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Application of Elliptic Fourier analysis to describe the lamina cribrosa shape with age and intraocular pressure

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ABSTRACT

The lamina cribrosa (LC) plays an important biomechanical role in the optic nerve head (ONH). We developed a statistical shape model of the LC and tested if the shape varies with age or IOP. The ONHs of 18 donor eyes (47–91 years, mean 76 years) fixed at either 5 or 50 mmHg of IOP were sectioned, stained, and imaged under a microscope. A 3D model of each ONH was reconstructed and the outline of the vertical sagittal section closest to the geometric center of the LC extracted. The outline shape was described using Elliptic Fourier analysis, and principal components analysis (PCA) employed to identify the primary modes of LC shape variation. Linear mixed effect models were used to determine if the shape measurements were associated with age or IOP. The analysis revealed several modes of shape variation: thickness and depth directly (PC 1), or inversely (PC 2) related, and superior–inferior asymmetry (PC 3). Only PC 3 was associated with IOP, with higher IOP correlating with greater curvature of the LC superiorly compared to inferiorly. Our analysis enabled a concise and complete characterization of LC shape, revealing variations without defining them *a priori*. No association between LC shape and age was found for the relatively old population studied. Superior–inferior asymmetry of LC shape was associated with IOP, with more asymmetry at higher IOP. Increased IOP was not associated with LC thickness or depth.

1. Introduction

The characteristic morphological changes of the optic nerve head (ONH) that occur concurrently with glaucoma have long been regarded as an important clinical biomarker of disease progression. Mounting evidence suggests that injury to the retinal ganglion cell axons at the level of the lamina cribrosa (LC) causes the surface morphology changes associated with ONH cupping and the ensuing vision loss (Quigley and Cone, 2013).

The LC is shaped by the spatial distribution of dense collagenous trabeculae that provide structural and nutritional support to the ganglion cell axons within the anterior region of the scleral canal (Sigal and Ethier, 2009). Numerous studies have focused on the analysis of LC shape, hypothesizing that this might represent a biomarker useful for glaucoma, for example: i) LC shape could indicate the strength of the tissues to resist the biomechanical insult of elevated IOP, e.g. thicker LCs corresponding with stiffer, stronger LCs (Sigal et al., 2005a; Yang et al., 2009), ii) LC shape may mark progression of glaucomatous neuropathy, e.g. thinner LCs corresponding with more advanced disease (Ren et al., 2009), perhaps with thickened LCs early in the disease (Yang et al., 2007), iii) changes in LC shape could indicate the sensitivity to IOP, e.g. LCs that deform more under elevated IOP corresponding with increased sensitivity to IOP (Yang et al., 2007; Grytz et al., 2012). However, there is no standardized method for measuring and describing the LC shape. A robust method of characterizing LC shape would enable a more refined description and comprehensive understanding of regional anatomy, sensitivity to IOP and progression of disease.

LC shape has conventionally been described using the so-called 'traditional morphometrics' (Quigley et al., 1990; Jonas et al., 1991). These methods, however, bear significant limitations including low statistical power due to measurement collinearity and the requirement that features of interest be defined *a priori*. In order to

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0014-4835/

Fig. 1. Example of the LC section (left) and with the tissue segmentation overlaid (right). Five tissue regions were identified: pre and postlaminar neural tissue (yellow), LC (red), pia mater (green) and sclera (orange). The fiducial markers used for unwarping are the four dark dots. Segmentation was done using a combination of bright field (left) and dark field images (not shown).

circumvent these restrictions we developed a statistical model of the shape of the LC using the Elliptic Fourier analysis (EFA), a geometric morphometric technique that is commonly employed in the quantitative description of biological forms (Sanfilippo et al., 2009). The objective of this work was to characterize the shape of the LC and how it varies with IOP and age. We wanted to avoid preconceptions on the nature of the changes that would occur with age and IOP and therefore we used methods of geometric morphometrics that would help us find the changes in shape, rather than the methods of traditional morphometry.

2. Materials and methods

2.1. Eye and section preparation

Eighteen eyes from nine donors were obtained from the Eye Bank of Canada and managed in accordance with the provisions of the Declaration of Helsinki for research involving human tissue. A full description of the histologic techniques has been presented elsewhere (Sigal et al., 2010, 2012). Briefly, the eyes were perfusion fixed by rapidly exchanging the isotonic saline with fixative (2.5% paraformaldehyde/2.5% glutaraldehyde) and maintaining IOP for 24 h. In each set of eyes, one was fixed at 5 mmHg, and the other at 50 mmHg. After fixation the ONH and peripapillary sclera were embedded in JB-4 plastic using a special mold with collagen sutures as fiducial markers. Sagittal (superior–inferior) sections 2 μm thick were cut at right angles to the fiducial markers. Sections were then stained with picrosirius red to identify collagen, and solochrome cyanin to identify myelin, nuclei, and blood cells, and photographed under bright field and dark field illumination (Leica MZ6, Heerbrugg, Switzerland) using a Nikon Coolpix 990 digital camera (2048 × 1536 pixels, 8 RGB bits per channel per pixel; Nikon, Tokyo, Japan). The images were aligned and unwarped to correct deformations that occurred during the sectioning process using a custom modified version of TPSSuper (F. James Rohlf, SUNY, Stony Brook, NY) based on the known fiducial marker positions as cast into the histologic block during the embedding procedure. From the images a 3D model of the ONH was reconstructed following our iterative procedure described elsewhere (Sigal et al., 2010). The anterior boundary of the LC was defined by the termination of the laminar beams and the insertion points at the sclera, whereas the posterior boundary was defined by two features: the termination of solochrome cyanin staining, indicating a lack of myelination inside the LC, and the "stacked plate" morphology of the connective tissues typical of the LC. To ensure consistency across all eyes, all the segmentations were checked and adjusted by a single observer (JGF). Once a 3D model of the ONH had been reconstructed and optimized, as described elsewhere (Sigal et al., 2010) the sagittal section closest to the center of mass of the 3D LC geometric center was selected and its segmentation used to define the LC for analysis for that eye. If the section contained the central retinal artery or vein through the LC the neighboring more temporal section was selected. This only occurred in four cases. Orientation information was preserved using the fiducial markers from the mold (Fig. 1).

2.2. Measurement of LC shape

EFA was used to quantify the shape of each LC section (Kuhl, 1982). With EFA, coefficients of sine and cosine terms

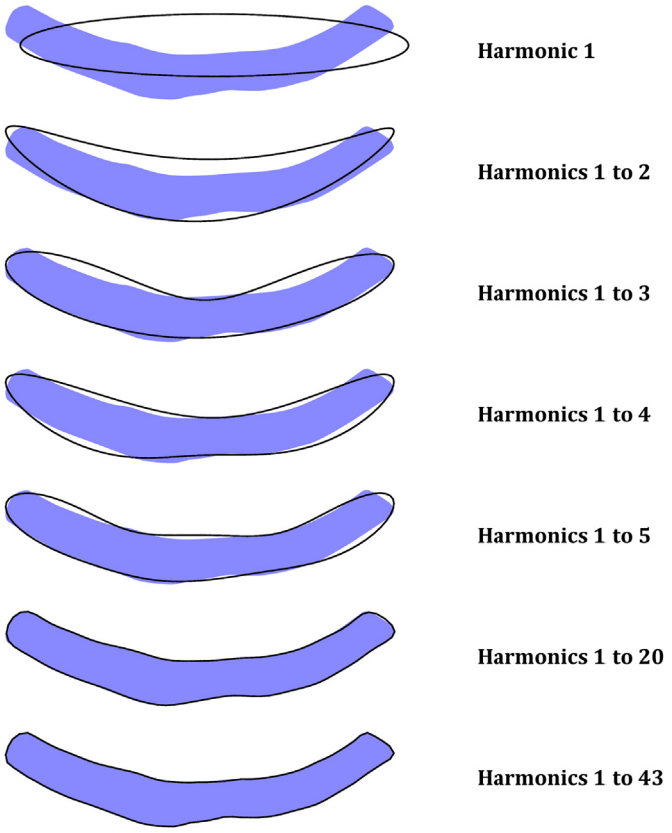

Fig. 2. Sequence of outline reconstructions from Elliptic Fourier analysis applied to the outline coordinates of the LC. The LC shape to be described is shown by the light blue shape, while reconstructed shapes are thin black outlines. Outlines are reconstructed using progressively more harmonics, from the simplest ellipse (one harmonic) at the top, to the highly accurate reconstructions with 20 and 43 harmonics. Note that each harmonic is described by four Fourier coefficients, two for the horizontal axis and two for the vertical axis.