

DR ALAN WILTON - ATAXIA RESEARCH PROGRAM

Update on Recent Developments September 2007 From Dr Alan Wilton

Microarrays are now available and as modern technology is advancing very fast we can possibly do this – it is the cutting edge of this sort of research. We look at several control dogs and several affected. We don't need a whole bunch of samples with all the relatives to make pedigrees. These particular microarray allow us to look at 20,000 different sites of the dog genome in one go. A microarray is very small, about 2 inches by 1 inch and it costs about \$500 an animal to do, but you only need about ten affected and ten controls - and we are looking for regions where there is very little variation in the affected individuals because that is where we will find the disease gene, They will have inherited both copies of the disease gene from the common ancestor and they will be the same defect in both copies of the gene. That is the idea of the whole genome scan microarray approach.

They are very expensive but would greatly reduce the time taken to discover the disease gene. We have been seeking additional funds to allow us to take this fast track approach. The Snow family, who own a Bench Kelpie, have made a large donation towards the research to allow us to take this approach so we can develop a test quickly (hopefully within 6 months).

This means we need as many samples as we can get quickly. We have a good start. Once we find the gene we need to develop a test and we hope to find further funding for that. Since the research time will be considerably quicker than first anticipated the WKC may be able to provide some funding that was committed for future years. We have approached the American Ataxia Foundation and are seeking tax deductible from other owners and breeders as well as sponsorships from companies.

An attempt to spread the word of these exciting developments through the media has resulted in some adverse publicity. (*See copy of the Victorian Weekly Times article above*) A rogue reporter has been very divisive. They have misquoted and manipulated information for sensationalism with total disregard for the facts. They have ignored all the work the WKC has put into understanding and controlling the disease in kelpies. At this vital time in the breed we need, now more than ever, to pull together to lick this dreadful affliction.

Lecture given at the WKC Annual General Meeting April 2007.

Dr Alan Wilton - Ataxia Research Program

We have been talking to the Working Kelpie Council about setting up a research program to look at the Ataxia problem in Kelpies. We have been working with genetic diseases in dogs over the past twelve or so years, mainly in Border Collies. There are three common genetic diseases in Border Collies. One is a nerve degenerative disease with an age of onset about eighteen months. It is a nasty disease because the pups have already gone to new homes and they have no idea that this devastating disease will be inflicted on them later. More recently, we have worked on an immune problem in puppies. It is widespread in both working Border Collies and the show Borders, and it is in the English lines as well as the Australian lines. So it is due to a mutation, which goes way back to the origins of the Border Collie breed.

I have brought along my assistant, Jeremy Shearman, who has done all the work on the immune problem in the Border Collies and he has done a great job. While it took something like ten years to find the gene for the nerve degenerative disease, it has taken him only two years to find the gene for the immune problem. He will be the person who will do the research on the ataxia in Kelpies. The other person listed on the grant proposal is Bill Ballard. He is another geneticist who has recently joined us at the University of New South Wales. He breeds Australian Cattle dogs. He shows them and has champion dogs - and he is interested in genetic diseases, particularly in cattle dog. He will also be working with us.

The aim of the project is to detect the genetic defect which causes this Ataxia in Kelpies which goes under the other name of Cerebellar Abiotrophy, which is the clinical name. Here is what we need to do. The dog genome is 2500 million base pairs and we have to find the one that is causing the problem. It is like looking for the needle in the haystack. Improvements in technology have greatly accelerated the ability for us to do that. The dog genome was published a few years ago and we can use that information to help us in the search. That is what we hope to do.

How are we going to do this? Well, together with the Working Kelpie Council and some Border Collie groups we are putting in a linkage grant application, which is a Government Grant where they will match the funding put in by industry partners, and industry is anybody who is non government. The reason that we combined the proposal with the Border Collie work is that we thought we would have a better chance at funding with a project more general than one dog breed. The Border Collie work that we are doing is nearing completion and within the next month or two Jeremy will have identified the genetic defect, then we can move on, spending most of the time on research into the Working Kelpies and

Ataxia.

The way the Granting process works is that the Government will match funding put in by the partners, and that can be either cash or in kind. The agreement we set up is not a strictly legal document, so if anything suddenly happens and one party does not come up with the money – everything doesn't fall apart. The proposal is that \$8,000 a year from the Working Kelpie Council will go towards buying the consumables and things that we need. We also cost into the income the contributions for the personnel who will be working on the project, so hopefully we will have people from the Council helping us, providing samples and information, and also informing breeders about the problem and the research that is going on, and what we need. So all those things have been factored into an application between the Border Collie groups and the Working Kelpie Council. We have asked for \$70,000 a year but they never give you what you ask for, even if we are successful. We might get \$30,000 or \$40,000 a year, so that might be enough to allow us to undertake the project.

Even without the money from the Government, we will still undertake the project – it just means we won't have quite as much money to spend on lots of things. Part of that money is a scholarship for Jeremy – he is working a couple of days a week at the moment somewhere else to support himself – so these things have to be taken into account. That is how linkage grants are set up. The results of the application will be known sometime in May, and if successful it will start in July – and whether successful or not Ataxia is something we definitely can make progress towards a DNA test and we will undertake the project whether the grant is successful or not. [Addendum: The grant was not successful but funds are being sort from other sources and the project is already well underway]

We need samples from animals that have the disease, and from their relatives. So that if any litters come up with ataxia affected individuals - we would like to get blood samples from all of the members of the litter, the affected individuals and all the unaffected individuals, as well as the parents and the all the grandparents that we can get. We will use the samples from these individuals to look at their DNA and try to work out the whereabouts of the ataxia mutation amongst all the genes in the dog.

We also need to have samples from animals that have nothing to do with Ataxia cases because we need to compare affected individuals and unaffected individuals to identify what is normal variation - like the differences in DNA between you and I – except in the dogs. We need to know what are just normal differences and which changes are the important mutations. We need to get at least an equal number of controls as affecteds.

As I said, the completion of the dog genome will allow us to do a lot of this work and is very useful to us. We also have a lot of information on organization of human disease genes which can be used for dog gene research – many of us go to the doctors a lot and there is a lot of information available about human disease. And there are a lot genetic diseases in humans, which are like Ataxia. We use information from diseases in people to try to work out what genes might be involved in dogs. They are the target genes or candidate genes. The reason for doing this research is to help the dogs [the breed]. There are many disease genes that occur in dogs and ataxia is just one of them. Most breeds have their problems – usually they are hidden under the carpet. We applaud the efforts of individuals who report disease cases because it is through these people who are very keen and concerned that something can be done about it. Without the realisation, that there is a problem, nothing can be done about it. So there shouldn't be any stigma attached to the breeders of animals who have this problem - you have no control over this sort of thing. It is only by making information about disease cases public that something can be done about it.

Besides information on Kelpies, there are a lot of benefits for looking at new diseases, such as working out the function of genes and understanding normal development. They are a sort of spin off from this type of work and we try to sell it to the granting agencies and people interested in human genetics instead of dog genetics as well.

As I said, humans are a good model for these genetic diseases. The human genome is very advanced in identifying the genes and what they do. There are many diseases identified and they make good candidates when we are looking for dog disease genes. As an example, there can be mutations in a particular gene, doesn't matter what it is, called Syn1, that result in a form of cerebellar atrophy (ataxia) in humans. So that makes a good candidate gene for the kelpie ataxia. We can see if that is what is the problem in kelpie ataxia. So we go to find this gene in the dog – and Jeremy is very adept at doing it - in the Border Collies he checked fifteen candidate genes before he found the right one. Here are some possible candidates. Number thirteen (on this list) could be a good candidate to start with. There are many different candidates here - a whole bunch of them – and Jeremy has a paper listing some, and if anybody is interested I've brought photocopies of the front page to hand around. There are many of these candidate genes we can look at to see if they are useful.

How do these sorts of things occur – the breeding structure in dog genetics, and in any domestic animal, are what results in these genetic problems occurring, and they will continue to occur while people still use the same sort of breeding structure. Basically, take a champion dog and breed from it

extensively, then do what is called 'line breeding', basically inbreeding – breeding related animals, and the result is that the same genes are passed down from both parents. So this is an example of a breeding of Border Collies (shows pedigree), so we have a pedigree here with q genetic disease and the data shows a mutation must have been present in the common ancestor. And if inherited down two different lines to an individual, you get two identical copies in these individuals. Basically, the problem is that the genes aren't working. A protein is not being made. It is not functioning and this will result in genetic diseases.

There have been a lot of genetic problems in people in countries like India where mating between relatives is quite common. There is little you can do about it other than avoiding inbreeding, but if you identify the genetic defect we can do DNA testing to find individuals which are carriers and which ones aren't. In addition, we can then slowly remove the condition from the breed by doing DNA testing and selective breeding. That is the plan.

Basically, all individuals who are affected with the gene come from the common ancestor who was carrying the gene. It happens in all breeds of dogs, and they all have different genetic diseases. It is very useful for human genetics because we can use these conditions in dogs that are very rare in humans, and we can't find a great deal out about them - we can actually use the dogs as models by these human genetic diseases. So it works both ways..

There are two approaches we can take. The first approach is the candidate gene approach. We can look through the literature and find genes we think are likely to be involved. We can have a list of symptoms and the genes that cause ataxia in humans or other dogs. One of the things which is wrong in the ataxic Kelpie is they are missing a particular type of cells in the brain, the Purkinje cells. So we look for loss of Purkinje cells in human ataxias and those conditions are the possible problem in the dog. We can look for those genes and we can test them. And that is what Jeremy is very good at.

The second approach is to look everywhere in the dog. Look at every chromosome. There are thirty-nine chromosomes in the dog and we can look at each one until we work out where the gene is. We can narrow down the possibilities. We can use mice as models and pick out all the genetic conditions with Purkinje cell degeneration. There are forty-one different genetic defects in mice that cause this sort of degeneration. That is forty-one candidates we can look at. There are other dogs which have ataxias – it occurs in the kelpies and in other breeds of dogs – they all make good models for kelpie ataxia. So that is one approach.

Another approach is trying to identify the whereabouts of the disease gene in the genome without knowing anything about its function. For this the technology used is DNA fingerprinting as used in paternity testing, the things you see on CSI or crime shows on television. They are very useful for us, as they are spread all over the genome and we can use them to try to locate the diseased gene. We will try to do that

To do that we need pedigrees, we need information about affected dogs and relatives, and we have all the thirty-nine chromosomes, we have to look at each one of those. The idea is we have genetic markers spread along all of the chromosomes, and we can use these genetic markers to examine those ataxia pedigrees and try to look for the disease gene there.

So even if you don't think the animals are involved in Ataxia it is still very useful for us to get samples from the animals because once we find something we can start eliminating different lines, and determine whether they carry the Ataxia or not. Being able to place the information together, the genetic information we gather, we need to put it together in the pedigree.

The most important things are samples related to cases, but samples from important dogs in breeding are needed also as they will be very useful for us in the future. We need to get them now while they are still alive – the older dogs are not likely to be around in the near future.

I can't give you a time schedule when this will occur – it is a bit like rolling a dice. If any of you are unlucky, you have any special lucky charms or something, use them. As I said the disease Jeremy was looking for in Border Collies was the fifteenth. It can be the first one or it can be the one hundredth you look at. So that is why there are several different approaches going at the same time. But none of the approaches work unless we have samples.

The best samples for us are blood samples because when we are doing the research we need a fair bit of DNA. We may have to do hundreds of tests on the research sample. When we have a developed a test, then it doesn't take very much DNA to do the test, you only need to do it once. The best samples for us are blood samples. Swabs can be taken from the mouth and that will provide us with some DNA. Swab can be taken with cotton buds, or we have special little nylon brushes which you rub just inside the dog's mouth for about twenty seconds or so. I think the Council has bought some sample kits which will allow people to take small blood samples – I see they are here. That will allow people to take a sample from their dogs at home and send it in rather than take it to the Vet – who will take blood from a vein in the leg, like they do to humans in the arm. This will give plenty of information. But you can take a sample at home. Basically you can clip the ear – lance it to put a little hole in the ear to make it bleed a little bit. We have some blotting paper cards you put against it to absorb some of the blood, and that

just comes into the lab, with information about pedigree. We need to know the names of the dogs and who they are related to, so we have a copy of the pedigrees on disc so we have most of the information. If new puppies are born we need to know who their parents are so we can put them into relationship with the pedigrees.

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**Sample Kits** for collection of small blood spots are available free of charge from the WKC

email: [admin@wkc.org.au](mailto:admin@wkc.org.au)

. Snail Mail

The Working Kelpie Council

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A **free CD** copy of Dr Don Robinson's original Video showing Ataxia affected pups and dogs is available free from the WKC. If you have not seen affected animals this CD would help you identify the problem- if you would like a copy and are enquiring from Australia we would welcome receipt of a self addressed A5 sized \$1.00 stamped envelope to help offset cost alternatively you could ask to be invoiced for say \$1.50 Australian Members \$2.00 Overseas to cover envelope and postage costs.

In the near future another CD or DVD will also become available free of charge- this is a professional produced CD about Ataxia and its effects, the research being undertaken and how your can help organised by Mr Terry Snow in conjunction with Dr Alan Wilton.

### **Donations:**

**Australian members** wishing to make donations should send them to Dr Alan Wilton,

**Overseas US members:** To avoid currency conversion fees at this end it is suggested that you contact WKC representative Linda Leyman who may be able to organise payment to the NSW University from her account arrangements with the WKC.

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