# Reduced Human and Murine Corneal Thickness in an Axenfeld-Rieger Syndrome Subtype

*Mika Asai-Coakwell,*[1,2] *Christopher Backhouse,*[3] *Ronald J. Casey,*[1] *Philip J. Gage,*[4] *and Ordan J. Lehmann*[1,2]

**Purpose.** Axenfeld-Rieger malformations of the anterior segment are clinically heterogeneous, and up to 50% of cases are attributable to *PITX2* or *FOXC1* mutation. In view of *PITX2*'s contribution to corneal development and the altered CCT in some *FOXC1*-related cases, this study was undertaken to investigate whether a related phenotype is associated with the *PITX2/Pitx2* mutation.

**Methods.** Central corneal thickness (CCT) was measured in patients and mice with *PITX2/Pitx2* mutations. CCT in affected individuals and unaffected first-degree relatives from a large *PITX2* mutation pedigree was measured with ultrasonic pachymetry. For murine measurements, the optical coherence tomogram (OCT) was calibrated against plastic films whose thickness had been determined with scanning electron microscopy (SEM). Subsequently, CCT was measured in ex vivo eyes from $Pitx2^{+/-}$ and wild-type murine littermates by using OCT.

**Results.** CCT in individuals with the *PITX2* mutation (mean 484 $\mu$m; range, 425–519; $n = 8$) was significantly lower than in their unaffected first-degree relatives (mean 582 $\mu$m; range, 550–590; $n = 5$; $P = 0.0002$, *t*-test). Scanning electron microscopy (SEM) and OCT measurements of reference films correlated closely ($r = 0.9995$) and subsequent OCT analysis of murine eyes revealed a significant reduction in CCT in $Pitx2^{+/-}$ compared with wild-type littermates ($Pitx2^{+/-}$: mean, 72 $\mu$m; range, 57–87, $n = 6$; wt: mean, 88 $\mu$m; range, 63–100; $n = 6$, $P = 0.035$, *t*-test).

**Conclusions.** The results show that *PITX2/Pitx2* mutation results in reduced corneal thickness and provides the first example of reduced CCT in a genetic subtype of glaucoma. These data will facilitate management of developmental glaucoma and offer potential for guiding molecular genetic testing in patients with Axenfeld-Rieger. The similar CCT reduction observed in patients and mice with comparable mutations emphasizes the utility of this murine model. The technical advance of optical murine CCT measurement also provides scope for serial in vivo imaging of the developing anterior segment and determining the effects of altered CCT on measured IOP. (*Invest Ophthalmol Vis Sci.* 2006; 47:4905–4909) DOI:10.1167/iovs.06-0457

The glaucomatous optic neuropathies represent one of the most prevalent causes of irreversible visual loss,[1,2] and as with other common diseases, treatment is seldom curative. Lowering intraocular pressure (IOP) remains the sole therapeutic strategy,[3] and in recent years it has been recognized that the accuracy of IOP measurements is affected by variations in ocular structure. Studies of the most readily measured parameter, central corneal thickness (CCT), revealed the IOP overestimation associated with increased CCT and the converse effect of decreased CCT.[4–7] Increased CCT has also been identified in types of anterior segment maldevelopment (dysgenesis) associated with glaucoma, including iris hypoplasia (*FOXC1*-encompassing duplications),[8] aniridia (*PAX6* and *Pax6*),[9,10] and dysgenetic lens (*Foxe3*),[8] a murine correlate of Peters anomaly.[11–13] Such examples emphasize the need for animal models to determine the cellular and molecular mechanisms underlying altered CCT, and their contribution to glaucoma. In view of the high risk of early-onset glaucoma present in anterior segment dysgenesis, determining whether other dysgenesis subtypes[14,15] have altered CCT, is of clinical and developmental significance.

Axenfeld-Rieger is a phenotypically heterogeneous group of anterior segment malformations in which dental, facial, cardiac, and umbilical anomalies may be present.[16] Axenfeld-Rieger is also genetically heterogeneous, with up to 50% of cases caused by *FOXC1* or *PITX2* mutations, whereas a third transcription factor (*MAF*) represents a potential cause of cases linked to one of the mapped Axenfeld-Rieger loci.[17–20] Although knowledge of the underlying genetic basis is prognostically important, with more severe glaucoma reported with *PITX2* mutation (Walter M, et al. *IOVS* 2000;41:ARVO Abstract 2809), attributing cases to *PITX2*, *FOXC1* or other genes has not been possible on phenotypic (clinical) grounds. Equally, the wide spectrum of genetic mechanisms underlying Axenfeld-Rieger, including chromosomal duplications and deletions (segmental and telomeric), chromosomal translocations, position effects as well as mutations[15,21–25] have made it impractical to identify the molecular basis in individual patients. Such impediments have combined to limit clinical application of the genetic basis of these disorders.

Normal corneal development is critically dependent on *PITX2*, neural crest cells that form the endothelium and stroma express *Pitx2* once they reach the future anterior segment,[26] whereas in *Pitx2*-deficient animals, corneal agenesis is observed.[27] Prompted by *PITX2*'s contribution to Axenfeld-Rieger and its key corneal developmental role,[26–28] we investigated whether alterations in corneal thickness were associated with the *PITX2* mutation. This involved CCT measurement of affected individuals and intrafamilial controls in an Axenfeld-Rieger pedigree of sufficient size to achieve statistical significance plus adaptation of human ocular imaging techniques to permit parallel measurements in a murine *Pitx2* mutation model. The similar results in patients and mice with comparable hypomorphic *PITX2/Pitx2* mutations, illustrate

From the Departments of [1]Ophthalmology, [2]Medical Genetics, and [3]Electrical and Computer Engineering, University of Alberta, Edmonton, Alberta, Canada; and the [4]Kellogg Eye Center, University of Michigan, Ann Arbor, Michigan.

Supported by Grants EY014126 and EY07003 from the National Eye Institute and funding from Research to Prevent Blindness (PJG); and the Alberta Heritage Foundation for Medical Research, Canadian Institutes of Health Research and Glaucoma Research Society of Canada (OJL). OJL is an AHFMR (Alberta Heritage Fund for Medical Research) Clinical Investigator and Canada Research Chair.

Submitted for publication April 23, 2006; revised June 16, 2006; accepted September 21, 2006.

Disclosure: **M. Asai-Coakwell**, None; **C. Backhouse**, None; **R.J. Casey**, None; **P.J. Gage**, None; **O.J. Lehmann**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Ordan J. Lehmann, Departments of Ophthalmology and Medical Genetics, University of Alberta, 829 Medical Sciences Building, Edmonton T6G 2H7, Alberta, Canada; olehmann@ualberta.ca.

the benefits of combined clinical and scientific investigation of ocular developmental genes and highlight the utility of the *Pitx2* mutation model. The findings have implications for glaucoma management in Axenfeld-Rieger, extend the range of structural changes associated with ocular developmental gene mutation, provide a new technique for studying anterior segment development in model organisms, and may facilitate identification of the genetic basis of the disease in an individual patient.

## METHODS

### Measurement of Human CCT

The previous identification and extensive characterization of a *PITX2* mutation in a local Axenfeld-Rieger syndrome pedigree provided an opportunity for more detailed investigation of the ocular phenotype associated with this sequence change (R69H).[29–31] Affected individuals and their unaffected first-degree relatives from this five-generation pedigree[29] were examined either at the University of Alberta Regional Eye Centre or the patient's home. The slit lamp examination included ultrasonic measurement of central corneal thickness (Pachmate; DGH Technology, Exton, PA) with the mean of 10 readings from the right eye of each individual used for analysis (two-tailed *t*-test assuming equal variance). Only a single large *PITX2* mutation pedigree was available for phenotyping. This study adhered to the tenets of the Declaration of Helsinki and was approved by the University of Alberta Hospital Health Research Ethics Board; informed consent was obtained from all participants.

### Calibration of OCT for Murine Range

To replicate the human findings and validate the clinical utility of an existing murine model, we investigated the effect of a murine *Pitx2*$^{+/-}$ mutation on corneal thickness.[27] This null mutation is comparable to R69H, which exhibits ~90% reduction in transactivation compared with wild-type *PITX2* as well as reduced DNA binding.[31] As histologic measurement of murine CCT is associated with appreciable inaccuracy,[8] the human optical coherence tomogram (Stratus OCT, software version 2.0; Carl Zeiss Meditec, Dublin, CA) was adapted for murine use. This involved calibrating OCT measurements of the thickness of uniform plastic films ($n$ = 9) against those provided by scanning electron microscopy (SEM; Electron Microscope Model 1430; LEO Electron Microscopy, Ltd., Cambridge, UK). For SEM analysis, portions of film were coated with gold (DESK-II cold sputter-etch; Denton Vacuum, Cherry Hill, NJ) before SEM measurements were recorded. For OCT, films were mounted vertically on a precision stand permitting $x$, $y$, and $z$-axis movement. The stand consisted of a rotatable holder on which the sample was mounted and attached via a series of stainless steel rods and a further clamp, to the side bar of the OCT (model numbers: ASC, PR, CR0.5 and MPR; Siskiyou Inc., Grants Pass, OR). The smallest thickness measurement obtained from three scans of each film was used for analysis. The correlation between the OCT and SEM measurements was subsequently determined.

### Murine CCT Measurement

A preliminary study of OCT measurements revealed no significant difference in CCT between fresh right and 4% paraformaldehyde-fixed left C57 murine eyes (Charles River, Wilmington, MA; data not shown). Accordingly, ex vivo eyes from *Pitx2*$^{+/-}$ mice and wild-type littermates aged 12 weeks, stored in phosphate-buffered saline after 24 hours of fixation with 4% paraformaldehyde and labeled with a numeric identifier, were used for subsequent analysis. For these experiments, the Pitx2 null allele had been backcrossed ($n$ = 7) onto an inbred C57BL/6J background, making each animal essentially identical genetically except at the *Pitx2* locus.

CCT was measured on the stage described earlier, by using the OCT's corneal function. To ensure perpendicularity of the OCT scan to the iris plane and hence measurement of central corneal thickness, all scans were centered on the pupil and were repeated after rotating the stage through 90°. PBS was periodically applied to keep the globes moist. CCT was determined with the OCT's integral software and the measurement from each eye that displayed the lowest CCT was included in the data analysis. A correction factor, equal to the mean discrepancy between OCT and SEM measurements, was applied to all readings. After the investigator was unmasked to genotype status, CCT data from the right eyes of *Pitx2*$^{+/-}$ and wild-type mice were compared (two-tailed *t*-test assuming equal variance). Histology using plastic (epoxy-resin) embedded sections was also performed on a small number ($n$ = 5) of *Pitx2*$^{+/-}$ and wild-type eyes. The study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

## RESULTS

### Human CCT Measurement

CCT in individuals with the *PITX2* mutation (mean, 484 μm; range, 425–519, $n$ = 8) was significantly lower than in their unaffected first-degree relatives (mean, 582 μm; range, 550–590, $n$ = 5; $P$ = 0.0002, *t*-test; Fig. 1). Decreased CCT was present in every individual with the *PITX2* mutation, all of whom exhibited the characteristic Axenfeld-Rieger anterior

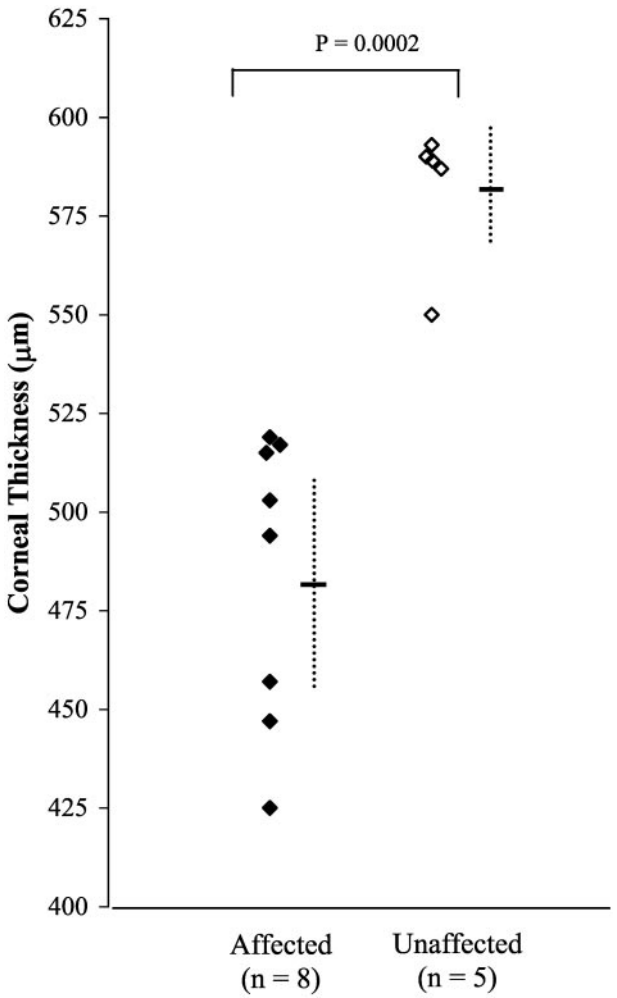


**FIGURE 1.** Corneal thickness of affected and unaffected individuals from the *PITX2* mutation pedigree. The statistical significance, the mean and 95% CI (*solid* and *dotted* lines; *right*) are displayed for each data set. The mean CCTs were 484 μm (affected) and 582 μm (unaffected); the affected or unaffected status of individuals has been confirmed by genotyping.[30]

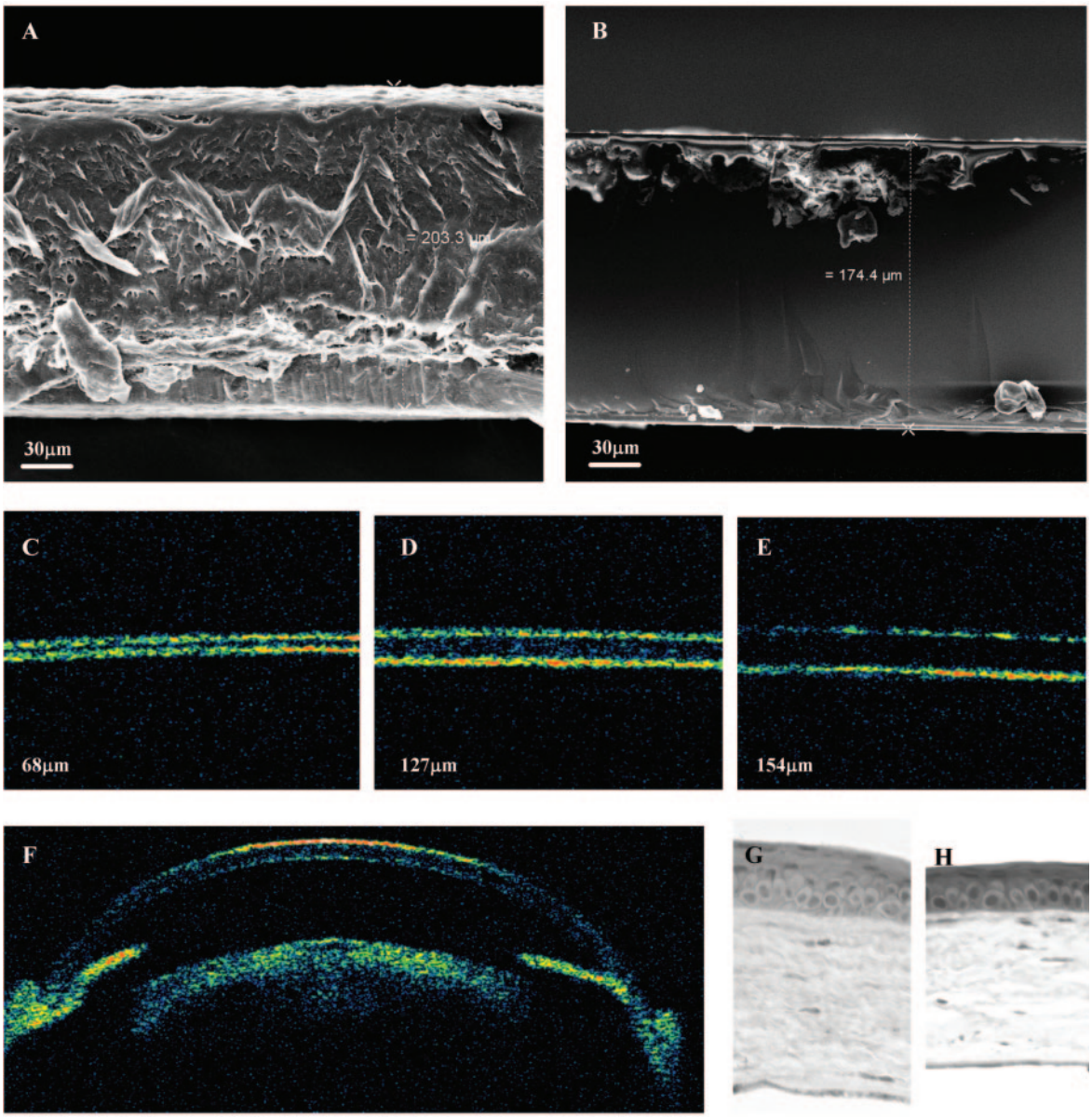


**FIGURE 2.** SEM (**A**, **B**) and OCT (**C**, **D**, **E**) images of reference plastic films used to calibrate OCT in the murine range (measurement scale or OCT reading, *bottom left*). (**F**) OCT images of the murine anterior segment (wild-type C57) demonstrating the high level of resolution achievable. (**G**, **H**) Photomicrographs of plastic-embedded sections from wild-type and *Pitx2*$^{+/-}$ corneas respectively; note the reduced stromal and epithelial thickness, and the number of epithelial cell layers, in *Pitx2*$^{+/-}$ cornea. Magnification, ×20.

segment phenotype,[29] even though some ($n$ = 4) had had neither glaucoma nor ocular surgery. Taken together with the observation of reduced corneal diameter (10–11 mm) in four affected individuals, this excluded the possibility of either iatrogenic or buphthalmic corneal thinning.

## Validation of OCT Measurements

SEM measurements of plastic films of various thicknesses (range, 43–318 $\mu$m; examples in Figs. 2A, 2B) were highly reproducible (data not shown), in keeping with the instrument's 50-nm resolution, and providing a reference scale against which to assess the OCT's accuracy. The OCT provided clear cross-sectional images of the reference films (Figs. 2C–E), from which thickness measurements were derived with the integral software (Table 1). Analysis of SEM and OCT plastic film thickness measurements revealed that these data were closely correlated ($r$ = 0.9995), with the OCT over-estimating the thickness by a mean of 16% (SD 2.7%, 95% CI 14.2–17.8; Table 1), compared with the SEM. As the OCT's measurement algorithm is based on the refractive index of cornea, one third of the overestimation is due to the differing refractive indices of cornea (1.37) and plastic (1.45), with the remainder attributable to the OCT's axial resolution. In view of this and SEM's 200-fold greater resolution than that of OCT (50 nm vs. 10 $\mu$m), SEM measurements were selected as the reference standard, and a 16% correction factor was applied to the subsequent OCT murine measurements.

## Murine CCT Measurement

The anterior segment OCT of ex vivo murine eyes provided detailed cross-sectional images of anterior chamber morphology.

In addition to optical sections through the corneal stroma, the conformation of the anterior chamber, iris, and angle were readily visualized (Fig. 2F). This level of resolution facilitated scan centration on the murine pupil and orientation of scans perpendicular to the corneal surface (by rotating the stand holding the globe by 90° around its *z*-axis). Analysis of murine CCT, corrected for the mean 16% overestimation of the OCT compared with the SEM, demonstrated a significant reduction in CCT in *Pitx2*$^{+/-}$ mice (mean, 72 $\mu$m; range, 57–87; $n = 6$) compared with wild type (mean, 88 $\mu$m; range, 63–100; $n = 6$; $P = 0.035$, *t*-test; Fig. 3). Qualitative analysis of the plastic-embedded histologic sections demonstrated decreased stromal and epithelial thickness, and a decreased number of epithelial cell layers (Fig. 2H).

## Discussion

This study demonstrates that *PITX2/Pitx2* mutation results in reduced corneal thickness and describes a technical advance that facilitates study of murine models of human anterior segment disease. The finding of decreased CCT in a large *PITX2* mutation pedigree ($P = 0.0002$) extends the known Axenfeld-Rieger phenotype and is supported by results from mice with a single functional copy of *Pitx2* ($P = 0.035$); it is also consistent with previous studies in which *Pitx2* was over- or underexpressed.[26,27,32] The similar ~20% CCT reduction in patients and mice with comparable mutations emphasizes the utility of this murine model and optical CCT measurement technique.

These data are also in accordance with the known evolutionary conservation of developmental genes such as *PITX2/Pitx2* where correct gene dosage is critical for normal morphogenesis.[27,28,33,34] Multiple embryological lineages contribute to ocular and dental development with neural-crest–derived cells forming most of the corneal stroma and dentine, respectively.[26,27,35] Observation of reduced corneal thickness in *PITX2*-attributable Axenfeld-Rieger thus parallels reduced tooth size (hypodontia or microdontia) caused by dentine hypoplasia. Such similarities between disparate neural crest–derived tissues add biological plausibility to the finding of reduced corneal thickness in mice and humans.

Exacting standards are needed to demonstrate that an allele is associated with a phenotype more frequently than would be expected by chance. Criteria include selection of appropriate controls, masking as to the underlying genotype, suitable statistical methodology, plausible biological context, low probabilities, and, above all, independent replication.[36] Such approaches, necessary to maximize the likelihood that association studies are replicated, were used in this study, with unaffected relatives used as intrafamilial control subjects. The rarity of pedigrees of sufficient size to permit statistically significant comparison, combined with the close relationship between orthologous genes, encouraged us to study a murine model to verify this clinical observation. However, the lack of murine pachymeters and difficulties inherent in accurate histologic measurement, especially cutting axial sections in <2-mm globes, required development of an alternative measurement technique. Accordingly the OCT, which uses optical interferometry, was adapted for murine CCT measurement and validated with scanning electron microscopy ($r = 0.9995$).

**Table 1.** Comparison of SEM and OCT Measurements of Plastic Reference Films

| SEM ($\mu$m) | OCT ($\mu$m) | Ratio of OCT to SEM Readings |
|---|---|---|
| 43.7 | 51 | 1.167 |
| 48.0 | 53 | 1.104 |
| 110.4 | 127 | 1.150 |
| 121.8 | 143 | 1.174 |
| 135.2 | 158 | 1.169 |
| 142.9 | 168 | 1.176 |
| 148.1 | 178 | 1.202 |
| 174.4 | 199 | 1.141 |
| 318.1 | 369 | 1.160 |

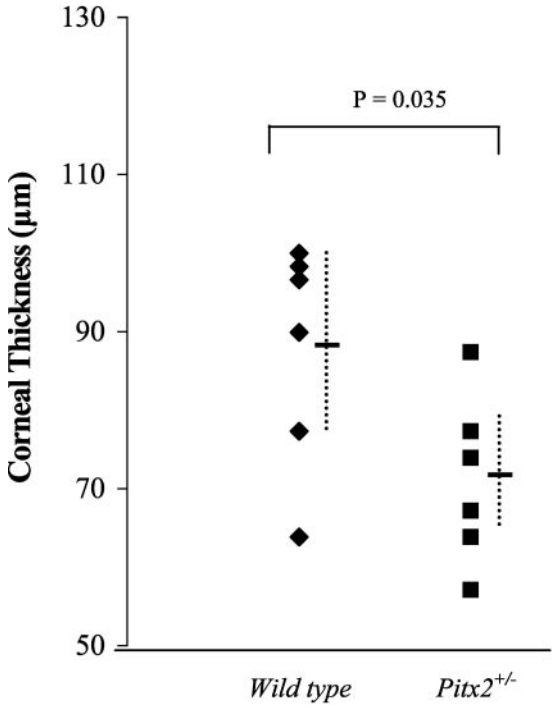


**Figure 3.** Corneal thickness of *Pitx2*$^{+/-}$ and wild-type mice. The statistical significance, the mean (*solid bar*), and the 95% CI (*dotted lines*) are displayed for each data set. The mean CCTs were 72 (*Pitx2*$^{+/-}$) and 88 (wild-type) $\mu$m.

The findings of reduced CCT in patients and mice with comparable hypomorphic *PITX2/Pitx2* mutations have several implications. Identifying the first genetic subtype of glaucoma with decreased corneal thickness extends the range of phenotypes associated with altered CCT and may facilitate setting appropriate target pressures for these cases. These findings are important, as ~20% reductions in CCT cause sufficient IOP underestimation (6.8 mm Hg; range, 5–8.6)[4] to provide *one* explanation for the more severe glaucoma reported in *PITX2*-attributable Axenfeld-Rieger cases (Walter M, et al. *IOVS* 2000;41:ARVO Abstract 2809). The observed genotype-phenotype correlation may also permit phenotypic identification of cases with *PITX2* mutation. If confirmed prospectively, phenotypic data could be used to guide molecular analyses in Axenfeld-Rieger and aid identification of genes that may cause up to 50% of cases. In view of the diversity of Axenfeld-Rieger corneal phenotypes (increased CCT with *FOXC1* duplication[8]; decreased CCT with *PITX2* mutation), and the greater glaucoma severity observed with *PITX2*-attributable cases (Walter M, et al. *IOVS* 2000;41:ARVO Abstract 2809), the ability to identify patients at increased risk of visual loss would have clinical applications.

From an imaging perspective, this study demonstrates that the OCT available in many ophthalmic units can be used to visualize and measure tissues in the murine anterior segment. Although the current axial resolution (10 $\mu$m) represents a (genotype-independent) limitation, the OCT provides a robust means of comparing CCT in different murine strains. Availability of higher-resolution instruments[37,38] is likely to enable detection of much smaller differences than the ~20% reduction identified in this study. The potential also exists to extend this

technique to in vivo measurement to determine the temporal effects of genetic mutation on anterior segment structure and development. It is anticipated that such approaches will permit analysis of a wider range of murine mutants. Subsequent study of patients with corresponding mutations may be a fruitful means of determining the effect structural changes have on IOP measurement.

In summary, this study has identified reduced corneal thickness associated with *PITX2/Pitx2* mutation and provided a simple method of measuring murine CCT. The findings have beneficial implications for clinical management, genetic analysis of Axenfeld-Rieger cases, and murine phenotyping. The complementary benefits of avoiding undertreatment of glaucoma and potential for identifying genetic Axenfeld-Rieger subtypes with a simple phenotypic marker, highlight the advantages of integrated clinical and scientific studies of developmental genes in species separated by many millions of years of evolutionary time.

## *Acknowledgments*

The authors thank the members of the family for assisting in the study, Michael Walter for considerable support that facilitated the work, Paul Dumais for performing the SEM measurements, and Stan Chan for advice regarding OCT imaging.

## *References*

1. Quigley HA. Proportion of those with open-angle glaucoma who become blind. *Ophthalmology*. 1999;106:2039–2041.
2. Quigley HA, Congdon NG, Friedman DS. Glaucoma in China (and worldwide): changes in established thinking will decrease preventable blindness. *Br J Ophthalmol*. 2001;85:1271–1272.
3. Leskea MC, Heijl A, Hyman L, et al. Factors for progression and glaucoma treatment: the Early Manifest Glaucoma Trial. *Curr Opin Ophthalmol*. 2004;15:102–106.
4. Doughty MJ, Zaman ML. Human corneal thickness and its impact on intraocular pressure measures: a review and meta-analysis approach. *Surv Ophthalmol*. 2000;44:367–408.
5. Brandt JD, Beiser JA, Kass MA, Gordon MO. Central corneal thickness in the Ocular Hypertension Treatment Study (OHTS). *Ophthalmology*. 2001;108:1779–1788.
6. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002;120:714–720; discussion 829–830.
7. Lee BL, Wilson MR. Ocular Hypertension Treatment Study (OHTS) commentary. *Curr Opin Ophthalmol*. 2003;14:74–77.
8. Lehmann OJ, Tuft S, Brice G, et al. Novel anterior segment phenotypes resulting from forkhead gene alterations: evidence for cross-species conservation of function. *Invest Ophthalmol Vis Sci*. 2003;44:2627–2633.
9. Ramaesh T, Collinson JM, Ramaesh K, et al. Corneal abnormalities in Pax6+/− small eye mice mimic human aniridia-related keratopathy. *Invest Ophthalmol Vis Sci*. 2003;44:1871–1878.
10. Brandt JD, Casuso LA, Budenz DL. Markedly increased central corneal thickness: an unrecognized finding in congenital aniridia. *Am J Ophthalmol*. 2004;137:348–350.
11. Blixt A, Mahlapuu M, Aitola M, et al. A forkhead gene, FoxE3, is essential for lens epithelial proliferation and closure of the lens vesicle. *Genes Dev*. 2000;14:245–254.
12. Brownell I, Dirksen M, Jamrich M. Forkhead Foxe3 maps to the dysgenetic lens locus and is critical in lens development and differentiation. *Genesis*. 2000;27:81–93.
13. Ormestad M, Blixt A, Churchill A, et al. Foxe3 haploinsufficiency in mice: a model for Peters' anomaly. *Invest Ophthalmol Vis Sci*. 2002;43:1350–1357.
14. Alward WL. Axenfeld-Rieger syndrome in the age of molecular genetics. *Am J Ophthalmol*. 2000;130:107–115.
15. Lines MA, Kozlowski K, Kulak SC, et al. Characterization and prevalence of PITX2 microdeletions and mutations in Axenfeld-Rieger malformations. *Invest Ophthalmol Vis Sci*. 2004;45:828–833.
16. Hjalt TA, Semina EV. Current molecular understanding of Axenfeld-Rieger syndrome. *Expert Rev Mol Med*. 2005;7:1–17.
17. Mears AJ, Jordan T, Mirzayans F, et al. Mutations of the forkhead/winged-helix gene, FKHL7, in patients with Axenfeld-Rieger anomaly. *Am J Hum Genet*. 1998;63:1316–1328.
18. Nishimura DY, Swiderski RE, Alward WL, et al. The forkhead transcription factor gene FKHL7 is responsible for glaucoma phenotypes which map to 6p25. *Nat Genet*. 1998;19:140–147.
19. Semina EV, Reiter RS, Murray JC. Isolation of a new homeobox gene belonging to the Pitx/Rieg family: expression during lens development and mapping to the aphakia region on mouse chromosome 19. *Hum Mol Genet*. 1997;6:2109–2116.
20. Jamieson RV, Perveen R, Kerr B, et al. Domain disruption and mutation of the bZIP transcription factor, MAF, associated with cataract, ocular anterior segment dysgenesis and coloboma. *Hum Mol Genet*. 2002;11:33–42.
21. Nishimura DY, Searby CC, Alward WL, et al. A spectrum of FOXC1 mutations suggests gene dosage as a mechanism for developmental defects of the anterior chamber of the eye. *Am J Hum Genet*. 2001;68:364–372.
22. Gould DB, Jaafar MS, Addison MK, et al. Phenotypic and molecular assessment of seven patients with 6p25 deletion syndrome: relevance to ocular dysgenesis and hearing impairment. *BMC Med Genet*. 2004;5:17.
23. Lehmann OJ, Ebenezer ND, Ekong R, et al. Ocular developmental abnormalities and glaucoma associated with interstitial 6p25 duplications and deletions. *Invest Ophthalmol Vis Sci*. 2002;43:1843–1849.
24. Lehmann OJ, Ebenezer ND, Jordan T, et al. Chromosomal duplication involving the forkhead transcription factor gene FOXC1 causes iris hypoplasia and glaucoma. *Am J Hum Genet*. 2000;67:1129–1135.
25. Kume T, Deng KY, Winfrey V, et al. The forkhead/winged helix gene Mf1 is disrupted in the pleiotropic mouse mutation congenital hydrocephalus. *Cell*. 1998;93:985–996.
26. Gage PJ, Rhoades W, Prucka SK, Hjalt T. Fate maps of neural crest and mesoderm in the mammalian eye. *Invest Ophthalmol Vis Sci*. 2005;46:4200–4208.
27. Gage PJ, Suh H, Camper SA. Dosage requirement of Pitx2 for development of multiple organs. *Development*. 1999;126:4643–4651.
28. Gage PJ, Suh H, Camper SA. The bicoid-related Pitx gene family in development. *Mamm Genome*. 1999;10:197–200.
29. Walter MA, Mirzayans F, Mears AJ, et al. Autosomal-dominant iridogoniodysgenesis and Axenfeld-Rieger syndrome are genetically distinct. *Ophthalmology*. 1996;103:1907–1915.
30. Kulak SC, Kozlowski K, Semina EV, et al. Mutation in the RIEG1 gene in patients with iridogoniodysgenesis syndrome. *Hum Mol Genet*. 1998;7:1113–1117.
31. Kozlowski K, Walter MA. Variation in residual PITX2 activity underlies the phenotypic spectrum of anterior segment developmental disorders. *Hum Mol Genet*. 2000;9:2131–2139.
32. Holmberg J, Liu CY, Hjalt TA. PITX2 gain-of-function in Rieger syndrome eye model. *Am J Pathol*. 2004;165:1633–1641.
33. Rankin CT, Bunton T, Lawler AM, Lee SJ. Regulation of left-right patterning in mice by growth/differentiation factor-1. *Nat Genet*. 2000;24:262–265.
34. Idrees F, Bloch-Zupan A, Free SL, et al. A novel homeobox mutation in the PITX2 gene in a family with Axenfeld-Rieger syndrome associated with brain, ocular, and dental phenotypes. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141:184–191.
35. Amendt BA, Semina EV, Alward WL. Rieger syndrome: a clinical, molecular, and biochemical analysis. *Cell Mol Life Sci*. 2000;57:1652–1666.
36. Freely associating (Editorial). *Nat Genet*. 1999;22:1–2.
37. Reiser BJ, Ignacio TS, Wang Y, et al. In vitro measurement of rabbit corneal epithelial thickness using ultrahigh resolution optical coherence tomography. *Vet Ophthalmol*. 2005;8:85–88.
38. Grieve K, Paques M, Dubois A, et al. Ocular tissue imaging using ultrahigh-resolution, full-field optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2004;45:4126–4131.