Noninvasive Detection of Retina Degeneration in Mice Using Diffusion MRI at 11.74T

Q. Wang[1], J. Chen[2], H. Zhang[2], S. Chen[3], and S-K. Song[4]

[1]Department of Mechanical, Aerospace and Structural Engineering, Washington University, Saint Louis, MO, United States, [2]Internal Medicine, [3]Ophthalmology and Visual Sciences, [4]Radiology, Washington University, Saint Louis, MO, United States

Introduction:

Retinitis pigmentosa is a family of inherited retinal diseases characterized by the progressive degeneration of rod and cone photoreceptors after birth. The retina degeneration-1 (*rd1*) mouse is a rodent model of retinitis pigmentosa. In *rd1* mice, nearly all photoreceptor nuclei have disappeared by four weeks, resulting in the significantly thinner retina than that of the age-matched control. We hypothesize that diffusion MRI is sensitive to the retina degeneration in *rd1* mice. The objective of this study is to quantitatively characterize the effect of photoreceptor degeneration on retina structures using directionally dependent and mean ADC in *rd1* mice. Diffusion weighted images were acquired along three orthogonal directions at 11.74T. Calculated directional and mean ADC in the retina from *rd1* mice were compared to those of the control.

Method:

Animal Model: Two to four months old *rd1* mice (n = 5) and age-matched control C57/BL6 mice (n = 5) were employed for the study. Mice were anesthetized with ketamine and xylazine by the initial single dose intraperitoneal injection followed by constant infusion during MRI. Body temperature was maintained at 37°C and the respiration was monitored using a small animal heating and monitoring system (SA instruments, NY).

MRI: MRI of the mouse eye was performed using a Varian 11.74T UNITY-INOVA scanner employing a custom-built solenoid coil for transmission and receiving. All images were acquired on a transverse slice through the optic nerve head of the mouse eye. A spin-echo sequence incorporating a pair of diffusion sensitizing gradients was used for DWI with the following parameters: slice thickness 400 μm, FOV 6 × 6 mm^2, in-plane resolution 47 × 47 μm^2, data matrix 128 × 128 (zero filled to 256 × 256), number of averages 4, TR 1.5 s, TE 35 ms, Δ 15 ms, δ 5 ms, b-value 0 and 955 s/mm^2 at three orthogonal diffusion weighting directions, i.e., in-plane parallel to the optic nerve (∥), in-plane perpendicular (⊥), and out-of-plane perpendicular to the optic nerve (⊙). In each direction, a pair of diffusion-weighted images was acquired with positive and negative diffusion gradients to minimize the background magnetic field gradient effect on the diffusion measurement. All diffusion-weighted images were acquired with respiratory gating.

Data Analysis: The combined layer of retina/choroid was clearly defined on diffusion weighted images. The identification of the MR-detected choroid, retina, and individual retina layers was performed based on the b_0 image (b-value = 0 s/mm^2) signal intensities. A pair of retina segments at each side of the optic nerve head were selected as the region of interest for quantitative analysis of the directional ADC and $\overline{ADC}$. The two ends of each segment were ~260 and ~790 μm away from the optic nerve head.

Histology: All mice were euthanized at the conclusion of MRI measurements. Eyes were enucleated and fixed with 4% paraformaldehyde. Four-μm thick slices sectioned through the optic nerve head and perpendicular to retina were stained with hematoxylin and eosin (H&E) for morphological analysis of the retina.

Statistics: Unpaired student t-test was performed to compare the mean of directional ADC and $\overline{ADC}$ from rd1 and control mice. Statistical significance was accepted at $p < 0.05$.

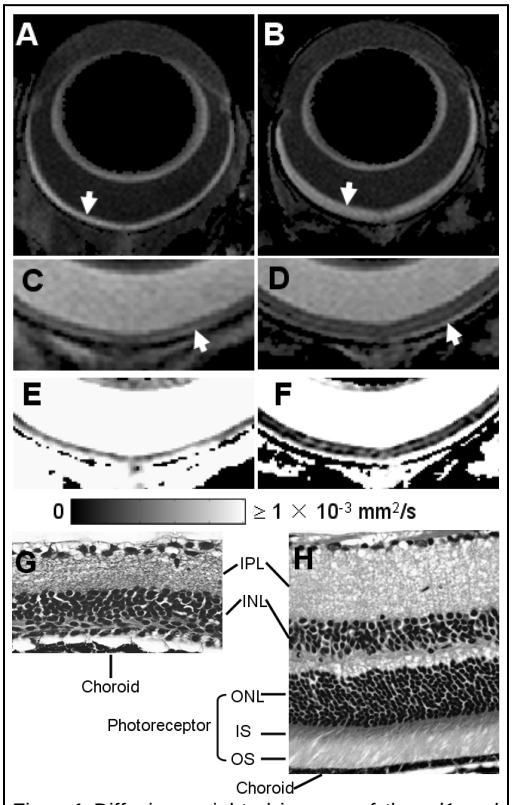

Figure1 Diffusion weighted images of the *rd1* and the control mouse (A, B); Expended views of the b0 images (C, D) and $\overline{ADC}$ maps (E, F) of the *rd1* and the control mouse; Histology maps of the *rd1* mouse and the control mouse (G, H)

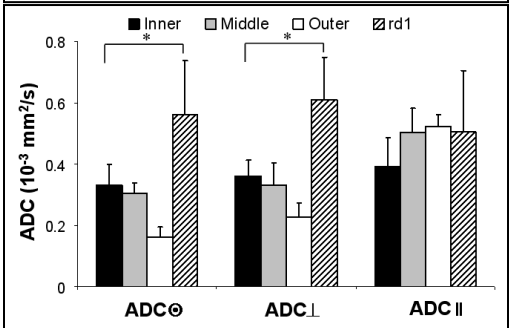

Figure 2 Quantified ADC⊥, ADC⊙, and ADC∥ in the three MR-detected retina layers of the control mouse and in the retina of the *rd1* mouse; * $p<0.05$

Results:

In both *rd*1 and control mice, the combined layer of retina/choroid was hyper-intense in the diffusion weighted images (Figs. 1A and B; downward arrows). The choroid adjacent to the sclera was hyper-intense on the non-diffusion weighted b_0 images (Figs. 1C and D; upward arrows). A single MR-detected retinal layer (dark) in the *rd1* mouse was observed. In contrast, three MR-detected retinal layers (dark-bright-dark) in the control mouse were seen on the b_0 images. The single MR-detected retina layer in the *rd1* mouse and three MR-detected retina layers in the control mouse were also observed on all directional ADC maps (images not shown) and the $\overline{ADC}$ maps (Figs. 1E and F). H&E stained slices of the retina from *rd1* mice demonstrated that the photoreceptor cells were lost (Figs. 1G and H), suggesting the single MR-detected retinal layer (dark) in *rd*1 mice correlates with the MR-detected inner layer (dark) in the control.

The quantified directional ADCs in the single MR-detected retina layer from *rd1* mice were compared with those from the control (Fig. 2). ADC⊥ and ADC⊙ in the MR-detected retina layer of *rd1* mice were significantly higher than those in the MR-detected inner and middle layers of the control. ADC∥ in *rd1* mice is not significantly different from that in control mice. In control mice, the water diffusion shows high diffusion anisotropy in the MR-detected outer layer with less anisotropic middle layer and an isotropic diffusion in the inner layer. In contrast, the water diffusion in the retina of *rd1* mice is isotropic. Calculated $\overline{ADC}$ of the MR-detected retina layer in *rd1* mice was 0.56 ± 0.17 × 10^{-3} mm^2/s. It is significantly higher than 0.34 ± 0.04 × 10^{-3} mm^2/s of the MR-detected inner retina layer in the control mouse ($p < 0.05$).

Discussion and conclusion:

The retina thickness of the *rd1* mouse was thinner than that of the control due to the degeneration of photoreceptor cells, resulting in a single MR-detected retina layer observed in the *rd1* mouse. This layer exhibited significantly higher ADC⊥ and ADC⊙ than the corresponding MR-detected inner retina layer of the control mouse, suggesting the remaining retinal neuronal cells of the rd1 mouse may suffer some cellular damage. The current findings demonstrate that diffusion MRI can be a noninvasive tool to detect retinal degeneration in mice. **Acknowledgment:** Supported by NIH EY12543.