

ABSTRACT

Retinol dehydrogenase 5 (*RDH5*) catalyzes the final oxidation for regeneration of 11-*cis*-retinal as part of the visual cycle in the retinal pigment epithelium (RPE) needed for regeneration of the visual pigments. In humans, *RDH5* mutations cause fundus albipunctatus (FA) which is characterized by night blindness, accumulation of white-yellowish fundus spots and in a subset of patients, macular degeneration. Currently, the potential genetic cause of this variability of macular involvement is not well understood, leaving unmet needs for therapies for patients with *RDH5* mutations. Using a large-animal cat model of *RDH5*-associated disease (*RDH5*^{-/-}) with a spontaneously occurring Gly181Val loss-of-function missense variant in *RDH5*, this study aims to meet these needs through identification of modifier loci contributing to this heterogeneous phenotype, characterization of the functional and structural effects of macular (*area centralis*: cat) degeneration, and development of gene augmentation therapy to rescue the functional phenotype of *RDH5*-mutations. We hypothesized a genetic modifier is contributing to the variability in the *area centralis* (*AC*) degeneration phenotype in the *RDH5*^{-/-} cat. Whole-genome sequencing data for 18 *RDH5*^{-/-} cats was generated at 30X coverage on a Nova Seq S4 – PE150 flowcell. As the mode of inheritance for the modifier action was not known, different segregations were filtered to account for all modes of inheritance. Variants, including single nucleotide and structural variants were then sorted by potential deleterious effects and investigated in Integrative Genomics Viewer (IGV) for expected segregation. Variants in genes identified as potential candidates were tested for expected segregation of various modes of inheritance within a larger sample set after genotyping using polymerase chain reactions (PCR) and Sanger sequencing. Though unidentified during this study, variants continue to be tested to identify a genetic modifier responsible for phenotypic variation in *AC* degeneration in *RDH5*^{-/-} cats.

We hypothesized degeneration of the *AC* would result in structural and functional abnormalities in the *RDH5*^{-/-} cats. Full-field flash electroretinograms (ERGs), including scotopic, photopic, and rod recovery protocols assessed the impact of *AC* degeneration on whole retinal function between *RDH5*^{-/-} cats with and without *AC* degeneration. Cats were binned into three groups using K Means Clustering. Groups were based on outer nuclear layer thickness assessed by Spectral domain optical coherence tomography (SD-OCT); A: No Degeneration, B: Intermediate Degeneration, C: Severe Degeneration. Despite a heavily photoreceptor populated area of the retina degenerating (the *AC*), no statistical differences in ERG responses across degeneration statuses was identified. We hypothesized gene augmentation therapy would improve rod recovery and slow *AC* degeneration. An adeno-associated virus serotype 2 with triple capsid mutations (AAV2) vector packaged with feline *RDH5* cDNA (AAV-VMD2-RDH5) was subretinally injected into 11 *RDH5*^{-/-} cats. One eye was injected with the vector, while the other eye was a non-injected control. The primary outcome measures were rescue of recovery of rod function following light exposure and structural preservation. Following euthanasia, immunohistochemistry was performed to ensure therapy resulted in appropriate expression of the transgene in the cells of interest (RPE). Gene augmentation therapy resulted in statistically significant improvement in rod functional recovery in the treated vs. untreated eye up to 1.5-years post-injection. Additionally, two treated individuals with *AC* degeneration injected at the *AC* showed preserved outer nuclear layer structure in the treated vs. untreated eye. In addition to excluding potential variants as the genetic modifier, this study improved understanding of the effect of *AC* degeneration on retinal function through multiple ERGs assessing different parts of the retina and developed a successful gene augmentation therapy.