Introduction
Glaucoma is the leading cause of blindness worldwide. Although the pathogenesis of glaucoma is not fully understood, both the retinal ganglion cell and its axons, the optic nerves, are injured in glaucoma. In this study, in vivo diffusion basis spectrum imaging (DBSI) and manganese-enhanced MRI (MEMRI) were performed to assess axonal pathologies (inflammation, demyelination and axonal injury) and to determine the degree of axonal transport deficit in 12-month old DBA/2J mice, a rodent model of glaucoma which develops progressive degeneration of visual function mimicking human glaucoma. Our results suggest that DBA/2J mice developed inflammation, axonal injury, demyelination with significant axonal transport disruption in optic nerves compared with the age-matched controls.

Materials and Methods
Animal Model: DBA/2J (male/female = 3/3), and age-matched WT (male/female = 3/2) mice at 12 months old were examined. DBSI: A pair of 8-cm diameter volume and 1.7-cm diameter surface active-decoupled coils was used. DBSI was performed on a 4.7-T Agilent small-animal MR scanner utilizing a spin-echo diffusion-weighted sequence with a 25-direction diffusion weighting scheme (25-direction and 25-b-value) was performed. All images were obtained with following acquisition parameters: TR = 1.5 s, TE = 37 ms, Δ = 18 ms, δ = 6 ms, maximal b-value = 2,200 s/mm², slice thickness = 0.8 mm, FOV (field of view) = 22.5 × 22.5 mm², in-plane resolution = 117 × 117 μm² (before zero-filled). MnCl₂ injection: 3-4 days post-DBSI scans, an intravitreal injection of 50 nmol MnCl₂ (0.25 μL of 0.2 M, at 3 μL/min) was performed on one eye from both DBA/2J and WT mice with 34-gauge needle*. After injection, mice were returned to regular cages for one recover from the anesthesia. MEMRI: after one hour post-injection, experiments were performed on the same scanner as DBSI using a standard 3D gradient echo sequence with the following parameters: TR = 15 ms, TE = 2.63 ms, flip angle = 20°, FOV = 15 × 15 × 22 mm³, matrix size = 128 × 128 × 64 (zero-filled to 256 × 256 × 128), acquisition time = 32.8 minutes, number of signals averaged = 16, and ten successive sets of 3D-T1W images were captured – 1.55 – 5.4 hours post-injection. The active-decoupled coils for MEMRI were the same as DBSI. B1-inhomogeneity correction: 3D-T1W image of a 2% agar gel phantom was placed underneath surface coil using the same MEMRI acquisition parameters with 64 averages. The raw 3D-T1W image of mouse brain was divided by the 3D-T1W image of phantom voxel by voxel using ImageJ to correct the 3D-T1W image. Data analysis: DBSI: 25 diffusion weighted signals and one signal at b = 0 were fitted into a linear combination of one anisotropic diffusion and multiple isotropic diffusion tensor components to estimate λǁ, λ⊥, fractions of restricted (putative cellularity) and non-restricted isotropic diffusion (putative vasogenic edema) using in-house DBSI computation package. Transport rate calculation: The corrected 3D-T1W image set was adjusted and rotated to an oblique plane covering retina and optic nerves before optic chiasm (Fig. 2A). Line ROIs were drawn on Mn²⁺-loading and contralateral optic nerves for all oblique corrected T1W images (Fig. 2A). Arrival of Mn²⁺ was determined by intensity of voxel enhancement (A). For quantification, line ROIs were drawn on Mn²⁺-loading and contralateral nerves (B). Arrival of Mn²⁺ was determined by threshold and normalized by whole length of the optic nerve. Normalized displacement of WT and DBA/2J mice at each time point showed different axonal transport rates (C). A significant axonal transport disruption was seen in optic nerves from DBA/2J mice. * indicates p < 0.005 Scale bar: 100 μm

Results
Comparing to control mice, DBA/2J mice exhibited a statistically significant 16% lower DBSI-λǁ (p < 0.005) and 87% higher inflammation (sum of restricted and non-restricted diffusion fraction; p<0.005). Mild demyelination was seen in DBA/2J mouse optic nerves. Axonal transport rate was significantly decreased by 58% from that of control mice. Representative IHC images suggest axonal injury (decreased SMI-31), demyelination (less MBP positive area), and inflammation (increased number of DAPI positive nuclei) in DBA/2J mouse optic nerves.

Conclusion
Our results demonstrated that DBSI not only detects axonal pathologies but also reflect inflammation. MEMRI, as has been shown in the literature, is readily applicable for in vivo axonal transport assessments in animal models. The current finding suggests that DBSI could potentially play a role in assessing optic nerve pathology in patients to understand the role of axonal degeneration in glucoma related blindness.

Reference

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Figure 1 Representative DBSI maps of control and DBA/2J optic nerves demonstrated a decrease of DBSI λǁ and λ⊥, and an increase of restricted (cells) and non-restricted (vasogenic edema) diffusion fraction in DBA/2J optic nerve. Group averaged analyses showed significant axonal injuries (reduced DBSI λǁ) and inflammation (cells +edema) and slight but not significant demyelination (increased DBSI λ⊥). * indicates p < 0.005

Figure 2 A serial time-lapse oblique and intensity corrected T1W images from representative mice from WT and DBA/2J optic nerves were presented to demonstrate the different degree of Mn²⁺ enhancement (A). For quantification, line ROIs were drawn on Mn²⁺-loading and contralateral nerves (B). Arrival of Mn²⁺ was determined by threshold and normalized by whole length of the optic nerve. Normalized displacement of WT and DBA/2J mice at each time point showed different axonal transport rates (C). A significant axonal transport disruption was seen in optic nerves from DBA/2J mice. * indicates p < 0.005

Figure 3 Representative 50x SMI-31 (intact axon), MBP (myelin), and DAPI (cell nuclei) from WT and DBA/2J optic nerves suggest the presence of axonal degeneration, demyelination, and inflammation in DBA/2J optic nerves.