RESEARCH LETTER

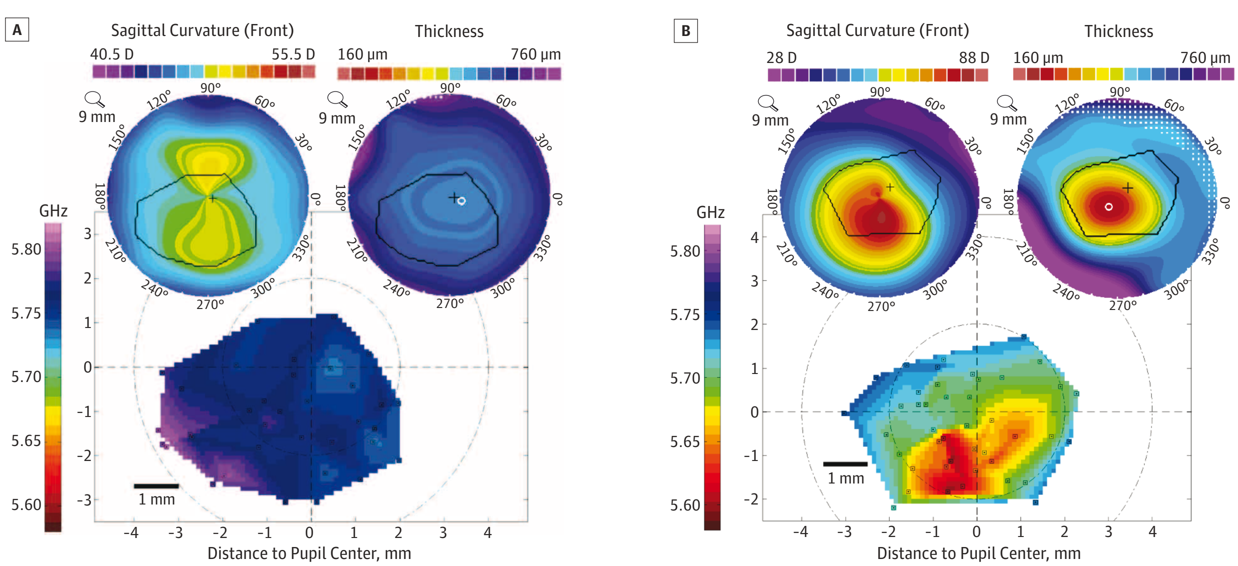
In Vivo Biomechanical Mapping of Normal and Keratoconus Corneas

Corneal mechanical strength is critical to withstanding intraocular pressure and maintaining normal shape.[1,2] In keratoconus, the mechanical stability is compromised,[3] which may lead to progressive morphological changes. Therefore, a noninvasive technique capable of accurately measuring the mechanical properties of the cornea may help us understand the mechanism of keratoconus development and improve detection and intervention in keratoconus. We previously developed Brillouin microscopy based on light scattering from inherent acoustic waves in tissues[4] and showed that this technique can provide quantitative estimates of local longitudinal modulus,[5] which correlate to the Young and/or shear moduli of the cornea.[2,6] Using a clinically viable instrument, for the first time, to our knowledge, we mapped the elastic modulus of normal and keratoconus corneas in vivo. We found distinctive biomechanical features that differentiate normal and keratoconus corneas and therefore have the potential to serve as diagnostic metrics for keratoconus.

Methods | The study recruited 6 volunteers with normal corneas (mean [SD] age, 37 [15] years) and 5 patients with advanced keratoconus (mean [SD] age, 43 [7] years). All participants signed an informed consent form approved by the Partners Human Research Committee (Partners Healthcare Institutional Review Board), in accordance with the principles of the Declaration of Helsinki. We constructed a laser-scanning confocal Brillouin microscope (wavelength, 780 nm; power, 1.5 mW; lateral/axial resolution, 5 μm/30 μm; sensitivity, approximately 10 MHz). The instrument was equipped with wide field-of-view imaging to allow real-time pupil detection and beam positioning (lateral accuracy of <0.5 mm). For participants with normal corneas, areas measuring about 5 × 5 mm in the central region of the cornea were scanned. For patients with keratoconus, similar regions, but including the center of the cone, were scanned as confirmed by their topographic images (Pentacam; OCULUS). To construct Brillouin maps, axial scans were taken at various transverse locations; the anterior mean Brillouin shift was computed from each axial scan by averaging the measured Brillouin shift values of the anterior portion of the corneal stroma. A color-coded elasticity map was obtained by 2-dimensional interpolation of the mean Brillouin shift in the anterior portion.

Results | Normal corneas were found to have relatively uniform anterior Brillouin shifts in the central region (**Figure 1**A). By contrast, keratoconus corneas presented strong spatial variations in Brillouin shifts (Figure 1B). **Figure 2** shows the average anterior Brillouin shifts of normal (n = 7) and keratoconus (n = 6) corneas in the cone region (<1 mm from thinnest

Figure 1. Brillouin Elasticity Maps



A, Representative maps of the mean anterior Brillouin shift for a 53-year-old with normal corneas. B, Representative maps for a 40-year-old patient with advanced keratoconus. Insets are the respective curvature (D indicates diopter) and pachymetry maps with outlined Brillouin-scanned areas.

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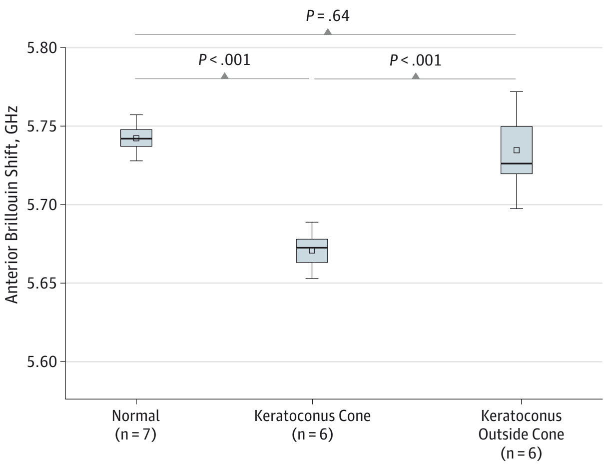
Figure 2. Focal Weakening in Keratoconus



The mean Brillouin shifts of the keratoconic corneas (n = 6) in the cone region vs outside the cone region compared with mean normal cornea values (n = 7). Bars represent standard error.

point) and outside the cone region (>3 mm away from thinnest point). A highly statistically significant decrease (unpaired *t* test, $P < .001$ was found in the keratoconic cone region with respect to normal corneas. Also, a highly statistically significant difference (unpaired *t* test, $P < .001$) was observed between the cone region and outside the cone region. The regions outside the cone showed no statistically significant difference compared with the normal corneas.

Discussion | We have described the distribution of elastic modulus in keratoconus and normal corneas in vivo. The elasticity maps show remarkable spatial variations around the cone. The reduction of 100 MHz in the keratoconus cone region (Figure 2) corresponds to an approximately 3% decrease in longitudinal modulus and approximately 70% reduction in shear modulus.[5] The regions away from the cone in the keratoconus corneas have similar Brillouin shifts as normal corneas, which is consistent with our ex vivo data.[5] This finding supports the longstanding hypothesis that keratoconus involves a spatially localized mechanical alteration in the cornea.[1] It also emphasizes the need for spatially resolved measurements for accurate analysis of the biomechanical anomalies in keratoconus. Future research is warranted to understand the relationship between the focal or heterogeneous mechanical weakening and morphological changes (ie, thinning and steepening) and to develop biomechanics-based metrics for improved diagnosis and prognosis of keratoconus, screening of at-risk patients for post-LASIK (laser in situ keratomileusis) ectasia, and monitoring the effects of corneal collagen cross-linking.

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