

PUBLIC ABSTRACT

CNGB1 MUTATION IN PAPILLON DOGS: THE IDENTIFICATION, CHARACTERIZATION AND CURE

By

Paige A. Winkler

Progressive retinal atrophy (PRA) is an inherited retinal dystrophy that affects over 100 breeds of dogs. Typically the disease begins with loss of dim-light vision and then progresses to total blindness. The loss of dim-light vision is due to a mutation in a gene that is necessary for rod photoreceptor development, maintenance or function. PRA is the canine equivalent of a human disease, retinitis pigmentosa (RP), which affects about 1 in 4000 people. Mutated genes that cause PRA in dogs are often the same genes that cause RP in humans. Information gleaned from PRA can be used to help understand RP in humans.

Papillon dogs are one of the 100 breeds of dogs that are affected with PRA. In this dissertation, I have identified a mutation in the gene CNGB1 which accounts for 70% of the PRA in the Papillon dogs. The CNGB1 gene codes for a protein that is necessary for rod phototransduction and olfactory transduction. Human patients with RP type 45 also have mutations in the CNGB1 gene.

The CNGB1 affected dogs have abnormal and decreased rod function at the earliest age tested. Normal day light (cone photoreceptor) vision is maintained until cone function decreases between 4 and 5.5 years of age. However, dogs maintain navigational day-light vision for many more years. These functional data, coupled with in vivo and ex vivo histological data, show that the disease results in a slow retinal degeneration that will eventually lead to complete blindness.

Gene replacement therapy is a method of supplying a cell with the means to produce a normal protein in place of the abnormal or absent protein caused by a mutation in a gene. Gene therapy has been proven successful in research and clinical settings, especially in the context of retinal degenerations. We used a viral vector to

introduce a copy of the normal dog CNGB1 gene back into the rod photoreceptors in the CNGB1 affected dogs. The gene therapy was able to rescue rod function and the rescue was maintained until the last data point was collected 9 months after injection. The olfactory involvement of the CNGB1 mutation was investigated in the CNGB1 affected dogs. The olfactory epithelium and the olfactory bulbs were abnormal in the CNGB1 affected dogs when compared to control dogs. I developed a behavioral test that could assess olfactory function in the CNGB1 dogs with very little training. The CNGB1 affected dogs had decreased but not absent olfactory function. The detailed descriptions of the retinal and olfactory phenotypes, in addition to the successful gene replacement therapy trial, in the CNGB1 affected dogs have laid the ground work for future studies including working with clinicians to advance gene replacement therapy trials in human patients with mutations in the CNGB1 gene.

ABSTRACT

CNGB1 MUTATION IN PAPILLON DOGS: THE IDENTIFICATION, CHARACTERIZATION AND CURE

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Progressive retinal atrophy (PRA) is an inherited retinal dystrophy that affects over 100 breeds of dogs. It is characterized by a bilateral retinal degeneration commonly resulting in blindness. Affected dogs typically present with loss of dim light vision, attenuation of retinal blood vessels and tapetal hyperreflectivity. The purpose of this study was to identify the underlying cause of PRA in Papillon dogs and to characterize the phenotype at the cellular and molecular levels.

I identified a mutation in the gene CNGB1 which accounts for 70% of the PRA in the Papillon dogs. The CNGB1 mutation involves a 6 base pair insertion and a 1 base pair deletion resulting in exon skipping and a premature stop codon cause by a frameshift. CNGB1 encodes the β -subunit of a cyclic nucleotide-gated ion (CNG) channel. CNGB1 has multiple splice variants expressed in rod photoreceptors, olfactory sensory neurons and other tissues. CNG channels are directly involved in rod phototransduction and olfactory transduction.

The retinal phenotype of the CNGB1 affected dogs was characterized by in vivo and ex vivo analyses. Electroretinograms (ERGs) and behavioral vision testing were conducted to assess retinal function throughout the course of the disease. The CNGB1 affected dogs had decreased and abnormal rod function at the earliest age tested but cone function was preserved until 5.5 years of age. Histological analyses showed that the morphology of rod photoreceptors deteriorate slowly over the first ~1.5 years of life while cone photoreceptor morphology is preserved for longer.

Adeno-associated viral (AAV) vector therapy was used to treat five CNGB1 affected dogs with a wild-type copy of canine CNGB1 cDNA. One eye was injected with a low titer (1×10^{12}) of an AAV vector delivering CNGB1 cDNA, six eyes were injected

with a higher titer (5×10^{12}) and one eye was injected with a GFP-expressing construct as a vehicle and procedural control. All dogs treated with the AAV vector containing the wild-type copy of CNGB1 showed rescue of rod function that was maintained throughout the time course of the study (9 months).

The CNGB1 affected dog olfactory phenotype was investigated using in vivo and ex vivo techniques. The olfactory epithelium and the olfactory bulbs were abnormal in the CNGB1 affected dogs when compared to control dogs. I developed a behavioral test that could assess olfactory function in the CNGB1 dogs. The CNGB1 affected dogs had decreased but not absent olfactory function.

The detailed descriptions of the retinal and olfactory phenotypes, in addition to the successful gene replacement therapy trial, in the CNGB1 affected dogs have laid the ground work for future studies including working with clinicians to advance gene replacement therapy trials in human patients with mutations in the CNGB1 gene.

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