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Bestrophin-Like Lesions Described in Dutch Belted Rabbits

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INTRODUCTION

- Baseline ophthalmic examination including slit lamp and indirect ophthalmoscope is critical in all species involved in ocular toxicity studies.
- Spontaneous ocular lesions identified during prescreening have been reported in mice[1], rats[2], dogs and nonhuman primates[3], involving cornea, iris, lens, vitreous, and retina.
- Laboratory rabbits (Dutch Belted or New Zealand white) are another common species used in ocular toxicology studies, with limited data reporting spontaneous ocular lesions involving cornea, iris, lens and vitreous[4].
- Our purpose is to describe the *in vivo* retinal microanatomy findings observed during pretest ophthalmic examination in Dutch Belted laboratory rabbits.

METHODS

- Total of 10 (8 males and 2 females) Dutch Belted rabbits, ranging in age from 5 to 27 months were used in this study.
- Ophthalmic examinations were performed as baseline prior to ocular studies.
- Fundus images from retinal changes were documented using a RetCam Shuttle.
- *In vivo* retinal microanatomy was evaluated under general anesthesia by non-invasive imaging with a Confocal scanning laser ophthalmoscopy (cSLO)/Spectral domain optical coherence tomography (sdOCT) instrument (Spectralis® HRA/OCT, Heidelberg Engineering). The cSLO images were taken using the near-infrared (IR), and autofluorescence (AF) modes. The sdOCT images were acquired as raster scans (9 automatic real-time tracking [ART]) or single scan (21 ART).
- At termination eyes were collected and fixed in Davidson's fixative and sent to StageBio for histological evaluation.
- At StageBio: Eyes were sectioned serially (50 µm apart) using in-life images to guide the sectioning through the area(s) with identified lesions. For each level, 4-5 µm thickness sections were generated, and stained with Hematoxylin and Eosin (H&E).

Table 1. Ophthalmic Findings and Procedures Performed

Rabbit # (age in months)	Sex	Eye	Ophthalmic finding	Fundus images	OCT	Fluorescence	Histology
1	male	OD	Single round pinkish color ~ 1/2 ONH size superior nasal to optic nerve head	7 and 11 months	7 and 11 months	yes	11 months
2	male	OS	Single round pinkish color ~ 1/4 ONH size superior nasal to optic nerve head	7 and 11 months	7 and 11 months	yes	11 months
3	male	OD	Single round pinkish color ~ 1/2 ONH size superior temporal to optic nerve head	7 and 11 months	7 and 11 months	yes	11 months
4	male	OS	Single round pinkish color ~1/4 ONH size superior temporal to optic nerve head	14 and 16 months	14 and 16 months	yes	16 months
5	male	OS	Single round pinkish color <1/4 ONH size superior nasal to optic nerve head	6 months	6 months	yes	6 months
6	male	OD	Single round pinkish color <1/4 ONH size superior to optic nerve head	27 months	27 months	yes (mild)	27 months
7	male	OS	Single round pinkish color ~ 3/4 ONH size superior nasal to optic nerve head	27 months	27 months	yes (mild)	27 months
8	male	OD	Single round pinkish color ~ 1/8 ONH size superior nasal to optic nerve head	27 months	27 months	yes (mild)	27 months
9	female	OS	Single round pinkish color <1/4 ONH size superior nasal to optic nerve head	5 and 7 months	5 and 7 months	yes	7 months
10	female	OU	Single round pinkish color <1/4 ONH size superior temporal to optic nerve head OD and ~ 1/4 ONH size superior temporal OS	5 and 7 months	5 and 7 months	yes	7 months

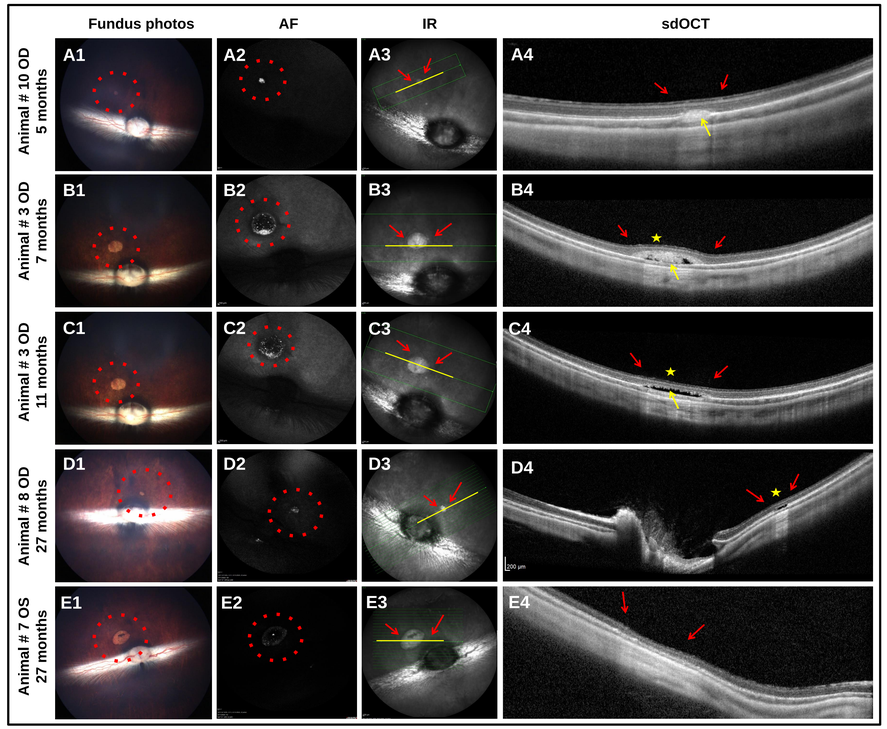

Figure 1. *In Vivo* Images Shows Alterations in Retinal Morphology
Representative fundus images of retinal abnormalities. **A1 - E1:** Fundus color images of round pinkish lesions. **A2 - E2:** Images showing fluorescent lesions in AF mode. **A3 - E3:** IR images showing lesions as discolored areas with yellow horizontal line representing the level of B-scan shown on OCT images A4 – E4. **A4 - E4:** sdOCT B-scans showed a detached retina (yellow asterisks), with some material accumulation in the subretinal space (yellow arrows) and area of retinal thinning (yellow arrow-head). Red circles represent the same area in fundus photos and AF images, red arrows represent same region in IR and sdOCT images.

RESULTS

- Nine rabbits presented unilateral and one rabbit presented bilateral, single, oval-pinkish lesions superior to the optic nerve head.
- Lesions in animals ranging from 5 to 16 months of age were fluorescent under AF mode. OCT showed focal retinal detachment, retinal thinning, and auto fluorescent material, likely lipofuscin, accumulated between the photoreceptor and RPE layers.
- In older animals at 27 months of age, lesion fluorescence decreased under AF mode. OCT in larger lesion showed no material accumulation between photoreceptor and RPE layers with retinal thinning, while small lesions showed no evident material accumulation between photoreceptor and RPE layers and minor retinal detachment.
- Clinical imaging findings correlated well with microscopic observations which included focal retinal detachment (with outer retinal atrophy and subretinal accumulation of amorphous amphophilic material, sloughed RPE cells, and/or melanophages) and focal retinal thinning (decreased cellularity of the retinal layers).

CONCLUSION

- This is the first report correlating *in vivo* microanatomical and histological description of retinal findings in Dutch Belted rabbits.
- Progression of lesions resembles Bestrophin-like lesions previously described in humans and dogs[5].
- Baseline ophthalmic examinations in animals enrolled in ocular toxicology studies are important to identify potential ocular lesions and determine their inclusion or exclusion in ocular studies.
- We suggest that a different name for changes broadly categorized as chorioretinal scars be considered, as lesions reported do not show fibrotic areas involving the retina or choroid, and present different anatomical changes in the retina / choroid

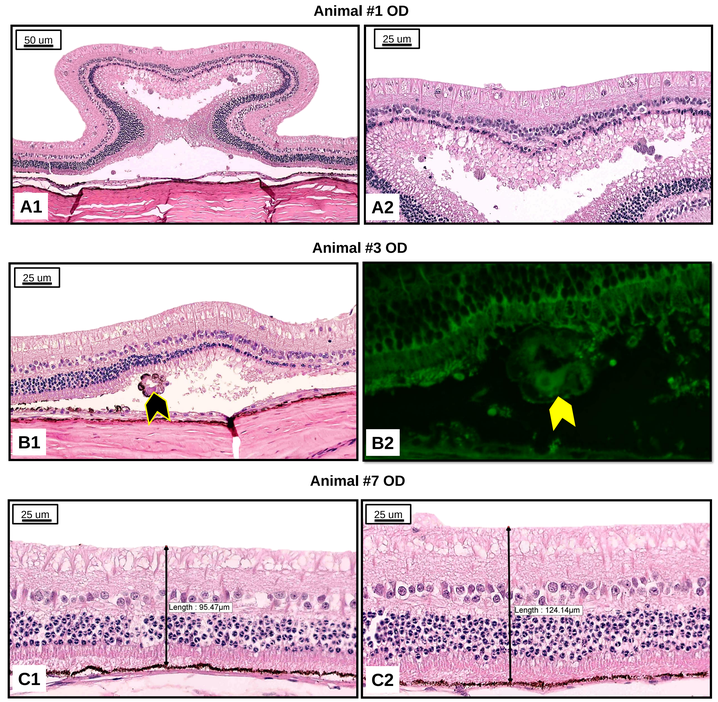

Figure 2. Histological Images

Photomicrographs of the clinically observed lesion noted in Animal #1. **A1**: Low magnification image showing focal retinal detachment, outer retinal atrophy, and subretinal accumulation of cellular debris. **A2**: Higher magnification image of A1.

Photomicrographs of the clinically observed lesion noted in Animal #3. **B1**: High magnification image showing focal retinal detachment, outer retinal atrophy, and subretinal accumulation of amorphous amphophilic material (black arrow-head) surrounded by sloughed RPE cells and melanophages. **B2**: The amorphous amphophilic material (yellow arrow-head) exhibits autofluorescence in the green channel.

Photomicrographs of the clinically observed lesion noted in Animal #7. **C1**: High magnification image showing focal retinal thinning, characterized by decreased cellularity of the retinal layers. **C2:** High magnification image of the retina immediately adjacent to C1, showing a lack of retinal thinning.

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Acknowledgments

- Brandon Ossont, Carrie Krowiak and the staff at Altasciences Preclinical Scranton for animal care support.
- Kristin Mays and the staff at StageBio for histology support.
- CR: None.