



# FARA NEWS

Friedreichs Ataxia Research Association of Australasia newsletter

Issue 1 August 2004

## FROM THE PRESIDENT

### ***A NATIONAL ORGANISATION***

It is with much pleasure that I write this introduction to the first newsletter of the Friedreich Ataxia Research Association (Australasia). FARA was formed in 2003 as a result of discussions between representatives of the various state Friedreich Ataxia state organisations. The view was that the time had arrived for the formation of a strong, unified body able to interact with research and funding organisations at the national level.

In New South Wales the new national body has replaced the state organisation. However, all members of the former FAA (New South Wales) automatically become members of the national body, as do members of other state organisations. A feature of FARA (Australasia) is that New Zealand becomes a member as do New Zealanders currently members of other New Zealand organisations.

### ***In This Issue***

From the President	1
Carrie's Words	4
Report from the Bruce Lefroy Centre	5
Friedreich Ataxia Clinic, Monash Medical Centre	7
Research project in FA – the development of MitoQ	7
Extracts prepared by Tim Holloway, CAGT Research Group, MCRI	8
Friedreich ataxia-update on pathogenesis and possible therapies.	11
Progress on Understanding the Role of Iron in Friedreich's Ataxia and the Development of Potential Iron Chelators for Treatment	13
On Frataxin Expression	15
Recessive Ataxias: Update	18
CONTACTS	22
Membership Form	23
Invitation to participate in research study	24

The principal objective of FARA is to raise funds for research leading to the treatment of Friedreich Ataxia and associated ataxias. With this in mind it aims to provide funds for a variety of activities including:

- Assistance to young researchers pursuing studies in the field of ataxia;
- Research into ataxia generally;
- Assistance to FARA approved organisations in their establishment and ongoing development.

As a means of achieving its objective FARA assists in the organisation of relevant seminars, forums and conferences relevant to the ataxias by providing funds that will assist in defraying costs associated with such activities.

### **COMMITTEES**

FARA has an executive committee which is responsible for the allocation of funds to applicants. This committee comprises four members drawn from the state and New Zealand associations. The current executive comprises Rodger Alexander (New Zealand), Steve Beetham (Victoria), Mike Dwyer (Queensland) and Peter Rousch (New South Wales).

FARA also has a Scientific Advisory Committee whose responsibility is to advise on the allocation of funds to applicants. The present committee is Professor Bob Williamson, Director of the Murdoch Children's Research Institute, Professor Ed

Byrne, Dean of the Faculty of Medicine at Monash University and Professor Bronya Keats, Head of the Department of Genetics, Louisiana State University, U.S.A. We are grateful to these distinguished researchers for accepting this role.

The Friedreich Ataxia Research Association is registered with the appropriate authorities and is endorsed by them as a Deductible Gift Recipient and an Income Tax Exempt Charitable Entity.

### ***FARA TO DATE***

*Although in our early stage of development, we have been able to undertake the following:*

Provide recurrent funding of the Bruce Lefroy Centre in Melbourne as agreed prior to 2004. This new Centre has a particular orientation to Friedreich Ataxia and has been established through the special generosity of the Lefroy family.

Ongoing meetings with the developers of, and researchers associated with, the drug Mitoquinone with a view to hastening the first world trials of the drug.

Assisting with the organisation and funding of the first Friedreich Ataxia Symposium to be held at the Gold Coast from 3<sup>rd</sup> to 4<sup>th</sup> November 2005. The symposium will be an adjunct of the World Neurological

Conference set down for Sydney on 5<sup>th</sup> November.

### ***THE FUTURE***

In welcoming all Fa'ers and their supporters to FARA I must stress that our efforts can succeed only with your help. We are a new organisation that must depend on external financial assistance. It is not easy to obtain large funds for what we call "orphan diseases" despite our contacts and best efforts. It is important that our objectives and needs are brought to the attention of government and non-government entities alike. You can all help in making others aware of what we are attempting to achieve. A feature of FARA is that all funds are used for the benefit of Friedreich Ataxia sufferers. Your executive members pay all their own expenses, including costs of accommodation and interstate/international travel to meetings. Newsletters, printing and postage are similarly donated. We don't employ anyone to harass potential donors by telephone. In brief, we are dependent upon ourselves. What drives us is the belief that we will find a treatment and then a cure for FA during the lifetime of our sufferers.

Peter Rousch AM  
*Emeritus Professor*  
*President*

### ***SOME COMMENTS ON THIS EDITION***

FARA(Australasia) wishes to thank the following for their input to this first

newsletter:

The various scientists who are leading the way in FA research.

The Murdoch Children's Research Institute.  
The Euro-Ataxia Association.

Jenni Callaghan, who has been responsible for the compilation of the newsletter and the development of the forthcoming FARA website.

As is to be expected from a research body, the main emphasis is on current research and recent publications. However, we have taken the view that it is important to keep in mind the mental and physical suffering endured by our people. Accordingly, we have included an extraordinary piece of writing by Carrie Beetham of Melbourne.

Readers will note from the research reports that knowledge has advanced apace in the past year.

Although the drug Cisplatin is unsuitable for use with Friedreich Ataxia sufferers, it exemplifies the possibilities for uplifting Frataxin levels, thus indicating that there should be other drugs that are non-toxic yet have a similar capacity. The research at the Murdoch develops this concept.

The continuing work on Frataxin reflects growing knowledge on its levels in carriers and patients and the consequences of this knowledge for the amount of increase in Frataxin required by patients.

The work on mouse models continues and a model that conforms to the neurological features of FA as found in humans is now a reality.

Unique iron chelators developed at the University of New South Wales exemplify significant advances in the possibilities in this aspect of treatment for Friedreich Ataxia.

While Mitoquinone is still to be trialled on humans, research is already underway to refine the existing drug.

The fine work carried on in the field of ataxia in Australia through such centres as the Murdoch Children's Research Institute, the Bruce Lefroy Centre for Genetic Health Research, the research centres at the Prince of Wales Hospital and the Westmead Children's Hospital in Sydney and the Friedreich Ataxia Clinic at the Monash Medical Centre provides great confidence in our ability to ultimately win the battle against the ataxias generally. Allied with these endeavours is the New Zealand work on Mitoquinone, the trials of which we look forward to with great interest.

### ***Carrie's Words...***

*By Carrie Beetham, 2004*

The day I was diagnosed does not stand out in my memory as being especially traumatic. Probably because I didn't believe what the doctors were saying. I was a healthy teenager with dreams and aspirations, not a

sufferer of a neurological genetic disease, facing life in a wheelchair with progressing physical disabilities.

I remember a day, later in the year in the lunchroom at school. All the girls were sitting in circles on the floor, where you would maneuver a path around each group in order to reach your own friendship circle. This should have been no big deal, I had been doing this every day for three years. But on this particular day I started to lose my balance. My steps were so wobbly and I had to lean on the girls' heads in order to steady myself. Of course everyone thought that I was joking around and laughed hysterically as I pretended to fall on top of my friends. But on the inside, my world had ended. 'Oh my god, this is real, what am I going to do?' I sat there in the lunchroom that day, petrified. My life was now completely out of control, this disease was really inside my body. It was an unimaginable feeling of fear, a feeling that has been with me everyday since.

That day in the lunchroom was 11 years ago now, during which time FA has left many devastating effects on my body. I cruise around in my wheelchair still disbelieving that this is happening to me, I guess everyone with a debilitating disease says that. But the unusual thing about FA is that there is no treatment at all. We can't even slow the progression down even though scientists have recently located the faulty gene and identified the problem. The ironical

thing about this disease is that there is currently enough knowledge to find a cure but because it is so rare, there is simply not enough funding and awareness to research possible remedies. Frustrating-yes, but I consider myself fortunate to be in the position that at least I know that a real cure is only a matter of time. There are FA sufferers of all ages, I am 26 and believe that there is a long healthy life ahead for all of us living with such cruel illnesses that intrude on our young lives.

I can't pretend it's not hard at times. I wish I could walk, dance or swim at the beach. I wish I could travel the world with a backpack on my back or enjoy a day without complete exhaustion. I wish I could drive a car or take a shower without a struggle. It's unbearably hard at times. But what I am sure of is that the good outweighs the bad. Nothing demonstrates the fragility of life like falling sick. And in this position you realize how magical and precious life really is, and whatever happens we have to make the most of it and appreciate everything that we have. I feel so lucky to be surrounded by the most beautiful people. The love and support of my family and friends gives me the courage to fight this disease. And the groundbreaking research that is discovering answers everyday, is supplying real hope that the cure is very close - and *YAFFA is the key*.

I was watching the fireworks on New Years Eve this year and I thought, that money

could fund research that could potentially cure Friedreichs Ataxia. A chilling thought considering there are 500 Australians sitting at home in our wheelchairs tonight, and I'm sure there aren't many who remember those costly firecrackers that ran for 15 minutes. All I'm saying is that money will be very appreciated at YAFFA! A small gesture can make such a significant difference, I know this because I've been lucky enough to see it happen during my 11 years of suffering FA.

We will never replace the lives, years and moments this disease has stolen from us. I'll never be a 15 year old girl who can have lunch with her friends and not have to worry that her body is becoming disabled. We can't erase the past but we can support research to find the cure now.

*FA has no place in our future.*

**REPORT FROM THE BRUCE LEFROY  
CENTRE FRIEDREICH ATAXIA CLINICAL  
RESEARCH PROGRAM TO THE  
FRIEDREICH ATAXIA RESEARCH  
ASSOCIATION (AUSTRALASIA)  
NEWSLETTER**

The Bruce Lefroy Centre for Genetic Health Research is a new research centre based at the Murdoch Childrens Research Institute at Melbourne's Royal Childrens Hospital. Friedreich ataxia clinical research is our major research focus

The Bruce Lefroy Centre research relates to the development of accurate outcome measures for Friedreich ataxia and clinical trials. To this end a number of research projects are under way that are looking at all aspects of Friedreich ataxia. These include:

1. Measurement of ataxia
2. Measurement of speech
3. Measurement of eye movements
4. Measurement of hand movements
5. Measurement of heart involvement in Friedreich ataxia.

Through the measurement of these parameters we hope to develop a very accurate measuring scale that can detect progression of Friedreich ataxia. This will allow accurate measurement of the benefits or otherwise of treatments for Friedreich ataxia.

The key members of our research team are Louise Corben, Michael Fahey and Veronica Collins. Louise is an occupational therapist by training and she is Coordinator of Friedreich Ataxia Clinical Research. In addition, Louise is undertaking a part-time PhD examining hand movements in Friedreich ataxia using very sophisticated computer-based equipment. In addition, Louise will be examining accurate measures to define an individual's quality of life in Friedreich ataxia.

Michael Fahey is a qualified paediatric neurologist and clinical geneticist. Michael

is undertaking his PhD on the development of scales that measure ataxia and its progression in individuals with Friedreich ataxia.

Dr. Veronica Collins is an epidemiologist, and Veronica is involved in statistical analysis that is critical to all of this work.

We are collaborating with experts based both in Australia and overseas these people include

- Dr. Owen White, Melbourne, and Dr. Philip Cremer, Sydney, who are experts in the measurement of eye movements
- Professor Bruce Murdoch and Dr. Louise Cahill from the University of Queensland who are world leaders in the measurement of voice
- Dr. Roger Peverill, research cardiologist, Monash Medical Centre, Melbourne
- Dr. Nellie Georgiou-Karisianis who is a neuropsychologist with international expertise in the measurement of hand movements in people with neurological disorders
- Associate Professor Elizabeth Waters, Community and Child Health Research Centre, Royal Children's Hospital, who is an expert in measurement of quality of life.

We have been extremely fortunate to have wonderful support from the Friedreich Ataxia Research Association (Australasia), and the individual State Associations that preceded

it. We have also been very fortunate to have support from the Friedreich Ataxia Research Alliance USA for our work. Finally, none of this work could happen without the individual support of people with Friedreich ataxia who so willingly respond to our calls for assistance with our research. We are exceedingly grateful for this.

A/PROF. MARTIN DELATYCKI

*Director—The Bruce Lefroy Centre for Genetic Health Research*

## **FRIEDREICH ATAXIA CLINIC, MONASH MEDICAL CENTRE**

**The Friedreich Ataxia Clinic at Monash Medical Centre continues to be held on the 4th Tuesday afternoon of each month.**

The staff of the clinic are:           Dr. Andrew Churchyard, Neurologist  
Ms. Melanie Toy, Physiotherapist  
Louise Corben, Occupational Therapist  
Nicki Dann, Speech Pathologist  
Dorothy Patrick, Social Worker  
Brian Hoare, Paediatric Occupational Therapist  
Christine Blackburn, Paediatric Physiotherapist  
A/Prof. Barry Rawicki, Rehabilitation Specialist  
Dr Michael Fahey, Paediatric Neurologist/  
Clinical Geneticist  
A/Prof. Andrew Kornberg, Paediatric Neurologist

A/Prof. Martin Delatycki, Clinical Geneticist

We have seen over 70 individuals with Friedreich ataxia at the Clinic. We are very conscious of the importance of being accountable for the outcomes of our clinic. The research described above is relevant not only to drug trials but also to all management outcomes

Through the Friedreich Ataxia Clinic and the Friedreich Ataxia Clinical Research Program we are absolutely committed to helping individuals with Friedreich ataxia achieve the best possible quality of life. We once again thank the Friedreich Ataxia Research Association for supporting the clinic and our research.

A/PROF. MARTIN DELATYCKI

*Director—The Bruce Lefroy Centre for Genetic Health Research*  
*Co-head—Friedreich Ataxia Clinic, Monash Medical Centre*

## **RESEARCH PROJECT IN FA – THE DEVELOPMENT OF MITO Q**

Mitoquinone (Mito Q) was developed by Drs Mike Murphy and Rob Smith as part of their research programme into targeted anti-oxidants. It is now being developed as a medicine with funding and expertise from Antipodean Biotechnology Ltd, currently based in Auckland. It is hoped that Mito Q will slow the progression of FA. Mito Q is a targeted anti-oxidant – it is a chemical very

similar to Coenzyme Q10 which is linked to another chemical causing it to accumulate inside the mitochondria of cells. This is important in treating FA, because it is the mitochondria that suffer the damage that ultimately causes the symptoms of FA.

It is not yet known whether MitoQ will help people with FA. At present the compound is still in the laboratory and animal testing stage. In addition, research is underway into making a tablet that is stable when stored appropriately. The drug development process needed to make a medicine available to patients is quite involved. Mitoscience is working with the Food and Drug Administration in the USA and the regulatory agencies of other countries, including Australia, to ensure that Mito Q is developed as quickly and safely as possible.

It is hoped to start clinical trials of Mito Q in FA patients in Australia and the US in 2005. The trials will involve patients receiving either Mito Q or a placebo (a tablet that looks the same as the mitoQ tablet but does not have an active ingredient). Neither the patient nor the doctor knows which treatment each patient is receiving. The studies will involve treatment for about a year and various tests and questionnaires will be done quite frequently so that any benefit, or harm, of the treatment can be assessed. If there is sufficient benefit, then we hope Mito Q will become a registered medicine and available for FA patients.

In Australia associate Professor Martin Delatycki will run the clinical trial through the Royal Children's Hospital in Melbourne. Not all FA patients will be suitable for this study, but if you would like to be included in a confidential database of potential trial participants, please send your name and contact details, date of birth and year of diagnosis of FA to:

Louise Corben, Friedreich ataxia clinical research coordinator, Bruce Lefroy Centre for Genetic Health Research, 10th Floor, Royal Children's Hospital, Flemington Road Parkville, 3052, Victoria. Fax +61 3 8341 6390, email- [louise.corben@ghsv.org.au](mailto:louise.corben@ghsv.org.au).

You will then be contacted before the study starts to confirm whether you are still interested and assess whether you might be suitable.

**Recent abstracts from the  
scientific literature on Friedreich  
ataxia Prepared by Tim Holloway,  
CAGT Research Group, MCRI**

**Real time PCR quantification of  
frataxin mRNA in the peripheral  
blood leucocytes of Friedreich  
ataxia patients and carriers.**

*Pianese L, Turano M, Lo Casale MS, De Blase I, Giacchetti M, Monticelli A, Criscuolo C, Filla A, Coccozza S.*

*J Neurol Neurosurg Psychiatry. 2004*

*Jut7S(7): 106 1-1063.*

The most common causative mutation of Friedreich ataxia (FRDA) is the unstable hyperexpansion of an intronic GAA triplet repeat that impairs frataxin transcription. Using real time quantitative PCR, we showed that FRDA patients had residual levels of frataxin mRNA ranging between 13% and 30% and that FRDA carriers had about 40% of that of controls. Asymptomatic carriers also showed reduced frataxin mRNA levels. We found an inverse correlation between the number of GAA repeats and frataxin mRNA levels. Real-time quantitative PCR may represent an alternative assay for FRDA molecular diagnosis.

**Comment:**

*This is a very recent report regarding the measurement of mRNA or message resulting from the FRDA gene which eventually codes for the Frataxin protein. The measurement of mRNA was performed using Real time PCR that is a fairly new technique that provides for much more sensitivity therefore giving a more accurate determination of expression or productivity from the FRDA gene. The data presented showed an inverse correlation between the residual Frataxin protein and the size of the GAA repeat thus confirming previous reports. In addition, they present new data regarding the accurate measurement of Frataxin mRNA in carriers which is reported as being 40% of controls. This indicates that potentially useful pharmaceuticals given to persons with FA may only need to raise expression of the FRDA gene to this level to allow full functionality of Frataxin. The actual technique should be useful in the diagnostic setting and also for monitoring increases in expression from the Friedreich ataxia gene once potential beneficial drugs are given to persons with FA.*

**Human BAC-mediated rescue of the Friedreich ataxia knockout mutation in transgenic mice.**

*Sarsero JP, Li L, Holloway TP, Voullaire L, Gazeas S, Fowler KJ, Kirby PM, Thorburn DR, Galle A, Cheema S, Koenig M, Williamson R, Ioannou PA*

**Mamm Genome. 2004 May;15(5):370-82.**

Three independent transgenic mouse lines were generated with the human Friedreich ataxia gene, *FRDA*, in an 188-kb bacterial artificial chromosome (BAC) genomic sequence. Three copies of the transgene per diploid mouse genome were integrated in a single site in each mouse line. Transgenic mice were mated with mice heterozygous for a knockout mutation of the murine *Frda* gene, to generate mice homozygous for the *Frda* knockout mutation and hemizygous or homozygous for the human transgene. Rescue of the embryonic lethality that is associated with homozygosity for the *Frda* knockout mutation was observed in all three lines. Rescued mice displayed normal behavioral and biochemical parameters. RT-PCR analysis demonstrated that human *FRDA* mRNA is expressed in all the lines. The relative expression of the human *FRDA* and mouse *Frda* genes showed a similar pattern in different tissues in all three lines, indicating position-independent control of expression of the human *FRDA* transgene. However, large differences in the human:mouse mRNA ratio were observed between different tissues in all three lines. The human transgene is expressed at much higher levels in the brain, liver, and skeletal muscle than the endogenous gene, while expression of the human transgene in blood is only 25-30% of the mouse gene. These studies will facilitate the development of humanized mouse models of Friedreich

ataxia through introduction of a GAA trinucleotide expansion or specific known point mutations in the normal human *FRDA* locus and the study of the regulation of gene expression from the *FRDA* locus.

**Comment:**

*This paper describes work from the Friedreich ataxia team in the Cell and Gene Therapy group at the Murdoch Childrens Research Institute. Several years ago, a research group in France, led by Dr Michel Koenig produced a knock-out mouse model for Friedreich ataxia (FA) that resulted in embryonic lethality (the pups die before birth). Frataxin protein is completely absent in these mice. The work in the above report describes the generation of transgenic mice containing a large piece of human DNA carrying the entire *FRDA* gene and possible surrounding regulatory components. These mice were then bred with the knock-out mice and the progeny were able to survive due to the presence of human frataxin. In addition to rescue, the mouse model also demonstrated tissue specific differences in *FRDA* expression between the human and mouse giving insight into species dependent differences in *FRDA* gene regulation. Therefore, this particular mouse model will facilitate the development of future models that will contain the GAA trinucleotide repeat or other markers of gene expression that will allow testing of possible therapies for FA.*

**Friedreich ataxia mouse models with progressive cerebellar and sensory ataxia reveal autophagic neurodegeneration in dorsal root ganglia.**

Simon D, Seznec H, Gansmuller A, Carelle N, Weber P, Metzger D, Rustin P, Koenig M, Puccio H.

J Neurosci. 2004 Feb 25;24(8):1987-95.

Friedreich ataxia (FRDA), the most common recessive ataxia, is characterized by degeneration of the large sensory neurons of the spinal cord and cardiomyopathy. It is caused by severely reduced levels of frataxin, a mitochondrial protein involved in iron-sulfur cluster (ISC) biosynthesis. Through a spatiotemporally controlled conditional gene-targeting approach, we have generated two mouse models for FRDA that specifically develop progressive mixed cerebellar and sensory ataxia, the most prominent neurological features of FRDA. Histological studies showed both spinal cord and dorsal root ganglia (DRG) anomalies with absence of motor neuropathy, a hallmark of the human disease. In addition, one line revealed a cerebellar granule cell loss, whereas both lines had Purkinje cell arborization defects. These lines represent the first FRDA models with a slowly progressive neurological degeneration. We identified an autophagic process as the causative pathological mechanism in the DRG, leading to removal of mitochondrial debris and apparition of lipofuscin deposits. These mice therefore represent excellent models for FRDA to unravel the pathological cascade and to test compounds that interfere with the degenerative process.

Comment: *Since the causative gene for FRDA was discovered in 1996 several groups have been attempting to generate mouse models that will recapitulate the*

*symptoms of FRDA, in order for therapies to be pre-tested before human use. This paper describes the characterisation and analysis of two such mouse models generated by the group of Dr Michel Koenig (Strasbourg, France). These mice actually develop the most prominent features of FRDA: which is predominantly a slow progressive neurodegeneration and accompanying pathology. Hopefully the models can be used to unravel the mechanisms of FRDA and perhaps test useful therapies that may be relevant for human use.*

### **Friedreich ataxia-update on pathogenesis and possible therapies.**

*Voncken M, Ioannou P, Delatycki MB. Neurogenetics. 2004 Feb;5(1):1-8. Epub 2003 Dec 19.*

Friedreich ataxia is the most-common inherited ataxia. Since the causative genetic basis was described in 1996, much has been learnt about the pathogenesis from human, animal, and yeast studies. This has led to the development of rational therapeutic approaches. In this review, the current state of knowledge regarding the pathogenesis of Friedreich ataxia is presented and possible therapeutic strategies based on this knowledge are discussed.

*Comment: Members of the CAGT research group have authored this review. Max Voncken, a visiting student from Holland with FRDA played a major part in preparing*

*this review. A wide range of literature is analysed focussing on studies in yeast, animals and humans leading to speculation on the pathogenesis of the disease, and the probable role of frataxin in iron-sulfur cluster biosynthesis. The majority of the review comments on possible therapies for FRDA, ranging from the use of antioxidants and iron chelation through to pharmacological therapies, giving an optimistic vision of novel therapies that may be used to potentially halt the progression of FRDA.*

The Cell & Gene Therapy (CAGT) Research Group at the Murdoch Children's Research Institute (MCRI) hails the formation of the Friedreich Ataxia Research Association (Australasia) (FARAA) as a major step forward in this part of the world in the struggle against Friedreich Ataxia (FA). We hope to work closely with FARAA over the coming years to develop a two-legged approach in the struggle against this disease, i.e. an approach that combines research for immediate improvements in clinical management and patient follow-up, with basic research for the effective therapy of the disease.

As a laboratory group working for the effective therapy of FA, we have based the development of our strategy on the following facts:

a) All FA patients have at least one FA chromosome with a large GAA expansion. A therapy that overcomes the effects of the

GAA expansion can therefore benefit all patients;

b) Even the largest GAA expansion allows a small amount of normal frataxin mRNA to be produced, while experiments in mice show that production of about 25-30% of the normal level of frataxin may be therapeutic. Thus in most patients a 5-10 fold increase in the production of normal frataxin may be therapeutic.

c) Frataxin appears to be an essential mitochondrial protein in most cell types, yet sensitivity to frataxin loss varies substantially between tissues, indicating reduced dependence on frataxin of some tissues, or the presence of alternative factors that may complement or compensate for the loss of frataxin.

d) Given the requirement for frataxin in most cell types and tissues, we do not anticipate that gene therapy or stem cell therapy can make any substantial contribution towards effective therapy for FA in the foreseeable future.

e) The frataxin gene has been fully sequenced as part of the Human Genome Project, but we still do not know much about its mechanisms of regulation in different tissues.

Under these circumstances, the basic strategy of the CAGT Research Group is to identify pharmacological agents that can

increase the rate of production of frataxin to potentially therapeutic levels. To achieve these objectives, the CAGT Research Group has used a human DNA fragment that contains the intact FRDA locus in a number of ways:

a) Show that the human FRDA locus can fully compensate for the deletion of the mouse frataxin gene in homozygous knockout embryos, i.e. human frataxin appears to be properly regulated and produced in transgenic mice.

b) Fuse the human FRDA gene to a fluorescent gene (EGFP) and use the fusion construct to develop cellular assays and frataxin-green mice. In both these systems, measurement of fluorescence intensity can be used as a direct and convenient method to measure the production of frataxin.

c) Frataxin/EGFP cellular assays are being used to identify potential inducers of frataxin. We hope that the assay will soon be used in high throughput screening of 100,000 chemicals and 2000 FDA-approved drugs.

d) Frataxin-green mice may be used to evaluate in vivo some of the most promising chemical agents.

e) The normal human FRDA locus is also being used to introduce into it a large GAA expansion and to generate a humanised mouse model for the GAA expansion. It is hoped that unlike other GAA mouse models

with smaller GAA expansions, our mouse model will provide an accurate model of the disease at both the phenotypic and genotypic levels. Such a model will greatly facilitate the preclinical testing of promising inducers of frataxin expression.

In conclusion, despite the discovery of the FRDA gene less than 10 years ago, substantial progress towards the development of effective therapeutic strategies has been achieved. We hope that by working closely with FARRA and other patient and philanthropic organisations in Australia and overseas over the next few years, we will overcome the remaining challenges and develop an effective pharmacological therapy that could benefit all patients with FA.

*Panos Ioannou, PhD*

*Head, Cell & Gene Therapy (CAGT)*

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## **Progress on Understanding the Role of Iron in Friedreich's Ataxia and the Development of Potential Iron Chelators for Treatment**

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As many in the Friedreich's ataxia (FA) community are aware, my laboratory has been concentrating on the role of iron in the development of FA and the design and use of iron chelators for treatment. We have been very intrigued by this line of study as patients with FA have been shown to accumulate iron within the "powerhouse" of the cell called the mitochondrion. Unfortunately, such accumulations of toxic iron can lead to marked damage to cells and could potentially play an important role in the development of FA. Below, I have briefly described some of the most important findings from my laboratory.

### **1. Our Successful Ph.D Students Have Made Significant Progress in Understanding How Frataxin Works:**

Our studies on FA have been spear-headed by the projects of 2 successful Ph.D students, namely Dr. Erica Becker, and soon to be Doctor of Philosophy, Tim Chaston. Our previous investigations with Erica showed that the molecule defective in FA, known as frataxin, could play an important role in how the mitochondrion handles its iron. These studies were published in the prestigious haematology journal "BLOOD" and have recently been confirmed and extended by other scientists in the United

States. Indeed, this latter work was published in another prestigious journal, namely the Journal of Biological Chemistry (Yoon T. and Cowan JA (2004) J. Biol. Chem. Apr 27 "Frataxin-mediated iron delivery to Ferrochelatase in the final step of Heme biosynthesis"). These developments are surely encouraging for my little group of FA researchers, as they demonstrate that we are on the "right track" to determining how frataxin works.

It is with great pleasure that I announce that Tim Chaston has recently submitted his Ph.D thesis for examination after 3 years of study within my laboratory. In a nutshell, Tim has shown that our chelators show appropriate properties for the treatment of FA. That is, they are non-toxic and do not induce damage to important molecules in the body such as DNA. These studies were crucial in terms of investigations in mice that are described below.

## **2. For the First Time Studies Using Mice Demonstrate That our Chelators are Orally Effective at Inducing Iron Excretion.**

Recent investigations in my laboratory have examined the ability of one of our best iron chelators at inducing iron excretion in mice. These experiments are important, as they set the stage for further research and eventually clinical trials. Clearly, before these drugs are used in humans they must be tested in animals.

The novel iron chelator, PCTH, that was developed in my lab was shown to have high Fe chelation efficacy (Becker, E.M. and Richardson, D.R. (1999) J.Lab. Clin. Med. 134:510-521; Richardson, D.R. et al. (2001) Biochim.Biophys. Acta 1536, 133-140) and its activity was important to determine in heart cells in culture and also in the mouse animal model. We showed that PCTH was significantly more effective than the chelator in clinical use (DFO) at mobilizing iron from heart cells. Moreover, PCTH prevented the uptake of iron into ferritin which stores iron in the heart. This was important to initially assess as cardiac complications are a major cause of death in FA patients. Further studies showed that PCTH was orally-active and well tolerated by mice at doses ranging from 50 - 200 mg/kg, twice daily for two days. The level of Fe excretion at 200 mg/kg was similar to the same dose of the orally effective chelators, pyridoxal isonicotinoyl hydrazone (PIH) and deferiprone. Effective chelation of iron in the liver by PCTH was shown via its ability to reduce the ferritin-iron accumulation. Mice treated for 3 weeks with PCIH at doses of 50 and 100 mg/kg twice daily showed no signs of toxicity as determined by weight loss and a wide variety of biochemical measures. This study demonstrated that PCTH was well tolerated at the doses used and induced considerable iron excretion by the oral route suggesting its potential as an iron chelator for the treatment of FA.

These studies using normal mice set the scene for examining the effectiveness of the PCTH chelator at preventing the development of FA in the knockout mouse model obtained from Prof. Koenig (France). These latter animals have taken nearly 2 years to breed, and certainly, the next 12 months will be critical in terms of developing Fe chelators for the treatment of FA.

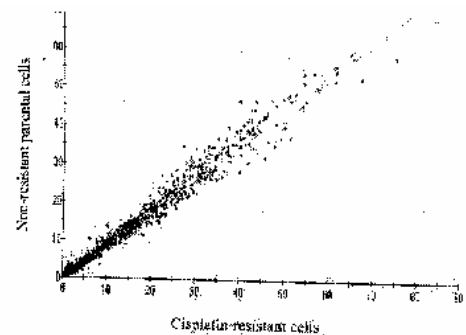
Finally, I would like to kindly thank the FA Associations of New South Wales and Victoria for generously supplying a Scholarship to Ph.D student, Tim Chaston.

### ON FRATAXIN EXPRESSION

*M. Ghazizadeh, MD, Nippon Medical School, Tokyo, Japan\**

Cisplatin is a drug that is used in the treatment of a wide variety of cancers involving various organs in humans, however the tumor cells frequently become resistant to its effect and no longer regress. Understanding the mechanism(s) behind the acquisition of resistance to cisplatin is the principal step that opens the ways to cope with the resistancy and maintain the beneficial effect of cisplatin. In an attempt to achieve this goal, we performed a screening of genes that participate in the process of resistancy by comparing the gene expression pattern of the cisplatin-resistant ovarian cancer cells with the gene expression pattern of the same cancer cells before they become resistant to cisplatin.

Recent progress in gene expression analysis has enabled screening of thousands of human genes by a method termed cDNA microarray analysis. In this method, nucleotide sequences of human genes are stuck to special plastic membranes or glass slides as single spots by robotic machines. It is possible to spot thousands of genes on small-size glass slides (gene chip) or membranes. Thus, two identical membranes carrying gene spots can be used for screening two samples, in this case the cisplatin-resistant ovarian cancer cells and the same cancer cells before they become resistant to cisplatin. Ribonucleotide acid (RNA) extracted from each sample is hybridized to a gene membrane and the intensity of hybridization of each gene on the membrane is measured and compared to the intensity of the same gene on the second membrane. This analysis is performed by special computer software that has been manufactured for the gene membranes.



**Figure 1** demonstrates a representative scatter plot of comparing the gene expression patterns between the cisplatin-

resistant cancer cells and the same cells before they become resistant to cisplatin. Each point represents one gene. Points that lie along the diagonal line represent genes expressed at equal levels in both samples, whereas points that lie off the diagonal line represent genes expressed at greater levels in the sample identified by the nearer axis. A two-fold or more variation in gene expression is used as a reliable limit of estimation. By this comparison, we see that many genes show increased expressions and many others show decreased expressions between the two samples. These changes are thought to be mainly responsible for the development of resistance to cisplatin.

One of the genes that frequently showed overexpression was FRDA gene. This finding led us to think that the expression of FRDA gene messenger RNA (mRNA) and its encoding protein, frataxin, may have been increased in cisplatin-resistant ovarian carcinoma cells. We performed experiments to check the levels of frataxin mRNA and protein in cisplatin-resistant ovarian carcinoma cells and their parental cells before acquisition of resistance. Frataxin mRNA levels were measured by a molecular biologic method termed reverse-transcriptase polymerase chain reaction (RT-PCR). The mean value of frataxin mRNA expression in cisplatin-resistant cells was higher than that in the parental cells. Frataxin protein levels were measured by Western blot analysis. The relative frataxin protein level in cisplatin-resistant cells was

higher than that in the parental cells. To confirm the intracellular expression of frataxin in the two cell groups, immunofluorescence staining for frataxin and microscopic observation was performed. An enhanced expression of frataxin protein was detected in cisplatin-resistant cells as compared with the parental cells, and the staining appeared to be mainly associated with the mitochondria. These findings suggested that in cisplatin-resistant cells the expression of the FRDA gene and its encoding protein frataxin are increased. This was the first time that an association between frataxin expression and cisplatin resistance could be established. We therefore concluded that cisplatin may have induced the FRDA gene to produce higher levels of frataxin expression. Although the mechanism(s) underlying this effect is yet to be defined, several lines of evidence suggest a protective role for it against cellular damage.

Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disease causing limb and gait ataxia and cardiomyopathy. This disease is caused by the expansion of a GAA trinucleotide repeat located in the first intron of the frataxin gene, resulting in decreased levels of frataxin mRNA and protein. Frataxin deficiency involves reactive oxygen species (ROS)-mediated cellular damage. Recent evidence suggests that frataxin might detoxify ROS via activation of glutathione peroxidase and elevation of other substances that reduce

ROS, a mechanism similar to that utilized by cisplatin-resistant cells.

Normal cellular metabolism is associated with the production of reactive oxygen species (ROS) by mitochondria, and consequently damage to DNA and proteins, which under certain conditions induces cell death and lysis. Mitochondria are dependent on glutathione to detoxify ROS and prevent oxidative damage. ROS-mediated cell damage has been associated with both cell death and cell lysis. Excessive formation of ROS as well as depletion of cellular antioxidants resulted in cell death. ROS has been shown to be associated with other forms of cell death as well, such as those that occur through interactions with specific molecules on the cell surface termed Fas ligands. These data suggest that ROS damage induces cell death.

In ovarian cancer cells, multiple mechanisms of cisplatin resistance have been proposed, including decreased drug accumulation, increased drug inactivation, enhanced repair of the damage caused by binding cisplatin to DNA in the cell nucleus, and tolerance to DNA damage. A mechanism by which cisplatin exerts its toxicity to living cells is through the generation of ROS. On the other hand, intracellular inactivation of cisplatin by glutathione has been proposed as a mechanism of cisplatin resistance.

Glutathione is the most abundant intracellular substance that reduces ROS and acts as a crucial cellular antioxidant. The reduction-oxidation (redox) state of a

cell is largely determined by the balance between generated ROS and endogenous expression of buffer substances that reduce ROS such as glutathione. Glutathione is necessary for resistance to oxidative stress through detoxification of ROS. It can also detoxify many endogenous toxins, including cisplatin, through the formation of glutathione adducts. Inhibition of intracellular glutathione by a special chemical inhibitor in cancer cell lines was shown to increase cisplatin sensitivity, but induction of glutathione production by a special chemical inducer led to increased cisplatin resistance. In addition, the glutathione content of tumor cells has been correlated with cisplatin resistance. These results indicate that glutathione may protect cells from ROS damage.

Increasing evidence suggests a role for frataxin in promoting cellular defense against ROS and that frataxin might detoxify ROS. Mutations in the frataxin gene leading to the decreased expression of frataxin conferred cellular sensitivity to oxidant stress which was rescued by chelators of iron and calcium and inhibitors of apoptosis (cell death). In a mouse model, transgenic overexpression of human frataxin increased cellular antioxidant defense via activation of glutathione peroxidase and elevation of substances that reduce ROS. These data suggest that frataxin may activate glutathione to protect cells from ROS damage. Taken together, these observations demonstrate that glutathione shares a role in both mechanisms of frataxin

function and cisplatin resistance. It is likely that repeated oxidative stress during acquisition of resistance to cisplatin might have regulated the glutathione level at a higher threshold to cope with the cellular ROS detoxification need. To accomplish this task, the frataxin gene might have been recruited.

Although cisplatin is a drug which is toxic to the living cells and has several undesirable adverse effects that makes it unsuitable for use in non-cancer diseases such as Friedreich's ataxia, the finding that it may induce frataxin expression is of particular interest. One major approach in drug discovery for the treatment of Friedreich's ataxia is to screen the existing drugs that are currently used in humans for their effects to increase frataxin expression. As the mechanisms of action of such drugs are already most clarified, the identification of such agents would open the avenue for further analysis of the mechanism(s) by which they increase frataxin. This endeavor will lead to the disclosing either of a safe drug that is already in use for the treatment of other diseases in humans and can be used for the treatment of Friedreich's ataxia as well, or of a drug with undesirable side effects whose mechanism of action can be scrutinized for searching or designing a new safe drug for the treatment of Friedreich's ataxia. In either cases, this approach facilitates more rapid drug discovery for the treatment of Friedreich's ataxia.

It should be noted that our finding of the possibility that cisplatin may have an effect

to increase frataxin expression is based on an indirect observation found in cisplatin-resistant tumor cells. The direct effect of cisplatin on the FRDA gene and whether this effect actually increases the frataxin mRNA and protein expression await further investigations using for example the recently developed innovative gene fusion constructs in which enhanced green fluorescent protein (EGFP) has been fused to the FRDA gene and this complex has been inserted into the living cells (Sarsero JP, Li L, Wardan H, Sitte K, Williamson R, Ioannou PA. Upregulation of expression from the FRDA genomic locus for the therapy of Friedreich ataxia. *J Gene Med.* 2003 Jan;5(1):72-81). Treating these cells with cisplatin or other promising agents in vitro and measuring the degree of increase in fluorescence intensity (indicative of the FRDA gene) and also in the frataxin protein expression will provide more definite clues for the effect of such agents on frataxin expression. In addition, the development of transgenic animals carrying the FRDA-EGFP fusion constructs will facilitate in vivo evaluation of cisplatin or other agents in terms of their effect on frataxin expression. There is no doubt that with these advances at disposal, one would expect a closer future for the discovery of an effective therapy for Friedreich's ataxia.

## **RECESSIVE ATAXIAS: UPDATE**

*Pr Michel Koenig, University of Strasbourg*

## 1. NEW RECESSIVE ATAXIAS

The availability of the genetic test for Friedreich ataxia in 1996 soon made clear that about half of the juvenile and adolescent onset recessive ataxia cases were caused by mutations in other genes. This prompted us to study frontier cases between Friedreich ataxia, a disease mostly affecting the spinal cord, and Ataxia-telangiectasia, another well-known recessive ataxia affecting primarily the cerebellum, and described since 1923 with non-neurological features such as chronic infections, immune deficiency and susceptibility to leukemia. We recently characterized two of these forms that are caused by a primary cerebellar degeneration and, like ataxia-telangiectasia, are associated with specific eye movement impairment called "ocular apraxia". Ocular apraxia is a difficulty or inability to make lateral eye movement on command. When requested, the patients turn their head

to look sideways (head thrust). Exaggerated eye blinking is often associated. Ocular apraxia is caused by eye movements of small amplitude (called "hypometric saccades").

In an earlier *euro-ATAXIA* Newsletter (issue 21, April 2002), I reported on the identification of the gene for one of the forms, called ataxia + ocular apraxia form 1 (AOA1), by my collaborator Maria-Ceu Moreira. Maria-Ceu has now identified the gene of the second form as well, AOA2 (Moreira et al. February 2004). The genetic studies have revealed that not all patients have ocular apraxia (which is sometimes difficult to recognize at late stages of the disease). Therefore, the AOA1 and AOA2 forms may account for a substantial proportion of all non-Friedreich recessive ataxias. Both forms appear to exist all around the world, unlike Friedreich ataxia which is mostly restricted to populations of European and Arabic origin.

### COMPARISON OF THE DIFFERENT FORMS OF AOA

	ataxia-telangiectasia	AOA1	AOA2
age of onset (years)	2-6	2-6	11-22
cerebellar atrophy	+	+	+
peripheral neuropathy	Limited	sensori-motor n.	Sensory n.

serum markers :			
alpha-fetoprotein	High	normal	high
albumin	Normal	low *	normal
cholesterol	Normal	high *	normal
chromosome	11q22	9p13	9q34

**\* only after 15 years of disease duration**

In both cases, the genes were novel. The proteins defined by the genes were named aprataxin for AOA1, and senataxin for AOA2 (owing to similarities of the AOA2 protein with a yeast protein named SEN1). The initial hints that aprataxin might be involved in DNA single strand break repair were confirmed by the discovery of a direct interaction between aprataxin and proteins of the single strand break repair pathway (work of the groups of H. Date, Japan, and K. Caldecott, England). The exact function of aprataxin is not clear yet. All of this suggests a link with ataxia-telangiectasia which is due to a defect in DNA double strand break repair. Senataxin also possesses a feature, called a "helicase domain", that suggests interactions with the nucleic acids (DNA or RNA). However, senataxin resembles more the RNA helicases, such as SEN1. The mechanisms that lead to cerebellar degeneration might be multiple and additional work is needed before general conclusions can be drawn.

The genetic test for AOA1 is now available on a routine basis, since it is a small gene and all mutations were so far clustered in only three gene fragments (called "exons"). The availability of a genetic test for AOA2 will be more problematic, since the gene is very large (24 exons) and one of these exons is strikingly large. However, all AOA2 patients so far appear to have one specific biochemical feature: an increase in serum alpha-fetoprotein (on repeated measurements). This feature, associated with the exclusion of an ataxia-telangiectasia diagnosis, based on other criteria, should help to restrict the number of genetic tests for AOA2 within reasonable limits.

**2. FRIEDREICH ATAXIA**

The identification in 1996 of the defective gene causing Friedreich ataxia prompted substantial work to understand the mechanism of the disease, construct mouse models and design tentative therapies. Friedreich ataxia is caused by a GAA

trinucleotide repeat expansion located outside the coding region of the gene. For this reason, the encoded protein which we named frataxin is of normal size but produced in very reduced amounts. We showed in a mouse model that having no frataxin at all is lethal very early in embryonic development. The disease in patients is therefore the consequence of having very small amounts of the normal frataxin protein.

Frataxin is a protein of the mitochondria, which is a compartment of the living cell. Energy is released there from the food and transferred to the cell's fuel, ATP, by electron transport in a structure called "respiratory chain". The function of frataxin was clarified last year by the identification of frataxin being the main protein binding partner of Isu1, a major protein involved in a process called "iron-sulfur (Fe-S) cluster assembly" (work of the groups of R. Lill, Germany, F. Foury, Belgium and J.A. Cowan, USA). These Fe-S clusters are, among other things, involved in the electron transport in the respiratory chain mentioned above. The specific deficiency of Fe-S clusters in the affected tissues of Friedreich ataxia patients (identified as early as in 1997 by the group of P. Rustin, Paris) is therefore the direct consequence of very low amounts of frataxin. The precise role of frataxin is not yet known, but it might well be to provide iron for Isu1 for Fe-S cluster assembly. The idea that I supported two years ago in the *euro-ATAXIA* Newsletter, that Fe-S cluster deficiency is secondary to oxidative stress

(production of toxic molecules called free radicals) is probably NOT correct. The targeting of Isu1, which is present in Friedreich patients and is desperately seeking for its protein partner, with pharmaceutical compounds, is, in my opinion, certainly a valuable research strategy for a future therapy of Friedreich ataxia.

Current therapeutic approaches for Friedreich ataxia, namely the use of anti-oxidants such as idebenone, remain valid as a means to fight secondary (and not primary) consequences of frataxin deficiency. As indicated two years ago, we have successfully demonstrated that idebenone prolongs a mouse's life in our Friedreich cardiomyopathy mouse model, even with a complete absence of frataxin in the heart of our models. We characterized and confirmed this protective effect by echocardiography and finally got the results to publication (Seznec, Puccio, Simon et al. April 2004, at last). This is a small but significant contribution to the delivery of market authorisation for idebenone for Friedreich ataxia therapy, yet most countries still demand positive results of a double blind study in patients for this crucial delivery.

In the mean time, Delphine Simon, H  l  ne Puccio and Herv   Seznec of my group constructed a mouse model that develops only the neurological symptoms of Friedreich ataxia. This is achieved by inducing the frataxin gene mutation (a deletion) into the nervous system shortly

after birth. The procedure resulted in a very progressive disease, with disease onset several months after the induction of the deletion and an almost normal life span. This model is therefore very similar to the disease in patients and it was rapidly published (March 2004). A therapeutic trial with idebenone using this model is already on its way. However, given the very progressive nature of the disease in this model, the trial will probably continue for quite some time.

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## SUBSCRIPTIONS

Subscriptions are now due for members of the former Friedreich Ataxia Association (New South Wales) and those who are not currently members of a state FA organisation. The New South Wales body has been replaced by the new national body. All members of the former New South Wales Association have been included as members of the new national body. All financial members of other state FA bodies are also included as members of the national body and do not need to pay an additional annual subscription to the national association.

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### PLEASE RETURN THIS FORM TO:

*Mr Mike Dwyer*  
Treasurer  
Friedreich Ataxia Research Association (Australasia)  
32 Karema Crescent  
Runaway Bay  
Queensland 4216

My cheque/money order for \$15 for my annual subscription to FARA is enclosed.

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**INVITATION TO PARTICIPATE IN RESEARCH STUDY.**

You are invited to take part in a research study into eye movements in Friedreich Ataxia. The aim of this study is to obtain more accurate measures of vision and eye movements in people with Friedreich Ataxia. This study is being conducted by Dr Swee Aw (Senior Hospital Scientist, Neurology, RPAH) and Dr Philip Cremer (VMO Neurologist, RNS), A/Prof Martin Delatycki (Bruce Lefroy Centre for Genetic Health Research, Melbourne) & Dr Michael Fahey (Bruce Lefroy Centre for Genetic Health Research, Melbourne).

If you agree to participate in this study, you will be asked to complete a range of tasks that assess how well your eye movements are functioning and answer a number of questions regarding the impact of your vision on day to day tasks. This testing will take approximately 3 hours. We would like to repeat the testing on three occasions over the next two years in order to understand better how the condition progresses. The questionnaires take about half an hour to answer and will be sent to you to complete prior to your appointment.

We are planning to conduct the first part of this study on **Friday 27 August, 2004 and Saturday 28 August, 2004**. The study will be conducted at the **Royal Prince Alfred Hospital**.

If you are interested in participating in this study please contact Louise Corben, Coordinator, Clinical Research, Bruce Lefroy Centre for Genetic Health Research (Melbourne) on 03 8341 6228 or [louise.corber@ghsv.org.au](mailto:louise.corber@ghsv.org.au) as soon as possible.