

SUMMARY OF FINAL REPORT

Development of an improved GAA repeat expansion mutation-based mouse model of Friedreich ataxia for therapeutic testing.

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Background and aims:

We previously established a GAA repeat expansion mutation-based FRDA transgenic mouse model, which exhibits a representative FRDA-like phenotype, including similar DNA methylation and histone modification epigenetic changes. This model is a good system in which to test novel therapies, but the FRDA-like phenotype is currently rather mild, with no overt ataxia and no reduction in life span. Therefore, the aim of this project was to develop an improved model by increasing the number of mutational GAA repeat sequences and hence increasing severity of the symptoms. This was done by performing genetic crosses of our existing FRDA mouse model with (a) different inbred strains of mice, and (b) genetically modified mice that are deficient for the DNA methyltransferase 1 gene (*Dnmt1*).

This new mouse model was then used for translational therapeutic research. Primary fibroblasts and neuronal cells from the new FRDA mouse model, human FRDA primary fibroblasts, and the FRDA mouse model itself were all used to test the effects of a number of potential frataxin-increasing compounds, including the class III histone deacetylase (SIRT1) inhibitor, splitomicin, the histone methylation reducing drug, mithramycin, the DNA methylation inhibitor, zebularine, and GAA-interacting compounds, pentamidine and DB221. The data from these preclinical studies provides valuable information for future FRDA clinical studies.

Results:

Aim 1: To develop an improved GAA repeat expansion-based FRDA mouse model.

The first aim of the project was to increase the severity of the phenotype of YG8 and YG22 *FXN* transgenic mice by increasing the size of the GAA repeat expansion mutation. This was done by breeding experiments. YG8 and YG22 mice (C57BL6/J genetic background) with the largest of the lower GAA bands within any mouse in the colony were bred to produce offspring that carry a higher number of GAA repeats than any other mice within the colony. This was then repeated to produce further offspring with increased numbers of GAA repeats (originally, YG8 mice had 90-190 repeats and YG22 mice had 190 repeats).

To try and effect comparatively large jumps in the size of GAA repeats from parent to offspring, the GAA repeat expansion-containing *FXN* transgenic mice were bred with mice from different genetic backgrounds (129, CBA, FVB) and also with genetically modified mice (*Dnmt1* heterozygous knockout mice). The YG22 mice were chosen for these further breeding studies because they have a higher number of GAA repeats.

Repeated breeding of YG8 mice over successive generations has enabled us to progressively increase the size of the lower GAA band in these mice (from 90 to 135 repeats). Repeated breeding of YG22 mice with 129 and FVB mice did not produce any obvious further increase in GAA repeat size. However, breeding of YG22 (C57BL6/J) mice with two rounds of CBA mice produced offspring with the largest GAA repeats thus far identified (230-260).

Breeding of YG22 mice with *Dnmt1* heterozygous knockout mice gave double-genetically modified mice with GAA repeat instability, with a preference towards expansions. Similar studies with *Pms2* knockout mice have shown that there is also a bias towards GAA repeat expansions in the offspring of YG8 or YG22 *Pms2*^{+/-} or *Pms2*^{-/-} parental mice (Ezzatizadeh et al 2012). Therefore, further breeding with both *Dnmt1* and *Pms2* genetically modified mice will be carried out in our continued efforts to establish colonies of larger GAA repeat expansion FRDA mice.

The different types of FRDA mice have been crossbred with heterozygous *Fxn* knockout mice to produce „YG8R“ and „YG22R“ (R = rescue) mice for the establishment of fibroblast and neural stem cell cultures, molecular and functional characterisation and subsequent therapeutic testing. *FXN* mRNA expression levels in cerebellar tissue of YG22R mice showed that levels are approximately 70% those of YG8R. However, YG8 mice (primarily the older female mice) showed a more severe phenotype in initial functional tests (including rotarod analysis and beam-breaker locomotor tests) (Sandi et al 2011). As a control line, we are currently also breeding the Y47 transgenic mice, which contain the same YAC *FXN* transgene, but with a normal number of nine GAA repeats (Pook et al 2001). In preparation for further phenotypic characterisation and long-term therapeutic studies, further groups of YG22R, YG8R and Y47R mice have now been established.

Aim 2: To test the effects of potential frataxin-increasing therapies.

Cultured mouse fibroblast and neuronal stem cell (NSC) lines have been established from many different types of mice (wild-type (WT), Y47 rescues (Y47R), YG8 rescues (YG8R), YG8 transgenics (YG8Tg), YG22 rescues (YG22R) and YG22 transgenics (YG22R)). These cells, along with human FRDA fibroblasts, have been used to measure the ability of potential therapeutic agents to increase frataxin expression. The class III histone deacetylase (SIRT1) inhibitors, splitomicin and sirtinol increased frataxin by up to 1.2X and 1.7X respectively. The DNA methylation inhibitors, zebularine and hydralazine, produced frataxin increases of up to 1.1X and 1.7X, respectively. The GAA-interacting compound pentamidine did not increase frataxin and showed a high level of cell toxicity. Whereas, the novel GAA-interacting compound DB221 produced frataxin increases of up to 3X and with little toxicity at low concentration (1uM), but less frataxin increase and some cell toxicity at higher concentration (5uM).

Short-term (4-5 days) therapeutic drug studies:

YG8R and YG22R FRDA mice were used and *FXN* mRNA and frataxin protein levels were measured in the cerebellum, brain, spinal cord, DRG and non-CNS tissues. The novel GAA-interacting compound DB221 did not significantly increase *FXN* expression in either brain or liver tissue. The histone deacetylase (HDAC) inhibitor, nicotinamide (NAM), similarly did not significantly increase *FXN* expression in either cerebellum or spinal cord, but YG22R mice are undergoing long-term NAM treatment as part of the European-funded EFACTS study.

Long-term (up to five months) therapeutic drug studies:

A five month treatment of YG8R mice with the Repligen HDAC inhibitors, 106, 136 and 109, produced amelioration of the FRDA-like phenotype (Sandi et al 2011). No functional improvements were seen in YG22R FRDA mice treated with a sirtuin for three months, or a general neuroprotective compound for five months. However, preliminary studies of the neuroprotective compound-treated FRDA mouse tissues have revealed a 1.6X increase in *FXN* expression levels in DRG, so further studies with this compound are now planned.

Recently, in collaboration with Dr. Roberto Testi (Rome), YG8R mice have been treated with another frataxin-increasing compound for a period of 14 weeks and significant improvements in rotarod and locomotor functional tests were seen, together with increased frataxin expression in DRG and amelioration of DRG neuropathology. These findings have been submitted for publication.

References:

Al-Mahdawi, S., Pinto, R.M., Ruddle, P., Carroll, C., Webster, Z., and Pook, M. (2004) GAA repeat instability in Friedreich ataxia YAC transgenic mice. *Genomics* 84: 301-10.

Ezzatizadeh, V., Mouro Pinto, R., Sandi, C., Sandi, M., Al-Mahdawi, S., te Riele, H. and Pook, M.A. (2012) The mismatch repair system protects against intergenerational GAA repeat instability in a Friedreich ataxia mouse model. *Neurobiol. Dis.* In Press

Pook, M.A., Al-Mahdawi, S., Carroll, C., Cossé, M., Puccio, H., Lawrence, L., Clark, P., Lowrie, M.B., Bradley, J.L., Cooper, J.M., Koenig, M. and Chamberlain, S. (2001) Rescue of the Friedreich's ataxia knockout mouse by human YAC transgenesis. *Neurogenetics* 3: 185-193.

Sandi, C., Pinto, R.M., Al-Mahdawi, S., Ezzatizadeh, V., Barnes, G., Jones, S., Rusche, J., Gottesfeld, J. and Pook, M. (2011) Prolonged treatment with pimelic *o*-aminobenzamide HDAC inhibitors ameliorates the disease phenotype of a Friedreich ataxia mouse model. *Neurobiol. Dis.* 42: 496-505

Lay summary of the results:

Friedreich ataxia (FRDA) is an inherited neurological disorder caused by both parents passing on a DNA mutation, known as a 'repeat expansion'. This leads to reduced levels of an important protein, frataxin, within cells. Although potential treatments of some later symptoms are now being investigated, it may be more effective to treat the early stages of the condition, producing an increase in frataxin protein. Within this project we aimed to obtain some indication of the effectiveness of potential FRDA therapies from studying cells that are cultured in the laboratory. However, ultimately this is an artificial situation that does not necessarily relate to how the therapy will work on a whole complex organism. Therefore, the use of a mouse model of FRDA to study potential therapies is considered essential.

We have recently established a good FRDA mouse model that is useful for therapeutic studies. However, the symptoms in this model are rather mild, so our aim was to develop an improved model that has more severe symptoms, thereby increasing the effectiveness of preclinical therapeutic studies.

We have now performed matings of the FRDA mice with other genetically modified mice and we have thereby produced novel types of FRDA mice that may be more useful for future drug studies. We are currently characterising different types of mice that we have produced to see which will be the most effective mice to use in future.

We have also established cultures of skin and nerve cells from the mice that can be grown artificially in the laboratory. These cells can be used to test potential new FRDA therapeutic compounds, and we have performed some such studies as initial screening procedures to identify hopeful therapeutic compounds, before testing on mice.

We have also tested our FRDA mice with several different compounds that have good potential for frataxin-increasing FRDA therapy. The results that we have obtained have been helpful when considering which drugs may be suitable for future clinical trials. Thus, in collaboration with Dr. Roberto Testi from Rome, we have identified another frataxin-increasing compound that should be studied further as a potential FRDA therapeutic compound.

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

These studies have provided important translational preclinical information regarding FRDA therapy. All FRDA families should benefit from these studies, which have led to the development of an improved GAA repeat-based FRDA mouse model that will provide very useful information for investigations of FRDA therapy.

Publications arisen from this project:

Sandi, C., Pinto, R.M., Al-Mahdawi, S., Ezzatizadeh, V., Barnes, G., Jones, S., Rusche, J., Gottesfeld, J. and Pook, M. (2011) Prolonged treatment with pimelic *o*-aminobenzamide HDAC inhibitors ameliorates the disease phenotype of a Friedreich ataxia mouse model. *Neurobiol. Dis.* 42: 496-505

Ezzatizadeh, V., Mouro Pinto, R., Sandi, C., Sandi, M., Al-Mahdawi, S., te Riele, H. and Pook, M.A. (2012) The mismatch repair system protects against intergenerational GAA repeat instability in a Friedreich ataxia mouse model. *Neurobiol. Dis.* In Press.

Conferences/ meetings where this research has been presented:

- Friedreich's Ataxia research Alliance Therapeutics Symposium, July 2009, Philadelphia, USA.
- Euro-Ataxia Conference, September 2009, Valladolid, Spain.
- International Symposium on Epigenetics, Chromatin Remodelling and Disease, July 2010, Valencia, Spain.
- 4th International Friedreich's Ataxia Scientific Conference, May 2011, Strasbourg, France

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

The PhD student who carried out the work, Vahid Ezzatizadeh, continues to work in the lab and will submit his thesis by the end of 2012.

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