Refreshed ICR Mouse Colony to Eliminate Retinal Degeneration Associated with pde6b Mutation

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Introduction

Moderate retinal degeneration was identified in up to 80% of male and female Hsd:ICR (CD-1) mice housed at Envigo vivariums in the USA. The presence of background ocular lesions greatly impact the interpretation of test article safety in a toxicology study,[1] and thus reduction of the incidence of this finding in Inotiv colonies was desired. A homozygous mutation in the pde6b gene (*pde6b*^{rd1}) is associated with retinal degeneration, which may be severe.[2,3] This mutation was common in Envigo Hsd:ICR (CD-1) mice and was removed from the colony at the Envigo Netherlands location using selective breeding methods. This colony was then used to refresh the remaining colonies of Hsd:ICR (CD-1) in the UK and the USA, which had been discontinued. The purpose of this study was to confirm retinal health in the refreshed USA colonies.

Methods

Mice were divided into two study groups. Group 1 was comprised of 5 male and 5 female CD-1 mice from the discontinued USA colonies. Group 2 was comprised of 10 male and 10 female CD-1 mice from the refreshed USA colony. The animals were housed at AAALAC-accredited CRO facilities in compliance with all federal regulations and Inotiv policies on the care, welfare, and treatment of laboratory animals. The animal rooms were on a 12-hour light/dark cycle. Observations and body weight measurements were recorded once at the time of assignment to the study and once prior to necropsy. At termination, ear punches were collected and sent for genotyping by Transnetyx (Cordova, TN). Animals were humanely euthanized according to AVMA standards at approximately 18 weeks of age, and eyes with optic nerve were collected in modified Davidson's fixative and processed routinely for microscopic examination by a pathologist.

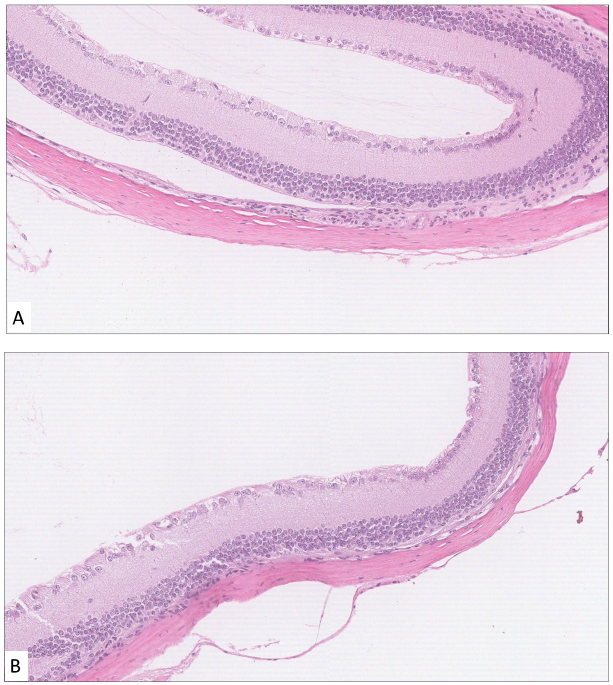
Results

- In Group 1 (discontinued colony), 8/10 mice were homozygous mutant, 1 female was heterozygous, and 1 male was wild type for pde6b-3. In Group 2 (refreshed colony), 19/20 mice were wild type, and 1 female was heterozygous mutant.
- There were no notable differences between the groups in body weight, clinical observations, or macroscopic observations. One male in Group 1 (discontinued colony) had a crusty right eye which was discolored at necropsy. This correlated microscopically to a unilateral minimal focal scleral neutrophilic infiltrate at the limbus and was considered incidental and not related to genotype due to the low incidence, unilateral distribution, and consistency with background findings in rodents. One female in Group 2 (refreshed colony) was euthanized early due to an imperforate vagina, a common incidental lesion in mice.[4] All other animals were normal.
- Microscopic findings of bilateral, diffuse, moderate outer retinal atrophy occurred in 4/5 homozygous mutant animals from each sex in Group 1 (discontinued colony). The retinal atrophy consisted of diffuse, bilateral, generalized loss of the photoreceptor, outer nuclear, and outer plexiform layers (Figure 1). Occasionally, there were small segments of focally retained outer nuclear or outer plexiform layers, as well as focal decreases in cellularity of the inner nuclear cells. The single wild type male from the discontinued colony had no microscopic lesions. There were no microscopic findings in the eyes of Group 2 (refreshed colony) animals in either sex, which were wild type or heterozygous mutant (Figure 2). All optic nerves were microscopically normal in both groups.

Table 1. Incidence of pde6b Genotype and Microscopic Findings

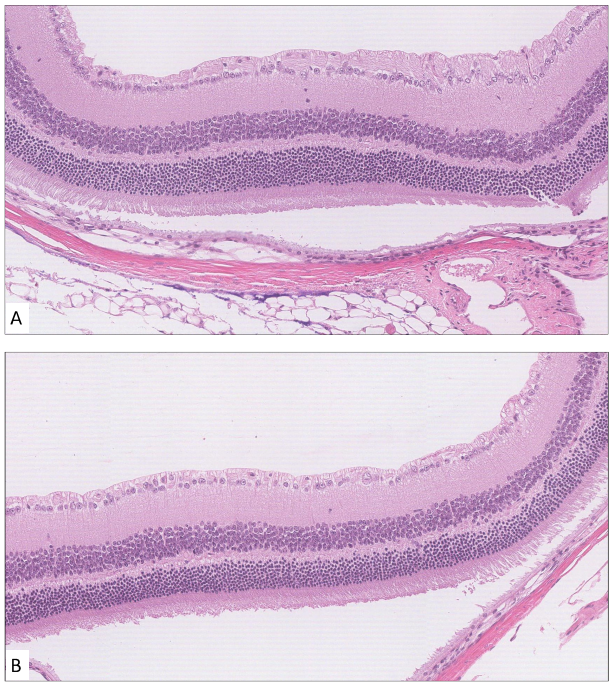
	Group 1 (Discontinued Colony)		Group 2 (Refreshed Colony)	
	Male (5)	Female (5)	Male (10)	Female (9)
Genotype (pde6b)				
Wild Type	1	0	9	9
Heterozygous rd1 Mutant	0	1	1	0
Homozygous rd1 Mutant	4	4	0	0
Microscopic Findings				
Retinal Degeneration (Moderate)	4	4	0	0

Figure 1. Retina from pde6b Mutant Animals from Discontinued Strain



A) Retina from Group 1 Male (homozygous mutant) 20X. H&E. B) Retina from Group 1 Male (homozygous mutant) 20X. H&E. The outer retina is diffusely and bilaterally atrophied characterized by loss of the photoreceptor layer, outer nuclear layer, and outer plexiform layer. The inner retinal layers are retained.

Figure 2. Retina from Wild Type Animals from Refreshed Colony



A) Retina from Group 1 male (wild type) 20X. H&E. B) Retina from Group 1 female (wild type) 20X. H&E. The retina in both animals is normal with a clearly visible photoreceptor layer, outer nuclear layer, and outer plexiform layer. Occasional artifactual retinal separation (as pictured here in wild type animals) occurred in animals in both groups.

Conclusions

- Microscopic lesions of retinal degeneration are absent in the refreshed Hsd:ICR CD-1 colony animals with wild-type or rarely, heterozygous rd1 mutant genotype for pde6b.
- This colony provides enhanced model quality of Envigo Hsd:ICR CD-1 mice, allowing for greater nuance in interpretation of ocular findings in toxicity studies.
- Additional work that has been performed since this study includes ophthalmologic exams of 18-week-old breeding animals from the refreshed colony which were all normal.
- Additional genotyping was performed to remove heterozygotes from the colony. Ongoing genotyping will be performed, with removal of newly discovered mutant animals from the refreshed colony. Accumulation of historical control histopathologic data will also be performed on an ongoing basis.

References
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