Yield of Investigations in Young Patients Presenting with Transient Monocular Vision Loss

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Introduction: The quest for non-invasive imaging biomarkers to detect axonal pathology in amyotrophic lateral sclerosis (ALS) remains unfulfilled. Prior research identified axonal spheroids, indicative of altered axonal transport, in the retinal nerve fiber layer (RNFL) of post-mortem retinas of ALS patients. This study aims to determine if a mouse model of ALS exhibits a retinal phenotype that increases over time through eye imaging.

Methods: We clinically monitored SOD1G93A mice (n=28, 10 males/18 females) and age-matched controls (n=28, 10 males/18 females), at 8 weeks of age for a duration of 12 weeks. Longitudinal in vivo retinal imaging was conducted every two weeks using Infrared Reflectance (IR-815 nm) and Blue Reflectance (BR-486 nm) fundus imaging, and Optical Coherence Tomography (OCT-870 nm) (Spectralis, Heidelberg Engineering). Hyperreflective puncta within the RNFL identified via IR-cSLO and confirmed with OCT were quantified. At 20 weeks, mice were euthanized, and wholemount retinas were processed for immunofluorescence staining with markers for axons (phosphorylated neurofilament, P-NF) and mitochondria (voltage-dependent anion channel 1/2, VDAC1/2). Statistical analysis was conducted using Generalized Linear Mixed Models (GLMMs).

Results: Hyperreflective puncta localized to the RNFL were observed in both female and male ALS mice via in vivo IR-cSLO and OCT. The number of puncta in ALS mice significantly increased over the study period ($p<0.001$), with ALS mice exhibiting significantly more puncta at 20 weeks compared to controls (mean ± SD: 5.2 ± 7.2 vs 0.9 ± 1.8, $p<0.001$). Female ALS mice displayed a significantly higher puncta count than female control mice (7.2 ± 8.3 vs 0.6 ± 0.9, $p<0.01$), and male ALS mice had significantly more IR-puncta than male control mice (1.6 ± 1.7 vs 0.6 ± 0.5, $p<0.05$). There was no correlation between clinical scores and puncta count. Immunofluorescence staining revealed the presence of P-NF-positive axonal spheroids in the RNFL and a pronounced VDAC1/2 signal in ALS retinas, with no colocalization observed in axonal spheroids.

Conclusion: Longitudinal in vivo imaging has uncovered a novel, progressive, and sex-dependent retinal phenotype in SOD1G93A mouse, characterized by RNFL axonal pathology. These findings underscore the potential of eye imaging as a valuable, non-invasive biomarker for ALS, offering prospects for early detection, monitoring disease progression, and evaluating treatment efficacy in clinical trials.