

Research into Genetics of Trapped Neutrophil Syndrome in Border collies.

Dr Alan Wilton at the Canine Research Lab at University of New South Wales is investigating the cause of Trapped Neutrophil Syndrome (TNS) with the aim of developing a DNA test. TNS is a fatal genetic disease in Australian and New Zealand Border collies. The Canine Research Lab developed the DNA test for CL, another fatal disorder, and now provides testing for carriers.

Background

TNS was first described in Border collies in New Zealand by Frazer Allen and Boyd Jones [1]. The physiological defect in TNS is failure of the neutrophils to migrate out of the bone marrow into the blood stream where they can protect against infection. The consequence of the low neutrophil count is high susceptibility to infections. Gut infections are common in some TNS pups resulting in lack of nutrients and poor growth. Some animals show a poor response to vaccination as the first sign of the problem.

TNS has since been reported in most Australian states as well as the USA. At least 15 affected litters have been reported and it is suspected that a large number have gone unreported. The disease is inherited as a recessive disorder, just like CL, with asymptomatic carriers producing litters with some pups affected. In the pedigrees that we are using for our research into the genetic cause of TNS, 5 out of 15 pups were affected. This is close to the ratio expected of 1 in 4 for a recessive disorder. Most TNS pedigrees have a common ancestor on both sides of the pedigree. This is often the case for recessive diseases and is consistent with the common ancestor being a carrier for the defect and transmitting it down both lineages. The mutation may have been in the population for some time but has become more noticeable when a carrier was extensively used in breeding. This has made the disease more common.

Until a DNA test can be developed, accurate estimates of the frequency of carriers are difficult. Perhaps 3% of Border collies are carriers for TNS which would mean only 1 in 1000 matings on average would produce TNS cases. Of course, some lineages will show a high incidence and some none at all. TNS occurs at a similar rate to CL in Border collies. Our typing of the general population for CL shows that 3% of animals with no known carriers for CL in the last three or more generations are carriers for the CL mutation. This is why DNA testing is recommended for all lineages.

A DNA test for TNS is the best way to control the disease by identifying carriers and selecting matings to avoid affected progeny and eliminate the defect. Development of a DNA test is possible. There are now available extensive genetic tools for identifying genetic defects in the dog. [2]. The sequencing of the dog genome means that the code for every gene is readily available. Many genetic markers are available to help locate genetic diseases as they are passed from one generation to the next [3, 4]. So, what has taken 10 years to do for CL can now be done much more efficiently.

Research at UNSW

The first resource required to identify a disease gene is samples from the affected subjects and their immediate family, parents and siblings. No progress can be made without DNA extracted from affecteds for the disease of interest. So to encourage reporting of cases for research we have a policy of confidentiality. We will not release the pedigrees of affecteds or the providers of samples. (They should still be reported by the owners to Border Collie Clubs for listing.) We have now collected families of 6 affected litters which gives us a good resource to start the research. The more cases we can gather the easier the task will be.

There are two approaches to identify disease genes. The candidate gene approach looks at individual genes that might be involved and tests them one at a time. Jeremy Shearman, who is doing his PhD in the lab, has been using this method. The other approach is gene mapping, which tests hundreds of regions of the dog genome looking for a link to the disease. This method requires samples from extensive pedigrees with numerous affecteds, siblings, parents and grandparents. Through the cooperation of some conscientious breeders, we now have the samples we need for this approach. When funds become available, Jeremy will start the process of whole genome scan, which involves testing hundreds of sites looking for one close to the TNS gene.

Once a location has been found then the mutation that is causing the problem can be identified. This requires DNA sequencing of genes in affected animals and comparing the sequence to unaffecteds. Looking for a genetic defect is like looking for a needle in a haystack. Dogs have 39 pairs of chromosomes made up of about 2,300,000,000 DNA bases. The gene defect is likely a change in a single one of those bases. That is why clues on where to look are needed.

Our initial work has focused on a few candidate genes involved in neutrophil release but it did not identify any promising leads. The work has been published in the scientific journal, *Animal Genetics* [5]. Jeremy has tested 12 different candidate genes without success. Many other gene products are known to be involved in the maturation of neutrophils and we are testing further candidates. The list of gene names is long and meaningless. There is also a Japanese group who have been sent samples from TNS affected cases to duplicate our research and they have published their sequences of the genes that our lab already tested on DNA databases.

Summary

The progress has been limited to collecting samples from TNS affected pedigrees that will allow a linkage analysis. The candidate gene approach has not yet identified the TNS gene and a whole genome scan will be undertaken when funds are available.

It is still hoped that we will develop a DNA based test for TNS so that breeders can remove this problem from the breed but we cannot estimate how long it will take. With luck it could be soon (by 2007) but it could also take longer..

Funding

This work was partially supported by Canine Research Foundation in 2005. Funds were withdrawn in 2006. The Victorian BCC donated \$1000 in 2005 to pay for pathology testing of new cases. Jeremy Shearman is supporting himself while he works on TNS for his PhD. It is hoped that, when the patent for CL testing is granted, funds may trickle in from royalties that will have to be paid by other labs that are offering our CL test, and that those funds can be used for TNS research. Funds can be applied for from government through Linkage grants, similar to the SPIRT grant that funded the CL research. These applications require extensive coordination and writing as well as a commitment to cash contributions from industry partners of around \$6,000 per year. The applications are due in late October.

References

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4. Ostrander EA, Krugylak L. (2000). Unleashing the canine genome. *Genome Research*. **10**: 1271-1274

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DNA banking,

DNA banking is storage of DNA from your important breeding animals. It may be useful in the future for tracing genes in your lineages. These could be disease genes like CL or TNS, physical trait genes like coat colour, or even genes for behaviour. We do not know what the future will bring but we can be assured testing will be available for many things using DNA.

FTA cards are a way to permanently store samples of blood or buccal swabs on a flat card. The DNA is preserved on the card which is stored at room temperature. The cards can be stored at home or at a central laboratory.

Cards for DNA banking can be obtained from Alan Wilton at UNSW for \$15 ea. Storage at UNSW is also available. Breeders/owners can be assured that any DNA stored at UNSW will not be used or made public without the breeders/owners consent.