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Study concept and design: Stager, Birch.
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In Vivo Biomechanical Mapping of Normal and Keratoconus Corneas

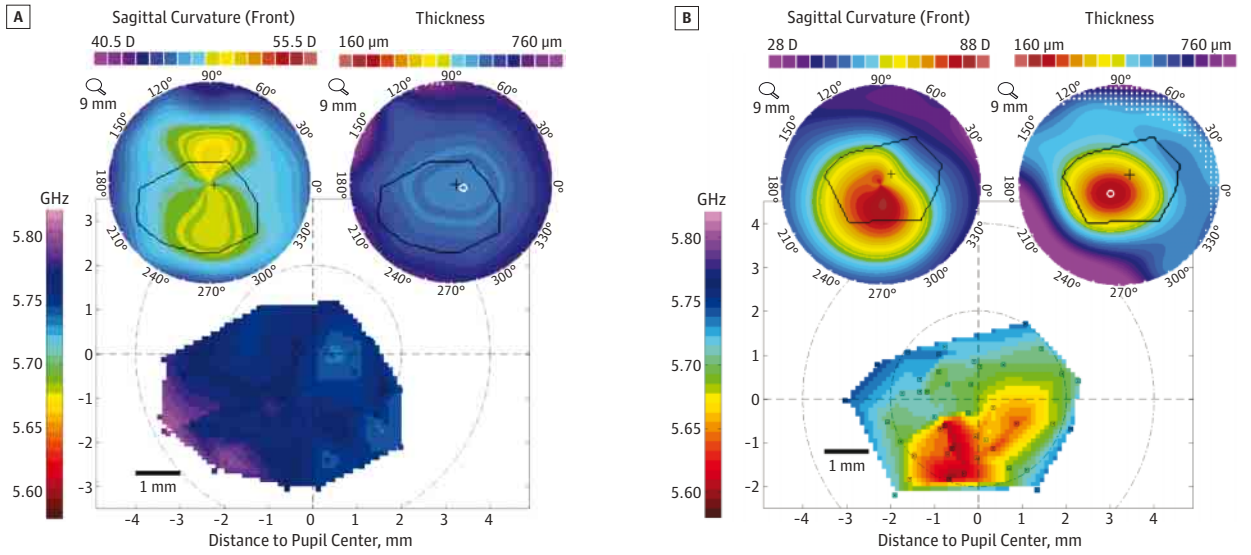
Corneal mechanical strength is critical to withstanding intraocular pressure and maintaining normal shape.[1,2] In keratoconus, the mechanical stability is compromised,[3] which may lead to progressive morphological changes. Therefore, a noninvasive technique capable of accurately measuring the mechanical 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 keratoconic corneas in vivo. We found distinctive biomechanical features that differentiate normal and keratoconic 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 and the study was 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, keratoconic 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 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 (paired *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.

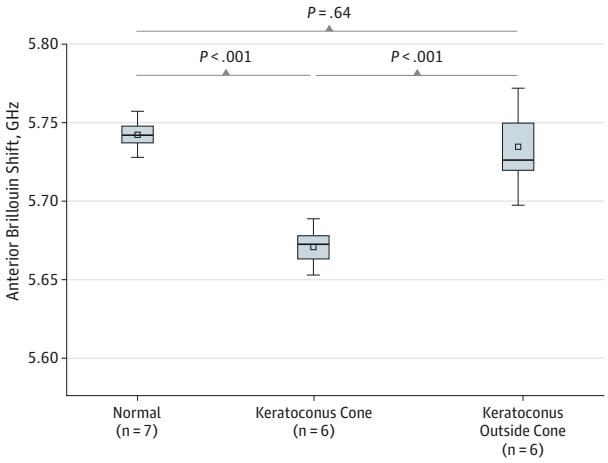
Discussion | We have described the distribution of elastic modulus in keratoconus and normal corneas in vivo. The elasticity maps show remarkable spatial variations around the cone. The reduction of 100 MHz in the keratoconic cone region (Figure 2) corresponds to an approximately 3% decrease in longitudinal modulus and approximately 70% reduction in shear modulus.[5] The regions away from the cone in the keratoconic corneas have similar Brillouin shifts as normal corneas, which is consistent with our ex vivo data.[5] This finding supports the long-standing 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

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.

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 SD.

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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Study concept an design: Scarcelli, Besner, Yun.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Scarcelli, Besner, Yun.
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Possession of the *HLA-DRB1*1501* Allele and Visual Outcome in Idiopathic Intermediate Uveitis

Idiopathic intermediate uveitis (IIU) is a potentially sight-threatening inflammatory disease characterized by breakdown of the blood-retina barrier with consequent leukocytic infiltration of the vitreous and retina. Poor visual outcome has been associated with cystoid macular edema, poor vision at presentation, and male sex.[1] The human leukocyte antigen (HLA) allele *DRB1*1501* has long been associated with multiple sclerosis (MS). Tang et al[2] prospectively analyzed 18 patients and found that *HLA-DRB1*1501* conferred increased risk of developing IIU associated with MS in some patients.

The purposes of this study were to prospectively evaluate the association between the *HLA-DRB1*1501* allele and IIU in patients and to determine whether *HLA-DRB1*1501* might be a separate independent risk factor for visual loss.

Methods | Participants included 85 patients with IIU and 300 healthy, demographically matched controls. Idiopathic intermediate uveitis was classified on the basis of ophthalmological examination and fluorescein angiography findings. Patients with systemic inflammatory, neoplastic, or infectious diseases were excluded, as were patients with a history of optic neuritis. Written informed consent for HLA typing was obtained from all individuals. This study received approval from the St Thomas' Ethical Committee and adheres to the Declaration of Helsinki.

Patients were reviewed at 3 months, 5 years, and 10 years (where possible) between 2000 and 2014. Additional appointments were given as required to adequately maintain control of intraocular inflammation.

Visual acuity (VA) of 6/12 or better was defined as a good outcome. Genomic DNA was extracted and genotyped for the HLA class II allele *HLA-DRB1*1501* using polymerase chain reaction amplification with sequence-specific primers.[3] Polymerase chain reaction products were electrophoresed and read using ethidium bromide and UV illumination.

Results | The **Table** shows VA and demographic data. Thirty-eight cases (45%) were *HLA-DRB1*1501* positive compared with 90 controls (30%) (15% difference; $\chi^2 = 6.45$; $P = .007$; odds ratio = 1.89; 95% CI, 1.15-3.09).

We found no association between VA and possession of the *HLA-DRB1*1501* allele. Twelve *HLA-DRB1*1501*-positive patients (31%) had a VA less than 6/12 at the end of the study, compared with 8 *HLA-DRB1*1501*-negative patients (17%) (14% difference; $P = .15$; relative risk = 1.52; 95% CI, 0.94-2.47). There was no identifiable difference in sex between *HLA-DRB1*1501*-positive and *HLA-DRB1*1501*-negative patients (2.5% difference; $P = .65$; odds ratio = 0.77; 95% CI, 0.31-1.91). The mean (SD) age at presentation was 62.09 (11.6) years for the *HLA-DRB1*1501*-positive patients and 59.72 (16.04) years for the *HLA-DRB1*1501*-negative patients ($P = .46$).

Discussion | Our findings are in agreement with the previously reported association between IIU and *HLA-DRB1*1501*. However, we were unable to identify any association between possession of the *HLA-DRB1*1501* allele and sex or age at presentation, as has been found in MS. We also did not find an association between *HLA-DRB1*1501* and final VA.[4]

Our exclusion criteria, prospective design, and extended follow-up distinguish this study from those previously reported. The patient population was comparable to those in other studies in terms of race, sex, and age.[2,5,6]

The results reflect the relatively benign nature of IIU in that 76% of our patients had good vision at 10 years. Similarly, Raja et al[5] reported a VA higher than 6/12 in 82% of their patients after 4 years.

The prediction of visual outcome from haplotype analysis has not been supported by this study. However, our findings cannot rule out the possibility that IIU is made up of a number of separate disease processes, of which some affect all age groups and others (*HLA-DRB1*1501* related) represent a *forme fruste* of MS. It is possible that patients in this study developed an associated systemic disease after their second review at 5 years but were not seen again at 10 years.

Finally, our data suggest that VA at 3 months reliably predicts vision at 5 and 10 years. This is important for future

Table. Patient Demographic Characteristics and Visual Acuity During the Study Period[a]

Characteristic	Follow-up, No. (%)		
	3 mo (n = 85)	5 y (n = 85)	10 y (n = 34)
Sex			
Male	31 (36)	31 (36)	8 (24)
Female	54 (64)	54 (64)	26 (76)
Race			
White	83 (98)	83 (98)	32 (94)
Nonwhite	2 (2)	2 (2)	2 (6)
Visual acuity			
≥6/12	73 (85)	67 (79)	26 (76)
<6/12	12 (15)	18 (21)	8 (24)

[a] The mean age at presentation was 40 years (range, 14-74 years).