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Microstructural Changes in Leber Hereditary Optic Neuropathy (LHON) Revealed by Adaptive Optics Ophthalmoscopy

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1. Main Text

Optic neuropathies (ON) are a group of neurodegenerative diseases affecting the optic nerve, including glaucoma, multiple sclerosis, and hereditary conditions such as Leber Hereditary Optic Neuropathy (LHON). LHON is one of the most common inherited optic neuropathies and is caused by point mutations in mitochondrial DNA, which lead to mitochondrial dysfunction[1], [2], [3]. Although mitochondria are present in nearly all cells, retinal ganglion cells (RGCs), particularly those in the papillomacular bundle, are selectively vulnerable. This results in rapid bilateral central vision loss. The penetrance of the disease is incomplete, and the mechanisms underlying this tissue specificity remain poorly understood. The pathological process is believed to involve impaired mitochondrial energy metabolism and increased oxidative stress [4].

Although enzyme replacement and gene therapies are under investigation for LHON, no effective treatment is currently available, and most patients progress to blindness [3]. The sudden and severe clinical onset of LHON contrasts with the gradual progression typical of other neurodegenerative diseases, highlighting the need for acute, high-resolution diagnostic tools. Building on our development of phase Adaptive Optics Scanning Laser Ophthalmoscopy (AO-SLO) and prior detection of inflammatory cells in multiple sclerosis, we now propose to investigate the RGC layer in LHON patients. Our goal is to extract cellular biomarkers of inflammation and neurodegeneration and perform cell quantification to advance understanding of LHON pathophysiology.

2. Methods

We used a custom-modified AOSLO system MAORI (PSI, Andover MA, USA), which has been previously described by Grieve et al[5]. This device uses adaptive optics to correct ocular aberrations with a 69-actuator deformable mirror operating in a closed loop at 10 Hz, enhancing SLO images to resolve individual cone photoreceptors. The system includes a confocal reflectance channel and four off-axis channels, with optical fibers relaying light to avalanche photodiodes. Off-axis detection generates phase contrast by capturing multiply scattered light refracted by transparent retinal structures. High-contrast images are produced by subtracting and normalizing signals from opposing off-axis apertures. Confocal and off-axis images are acquired simultaneously, corrected for eye motion, and averaged to improve signal-to-noise ratio. Imaging uses a 757 nm source for AOSLO and 840 nm for wavefront sensing.

We acquired retinal images at 4° temporal and nasal eccentricities using our phase contrast AOSLO in a cohort of 38 LHON patients, 15 of whom have received gene therapy. A total of 65 eyes were imaged, including 31 treated eyes, 13 of which were imaged both before and after treatment. Image sequences were registered to generate confocal and phase contrast images. Several patients returned for multiple visits over several months, allowing us to create timelapse videos. Finally, we computed quantitative biomarkers such as cell density and cell size. Patients were also imaged using the commercially available AO corrected camera rtx1 (Imagine Eyes, Palaiseau, France), providing a field of view of 4°x4° at the same eccentricities and focalised in the vascular plexus. We computed qualitative and quantitative biomarkers such as cell density and size.

3. Results

Imaging of the retinal ganglion cell layer in LHON patients revealed the presence of inflammatory cells (Fig.1), with differences in their density and distribution observed before and after gene therapy. Using our high-resolution AOSLO system, we documented these cells in depth with high precision, along with other structures such as stable cysts. While inflammatory cells were mobile and shifted between visits, cysts remained fixed over time. These cells were round with an average size of 12.1 ± 3.4 μm. These cells exhibited a compact cytoplasm and typically contained a round intracellular feature, presumably the nucleus, although multiple features were occasionally observed. Comparison with histological data supports their identity as immune infiltrates, likely lymphocytes, based on their morphology and behavior.

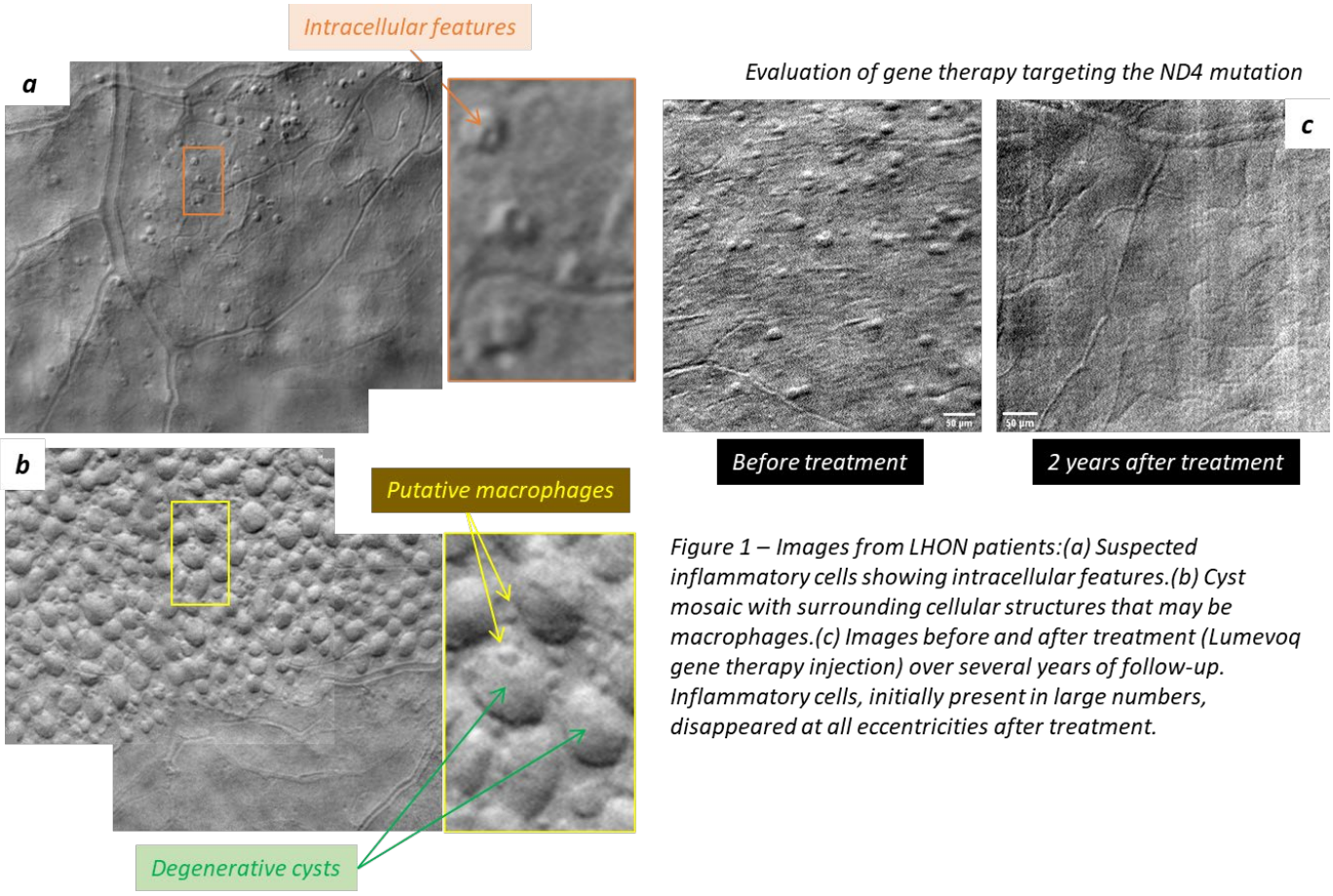

Figure 1 – Images from LHON patients:(a) Suspected inflammatory cells showing intracellular features.(b) Cyst mosaic with surrounding cellular structures that may be macrophages.(c) Images before and after treatment (Lumevoq gene therapy injection) over several years of follow-up. Inflammatory cells, initially present in large numbers, disappeared at all eccentricities after treatment.

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4. References

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