cells. These mouse models displayed an inactivation of mitochondrial iron-sulphur cluster proteins and an accumulation of iron in the mitochondria, as seen in human patients, but developed a much more severe disease than humans with FA with a rapidly progressing, lethal **cardiomyopathy**. Administration of idebenone delayed the progression of the disorder in the mice and delayed death by 1 week, suggesting that idebenone can be cardioprotective even when there is a complete lack of frataxin, but cannot overcome the primary deficiency of iron-sulphur proteins (Seznec et al 2004).

Small, **open-label** trials in various countries have since tested idebenone in patients with no serious side effects detected (Atuch et al 2002; Buyse et al 2003; Di Prospero et al 2007; Hausse et al 2002; Ribai et al 2007; Rustin et al 2002). In the case of cardiomyopathy in FA, there have been small scale studies completed thus far that show modest benefits from idebenone therapy (Hart et al 2005; Mariotti et al 2003; Rustin et al 2002; Artuch et al 2002; Buyse et al 2003; Hausse AO et al 2002).

A large, multicentre, **phase III trial** began at the end of 2007, sponsored by the pharmaceutical company Santhera. The researchers will be testing the safety and tolerability of idebenone at doses ranging from 450-2250mg/day, and its efficacy at treating the neurological symptoms of FA. For information on the worldwide trials visit <http://www.clinicaltrials.gov> or for more on the trial in the UK visit our [taking part](#) section of the website.

Another antioxidant that has been put forward for testing in FA is **mitoquinone (MitoQ)**, a compound which is selectively targeted to concentrate antioxidant action in the mitochondria. New Zealand's Antipodean pharmaceutical company is developing the compound to take it to phase II trials in patients with FA, whilst currently investigating the treatment in Parkinson's disease. Meanwhile, a compound called **EPI-A0001** has reportedly been shown to improve mitochondrial energy production and reduce oxidative stress in yeast cells and the Friedreich's Ataxia Research Alliance (FARA) report that the compound has been accepted onto the National Institute of Health's 'fast-track' program to advance into early clinical trials by 2008/2009. For more information on this research see the FARA website (www.curefa.org.)

The antioxidant 5-carboxy-1,1,3,3-tetramethylisoindolin-2-yloxy (**CTMIO**) is being tested by Dr Nuri Gueven and a team of Australian researchers, with assistance from Dr Mark Pook's team in the UK for testing in animal models. The researchers

previously tested this compound in mice models of ataxia telangiectasia, an inherited condition that shares some similar symptoms with FA, such as loss of balance and slurred speech, but also causes some very different symptoms such as weakening of the immune system and a high incidence of cancers. They found that CTMIO slowed the progression of tumour formation and reduced damage to nerve cells, causing them to hypothesise that the antioxidant might be useful for other neurodegenerative conditions (Gueven N et al 2006).

Because of the abnormal accumulation of iron seen in FA, another potential approach to treatment is **iron-chelating therapy**- using compounds which bind to iron and remove it from the mitochondria. The main challenge in this approach is to find compounds which prevent iron building up to excessive levels and increasing free radical formation and oxidative stress, whilst not removing iron from other parts of the body where it has an essential role (e.g. the blood). The iron-chelating agent **Deferiprone** has received some attention lately, since in 2007 researchers in Paris published results of a six-month study conducted in 9 adolescent patients. Their results showed that treatment with deferiprone reduced iron accumulation seen with MRI scanning in certain parts of their brain, and in the youngest patients improved their neurological symptoms (Boddaert N 2007).

There are some concerns about iron-chelating therapy, namely that iron removing agents could have toxic effects in people with FA who have iron accumulation in specific places instead of generalised iron overload, and that they may cause increased abnormalities in the iron-sulphur cluster proteins which are already deficient in FA.

A much larger trial is now being instigated at research centres across Europe, including the UK, and the USA to test the safety and tolerability of deferiprone for people with FA. For more information see the trial information page on www.clinicaltrials.gov

In April 2008 a study was published looking at a new iron-chelating agent called PCTH (2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone) (Lim et al 2008). In experiments on cells from patients with FA, researchers found that PCTH more rapidly penetrated cells inducing faster iron removal, and was more effective at protecting cells from free radical toxicity than other iron chelators and more effective even than existing antioxidants. The researchers hypothesised that the rate at which the iron-chelating agent penetrates the cell is the important factor in their effectiveness at protecting cells; therefore PCTH is posed as a potential treatment for iron-overloading conditions such as FA.

INCREASING FRATAXIN

Since we now know that FA causes a deficiency of frataxin, an essential protein for life, some researchers are looking at ways to directly stimulate the production of frataxin in FA cells.

On these lines, encouraging research published by Pianese et al in 2004 showed that people with FA had residual frataxin levels of between 13-30%, whilst asymptomatic carriers of the gene (people with one normal gene and one affected gene who have no symptoms, as opposed to people with the condition FA who have two affected genes) also had reduced levels at around 40% of normal. This suggests that any therapy aimed at increasing the levels of frataxin may only need to increase levels to 40% to prevent symptoms.

In Austria, trials have been carried out using **rhuEPO (recombinant human erythropoietin)**, a treatment known for its use in stimulating blood cell production in patients undergoing dialysis for kidney failure (Boesch S et al 2007). Its potential benefit for Friedreich's ataxia was recognised when patients receiving it during dialysis experienced unexpected **neuroprotective** and **cardioprotective** effects. It is still not properly understood how rhuEPO exerts this effect, but further research has revealed that rhuEPO injections appear to increase expression of frataxin protein in cells (including neurons) from FA patients and up-regulation of frataxin in 70% of patients in the trial who were given rhEPO for 8 weeks. The treatment also appeared to reduce DNA damage and oxidative stress, and the trial has been extended to investigate the effects over a longer period of time. A trial is now underway in Naples, Italy (see www.clinicaltrials.gov) to further assess the effect of rhu-EPO on frataxin expression in patients.

At the Euroataxia conference in November 2007, it was announced that a 2 year clinical trial looking at **pioglitazone** for FA is starting in France (Husson I, see details on www.orpha.net). Pioglitazone is a drug used as a treatment for diabetes, and is being tested for other neurological disorders (such as spinal injuries and MS) as well as for FA. A case study of an MS patient receiving pioglitazone had promising results as they noticed an improvement in coordination and strength, and improved memory, although there were no changes on the MRI changes which are seen in MS suggesting it was not correcting the pathology.

For FA, the potential beneficial actions are thought to be a combination of inducing proteins involved in energy production, conferring neuroprotection, an antioxidant action and increasing the stability of iron-sulphur clusters.

An alternative way to increase frataxin levels would be to deliver a version of the deficient protein straight to affected cells. Whilst this technique is still in the early stages of research, researchers in the USA are hoping to **synthesise frataxin** and target it to the mitochondria in cell models of FA. One of the researchers involved in this work will be attending Ataxia UK's Scientific Conference in autumn 2008 to provide an update on this work.

GENETIC APPROACHES

Since the gene causing FA has been found, it is apparent that an ideal therapy for the condition would be to replace or remove the underlying genetic defect. If the

abnormal GAA repeat expansion could be removed or shortened by a sort of genetic surgery, or a normal copy of the gene inserted, it may allow the gene may be read as normal and frataxin production to carry on uninterrupted.

In this way, it is thought to eventually be possible to 'infect' cells with bacteria or viruses containing DNA or RNA coding for frataxin. Scientists at the Children's Medical Research Institute in Sydney, Australia showed that it was possible to create viruses containing human frataxin DNA which would increase frataxin expression in cells from FA patients and make cells more resistant to oxidative stress (Fleming J et al 2005). In 2007, researchers then published a study showing that mouse models who have been modified to be deficient in frataxin, appear to recover from their symptoms after injection with Herpes Simplex virus type 1 (HSV-1) amplicon **vectors** expressing human frataxin complementary DNA (Lim F et al 2007). In more simple terms this means that modified viruses (such as Herpes Simplex) could be used as a carrier to infect cells with specific pieces of DNA (e.g. the frataxin gene) and cause them to express the protein encoded in that DNA (e.g. frataxin). In 2007 Ataxia UK awarded funding to a group of the researchers working in this area to further investigate the effects of using harmless versions of Herpes Simplex viruses to deliver a correct version of the frataxin gene to mice with FA. More information on this research can be found [here](#).

Another option, which has been investigated for Duchenne's Muscular Dystrophy, might be to use short pieces of DNA or RNA as a 'patch' to skip over the genetic defect (Arechavala-Gomez V 2007).

Another avenue of research which is being looked at is using drugs to directly target the structure surrounding the gene and this is described further below.

In FA the deficiency of the protein frataxin occurs because the gene responsible for producing the protein appears to be 'switched off'. A group of compounds called **Histone Deacetylase inhibitors (HDAC inhibitors)** are being talked about as a possible way of switching the gene back on. Research has demonstrated that the abnormal GAA repeat expansion in the FA gene led to the DNA becoming unusually packaged by dense heterochromatin structures, preventing the DNA from being read (Saveliev, A 2003).

The original theory was investigated by a team of researchers headed by Professor Festenstein in London, who have received funding from Ataxia UK (see [here](#) for their current projects receiving funding). researchers have been investigating whether substances which prevent the heterochromatin binding to DNA could reverse the silencing of the gene. In 2006 Joel Gottesfeld's team at the Scripps Institute in California published the results of a lab based study using cells from patients that showed HDAC inhibitors could reverse silencing of the frataxin gene (Herman et al 2006). In their experiments particular compounds from the HDAC inhibitor family were able to increase the level of frataxin up to normal levels in cells taken from FA carriers and up to 50% of normal in cells from people actually affected by FA, which may be enough of an increase to prevent symptoms.

The HDAC inhibitor group of compounds is now being looked at by several groups of researchers, including Professor Festenstein's, and Professor Massimo Pandolfo's group in Brussels, also funded by Ataxia UK, to try and identify potential treatments from this group that have the most favourable properties and to test them in animal models of Friedreich's ataxia. Very recently, favourable results have been produced with a HDAC inhibitor called compound 106; in mice with FA undergoing treatment with compound 106, frataxin levels were restored to normal levels, with no apparent toxic effects (Rai et al 2008). This suggests a possibility that these compounds could correct the pathological process underlying FA.

A pharmaceutical company called Repligen has become involved with these compounds, forming an exclusive commercial licensing agreement with the Scripps Institute. Repligen's input will be important for funding the necessary animal trials and safety tests which are the first stage in bringing a new drug to human trials and ultimately getting approval from necessary regulatory bodies to enable drugs to become available to patients. Meanwhile researchers are continuing to investigate other compounds in this class to find the most effective treatments.

Researchers in Italy have recently begun tests to assess the efficacy of HDAC inhibitors and rhuEPO in combination and individually, in cell models of FA. It is thought that the up-regulation of frataxin protein observed with these two drugs may be complementary because they act at different stages in the production of the protein (Cocozza 2008)

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