PUBLIC ABSTRACT

CELLULAR AND GENETIC CHARACTERIZATION OF OCULAR MELANOSIS IN THE CAIRN TERRIER

DOG

By

Ethan Dawson-Baglien

Ocular melanosis (OM) is an inherited eye disease seen in the Cairn terrier dog breed. The disease is very common in Cairn terriers – although the exact number of dogs affected by the disease is not known, surveys of Cairn terrier breeders and owners frequently rank it near the top of health concerns in the breed. The disease progresses through several stages, starting off as a thickening and darkening of the iris. Eventually, dark brown-black pigment begins to appear in patches in abnormal areas of the eye, such as the sclera (the whites of the eye). These patches gradually grow and expand over time. Within the eye, pigmented material is shed into the anterior chamber of the eye (the fluid-filled space between the iris and the front of the eye). This pigmented material clogs up the eye's internal drainage pathways, and fluid builds up within the anterior chamber, leading to an increase in pressure within the eye. This increase in pressure can lead to painful glaucoma, and eventually blindness, in the dogs with the most severe cases of OM. The underlying causes of OM are not currently known. Two different methods were used to attempt to find out more about the disease – a cell culture method and a gene sequencing method.

In the cell culture method, donated eyes from dogs with and without OM were used to isolate and grow uveal melanocytes – the pigmented cells of the eye which grow and migrate in OM. These cells were then tested using a variety of different cellular assays to determine how the melanocytes from affected eyes differed from those in unaffected eyes. The only tests where the melanocytes from the OM-affected dogs showed any difference from those in unaffected dogs were in pigmentation – the melanocytes from OM-affected dogs had much more pigment, and made new pigment more quickly, than those from unaffected dogs.

Gene sequencing methods were also used to try to find where in the genome the mutation that causes OM was located. To determine the general location of the mutation, a

whole-genome SNP array was used to test 94 dogs at 170k markers from all around the 2.8 billion base pairs of the canine genome, to see if any of the markers was associated with the disease. This identified a 7.5 million base-pair long region of chromosome 11 that was significantly associated with the disease. Next, the entire genome of 10 dogs was sequenced, 5 OM-affected and 5 unaffected, to look for the exact mutation causing OM. Analysis of the sequencing data failed to identify a likely causal variant, either within the identified region or in known genes related to pigmentation disorders. Finally, RNA sequencing was performed on eye tissues from 12 dogs; 7 OM-affected and 5 unaffected, to determine whether there were any differences in gene expression between the two populations. Six genes were identified that were expressed differently between the two populations that were in pathways known to be associated with cancer metastasis.

Although a causal variation for OM has not yet been discovered, several promising new clues have been identified that can be followed up on, including the general location of the causal DNA mutation on chromosome 11, and a number of genes whose expression are altered in OM-affected dogs. Following these leads may finally allow us to identify the underlying cause of OM.

ABSTRACT

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Ocular melanosis (OM) is an inherited eye disease characterized by a thickening of the iris root due to expansion of a melanocyte population and an abnormal progressive deposition of pigment in areas of the eye such as the sclera and episclera. The disease is inherited in an autosomal dominant fashion, and occurs almost exclusively in Cairn terrier dogs, in whom the disease is very common. OM is relatively recent in origin, leading us to suspect that it has a single causative underlying mutation, which spread through the Cairn population via a founder effect. In this work, two different approaches were used to attempt to characterize the OM phenotype – a cellular and a genetic approach.

A method for isolating and culturing canine uveal melanocytes without contamination from other cell types was initially developed. This was then used to isolate melanocytes from OM-affected and control dogs which were then compared using various assays of physiological behavior. These included a battery of standard cell behavior tests to evaluate doubling time, migration rate, anchorage independence, ability to migrate through a membrane, extracellular matrix preference, and melanin content and rate of production. In all aspects, melanocytes from OM-affected dogs and those from unaffected dogs were identical in vitro, except for melanin content and production rate – after initial culture, OM-affected melanocytes contained much more melanin than unaffected cells, and produced more melanin as well. These changes were eventually lost in later passages, but differences were statistically significant ($p<0.05$) in early-passage cells.

Attempts to identify a causal mutation for OM based using a candidate gene approach had not previously been successful, so a whole-genome SNP array was used to examine 94 Cairn terriers at ~170k evenly spaced markers throughout the genome. This identified a 7.5 megabase (Mb) region of chromosome 11 that was significantly associated with the disease.

Sanger sequencing of positional candidate genes selected from the region of interest did not reveal any variants associated with the disease. Whole-genome sequencing was performed on 10 dogs; 5 affected and 5 unaffected, but no genes either within the region detected via the SNP array or within a list of genes known to be associated with pigmentation disorders contained any polymorphism that segregated with the disease. Finally, RNA sequencing was performed on 12 samples, 7 affected and 5 affected, and the transcriptomes of OM-affected and unaffected dogs were compared. Although no individual genes had a statistically significant difference in expression level, pathway analysis of the genes with the lowest p-values revealed 6 genes with differential expression levels that were part of 1 of 2 pathways known to be associated with cell migration in metastatic cancer.

Although further studies are needed, the identification of both a region associated with OM and a putative pathway which may be involved in the migratory component of the disease functions are major steps toward identifying the underlying cause of the disease.