

DEVELOPMENT OF A RAPID DNA TEST FOR JACK RUSSELL ATAXIA Progress report

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Objective and Hypothesis: The objective of our research is to develop a rapid DNA test that will enable Jack Russell terrier breeders to identify asymptomatic carriers of the neurodegenerative disease, we call Jack Russell Ataxia (JRA). Such a test would allow breeders to make informed breeding decisions to eliminate the causative mutant gene from their lines. Our hypothesis is that a genetic defect is responsible for JRA in Jack Russells. We also hypothesize that the defect in the gene is inherited as an autosomal recessive trait and is not related to the late onset (spino-cerebellar) form of JRA. Our plan is to test this hypothesis by accomplishment of three specific aims. These are:

1. Assembly of DNA samples from an extensive Jack Russell pedigree suitable for analysis.
2. Determination of the canine gene responsible for JRA in Jack Russells.
3. Development of a rapid DNA test to detect the mutation in affected dogs and carriers

PROGRESS TO DATE

Objective 1.

We have examined 64 affected puppies over the course of this study; 3 affected puppies in 1995, 5 in 1996, 11 in 1997, 15 in 1998, and 19 in 1999, 8 in 2000, 8 in 2001 and, so far, 5 in 2002. We have collected blood samples and extracted DNA from 722 dogs

Objective 2.

We have not determined the gene responsible for this condition in Jack Russell Terriers but have eliminated several candidate genes. These include several coat color genes, including *A/a^t*, *T/t* and several genes associated with cerebellar development such as *bcl-2*, *bax* and *bat*. To date none of these genes appears to be mutated in affected or carrier Jack Russell Terriers.

Objective 3

The DNA test that we are working on will be identical to that done for HYPP in horses. We anticipate providing information on carrier status using a PCR-based DNA test that can be performed on 5mls of EDTA preserved blood. This test will be able to detect carriers prior to breeding and affected puppies before they show clinical signs. We are using a method developed using the genomic DNA extracted from leukocyte sonicates (see Objective 1). Amplicons produced from microsatellite marker primers will be purified by preparative PAGE and we are currently screening our large database of Jack Russell DNA for effective markers. An effective marker is one that co-segregates along with the causative gene and may not be the gene responsible for the disease. If the mutated allele of the selected marker involves restriction sites, routine PCR/RFLP assays will be designed

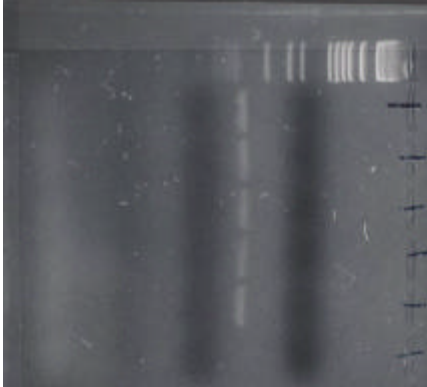
in which PCR primers constructed to flank the polymorphic sites and the amplicons obtained from these primers are digested with the appropriate restriction enzymes. These digests will be fractionated by submarine agarose gel electrophoresis and separated cut and uncut amplicons will be visualized by ethidium bromide fluorescence. In the event that the mutated allele does not contain a convenient restriction site, primers will be designed to anneal adjacent to, but not overlapping, the mutation site. This primer will contain a purposeful mismatch such that when incorporated into an amplicon, it creates an allele-specific restriction site. Primers of these designs will be the basis of a rapid DNA screening test.

Results

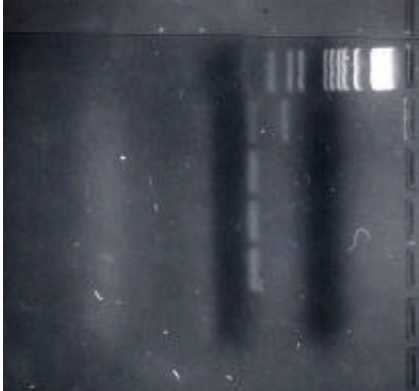
These gels are examples of several of the microsatellite markers we have been using and the results.

In the first two gels, the first lane is the molecular marker bands, the second lane is a carrier, the third lane is an affected and the fourth and fifth lanes (if present) are normal controls.

FH2309 – no differentiation between carriers, affected and normal animals

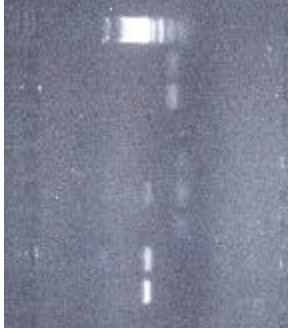


FH2313 – carrier animal (second lane) shows different banding



In these next two gels, the first lane is the molecular markers, the next two lanes are affected dogs, the second two are carriers and the third two are normal.

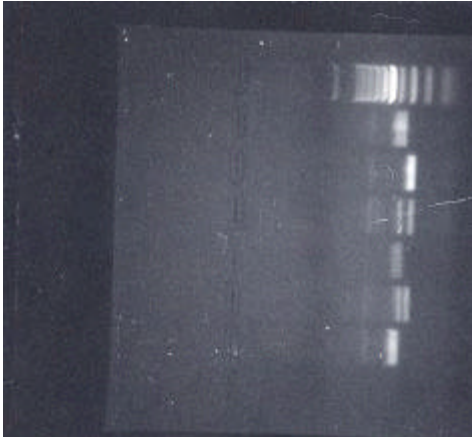
FH2541- this is one of the more promising markers. One of the carriers shows the expected double banding pattern while the other one does not. Although this result was at first interpreted as unsuitable, we have since realized that the other carrier may have been incorrectly identified and are repeating this experiment.



FH2531 – there is no difference noted between the carriers and affected dogs, although the normals appear slightly different.



FH2137 – in this gel, the first two bands are from normal animals, the second three are carriers and the last band is an affected pup. Again, two of the carriers are showing the expected double banding, however the carrier in the middle is not. In addition, the two normal animals have dissimilar banding patterns



Conclusion.

Our research so far has been slow, but successful. We have eliminated many candidate genes and are currently investigating others. We are also making progress in our use of microsatellite markers. We plan to continue this line of investigation using additional microsatellite markers and Jack Russell DNA until we develop a test for this disorder.