Eye Imaging to Develop a Non-Invasive Biomarker for Amyotrophic Lateral Sclerosis

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Background

- Amyotrophic lateral sclerosis (ALS) is an incurable rapidly progressive neurodegenerative disease that causes degeneration of motor neurons and death.[1]
- The pathological hallmark of ALS is the presence of axonal spheroids in motor neurons in the spinal cord.[2]
- Current biomarkers for ALS are expensive and difficult to implement in clinics and clinical trials.[3]
- The retina and optic nerve are extensions of central nervous system (CNS) and provide valuable insight into CNS diseases.
- Our team recently demonstrated existence of retinal spheroids in retina of post-mortem ALS eyes compared to age-matched controls.[4]
- 10% of ALS cases are familial and SOD1 mutations are present in about 19% of these cases.[5]
- SOD1 transgenic mice are a well-established animal model to study ALS, as they replicate the ALS in patients.[6]

Purpose

- To determine if hyperreflective profiles exist in SOD1 transgenic ALS mouse model.
- To characterize retinal hyperreflective profiles in ALS mouse models.

Materials and Methods

- Experiments were performed after Keenan Research Centre Animal Care Committee approval.
- SOD1 transgenic mice (n= 8, 4M/4F) and WT mice (n =35, 4M/4F) (C57BL/6J strain)
- Muscle weakness was monitored using a scoring system of ALS related clinical findings.
- At age of 2 months, *in vivo* imaging was performed weekly under general anesthesia (2% isoflurane) using Spectralis system (Heidelberg Engineering, Germany) that combines confocal laser scanning ophthalmoscope (cLSO): **Blue Reflectance-BR (488nm) InfraRed-IR (820nm),** and Optical Coherence Tomography (OCT, 870nm).
- A 25D lens and a contact lens with a curvature of 1.7 were used during imaging.

Statistical analysis

- Linear mixed model was used to compare increase of hyperreflective profiles over time in SOD1 mice with that of control mice.

Figure 1. Spectralis HRA + OCT, Heidelberg Engineering, Germany

Results

InfraRed (IR)

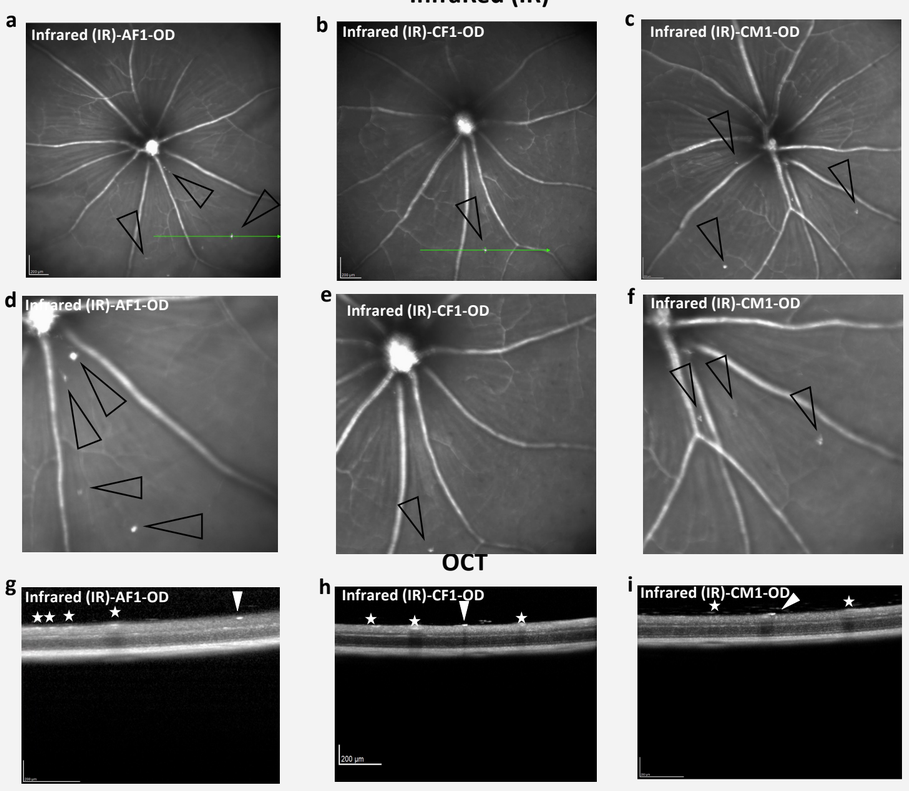

Figure 2. Retinal images of ALS and control mice (OD) at age 20 weeks . (a) Fundus image of ALS AF1 mouse shows Hyperreflective profiles (*Arrow heads*). **(b)** Fundus image of control CF1 mouse **(c)** Fundus image of Control CM1 mouse shows Hyperreflective profiles (*Arrow heads*) **(d-f)** Zoomed in fundus images of AF1, CF1 and CM1. **(g-i)** OCT scans of AF1, CF1 and CM1 retina. Hyperreflective profiles are in the inner most layer of retina (*arrowheads*). Blood vessels are shown by *stars*.

- At 20 weeks of age, all ALS mice and 6/8 control mice show hyperreflective profiles in IR mode and OCT.
- The number of retinal hyperreflective profiles significantly increases in female ALS mouse model compared to female controls (P<0.0001) and male ALS mice (p=0.0029).
- These profiles are seen in the innermost layer of the retina in OCT.

	ALS			Control		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Sample size	**4**	**4**	**8**	**4**	**4**	**8**
Puncta number	8	20	28	12	3	15
Mean	2	5	3.5	3	0.75	1.875
SD	2	3.16	2.93	4	0.96	2.95

Table 1. Number of hyperreflective profiles and statistical analysis in SOD1 mice compared with control mice at age 20 weeks.

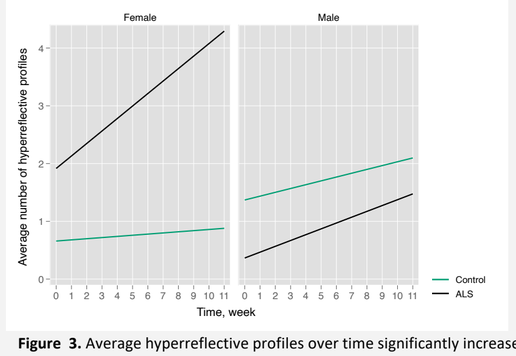

Figure 3. Average hyperreflective profiles over time significantly increase in female SOD1 mice compared to female control (P<0.0001)

Conclusion and Discussion

- We see hyperreflective profiles in ALS mouse in IR modality and OCT.
- ALS mice show similar retinal hyperreflective profiles as post-mortem ALS human eyes.
- The increase in retinal hyperreflective profiles is a promising result.
- The average number of hyperreflective profiles significantly increased in female SOD1 mice compared to female controls during the development of the diseases.
- SOD1 transgenic mouse model can be a promising model to study novel non-invasive ocular biomarkers for ALS.

Future Directions

- Using histopathological analysis of mouse retina sections, we will be able to validate the existence of these hyperreflective profiles and determine their molecular components.
- We will investigate the relationship between these puncta and the clinical progression of ALS in the mice.
- We will also perform Spectralis imaging and histopathological analysis on retina sections of post-mortem human ALS eyes from Human Eye Biobank for Research (HEBR).
- Combining these data, will determine if molecular and optical characteristics of retinal puncta can be used as a novel ALS biomarker.
- Using retinal hyperreflective profiles as a non-invasive biomarker can be relevant in diagnosis, monitoring progression and measuring drug effects in clinical trials searching for a cure for ALS.

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Acknowledgments

THE ALAYNE & RON METRICK FUND
Fondation Brain Canada Foundation
Health Canada Santé Canada
INNOVATION Canada Foundation for Innovation Fondation canadienne pour l'innovation
ALS SLA CANADA
Henry S. Farrugia Ophthalmic Research Fund