Canine Genetics Research Progress Report

Breed: Keeshond

Condition: Idiopathic Epilepsy (IE)

Date: July 2013

Current Funding:

Funding Body: Kennel Club Charitable Trust

Amount remaining: £8,739.77

Duration: Extended to 17th March 2014

The Animal Health Trust (AHT) staff members involved in investigating idiopathic epilepsy in the Keeshond are generously supported by the Kennel Club, as part of the Kennel Club Genetics Centre at the AHT. The whole genome scan, including laboratory reagents and consumables required to prepare DNA samples for processing, will be funded by the Kennel Club Charitable Trust.

What stage are we at with this research?

Scientists at the AHT, alongside Dr Barbara Skelly from the University of Cambridge, have continued to work hard on our challenge to unravel the genetic basis of idiopathic epilepsy in the Keeshond. In our last report we outlined our plans for an in-depth genetic analysis known as a genome-wide association study (whole genome scan). This update summarises the outcome of our investigations thus far and makes suggestions for the best way to take this research forward.

What did the whole genome scan involve?

The whole genome scan carried out earlier this year compared thousands of DNA markers in epileptic Keeshonds ("cases") with those in non-epileptic Keeshonds ("controls") looking for one or more regions of the genome that were consistently shared between the cases, but

that were different in the controls. Analyses of the resulting data were carried out in two stages. The first analysis compared DNA samples from 19 of the most robustly diagnosed epilepsy cases with 29 controls, affording us the best opportunity to identify any signals suggestive of an "association". In a second analysis we sought to boost the chance of finding a signal by increasing the number of cases to 27. However, although we normally anticipate that a larger number of cases will benefit the analysis, their inclusion introduced a trade-off in that the additional eight samples were from less well-defined cases - either because we were lacking clinical information, or because these dogs did not fit the classic clinical picture of epilepsy quite as closely as others in the study.

What were the results?

If epilepsy in all the cases we included in our analysis had been caused by a single, identical recessive mutation we would have expected the analysis to have identified the region of the genome associated with the condition (and that therefore harboured the mutation). Unfortunately the analysis did not reveal a single region of the DNA that was consistently shared between cases, meaning that it is very unlikely that epilepsy in the Keeshond is caused by a single recessive mutation. At this stage we cannot distinguish between other modes of inheritance, including multiple, different recessive mutations that cause clinically similar forms of epilepsy; dominant mutation(s); or indeed that the condition is genetically complex, being caused by variants at multiple positions in the genome, with or without the interaction of environmental factors.

What happens next?

We need to collect and investigate DNA from more dogs. In our last report we emphasised that the number of samples we were sending away for the first whole genome scan may not be large enough to give us a definitive result. We suggested that sample collection should carry on in anticipation of this being the case, and we have been very pleased to see samples continuing to arrive. Our aim is to collect a similarly-sized set of samples for a further whole genome scan which we can analyse in combination with the first dataset to boost our power to identify region(s) of the genome associated with epilepsy.

One effect that was seen to some degree in our first dataset is known as "population stratification", and this can sometimes inhibit a signal from showing itself as definitively as it might. While some of our epileptic Keeshonds were bred overseas, the majority of our control samples came from unaffected dogs born in the UK. Although we can allow for this

to a certain extent in the analysis, and we are aware that the Keeshond breed is a relatively international community, we can strengthen our chance of success by more closely matching the origin of our controls to those of our cases in the next set of samples we investigate.

What can we do to further this research?

We continue to encourage you to send us DNA from any Keeshond with a diagnosis of idiopathic epilepsy. It helps us considerably if you can include as much clinical information as possible with your submission as this helps us to robustly define our cases. If you prefer, we can get in touch directly with the veterinarian treating your Keeshond if you provide us with their contact details and your consent for us to do this. We always welcome samples from older Keeshonds, aged eight years or over, who have never suffered from seizures of any kind - to act as controls in our study. We are especially keen to gather control samples from Keeshonds that are not UK-bred - particularly those based in the USA, Canada and Australia. As always, we are grateful for any health updates you can give us on those dogs for which we currently hold DNA samples. For further information on how to submit a sample, or to send us updated health information, please email Bryan McLaughlin at **bryan.mclaughlin@aht.org.uk**

What is the timeframe for the proposed research?

This important initial phase of the study is funded until mid-March 2014. We can therefore continue to collect DNA from additional cases and controls for this stage of the research until Christmas 2013, which will give us time to gather as many samples as possible but still leaves us enough time to undertake the whole genome scan and analyse the data before the funding expires. After this time we will still welcome further samples as they will be needed for the next stage of the research, so please continue to send them in.

Acknowledgements

Our grateful thanks go to the Kennel Club Charitable Trust for extending the funding period for this study, giving us the opportunity to build on the hard work that has already been done. We also thank Anji Marfleet and Jane Saunders for their unwavering assistance, together with all those owners and their Keeshonds from around the world who continue to support this project.