

EuroAtaxia Conference Paris 9th-10th November 2007

Report by Laura Rooke

This year, EuroAtaxia's annual conference was hosted by the French organisation *Association Française Ataxie de Friedreich*. Here I summarise the scientific presentations that took place over the two day event.

Friday 9th November

Dr Alexandra Dürr started off the morning session, which was focused mainly on the dominant ataxias, by talking about the activities of the SPATAX (spastic paraplegia and ataxia) network. Researchers in this network in the UK are Nick Wood (London), Thomas Warner (London) and Evan Reid (Cambridge). Since 2001, the network has been assembling a collection of families and working on the mapping and refinement of several ADCA loci. They also plan to use clinical tools developed by members to analyse phenotype/genotype correlations. Some studies already have revealed variability in symptom manifestation with different types of mutations; for example, CAG repeat expansions seem to cause a more quickly progressing disorder, with greater pontic/vermial atrophy on MRI, whereas point mutations are associated with less severe disorders, and a larger variation in age of onset. Analysing the differences in symptom patterns could be helpful for deciding which genetic tests to perform when assessing ataxic patients e.g. if patient has had very slowly progressing symptoms for more than 10 years might be better to concentrate on testing for point mutations rather than CAG repeat length.

Dr Annie Sittler next gave a talk on research looking at the role of PML (promyelocytic leukaemia) nuclear bodies in SCA7. PML bodies are nuclear proteins which are involved in various processes including apoptosis and tumour suppression and certain ones (PML IV) have been implicated in the toxic aggregation of ataxin-7 in SCA7. When mutant ataxin-7 is present, PML IV recruits it into nuclear bodies and the mutant protein is degraded, destroying the normal fibrillar structure of PML aggregates. PML IV may therefore represent a new therapeutic target for SCA7, and the researchers are now going to look at the effects of Beta-interferon (which mimics the PML IV effect) in SCA7 mouse models. The mouse models that Sittler has have a very severe form of SCA7 (with 266 repeats) and have had problems with breeding.

The EUROSCA project and the current research taking place under the EUROSCA umbrella was presented by Prof Thomas Klockgether (Clinical Coordinator). He announced that the registry of ~4000 patients established by the project will be online in a few days and listed the trials currently being supported by researchers in the network; these were lithium in SCA1 (phase I), VPA in SCA3 (preparing for phase 1), 4-aminopyridine in SCA1 (phase II). Santhera have rejected a proposal to test idebenone in SCA3, but other drug targets in the pipeline included HDAC inhibitors for SCA, stimulation of autophagy (Rubinsztein) and inhibitors of protein aggregation.

Dr Paola Giunti introduced the clinical features of the newly identified SCA11, which include downbeat nystagmus and saccades (demonstrated by video), truncal ataxia, intention tremor and dystonia. Work on the gene identified a frameshift mutation in tau tubulin kinase 2 (TTBK2) gene which was likely to be pathogenic. Sequencing of the SCA11 gene in further families is ongoing and the plan is to sequence all available ADCA families to estimate the mutation frequency and collect data on genotype/phenotype correlation.

Another new SCA which has been identified was talked about by Dr Franco Taroni. SCA28 has been studied in 17 patients with an age of onset between 12 and 64 (mean 34.8). A region of 74 genes on chromosome 18 was studied, and after prioritising those expressed in the cerebellum to screen more thoroughly, a missense mutation was found which coded for a protein highly expressed in perkinje cells. The frequency of this SCA28 mutation was found to be 2.7% among ADCA in Italy, with variable penetrance of the gene (some members of families had no symptoms but had MRI changes). Non-expansion SCA genes now outnumber the polyQ expansion ones.

Prof Michel Koenig talked about a new recessive ataxia involving a mitochondrial gene ADCK3, which was identified in a family with secondary consanguinity (second cousins). The characteristics seen in the family were cerebellar atrophy and ataxia but without many other symptoms except for exercise intolerance in several members (due to lactic acidosis build up). ADCK3 protein appears to have an ancient function related to non-protein kinases and leading to partial CoQ10 deficiency (possible role in ATP level feedback loop). This is the 5th mitochondrial protein to be implicated in the recessive ataxias (others are frataxin, ABC7, Twinkle, POLG).

Olaf Reiss talked about transgenic animal models for SCA3. Ataxin 3 has many functions, including acting as a 'shuttling mechanism' for transporting misfolded proteins from the E.R, and protease activity. A mouse model was being created to help decipher the biological function of ataxin 3 further and to develop treatments. Reiss showed a fantastic video of an ataxic mouse and pointed out how the widespread legs and shakiness helped demonstrate ataxia in the animals. He showed how gait can be monitored in mice using footprints which looked chaotic in ataxic mice compared to normal mice.

A talk on biomarkers in neuro-degeneration was given by Prof Stefano Di Donato. He said that adenosine receptor signalling was found to be affected in brains of people with HD and was now being investigated in FA, SCA1 and SCA2. They found that the activity of the endocannabinoid enzyme FAAH is greatly decreased in HD, pre-HD and SCA (but not in FA) and that this led to much higher levels of anandamide being found in these patients than in controls. Therefore this enzyme (FAAH) could be a potential therapeutic target. He also talked about the decrease in cholesterol biosynthesis seen in brains of HD mouse models.

Hélène Puccio gave a summary of advances in FA research, concentrating on FE-S clusters. Deficiency of frataxin in FA seems to trigger deficiency of many FE-S containing proteins in cells (both mitochondrial and extra-mitochondrial proteins). This may be important for neurodegeneration as at least 3 enzymes of DNA repair are FE-S proteins. There are many more FE-S proteins to be identified- the ones identified so far are likely to be the most stable, but have also showed tissue-specific instability.

Pierre Rustin's talk followed on nicely from Puccio's, as he was talking about iron clusters as a target at risk in FA. He said that frataxin has a role in presenting iron to be incorporated into proteins, and when there is a frataxin deficiency, the iron accumulates abnormally and causes the production of radicals via the Fenton reaction (oxidation). This leads to the disruption of normal structure (e.g. actin fibres disorganized?). Aconitase is a major mitochondrial enzyme which has been found to be defective in FA patients, and which requires FE-S clusters, therefore this could be a major target in FA. Idefobone has been found to recover this protein up to almost normal levels, (in neuroblastoma cells), but iron-chelating treatment (e.g. deferiprone) appears to inhibit aconitase in a time-dependant fashion, which also appears to

inhibit the growth of cultured skin fibroblasts. Therefore Rustin suggested that it is perhaps not a good idea to use iron-chelating treatments in FA where aconitase is already low, and where there might be untold effects on other iron containing structures (such as FE-S cluster proteins).

Dr Joel Gottesfeld started his talk by describing the history of his research into HDAC inhibitors, which turned out to be a very clear explanation of the science behind these compounds. He started out by trying to create molecules which would bind to GAA repeats and prevent the silencing, and then (after Festenstein published research on the repressive chromatin structure around GAA repeats) looked at heterochromatin proteins which surround the frataxin gene. Studying this structure revealed a model for heterochromatin-mediated silencing of the gene, where histone deacetylases (HDACs) were being recruited to the chromatin causing an altered, condensed structure which could no longer be read for transcription. Thus, if a molecule could be found which would inhibit the deacetylases, the gene may be 'unsilenced'. Molecules identified with these properties have been studied (particular compound 4B) and shown to increase frataxin levels to normal levels in cells from FRDA carriers and to the level seen in carriers (~50% of normal) in FRDA patients. Perhaps most interestingly, mice models for another condition (with CAG repeat mutations) who were given compound 4B in drinking water had a reversal of neurodegeneration, less weight loss and improved motor performance compared with control mice. The pharmaceutical company Repligen, who hold a licensing agreement with the research institute to further develop these compounds, have already tested pharmacokinetics and metabolic stability and have found favourable drug-like properties but a less than ideal therapeutic index so are now looking at synthesising derivatives of these structures which might be more effective. Repligen are also applying for licenses for phase 1 trials.

Clinical trials in FA

Isabelle Husson kicked off this session by talking about the clinical trial with pioglitazone starting in France. Glitazone is a PPAR antagonist which is used as a treatment for diabetes. She reasoned that because diabetes is frequently a complication of mitochondrial disorders, the treatment must not be toxic to the mitochondria. As well as for FA, pioglitazone is being tested for other neurodisorders including spinal injuries and has shown "spectacular" results in an MS patient (case study- revealed improvement in coordination and strength, memory and but no effect on demyelination on MRI) which has informed about dose needed and the ability to cross the BBB. The action is thought to be a combination of the inducement of proteins involved in energy production, neuroprotection, antioxidant and increasing the stability of FE-S clusters. Because of the increased heart failure observed in diabetes patients, FA patients with cardiac insufficiency are being excluded from the trial at present. The trial is randomised, double blind trial with 15 patients in each group, and the trial will last 2 years. Patients are allowed to continue with current treatments such as idebenone if taken at the start and continued at the same dose.

Dr Susan Perlman from California talked about the US ataxia clinical trials network, which has its base at the coordinating centre in the University of Rochester, NY where the structures were already in place for HD. There currently are 16 projects in 7 categories (as seen in the FARA pipeline), with the majority in the research/pre-clinical phase. Idebenone is at the forefront of development with trials starting this month at UCLA and Children's Hosp Philadelphia. The mitioquinone trial which completed phase 1 testing in normal control subjects in 2006 has not progressed to clinical study due to the FDA and Australian regulatory bodies requiring more info on animal studies and safety. The pharmaceutical company concerned (Antipodean) is

looking at mitochondria for other indications (e.g. hepatitis) and developing additional mitochondrial disease targets, but seem to have dropped interest in developing for FA.

The deferiprone trial was described by Arnold Munnich (France). The idea for using the iron chelating treatment was stumbled upon when follow up of children with Friedreich's ataxia revealed that those who had been given oral iron for anaemia had rapid worsening of their gait ataxia. This raised the question of whether there was mis-compartmentalisation of the iron, and whether frataxin was involved in iron donation for heme synthesis? In addition, when the researchers carried out MRI visualisations of patient's brains, they found that there was iron accumulation in the dentate nucleus. The iron-binding compound deferiprone was suggested by haematologists who have used the treatment on thousands of children and because it can cross the BBB, but does not cause iron deficiency.

In the first small clinical trial using deferiprone, 11 adolescents/young adults were given 1 year treatment with 5-10mg/kg/day. The results were a decrease in iron in the dentate nucleus, with a greater effect in those who had the higher T2* value on MRI at the start. There was a maximum effect after 2 months treatment. Some unexpected side effects arose, including coldness, pain, tremor, voice changes, and there were 2 major adverse events (severe agranulocytosis (reversible) in one patient and an extreme neurological reaction to a very high dose in one patient). The new multi-centre trial will be a double-blind, placebo controlled trial to test safety and efficacy and patients will have their bloods monitored very closely. Munnich is now interested in considering whether other mineral deficiencies are important e.g. copper?

In an unscheduled talk, Barbara Schieber-Mojdehkar added a bit about the ongoing EPO trial which is now in the extension phase. So far the mean change in frataxin levels which has occurred in the trial is around 27% compared to individual baseline levels and there has been a significant improvement on ataxia rating scales (SARA scale), along with a significant reduction in markers of oxidative stress. The doses used were 5,000-20,000 IU rhuEPO 3x a week. It is still not known how the rhu-EPO is exerting its effect on frataxin levels.

After a brief coffee break, Paul Konanz from the Friedreich's Ataxia Parent's Group in the US spoke about how patient organisations can collaborate with researchers to remove barriers (such as time, money, and communication) to speed up research. A heated group discussion then took place about getting patients involved in clinical trials, with some patient representatives concerned those patients who were older or at a more advanced stage were being excluded from trials. Pandolfo chaired this discussion, and explained the point of view of researchers needing to conduct the trials in a scientific way and how this would be important for drug regulatory authorities. Susan Perlman also commented on the practise of researchers involved in trials being able to compassionately prescribe treatments under study to people not normally eligible to take part if a clear benefit is being seen.

Saturday 10th November

The conference split into two parts this morning, with the patient representatives having a timetabled series of talks on sharing information and how best to help people with ataxia and the scientists who remained getting together for informal updates on current research.

Richard Festeinstein talked about his ongoing research looking at position effect variegation around the GAA repeats in Friedreich's ataxia. He talked about the class 3 HDAC inhibitors (e.g. nicotinamide), which have shown small increases in frataxin

levels in cell lines, but with different results in mice cells and in human cells and which not been as effective as Gottesfeld's compound 4B. Festenstein will now look at other classes, particularly class 2.

He showed some graphs of the transcription activity around the FRDA gene, which demonstrated an increase of Negative Elongation Factor (NELF) over GAA repeats, suggesting there is a slowing down of transcription at particular sites, linked to RNA polymerase pausing. It has been observed that RNA pausing is not apparently dependent on repeat length, but on PEV, which may explain how phenotype can vary in individuals instead of correlating with number of repeats.

The ongoing need for an improved mouse model of Friedreich's ataxia was brought up by Mark Pook, who talked through some of his work on transgenic mice with and without GAA repeats. It is necessary to create these models because these repeats do not occur naturally in mice, and Pook has now been analysing the promoter regions and CPG 'islands' in the code. Normally CPG sites are non-methylated in promoter regions, but in the frataxin gene in humans there is an area (just before exon 1) of 4 CPG sites which have DNA methylation, to varying degrees in different tissues (e.g. heart and brain). His research has found the patterns are opposite in the mice models.

The afternoon was reserved for the EuroAtaxia AGM and election of officers.

The overall conference felt quite skewed towards Friedreich's ataxia, and that was the interest of the vast majority of the patient representatives present. However, the number and enthusiasm of people present showed that this is a time of big interest in Europe in ataxia research, and that there is a climate of willingness to share knowledge and materials with other researchers and with the wider community, such as patient organizations, to the benefit of all concerned in ataxia research.