



FARA NEWS

Friedreichs Ataxia Research Association of Australasia newsletter

Issue 3 December 2005

FROM THE PRESIDENT

This edition of the newsletter marks a turning point in the story of Friedreich Ataxia (FA) research. For the first time we are now talking about a treatment for the disorder in addition to finding a means of preventing deterioration.

FA sufferers are probably saying to themselves "We hear about these advances but no drug has made its way to me yet". This is an understandable reaction, but we need to understand the many hurdles that have to be negotiated with authorities before a drug can be approved for delivery to patients. This procedure can often take up to three years and, in some cases, even longer. However, a number of scientific discoveries in the past twelve months have exciting implications for FA treatment. The science that underlies these advances is a product of the work being undertaken in Australia, the U.S.A. and Europe. The sharing of advances across the world is responsible for the breakthrough that we are hoping will result in treatments in the short term.

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Antioxidants

The trial of Mitoquinone will commence in the first part of next year in Australia and the U.S.A.. Mitoquinone is an antioxidant that penetrates into the mitochondria and will, hopefully, prevent the build-up of free radicals that cause so much damage. At the same time the antioxidant Idebenone will be the subject of clinical trials in Europe.

Frataxin Levels

We know that the lack of sufficient quantities of the protein Frataxin is the basic cause of FA. For some years scientists have been searching for ways to overcome this lack in the view that the identification of a compound that will increase Frataxin levels will act as a therapy and, hopefully, reverse the symptoms of FA. Researchers at the Murdoch Children's Research Institute in Melbourne have developed a sophisticated mouse model with the human FA condition. They are also able to use their previously developed assay to test existing drugs and compounds to ascertain whether these can increase Frataxin levels. It is expected that this testing of existing drugs will be completed in the next two months. The advantage of finding an existing approved drug that resolves the Frataxin problem is that it would not have to be processed through the approval mechanism that is required for new drugs.

Occurring simultaneously with this work are two other projects. Dr Joel Gottesfeld, working with compounds at the Scripps Research Institute in California, has been able to elevate Frataxin levels in blood cell cultures of FA patients at least to the level of their non-affected siblings. In Austria, Dr Barbara Scheiber-Mojdehkar has used a drug (EPO) in her laboratory work to raise Frataxin levels from two to five-fold. The endeavour in this project is to fast-track a pilot study with FA patients.

The Future

We continue to be optimistic that a therapy for FA is close at hand.

While there is still work to be done since the current research has been undertaken only in the laboratories, the research is exciting and the prospects of a treatment are now so much closer. We can at last say that the treatment will not be a matter of "if" but "when".

Peter Rousch
President

FRIEDREICH ATAXIA IN AUSTRALIA

Recent Events

Gold Coast 3-4 November 2005

A two-day meeting of researchers from a variety of countries was the highlight of November. The abstracts of papers relevant to Friedreich Ataxia have been included in this newsletter. The presentations were most impressive and left the participants with the view that we had reason to be optimistic about the potential for treatments that would commence in 2006.

Melbourne 9 December 2005

This meeting brought together Australian researchers from wide backgrounds. We were able to consider the possibilities that now exist through the input of researchers involved in stem cell research, iron chelators and antioxidants. An overview of plans for clinical trials of various drugs was presented and the decision was taken to co-ordinate the various aspects of FA research through liaison between the research groups.

FA Treatments

These are, indeed, exciting times. There is a real chance that the

homestretch is at least in sight. Another way to say the same thing is that the scientific projects we are all nurturing together right now, including those that will be in clinical trials over the coming year, look so promising that many of us believe they will result in treatments. It may very well be that effective treatment will involve a cocktail and that the cocktail may very well include several of the drugs now in trial or approaching trial. Taking the drug candidates one at a time:

1. Idebenone — in Phase II at NIH right now.

Idebenone is also to be in a phase II — III in Europe within a few months. That trial is to be run by the drug company providing NIH with Idebenone — Santhera.

We have most often referred to Idebenone as an antioxidant — a beneficial scavenger of free radicals that would otherwise cause damage to the cells. While it may be an antioxidant to some extent, it seems more and more apparent that Idebenone is something more as well. It seems likely that Idebenone also helps mitochondria by moving electrons properly along the mitochondrial walls so that more energy and fewer free radicals are produced.

2. Mitoquinine

Planning is underway to get it into a phase II clinical trial in the first half of next year, probably in Australia (Murdoch Institute, Martin Delatycki) and California (UCLA, Sue Perlman) Mitog is CoQ10 plus a small addition that takes it right to the mitochondria.

The MitoQ scientists expect MitoQ to act as a very effective antioxidant because it will concentrate at much higher levels in the mitochondria so as to scavenge free radicals right where they are produced and before they cause damage.

3. A-0001 (Edison Pharmaceuticals)

Planning underway to get this into a phase II clinical trial near the middle of next year. You may have read that FARA has awarded a grant of \$3 million to this development Project. Leading this drug development effort are people like Dr. Guy Miller of Edison, Dr. Rob Wilson of the University of Pennsylvania and Dr. Sid Hecht of the University of Virginia. These scientists report that A—0001 inserts itself into the mitochondria where it assists in transporting electrons smoothly along the mitochondrial walls so that much more energy and far fewer free radicals are produced. Such electron flow is normally assisted by the iron—sulfur clusters that the frataxin protein is involved in assembling. Because our patients have much lower levels of frataxin protein, however, the electrons along their mitochondrial walls tend to jam up and escape prematurely so that free radicals are produced and the energy production process is not completed consistently. In sum, these scientists believe molecules like A—0001 stand a very good chance of compensating for the low levels of frataxin protein so that significantly more energy is produced and significantly less oxidative damage is caused.

4 Drugs that may be able to increase frataxin protein levels

FARA is working closely and hard on two exciting projects in this category. One is led by Dr. Joel Gottesfeld at the Scripps Research Institute in La Jolla, CA. under several RARA grants. His project, to which a good number of you (thank you) have donated blood samples (Mary—Lisa has helped assemble some and Sue Perlman has sent others from her UCLA clinic) , shows tremendous promise in that the compounds he is working with seem to elevate frataxin protein levels in the blood cell cultures of patients up to and above the frataxin protein levels in their carrier siblings. These compounds operate, not on the mitochondria, but within the cell nucleus on the chromosome that contains the FA gene itself. The other project is led by Dr. Barbara Scheiber—Mojdehkar in Austria, also under several grants from FARA and Seek A Miracle. This is the project in which a drug called EPO is being used on blood, heart and nerve cell cultures from FA patients with the apparent result of raising frataxin protein levels from two to five fold. Dr. Scheiber—Mojdehkar is not yet certain exactly how this compound works to obtain this promising outcome, but she is working hard to advance a pilot clinical study with FA patients. She wrote FARA this week and sent us her journal article and we have written her back asking how we might be of further help.

Both of these protein—elevating projects are using compounds that are already in use in other diseases or are in clinical trials in other

diseases. So, if they continue to show such promise in FA, the scientists should have a much easier, quicker time getting them into clinical trials for FA — they will have already been through animal safety studies and been administered to humans. The net result is that this protein elevation approach could be on a very fast track.

The potential benefit of the kind of frataxin protein increases involved in these two projects is tremendous. As you know, FA carriers have lower frataxin protein levels than non—carriers but are completely healthy. So, our patients would obviously benefit tremendously were we able to get their frataxin protein levels up to or above those of their carrier siblings and parents.

We need to keep in mind that cell cultures are not people. Much needs to be done to prove that these exciting cell culture results can be duplicated in our patients and that the drugs will be safe for them. But, these projects show tremendous promise and are on a fast track.

So, you can imagine how exciting it would be to see a ‘cocktail therapy’ developing that might include antioxidants that mop up free radicals, mitochondrial electron transport compounds that produce more energy and fewer free radicals — even when frataxin levels are unusually low— and compounds that increase those frataxin protein levels. These wonderful scientists are growing in the conviction that such a “cocktail” would stop this

disease in its tracks. What a Happy Hour we will have with that cocktail.

Working alone, there is little any of us can accomplish. Working together, there is little we can NOT accomplish. All of us working together will get this thing done. We will all be part of treating and curing this disease.

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PANOS IOANNOU

Associate Professor Panos Ioannou passed away earlier this year. He was a great loss to those who knew him as a person and to those of us who were fortunate to see his research into Friedreich Ataxia at first hand. Panos was the head of the Cell and Gene Therapy unit at the Murdoch Children's Research Institute where he headed a team that is a world leader in FA research. A brilliant academic, Panos had research interests in a variety of fields but it was his recent work in Friedreich Ataxia that attracted significant funding from funding sources in a number of countries.

Panos left us with a legacy that he believed would solve the Friedreich problem. His development of the assay that enabled the screening of drugs for frataxin potential was unique in the FA field. Despite the severity of his illness Panos continued his research to the very end of his life and found the strength to write a final letter to his colleagues

and friends only days before his death.

Panos's colleagues and co-researchers in the ataxia field are continuing his work. The eventual outcome of their research will reflect greatly upon the person who, through his wisdom, fine intellect and passion for the task in hand, ensured that a breakthrough would occur.

Friedreichs Ataxia, No Changes in Mitochondrial Labile Iron in Human Lymphoblast - A DECREASE IN ANTIOXIDATIVE CAPACITY?

Brigitte Sturm, Ute Bistrich~, Matthias Schranzhofer, Joseph P. Sarsero, Ursula Rauen, Barbara Scheiber-Mojdehkar, Herbert de Groot, Panos Ioannou, and Frank Petrat

Friedreich's ataxia (FRDA) is caused by low expression of frataxin, a small mitochondrial protein. Studies with both yeast and mammals have suggested that decreased frataxin levels lead to elevated intramitochondrial concentrations of labile (chelatable) iron, and consequently to oxidative mitochondrial damage. Here, we used the mitochondrion-selective fluorescent iron indicator/chelator rhodamine B-[(1,10-phenanthroline-5-yl)aminocarbonyl]benzylester (RPA) to determine the mitochondrial chelatable iron of FRDA patient lymphoblast and fibroblast cell lines, in comparison with age- and sex-matched control cells. No alteration in the concentration of mitochondrial chelatable iron could be observed in patient cells, despite strongly

decreased frataxin levels. Uptake studies with Fe-transferrin and iron loading with ferric ammonium citrate revealed no significant differences in transferrin receptor density and iron responsive protein/iron regulatory element binding activity between patients and controls. However, sensitivity to H₂O₂ was significantly increased in patient cells, and H₂O₂ toxicity could be completely inhibited by the ubiquitously distributing iron chelator 2,2-dipyridyl, but not by the mitochondrion-selective chelator RPA. Our data strongly suggest that frataxin deficiency does not affect the mitochondrial labile iron pool or other parameters of cellular iron metabolism and suggest a decreased antioxidative defense against extramitochondrial iron-derived radicals in patient cells. These results challenge current concepts favoring the use of mitochondrion-specific iron chelators and antioxidants to treat FRDA.

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Recombinant human erythropoietin: effects on frataxin expression in vitro

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Background Friedreich's ataxia (FRDA) is a neurodegenerative disorder caused by decreased expression of the protein frataxin, recently described to be an iron chaperone for the assembly of iron-sulphur clusters in the mitochondria, causing iron accumulation in mitochondria, oxidative stress and cell damage. Searching for compounds that could possibly

influence frataxin expression, we found that the cytokine recombinant human erythropoietin (rhuEPO) significantly increases frataxin expression by a still unknown mechanism.

Materials and methods Isolated lymphocytes from FRDA patients, isolated human cardiac cells (fibroblasts and myocytes) from patients undergoing heart transplantation and P19 mouse cells (neuronal typ), were incubated with different concentrations of rhuEPO, and immunoblot was carried out for the detection of frataxin.

Results We show for the first time that the cytokine recombinant human erythropoietin (rhuEPO) can, additionally to its reported neuro- and cardioprotective properties, increase frataxin expression *in vitro*. We show that rhuEPO significantly increases frataxin expression in primary lymphocytes from patients with Friedreich's ataxia. Further we show that rhuEPO can also increase frataxin expression in many other cell types; among them the most affected cell types in FRDA such as neurones and cardiac cells.

Conclusion Our results provide a scientific basis for further studies examining the effectiveness of this agent for the treatment of FRDA patients.

ATAXIA SEMINAR
GOLD COAST, AUSTRALIA
3-4 NOVEMBER 2005

The following abstracts relevant to Friedreich Ataxia have been included

in this edition of the Newsletter. FARA (Australasia) wishes to thank Dr Martin Delatycki and the various presenters for their contributions.

I-02 The Cerebellum and Cognition

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The cerebellum has traditionally been regarded as contributing exclusively to the motor system. However, converging lines of evidence increasingly support a role in cognition. Such evidence comes from neuroanatomical and functional neuroimaging studies in normals, as well as from neuropsychological studies in patients with focal cerebellar lesions or cerebellar degenerations.

The ventral (macrogyric) portion of the dentate nucleus and the related lateral aspects of the posterior lobe of the cerebellum have undergone enormous expansion in great apes, and especially humans, paralleled only by the increase in prefrontal cortex. Trans-synaptic tract-tracing methods have demonstrated that the lateral zones of the cerebellum are organised into a series of parallel cortico-ponto-cerebello-thalamo-cortical loops, and that the prefrontal regions (and temporal and parietal association cortex) are linked with the lateral posterior lobe and associated ventral dentate. A large number of functional neuroimaging studies – typically using subtractive designs to eliminate activation due to

motor response – have demonstrated cerebellar involvement in various non-motor domains, including sensory discrimination, attention, working memory, episodic memory retrieval, and aspects of language.

Several studies of patients with discrete cerebellar lesions have revealed deficits in working memory/executive functioning, and sometimes in visuospatial skills. Studies of patients with neurodegenerative disorders are harder to interpret, given the possibility of extracerebellar involvement and the more pervasive visual, motor and speech disorder that may affect test results. However, there is strong support in the literature for cognitive impairment in SCA's 1, 2 and 3. Our own studies have compared SCA 6 ("pure" cerebellar) and FRDA (motor deficit disproportionate to cerebellar involvement due to spinocerebellar tract involvement) with SCA's 1 and 2. Preliminary analysis reveals impairment in SCA 6 equal to that in SCA 1, and greater than that in FRDA, despite greater ataxia severity in the FRDA group as judged by ICARS scores.

I-03 Fragile X Tremor Ataxia Syndrome

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The classical Fragile X syndrome of mental retardation has been known since the late 1970s/early 1980s, and any Genetics Service deals with a number of 'fragile X families'. It is therefore surprising, in retrospect, that the syndrome of ataxia and

tremor associated with the fragile X 'premutation' (FXTAS) took so long to be recognised. Since its first description in 2001, there has been practically an annual doubling in the number of papers on the condition. The syndrome usually has an onset from late middle age though to elderly. The male premutation carrier is the typical subject, but females are not immune. The clinical features are rather non-specific, although the neuroradiology can be very indicative. The likely pathogenesis involves accumulation of the (slightly elongated) messenger RNA in the nucleus of neurones, with the subsequent formation of intranuclear inclusions, and compromise of neuronal function. Animal models support this as being the mechanism. The new knowledge of this syndrome complicates genetic counselling in 'fragile X families', with the discovery of the classical Fragile X syndrome raising the risk in other family members of FXTAS, and vice versa.

I-06 Friedreich ataxia mouse models and disease pathogenesis

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Friedreich ataxia (FRDA), the most common recessive ataxia, results from a generalized deficiency of mitochondrial and cytosolic iron-sulfur protein activity due to partial loss of function of frataxin, a mitochondrial protein involved in Fe-S cluster (ISC) biosynthesis. Iron-induced oxidative stress and hampered superoxide dismutases

signalling have been proposed to be involved in the pathogenesis of the disease. This has led to the use of antioxidants for FRDA therapy. However, to this date, FRDA remains a devastating disease for which there is no cure.

We have generated conditional mouse models that reproduce important progressive pathological and biochemical features of the human disease, including cardiac hypertrophy, mixed cerebellar and sensory ataxia, ISC enzyme deficiency, and intramitochondrial iron accumulation. These mice models have enabled us to demonstrate that, contrary to previous thoughts, mitochondrial iron accumulation is not a primary event in the disease but is preceded by mitochondrial Fe-S enzyme. Moreover, we demonstrate that complete frataxin-deficiency does not induce oxidative stress in neuronal nor cardiac tissues. We identified an autophagic process as the causative pathological mechanism in the sensory neurons of the dorsal root ganglia. Finally, we have used these mouse models for therapeutic testing of different antioxidant compounds, and showed that idebenone has a significant effect on the cardiac function and the life-span of the murine model. In conclusion, these models are excellent models for deciphering the physiopathology of the disease and for testing pre-clinical therapeutic protocols.

O-01 Defective p53 response and apoptosis associated with an

ataxia-telangiectasia-like phenotype

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ATM, the protein defective in ataxia-telangiectasia (A-T) plays a central role in DNA damage response and signalling to cell cycle checkpoints. Once activated ATM phosphorylates multiple substrates including p53 to regulate cell cycle checkpoints and apoptosis. A cell line (ATL2ABR) from a patient with an A-T-like clinical phenotype was characterized by its sensitivity profile to various DNA damaging agents. Surprisingly, intermediate sensitivity to ionizing radiation but marked hypersensitivity to H₂O₂ and Mitomycin C placed its cellular phenotype between A-T and other ataxia oculomotor apraxia syndromes (AOA). This specific sensitivity profile was associated with a defective repair of DNA single strand breaks. ATL2ABR cells showed normal ATM activation and efficient phosphorylation of several ATM substrates. However, ATL2ABR cells did show a defect in the p53 response to radiation. Since no mutations were detected in ATM cDNA and a normal level of interaction between p53 and peptidyl-prolyl-isomerase Pin1 were detected, post-translational modification appeared intact in these cells but operated at reduced level.

Defective p53 stabilization was accompanied by defective induction of p53 effector genes and a failure to induce apoptosis in response to DNA damaging agents. Continued association between p53 and Mdm2 occurred in irradiated ATL2ABR cells in response to DNA damage. Incubation with the Mdm2 antagonist, nutlins, increased the stabilization of p53 and its transcriptional activity in ATL2ABR cells comparable to control cells. However, while nutlins also induced apoptosis in control cells they failed to induce apoptosis in ATL2ABR cells. These results suggest that ATM-dependent stabilization of p53 and induction of apoptosis by radiation involves an additional factor that is defective in ATL2ABR cells.

P-01 Screening of SCA2 and SCA3 in the Patients with Parkinson Disease in Korea

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The clinical features of spinocerebellar ataxia (SCA) are variable. Recent genetic screening for patients with predominant parkinsonian symptoms and in some cases of typical Parkinson disease (PD) showed a few cases of increased number of trinucleotide repeats on SCA-2 and SCA-3. PET imaging showed reduced striatal fluorodopa uptake and normal raclopride binding in a SCA-2 family, which was similar to other PET findings in idiopathic PD. No pathologic examination was made in

these patients, which could determine the extent and interaction of usual SCA pathology and Lewy body pathology.

Encouraged by these findings, we typed the SCA-2 and SCA-3 repeats for expansion in a series of 416 and 365 patients diagnosed with PD by the PD society Brain Bank criteria, respectively.

We identified two cases of mild expansions (34 and 35 repeats) in SCA-2. They were apparently sporadic, and had typical levodopa-responsive parkinsonism without cerebellar dysfunction. Further screening for other familial members of the patients is in progress.

Our work indicates that SCA-2 expansion mutations could be a rare cause of parkinsonism in the Korean patients. Gene study for SCA-2 should be considered in autosomal-dominant cases of PD, and sometimes even in the sporadic cases of PD if our patients were truly sporadic.

P-02 Friedreich Ataxia: disease progression, natural history and tools to measure disease severity

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Background: *Friedreich ataxia (FRDA) is the most common genetic cause of ataxia affecting 1:30,000. FRDA is characterised by progressive neurological symptoms, functional decline and*

cardiomyopathy. There is no gold standard for evaluation of neurological status in FRDA. To assess the benefits of therapies it is critical to develop accurate clinical outcome measures.

Methods: Data was collected in 50 patients at a single time point and 12 patients at 12 months using the ordinal rating scales the Functional Independence Measure (FIM) the Barthel Index (MBI), the International Cooperative Ataxia Rating Scale (ICARS) and the Friedreich Ataxia Rating Scale (FARS). These tests were compared to continuous measures of function comprising the 25-foot walk test (25FW), the 9-hole peg test (9HPT) and the Sloan Low contrast Letter Chart (SLCLC).

Results: *Concurrent criterion validity of the FARS is indicated by high correlation with the FIM and MBI (Spearman's rho = >0.88 p < 0.001). A natural history of decline can be detected with both the functional measures and continuous measures. The FARS and MBI scales can detect a significant change at a 12-month time point. However, the responsiveness of the FIM and MBI is poor compared to the FARS. The FARS has high correlation with the 25FW (rho = 0.95 p < 0.001) and 9HPT (rho = 0.9 p < 0.001) but only mild correlation with the SLCLC (rho = 0.58 p < 0.001).*

Conclusion: The FARS has good concurrent validity with functional measures and continuous physical measures. Ongoing work is required to maximise responsiveness of clinical measures.

P-05 Clinical and Genetic Heterogeneity of Autosomal Recessive Ataxia in a Hispanic Population

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Background: Friedreich ataxia (FRDA) accounts for ~75% of all Indo-European recessive ataxia patients. Approximately 98% of pathogenic chromosomes have expansions of a GAA triplet-repeat in the *FXN* gene (E alleles), and strong linkage disequilibrium among polymorphisms spanning this locus indicates a common origin of Mexican Mestizo and European E alleles. Clinically, Harding's diagnostic criteria have a sensitivity and specificity of 0.72 and ~1.0 respectively.

Methods: We screened 151 Mexican Mestizo patients with recessive and sporadic ataxia for *FXN* mutations and correlated these with the phenotype.

Results: 14/151 (9.3%) patients carried homozygous GAA expansions in the *FXN* gene, and no compound heterozygotes were identified. Interestingly, 30% of the mutation carriers had atypical FRDA and more than 60% of those who fulfilled Harding's clinical diagnostic

criteria for FRDA did not have *FXN* gene mutations, indicating the existence of non-allelic genetic heterogeneity. The non-FRDA patients were further screened for mutations in other ataxia causing genes, but all of them remained undiagnosed. Harding's diagnostic criteria for FRDA have poor sensitivity and positive predictive value in the Mestizo population: 0.692 and 0.375 respectively, but good specificity and negative predictive value: 0.874 and 0.963 respectively. This indicates that the use of clinical diagnostic criteria in Mestizo patients is useful for ruling out FRDA rather than to detect true cases.

Conclusions: Our data indicate that the underlying cause for recessive/sporadic ataxia in the Mexican population remains largely unknown, warranting further investigation of non-allelic heterogeneity. This should be considered in the management of Hispanic populations.

P-06 Accurate Humanised Mouse Models of Friedreich Ataxia

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Friedreich ataxia (FA) is an autosomal recessive neurodegenerative disease caused by a GAA trinucleotide repeat expansion within the first intron of the *FRDA* gene. The expansion results in the reduction of *FRDA* mRNA and in an insufficiency of frataxin protein. Knockout mouse models of FA display important

phenotypes of the disorder but do not accurately recapitulate the molecular basis of the disease and thus cannot be used to evaluate strategies for overcoming the effects of the GAA expansion. Our strategy is to generate accurate humanised mouse models of FA, which contain the entire human *FRDA* genomic locus with a long GAA expansion. Such mice should not only manifest the main phenotypic symptoms of FA but also provide the correct underlying molecular cause of the disease. Using single copy BAC vectors we have cloned and stably maintained long GAA expansions without contraction or rearrangements. Using homologous recombination, a (GAA)₅₀₀ expansion was introduced into the first intron of the human *FRDA* gene present on a BAC clone. The modified BAC was used to generate transgenic mice. This is the longest GAA expansion to be stably maintained in transgenic mice. RT-PCR and Western blot analyses confirmed that the presence of the introduced GAA expansion results in decreased *FRDA* gene expression and in lower levels of frataxin. The modified human transgene is able to rescue the embryonic lethality of homozygous *Frda* KO mice. Rescue mice are being assessed by a series of behavioural, neurological and histological tests for phenotypic symptoms of FA.

P-07 High Throughput Screening Using a Genomic Reporter Assay for Friedreich Ataxia Therapy

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Friedreich ataxia (FA) is characterised by neurodegeneration and cardiomyopathy induced by a GAA trinucleotide expansion in the first intron of the *FRDA* gene that causes reduced synthesis of frataxin. As the coding sequence is unaltered and there is a correlation between expansion length, the amount of residual frataxin produced and the severity of disease, pharmacological upregulation of *FRDA* expression may restore frataxin to therapeutic levels in patients. To facilitate screening of compounds that modulate *FRDA* expression in a physiologically relevant manner, we have established a Genomic Reporter Assay (GRA) consisting of a stable human cell line containing an *FRDA-EGFP* fusion (in-frame fusion of the *EGFP* gene with the entire normal genomic *FRDA* locus on a BAC clone). Following exposure to test compounds, *FRDA* expression is analysed by flow cytometry and Real Time RT-PCR. The anti-cancer drug cisplatin elicits the greatest level of induction (2.5 fold) of *FRDA* expression. In contrast, antioxidants have shown a time-dependent decrease in *FRDA-EGFP* expression.

We have optimised the assay for use in a High Throughput Screening format by measuring EGFP levels and cell viability fluorometrically. The assay was evaluated using cisplatin as a control test compound. A 1.6 fold level of induction by cisplatin was consistently obtained. Any compound that increases *FRDA* expression by more than three-fold should be detectable in the assay. A

small chemical library is currently being screened and analysed. An increase in frataxin levels by several-fold in FA patients could be therapeutic.

P-08 FRDA-EGFP Genomic Reporter Transgenic Mice

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Friedreich ataxia (FA) is an autosomal recessive neurodegenerative disease caused by the expansion of a GAA trinucleotide repeat within the first intron of the *FRDA* gene. The GAA expansion results in the reduction of *FRDA* mRNA and in an insufficiency of frataxin protein. To elucidate the mechanisms regulating *FRDA* expression and to develop an *in vivo* assay for agents that might upregulate *FRDA* expression in a therapeutically relevant manner, we have generated transgenic mice with a BAC genomic reporter construct consisting of an in-frame fusion between the *FRDA* and *EGFP* genes. Production of full-length frataxin-EGFP fusion protein was demonstrated by immunoblotting. EGFP expression was observed as early as day E3.5 of development. Most tissues of adult transgenic mice were fluorescent. The level of *FRDA-EGFP* expression in peripheral blood, bone marrow and from enzymatically disaggregated tissues was quantitated by flow cytometry. A two-fold increase in EGFP expression was observed in mice

homozygous for the transgene compared to hemizygous mice. The *FRDA-EGFP* transgene also rescues the embryonic lethality of homozygous *Frda* knockout mice without any signs of behavioural or histological abnormality. The *FRDA-EGFP* transgene thus appears to produce a fully active bifunctional hybrid protein. These transgenic mice will permit the examination of spatial and temporal aspects of *FRDA* gene expression, and the preclinical evaluation of pharmacological inducers of *FRDA* expression in a whole animal model. Additionally, tissues from these mice should be valuable for stem cell transplantation studies.

P-09 Gene Regulation Studies of the Friedreich Ataxia Locus Using Genomic Reporter Assays

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Friedreich ataxia (FA) is a progressive neurodegenerative disease caused by a trinucleotide repeat expansion in the first intron of the *FRDA* gene resulting in insufficiency of frataxin protein. As the coding sequence of the *FRDA* gene is unaltered, targeted upregulation of gene expression may restore cellular frataxin to therapeutic levels in patients. Understanding the mechanisms controlling *FRDA* expression should enable a rational approach for the pharmacological restoration of gene expression and the therapy of FA. Currently no information is available about the

position of any long-range, *cis*-acting regulatory sequences that regulate human *FRDA* expression. We have established a system for the bioinformatic identification and experimental verification of regulatory mechanisms that direct the expression of the *FRDA* gene. Utilisation of data from the sequence assemblies of the human and other mammalian genomes for cross-species comparative genomics analysis has identified a number of conserved, non-coding regions surrounding the *FRDA* gene. The role of these sequences will be evaluated by their deletion in the context of an *FRDA-EGFP* genomic reporter, consisting of the in-frame fusion of the *EGFP* gene to the entire normal genomic *FRDA* locus on a BAC clone. To facilitate studies in human cells we have developed a dual-reporter enhanced BAC (EBAC) vector. The EBAC can be stably maintained episomally and includes the *FRDA-EGFP* genomic reporter and the gene encoding DsRed-Express fluorescent protein. The EGFP/DsRed-Express ratio will provide a sensitive and specific assay for detecting the effects of deletions of regulatory regions of the *FRDA* gene while concurrently allowing for correction of differing transfection efficiencies.

P-10 SCA 7 With Late Retinal Degeneration From India

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Background: Retinal degeneration constitutes an important component in early adult onset patients with Spinocerebellar Ataxia type 7 (SCA7), a neurodegenerative disorder caused by expansion of an unstable CAG repeat on chromosome 3p12-21.1. We report the phenotype of genotypically confirmed SCA 7 patient who did not show retinal degeneration till 2 years of disease onset.

Method: Size of the (CAG) array of expansion of SCA 7 locus was detected by gene scan analysis in ABI prism 377 automated DNA sequencer and it was 50 whereas normal alleles had 7 –17 repeats (200 chromosomes).

Results: The patient was a 29-year-old man who had progressive gait ataxia since the age of 27 years. His father suffered from similar problem since the age of 47 years. On examination he had visual acuity of 6/36 bilaterally, normal visual field and color vision. Fundoscopic examination revealed neither macular pigmentation nor disc pallor. There was no nystagmus or ophthalmoparesis but saccades were mildly slow. He had gait ataxia and mild incoordination in the upper limbs. All deep tendon reflexes were brisk and plantars were flexor. Visual evoked responses were normal in latency and had reduced amplitude bilaterally. MRI of brain revealed cerebellar and pontine atrophy. Nerve conduction studies in limbs were normal.

Conclusion: Phenotypically ADCA II or SCA 7 diagnosis was not thought.

This case emphasizes the need for genetic confirmation of type of SCA. It also reveals that some cases of early adult onset SCA 7 may have late appearance of visual symptoms and signs.

P-11 A novel saccin mutation in a Japanese showing clinical uniformity of autosomal recessive spastic ataxia of Charlevoix-Saguenay

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Background: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) was originally described among French Canadians in the Charlevoix-Saguenay-Lac-Saint-Jean region of Quebec (OMIM 270550). The gene responsible for ARSACS was identified as saccin, and a frameshift (8585 deletion T, 2805X) and nonsense (C7245T, R2355X) mutations were reported in Quebec.] Recently, patients with other mutations have been described in some specific countries.

Patient: A 39-year-old Japanese woman, who felt unsteadiness in her gait and clumsiness in her hands after age 35. The SNAP was not evoked in any of the limbs. MRI revealed marked atrophy of the cerebellar vermis limited to the portion above the pyramids.

Method: After informed consent was obtained, genomic DNA was extracted from leukocytes of the patient. The coding exons of the saccin gene were amplified by PCR and sequenced directly.

Result: A homozygous C-to-T transition at position 3774, which results in substitution of glutamine 1198 for a stop codon (Q1198X) was revealed. This mutation was absent in 100 chromosomes of the normal controls.

Conclusion: We found the nineteenth saccin mutation, which resulted in a shorter truncated protein than those of French Canadian patients. Except for the minor variations, all ARSACS patients showed almost uniform clinical features; spastic ataxia following progressive peripheral neuropathy. Most mutations are homozygous nonsense and frameshift leading to early stop codon in various positions, causing loss-of-function of saccin protein. Domain structure of saccin and the conformational change by mutations may clarify the mechanism of ARSACS.

FUNDRAISING – 2005

The year has been marked by significant fundraising efforts. We are especially grateful to those who participated in the annual golf day at Commonwealth Golf Club; to the participants in “Go the Tan” in Melbourne; to Renee Spruyt of South Australia for his wonderful donation of wine from his vineyards and to the Canning Family whose contributions are acknowledged elsewhere in this newsletter.

We also have to acknowledge the ongoing support of those who organize and attend our functions. In this regard the members of YAFFA

(Young Australians Fighting Friedreich Ataxia) and their friends have been wonderful contributors, particularly through their FA Ball.

We are very fortunate also that individuals and service clubs continue to support us to the extent that they have in the past. This has allowed us to make significant contributions to the latest research ventures in Australasia with a view to speeding up the dates for providing treatment to our sufferers.

Special Thanks to the Gleeson Family:

FARA has received generous support from many sources since its formation about three years ago. One family deserves special recognition for their generosity.

Jack & Joyce Gleeson from Townsville, and their family, including Patricia who suffers from FA, have donated an extraordinary \$115,000 to FARA. After an initial contribution of \$15,000, FARA has received a further \$50,000 per annum for the last two years.

Their Gleeson family contributions have made an enormous difference to the effectiveness of FARA, and to the amount and pace of research taking place.

On behalf of all FA sufferers and their families, I would like to say a very big thank you to the Gleeson family.

FARA is also pleased to extend to the Gleeson family FARA's first

Honourary Life Membership of FARA.

*Mike Dwyer
Treasurer.*

FRIEDREICH ATAXIA ASSOCIATION OF VICTORIA (FA VIC)

Event: Annual General Meeting (AGM)
Date: Sunday 13th November 2005 (2pm)
Venue: Murdoch Children's Research Institute (MCRI) at the Royal Children's Hospital

- 1. Welcome** – Steve Beetham – Approximately 20 people in attendance.
- 2. Acknowledgment** – Special thank you in remembrance of the important work done by Panos Iannou for FA.
- 3. Apologies** – Tim Curran (Treasurer) and Mick Coffey (Secretary).
- 4. Minutes** – Minutes of previous AGM accepted as read.
- 5. Treasurer's Report** - Presented by Steve Beetham in Tim Curran's absence. Financial statements circulated including independent auditors report.
- 6. Secretary's Report** – Presented by Steve Beetham in Mick Coffey's absence.

- Letter written to the minister for health regarding podiatry surgery.
- Letter written to Victorian Government offering comments from a FA perspective for inclusion in the Review of Disability Legislation.

7. President's Report – Presented by Steve Beetham

- Thank you for the great work done by Darren & Sandie Keeble stepping down from the FA Vic committee.
- Gordon Marriott and Tanya Coffey on FA survey
- Mention of a \$30,000 grant awarded to FA VIC by Justin Madden to participate in a gym program via Nunawading.
- FA VIC website to become a page on the FARA website
- Thankyou to the FA clinic and staff for their wonderful work.
- FA VIC remain the only state based representative body, but the majority of future fund raising and research activities to be conducted via FARA. FA VIC will remain for specific grant applications and fund raising purposes for Rehabilitation purposes.

8. Elections – Mr A Denson acted as returning officer. All positions were elected unopposed.

- Steve Beetham as President
- Mick Coffey to assume joint role as Secretary/Treasurer
- Tim Curran steps down as Treasurer to join FARA.

- FA VIC committee remains unchanged.

9. Presentation - Associate Professor Martin Delatycki (Director of the Bruce Lefroy Centre, Head of MCRI FA Research Program Clinical Arm)

10. Presentation - Dr Joe Sarsero – Head of MCRI FA Research Program Laboratory Arm

11. Friedreich Ataxia Research Association (FARA) Update – Presented by Steve Beetham

FARA has an executive committee which is responsible for the allocation of funds to applicants, and also a Scientific Advisory Committee whose responsibility is to advise on the allocation of funds to applicants.

- Research Update; A number of promising FA research /programs are currently underway including;
 - Ongoing research into the drug Mitoquinone. It is hoped the first world trials of the drug will commence in 2006.
 - A Drug screening program, to investigate other drug possibilities with regard to the Frataxin Protein. The program will screen approximately 2,000 FDA approved drugs and expected to commence in January 2006.
 - Indebenone Clinical Trials in the US; This study will

determine whether a drug called idebenone is safe and effective in reducing the level of oxidants that are believed to damage the nervous system and hearts in patients with Friedreich's ataxia.

- Fund Raising Update; Fund raising activities have gained momentum under full time employee Varli Beetham. Key events include
 - sponsored lunches
 - annual FA Golf Day
 - YAFFA ball
 - 'Go the Tan' fun run
 - wine donation
 - Dennis Family Homes Auction.
- FARA website will be the source of information on the research activities into FA (www.fara.org.au).

12. General Business & Questions

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