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The pressure-induced deformation response of the human lamina cribrosa: Analysis of regional variations

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ABSTRACT

The objective of this study was to measure the pressure-induced deformation response of the human lamina cribrosa (LC) and analyze for variations with age and anatomical region. The posterior scleral cup of 8 eyes from 6 human donors was mounted onto a custom inflation chamber. A laser-scanning microscope was used for second harmonic generation (SHG) imaging of the collagen structure in the posterior volume of the LC at pressures from 5 mmHg to 45 mmHg. The SHG volumes were analyzed by the Fast-Fourier Iterative Digital Volume Correlation (DVC) algorithm for the three dimensional (3D) displacement field. The components of the Green–Lagrange strain tensor and the in-plane principal and maximum shear strains were evaluated from the DVC displacement field for the central and peripheral regions of the LC and the nasal, temporal, inferior, and superior quadrants surrounding the central retinal artery and vein. Among the major findings were that older age was associated with lower strains, the maximum shear strain was larger in the peripheral than central region, and the maximum principal strain was lower in the nasal quadrant. The elliptical shape of the LC was also predictive of the biaxial strain ratio. Age-related and structure-related variations in the pressure-induced strains of the LC may contribute to the susceptibility and severity of optic nerve damage in glaucoma, and regional variations may explain the progression of axonal damage and tissue remodeling observed in the LC in glaucoma.

Statement of Significance

Glaucoma causes vision loss through progressive damage of the retinal ganglion axons at the lamina cribrosa (LC), the connective tissue structure that supports the axons as they leave the eye. Mechanical characterization of the LC is challenging because of the complex 3D shape and inaccessibility of the tissue. We present a new method using digital volume correlation to map the 3D displacement and strain fields in the LC under inflation. We report for the first time significant regional variations in the strains that are consistent with the pattern of optic nerve damage in early glaucoma. Thus regional strain variations may be predictive of the progression of axonal damage in glaucoma.

1. Introduction

The human lamina cribrosa (LC) is a connective tissue structure in the optic nerve head that forms a part of the eye-wall separating the intraocular cavity and the intraocular pressure from the retrocular space and the intracranial pressure. The LC serves to mechanically support the axons of the retinal ganglion cells as they exit the eye, the lamina capillaries that nourish the neural and cellular tissues of the optic nerve head, and the central retinal artery and vein entering and exiting the eye through the optic nerve head. Glaucoma is characterized by the progressive dysfunction and death of the retinal ganglion cells and remodeling of the connective tissue structure at the LC [1]. In glaucoma, defects in the visual field develop in the mid-periphery in the early stages of the disease and can grow into significant central and peripheral vision loss in later stages [2,3]. Glaucomatous optic axon damage occurs in all

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regions of the optic disk, and regional variations in damage depend on the stage of the disease [1,4–6]. Jonas et al. [4] showed that damage to the neuroretinal rim in moderate glaucoma occurred mainly in the inferotemporal and superotemporal regions [5], while the remaining portions of the neuroretinal rim in advanced glaucoma was found in the nasal region [5]. Quigley and Addicks [6] performed histological evaluations of optic nerve axons at the LC and found that axon loss occurred in all regions but was greater in the superior and inferior regions of the LC. Furthermore, the pattern of axon loss corresponded to regional differences in the structure of the LC, which contained larger pores and thinner beams in the superior and inferior regions [7].

The intraocular pressure (IOP) is strongly associated with the prevalence and severity of optic nerve axon damage in open angle glaucoma [8–11]. Higher IOP is associated with increased prevalence [12–14] and lowering IOP slows the progression of the disease [15,16]. Higher IOP is also associated with disruption of axonal transport [17–22], changes in gene expression and signaling, and dysfunction of the neurons and glia [23,24]. However, there is a wide overlap in the distribution of IOP among those with and without glaucoma. Nearly half of patients with open angle glaucoma (OAG) have normal IOP, lower than 21 mmHg, and the majority of ocular hypertensives do not develop glaucoma [10,25–27]. The stress and strain state of the connective and neural tissues of the optic nerve head are determined by the level of IOP and the mechanical behavior of the LC and the surrounding sclera [28–32]. Variations in the structure and mechanical properties of the LC and sclera may explain why some patients with low IOP develop glaucoma, while some patients with high IOP do not, and why in a few patients their glaucoma continues to progress after IOP lowering treatment.

The objective of this study was to measure the deformation response of the human LC to controlled pressurization and analyze for variations with age, LC shape, and anatomical region. Mechanical characterization of the LC is challenging because of the complex 3D geometry, small size, and inaccessibility of the tissue. Previous studies of LC deformation measured the displacements of landmarks in the LC [33–35] and adjacent tissue structures [36], and changes in the anatomical geometry of the LC using light microscopy [37] nonlinear optical microscopy [38], static histomorphometry [39,40], and spectral domain optical coherence tomography (SD-OCT) [41–43]. Albon et al. [38] measured the volume strain and perimeter strain of the normal human LC from second harmonic generation (SHG) imaging of collagen viewed perpendicular and parallel to the optic nerve axis, and found a stiffer response with age. Zeimer et al. [35] measured a stiffer displacement response of the LC in post-mortem human glaucoma eyes than in non-glaucoma eyes using laser Doppler velocimetry, while Belleza et al. [39] measured a more compliant response in early glaucoma monkey eyes using static histomorphometry.

Recent advances in volume imaging and post-processing methods have made possible full-field deformation mapping of the pressure-induced deformation in the optic nerve head. Sigal et al. [44] applied 2D digital image correlation to analyze the anterior-posterior projections of SHG z-stacks of the human LC with *ex vivo* inflation and reported a highly heterogeneous strain field with localized regions of large in-plane strains. Girard et al. [45,46] applied digital volume correlation (DVC) to analyze the deformation of *in vivo* SD-OCT scans of the visible anterior volume of the human LC and peripapillary sclera after IOP-lowering surgery. Results from 8 glaucoma patients showed that lowering IOP decreased the strain and displacement magnitudes in all tissues of the optic nerve head for all patients. Coudrillier et al. developed a phase-contrast micro-computed tomography (micro-CT) method that used DVC to measure the deformation response of the LC to inflation. The authors applied the method to pig eyes and reported that stiffening of the peripapillary sclera with glutaraldehyde increased the maximum principal strain in the LC [47].

We developed an *ex vivo* inflation test that uses a laser scanning microscope to acquire the backscattered SHG signal of the posterior volume of the human LC under an applied pressure, and the Fast Iterative DVC algorithm of Bar Kochba et al. [48] to analyze the SHG volumes and calculate the 3D displacement field. In DVC, the deformation field is obtained by correlating the image intensity distribution within subsets of voxels between a reference (undeformed) and deformed image volume [49,50]. First introduced by Bay et al. [51], DVC has been applied to a wide variety of imaging modalities to map the deformation of heterogeneous materials, including bone [51,52], wood [53], and 3D cell cultures [54,55], in addition to the more recent applications to the LC. The Fast Iterative DVC is a computationally efficient and high resolution DVC method that performs subset matching in the Fourier domain [48,56] and iteratively refines the subset size and spacing to resolve sharp gradients in the displacement field. We developed a method to compute the strain field from the gradient of the DVC displacement field and analyzed the strain components and strain invariants for correlations with age and the shape of the LC opening and for variations with the anatomical regions of the optic nerve head.

2. Methods

2.1. Specimen preparation

Eight human eyes from 6 donors (ages 26–93) were received from the National Disease Research Interchange (NDRI) 12–24 h post-mortem. The group included eyes from 3 male and 3 female donors with a mean age of 67.0 ± 26.6 years and no history of glaucoma (Table 1). The eyes were shipped wrapped in wet gauze in a closed container placed on ice. Upon receipt, the eyes were stored at 4 °C, and all testing was conducted within 48 h post-mortem.

The extraocular tissues were removed from the eye with fine scissors and the optic nerve was cut flush with a razor blade to the sclera. The depth of this cut varied among specimens (Fig. 1a), and multiple thin cuts were often required to remove the post-LC myelinated optic nerve to reveal the trabecular structure of the LC, which was visualized under a dissecting microscope after each cut.

The eye was glued to a polycarbonate ring, machined to fit the curvature of the globe, 2–5 mm posterior to the equator using cyanoacrylate (Permabond 910, Electron Microscopy Sciences, Hatfield PA). Three ring sizes were manufactured to account for variation in the globe diameter. The ONH was centered within the ring, and the exposed posterior sclera and LC were kept hydrated with 1 M phosphate buffered saline (PBS) as the adhesive dried. The cornea, anterior sclera, and intraocular components, including the retina and choroid, were removed with a scalpel leaving the posterior scleral shell. The anterior scleral edge was scored using a razor blade and the edges glued flat to the back of the polycarbonate ring to provide a more rigid seal (Fig. 1b).

2.2. SHG imaging

The specimen was mounted onto a custom inflation chamber with tilt-correcting gears about two orthogonal in-plane axes [57]. The pressure lines were flushed of air and filled with PBS and the specimen inflated briefly to ensure there were no leaks prior to testing (Fig. 1c). The inflation chamber was placed on the stage of a Zeiss laser-scanning microscope (LSM 710 NLO, Oberkochen, Germany) (Fig. 1d) and the exposed posterior surface was covered with PBS to maintain hydration for the duration of the