Cairn Terrier Ocular Melanosis Report March 2020

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The goal of our research is to identify the gene mutation that causes ocular melanosis in Cairn terriers. As described in our update in September 2019, we have identified a ~7 million base pair region within the Cairn terrier genome that is very likely to contain the gene mutation. However, after thorough investigation of genes within this region using multiple techniques, we have yet to identify the mutation. During our investigations we have found areas within this region that are incomplete in the canine "map" (the canine genome reference sequence) which we (and most other canine geneticists) use to help analyze our data. Without an accurate "map" we cannot use traditional methods of mutation discovery. For this reason, we used a new form of sequencing that can sequence very long strands of DNA and therefor has the capability of identifying large changes in a chromosome. The downside of using cutting edge technology is that tools and methods for analyzing the data are early in their development and analysis is not yet streamlined. We have started some strong collaborations with neighboring universities to work together on these new types of datasets. One method for evaluating this data is to make our own "map". Historically, this method would take years to do and take considerable computing power and resources. However, our collaborators have found a way to do this with our data using far fewer resources. A second method is to fill in the missing data in the original canine "map". It is likely that we cannot find the ocular melanosis mutation because it is located within the incomplete areas of the canine "map" (i.e. areas where the reference sequence is not complete and there is missing sequence data), there has recently been a gene identified for a different condition in another breed of dog in which this was the case. A third method recently became available. Two new canine "maps" have been generated and made publicly available.

We propose the following research goals for 2020:

1) Continue working closely with our collaborators to analyze our new long strand DNA sequences.
    a. We will create our own Cairn terrier "map" using an unaffected Cairn terrier and compare our affected Cairn terriers to this new "map".
    b. We will analyze our long strand sequencing data for large changes in the DNA that is difficult to detect using traditional sequencing
2) We will aim to fix the canine "map"
    a. We will purchase large pieces of the DNA sequenced to make the original canine "map" (this is a service from the Institute that created the original map where chunks of the actual DNA of the genome can be purchased – called bacmids). The pieces that we will obtain are the ones that will sufficiently span the chromosomal regions we have identified as missing or incorrect
    b. These pieces will be sequenced by traditional methods AND with long strand sequencing technology
3) We will use the newly available canine "maps" to analyze our current Cairn terrier data obtained from traditional sequencing methods.
    a. We will analyze this data for small mutations and for large DNA changes (for example duplications, inversions etc)
4) We will sequence more dogs using traditional methods

a. The cost of sequencing a genome has reduced significantly in the past 5 years. Sequencing additional dogs would greatly improve our data set. Targeting dogs with very severe ocular melanosis, dogs within family groups and affected dogs with any unaffected siblings would greatly improve the power of detection of a mutation.