Novel Anterior Segment Phenotypes Resulting from Forkhead Gene Alterations: Evidence for Cross-Species Conservation of Function

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Purpose. Mutations in murine and human versions of an ancestrally related gene usually result in similar phenotypes. However, interspecies differences exist, and in the case of two forkhead transcription factor genes (*FOXC1* and *FOXC2*), these differences include corneal or anterior segment phenotypes, respectively. This study was undertaken to determine whether such discrepancies provide an opportunity for identifying novel human–murine ocular phenotypes.

Methods. Four pedigrees with early-onset glaucoma phenotypes secondary to segmental chromosomal duplications or deletions encompassing *FOXC1* and 18 individuals from 9 *FOXC2* mutation pedigrees underwent detailed ocular phenotyping. Subsequently, mice with mutations in *Foxc1* or a related forkhead gene, *Foxe3*, were assessed for features of the human phenotypes.

Results. A significant increase in central corneal thickness was present in affected individuals from the segmental duplication pedigrees compared with their unaffected relatives (mean increase 13%, maximum 35%, $P < 0.05$). Alterations in corneal thickness were present in mice heterozygous and homozygous for *Foxe3* mutations but neither in *Foxc1* heterozygotes nor the small human segmental deletion pedigree. Mutations in *FOXC2* resulted in ocular anterior segment anomalies. These were more severe and prevalent with mutations involving the forkhead domain.

Conclusions. Normal corneal development is dependent on the precise dose and levels of activity of certain forkhead transcription factors. The altered corneal thickness attributable to increased forkhead gene dosage is particularly important, because it may affect the clinical management of certain glaucoma subtypes and lead to excessive treatment. The *FOXC1* and *Foxe3* data, taken together with the novel ocular phenotypes of *FOXC2* mutations, highlight the remarkable cross-species conservation of function among forkhead genes. (*Invest Ophthalmol Vis Sci.* 2003;44:2627–2633) DOI:10.1167/iovs.02-0609

Recognition that mutations in orthologous genes frequently cause similar phenotypes has allowed the field of comparative genetics to contribute to the understanding of human disease. As the human, murine, and *Drosophila PAX6* mutants (*aniridia*, *Small eye*, and *eyeless*) demonstrate, genotypic conservation can be mirrored in a phenotype extending across a considerable evolutionary time span. The same phenomenon is exhibited by members of the Forkhead Box (Fox) transcription factor gene family, as the thyroid, immunodeficiency, and *scurfy*-related phenotypes of *Foxe1/FOXE1*, *Foxn1/FOXN1* and *Foxp3/FOXP3* mutants illustrate. Forkhead genes also exhibit common functional characteristics, including sensitivity to altered gene dosage—a feature that is conserved between such evolutionarily divergent organisms as mammals and zebrafish.[1–4] Despite this close relationship, appreciable differences exist between the reported ocular phenotypes of certain human (*FOXC1/FOXC2*) and murine (*Foxc1/Foxc2*) forkhead orthologues, which may be attributable to real biological differences, variations in genetic background, and/or aspects of the phenotypes that had not been assessed.

Mutations in the *FOXC1* gene, located on chromosome 6p25, principally result in a range of Axenfeld-Rieger phenotypes that are strongly associated with glaucoma.[5–7] The natural murine *Foxc1* mutant, congenital hydrocephalus, in the homozygous state ($Foxc1^{ch/ch}$) dies in the perinatal period with cerebral, cardiac, ocular, renal, and skeletal defects.[8] The ocular abnormalities include iris anomalies similar to those in humans, and, in addition, profound corneal changes. These include failure of the corneal endothelium and lens epithelium to separate, resulting in absence of anterior chamber formation.[1,8] Milder corneal (iris and systemic) disease occurs in heterozygous ($Foxc1^{ch/+}$ and knockout $Foxc1^{+/-}$) mutant mice demonstrating a relationship between the severity of the phenotype and the dose of *FOXC1*.[2,9] However, such corneal phenotypes have not been a characteristic observation in either *FOXC1* mutations or cytogenetic abnormalities that alter *FOXC1* gene dosage.[8,10–12] Mutations in another forkhead gene, *Foxe3/FOXE3*, cause a failure of corneolenticular separation similar to $Foxc1^{ch/ch}$, resulting either in the (murine) dysgenetic lens (*dyl*) phenotype or a proportion of (human) Peters anomaly or anterior segment dysgenesis cases.[13–16] The corneal phenotypes of *Foxc1*, *Foxe3*, and *FOXE3* mutants plus the corneal expression of *Foxc1/FOXC1*,[8,17] suggested the existence of an as yet unidentified role for *FOXC1* in human corneal development.

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FOXC1 shares coordinated function and overlapping tissue expression with *FOXC2*, as well as 97% amino acid identity across their forkhead (DNA binding) domains.[2–4] Mutations in *FOXC2* cause an autosomal dominant disease characterized by lymphedema of the limbs and distichiasis (additional diminutive eyelashes).[18] In contrast, haploinsufficiency of *Foxc2* in knockout (*Foxc2*$^{+/-}$) mice causes iris, trabecular meshwork, and iridocorneal angle anomalies.[2] The presence of murine anterior segment anomalies raises the possibility of an unrecognized role for *FOXC2* in iris and trabecular meshwork development (analogous to that of *FOXC1* in corneal development).

The close functional and phenotypic relationship between forkhead orthologues suggests that scrutiny of differences between the phenotypes caused by mutations in *Foxc1/FOXC1* and *Foxc2/FOXC2* may provide a model for determining unrecognized phenotypes. The results from a detailed ocular assessment of patients or model organisms with altered *FOXC1* gene dosage or *FOXC2* mutations illustrate how this simple approach can elucidate aspects of gene function and have implications for the management of certain developmental glaucomas.

Methods

Four pedigrees (A–D) with 6p25 segmental duplications or deletions encompassing *FOXC1* have been identified by microsatellite marker genotyping or fluorescence in situ hybridization (FISH)[11,12] (Lehmann OJ, Jordan TL, Ebenezer N, et al., ARVO Abstract 2846, 2001). The pedigrees with segmental duplication (A–C) and deletion (D) exhibit glaucoma-associated phenotypes, iris hypoplasia, or Axenfeld-Rieger, respectively, which are attributable to increased or decreased *FOXC1* gene dosage. The extent of these cytogenetic abnormalities, determined primarily with FISH, has been reported in pedigrees A, B, and D.[12] Subsequent refinement with an additional microsatellite marker (*BA13-129*, forward CCACGCAAGTCACCTTCC, reverse AGGAACTGCGGCTTCTTCC, T_a 60°C) demonstrated that the duplications in pedigrees A and B encompass *FOXC1* and *FOXF2*. No other genes except exons of guanosine diphosphate (GDP)-mannose 4,6-dehydratase (GMDS) have been observed within the duplicated intervals. The deletion in pedigree D encompasses *FOXC1* but not *FOXF2*,[12] whereas the extent of the duplication in pedigree C has yet to be defined. Individuals from these pedigrees, and 18 patients with lymphedema-distichiasis from nine unrelated families with known *FOXC2* mutations[19,20] were carefully examined for the ocular features of naturally occurring and transgenic mouse models.

After examination of two affected members of pedigree A identified a potential corneal phenotype, additional family members were investigated (pedigrees A [$n = 20$], B [$n = 14$], C [$n = 11$], and D [$n = 3$]). The central corneal thickness (CCT) was measured ultrasonically (Altair 2000 pachymeter; Optikon, Rome, Italy), and the mean of the five lowest readings (corresponding to the center of the cornea) from the right eye was used for analysis (two-tailed *t*-test assuming equal variance). CCT data from two populations of ethnically matched unaffected ($n = 25$) and UK residents with glaucoma ($n = 119$), were also studied. Corneal endothelial cell morphology and density were documented in 17 representative individuals (pedigrees A–C), using an in vivo specular microscope (SP-1000; Topcon, Newbury, UK). Hematoxylin and eosin (H&E)–stained histologic sections from the right eyes of *Foxe3*$^{dyl/dyl}$, *Foxe3*$^{dyl/+}$, *Foxc1*$^{+/-}$, *Foxc1*$^{ch/+}$, and strain-matched wild-type mice were examined to determine whether changes were present comparable to those observed in humans. Additional histology using plastic (epoxy resin)-embedded sections was also performed on *Foxe3*$^{dyl/+}$ and wild-type mice. The dimensions of the corneal stroma, which constitutes approximately 95% of the corneal thickness, were measured from digital images of histologic sections (*Foxc1* [MetaMorph software version 4.6; UIC, Downingtown, PA] and *Foxe3* [AxioVision software version 3.0, Carl Zeiss Microimaging Inc, Oberkochen, Germany]). Slit lamp biomicroscopy, pachymetry, and specular microscopy of the cohort of patients with lymphedema-distichiasis was performed by an ophthalmologist (OJL) masked to the presence or nature of any *FOXC2* mutation. The iridocorneal angles, assessed by gonioscopy, were graded as either normal or abnormal, and abnormalities of the iris, cornea, and optic nerve were documented with a digital slit lamp–mounted camera (DXC-950P; Sony Corp., Tokyo, Japan). This study adhered to the tenets of the Declaration of Helsinki and to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Results

CCT was increased in affected individuals (mean 600 μm, $n = 27$) from each of the three segmental duplication pedigrees compared with their unaffected relatives (A, B, and C: $P = 0.008$, 0.044, and 0.003, respectively; Fig. 1). Elevated CCT was present in affected individuals who had neither had glaucoma nor required glaucoma surgery. Specular microscopy in an unselected subset of affected individuals (34/54 eyes) revealed normal corneal endothelial cell morphology and density (>2500 cells/mm^2) (Fig. 2). CCT measurements from unaffected individuals (pedigrees A–C; mean, 532 μm; $n = 20$), patients with segmental 6p25 deletion (pedigree D; mean, 540 μm; $n = 3$), and control individuals with unaffected or glaucomatous eyes were very similar (Fig. 1) and closely matched the published values for individuals in the United Kingdom.[21]

Mean corneal stromal thickness in *Foxc1*$^{+/-}$ (73 μm; range, 65–86, $n = 11$) and *Foxc1*$^{ch/+}$ mice (73 μm; range, 66–78, $n = 4$) was not significantly different compared with their strain-matched wild-type littermates (C57BL/6J: 67 μm; range, 58–79, $n = 10$, $P = 0.14$; CHMU/Le: 89 μm; range, 80–98, $n = 2$, $P = 0.09$ respectively; *t*-test). Homozygous *dyl* mutants exhibited marked thinning of the corneal stroma (to approximately two thirds that of the wild-type control), abnormal corneal endothelial and anterior lens epithelial morphology, subsequent failure of anterior chamber formation (Fig. 2),[22] and angle anomalies.[13,22] In heterozygous *dyl* mutants, the thickness of the central stroma relative to the peripheral corneal stroma was significantly increased ($P = 0.015$; Fig. 1), including in macroscopically normal eyes, and was associated with corneal edema, appearing as increased spacing between the stromal layers (Fig. 2). A trend toward increased corneal epithelial thickness in *Foxe3*$^{dyl/+}$ epithelium (31.4 μm) compared with wild type (24.6 μm), which was attributable to an increased number of epithelial cell layers (Fig. 2), did not reach statistical significance ($P = 0.09$) in the small sample studied (seven *Foxe3*$^{dyl/+}$, three wild type [BALB-c]). The absence of the anterior chamber in some sections from heterozygous *dyl* and wild-type mice (Fig. 2) was probably caused by compression during the cutting of the histologic sections. In view of this, additional histology was performed on plastic-embedded sections, which confirmed that the corneal thickness of *dyl* heterozygotes was increased when compared with wild-type control animals (data not shown).

Of the nine *FOXC2* mutations present in the patient cohort with lymphedema-distichiasis, four were within and the other mutations lay downstream of the forkhead domain (Fig. 3). Ocular anomalies were present in all 10 individuals with forkhead domain mutations. In contrast, those with mutations outside this motif (eight individuals) either exhibited milder ($n = 4$) or no ocular phenotype ($n = 4$). The iris anomalies included local or more generalized iris hypoplasia that was frequently associated with absence of sectors of the iris ruff. The other developmental anomalies, affecting the cornea, iridocorneal angle, pupillary shape, and anterior segment size (Figs. 2, 3), were associated only with forkhead domain muta-

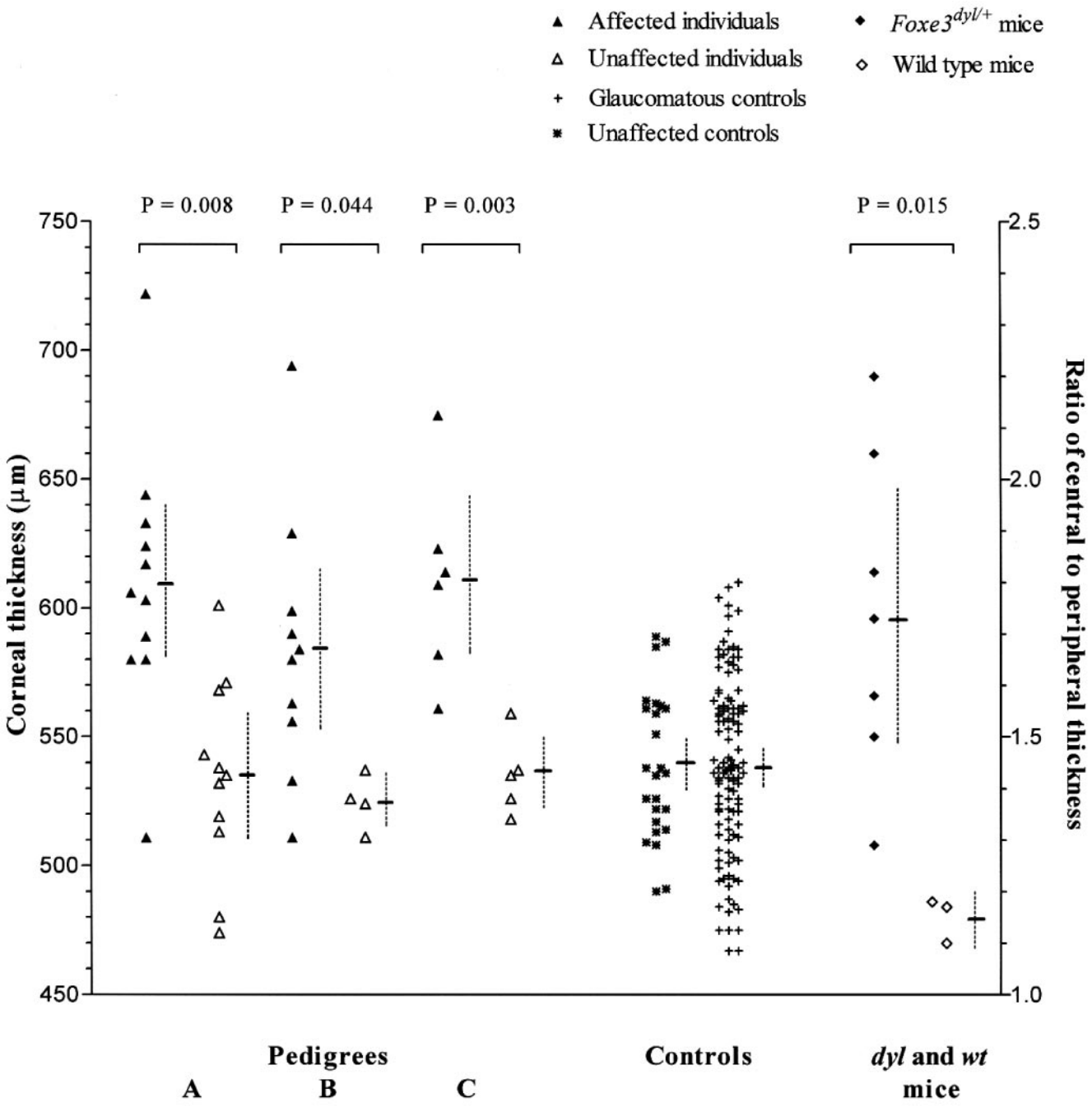


FIGURE 1. Corneal thickness of affected and unaffected individuals from the duplication pedigrees A to C (*left y*-axis) together with the ratio of central to peripheral stromal thickness in *Foxe3*$^{dyl/+}$ and wild-type mice (*right y*-axis). (The duplications in pedigrees A and B encompass *FOXC1* and *FOXF2*, whereas the extent in pedigree C has yet to be defined.) The mean CCTs were: pedigree A (610 μm [affected]), 534 μm [unaffected]); B [584 μm, 525 μm]); C [611 μm, 535 μm]). The statistical significance, the mean and 95% confidence intervals (*solid* and *dotted lines*; *right*) are displayed for each data set. CCT measurements from cohorts of individuals from the United Kingdom with unaffected or glaucomatous eyes are included for comparison. The affected/unaffected status of individuals from pedigrees A to D has been confirmed by genotyping.

tions. These phenotypes varied between affected relatives and in some cases between the eyes of the same individual (Fig. 2; A2, D2). Retinal or optic nerve anomalies were present in two individuals: unilateral optic nerve hypoplasia causing unilateral blindness with no perception of light (Fig. 2, A2) and situs inversus (aberrant course of retinal vessels as they exit from the optic disc; Fig. 2, D1). Despite the presence of angle anomalies, the intraocular pressure (IOP) was within the normal range, as were CCT measurements (data not shown).

DISCUSSION

A host of examples exist in which the identification of a human disease-causing gene has been guided by study of its animal orthologue. We hypothesized that the converse approach, comparing differences between the known phenotypes of human and murine forkhead genes might provide a simple means of identifying novel phenotypes in both species. The broader phenotypes associated with chromosomal duplications encom-

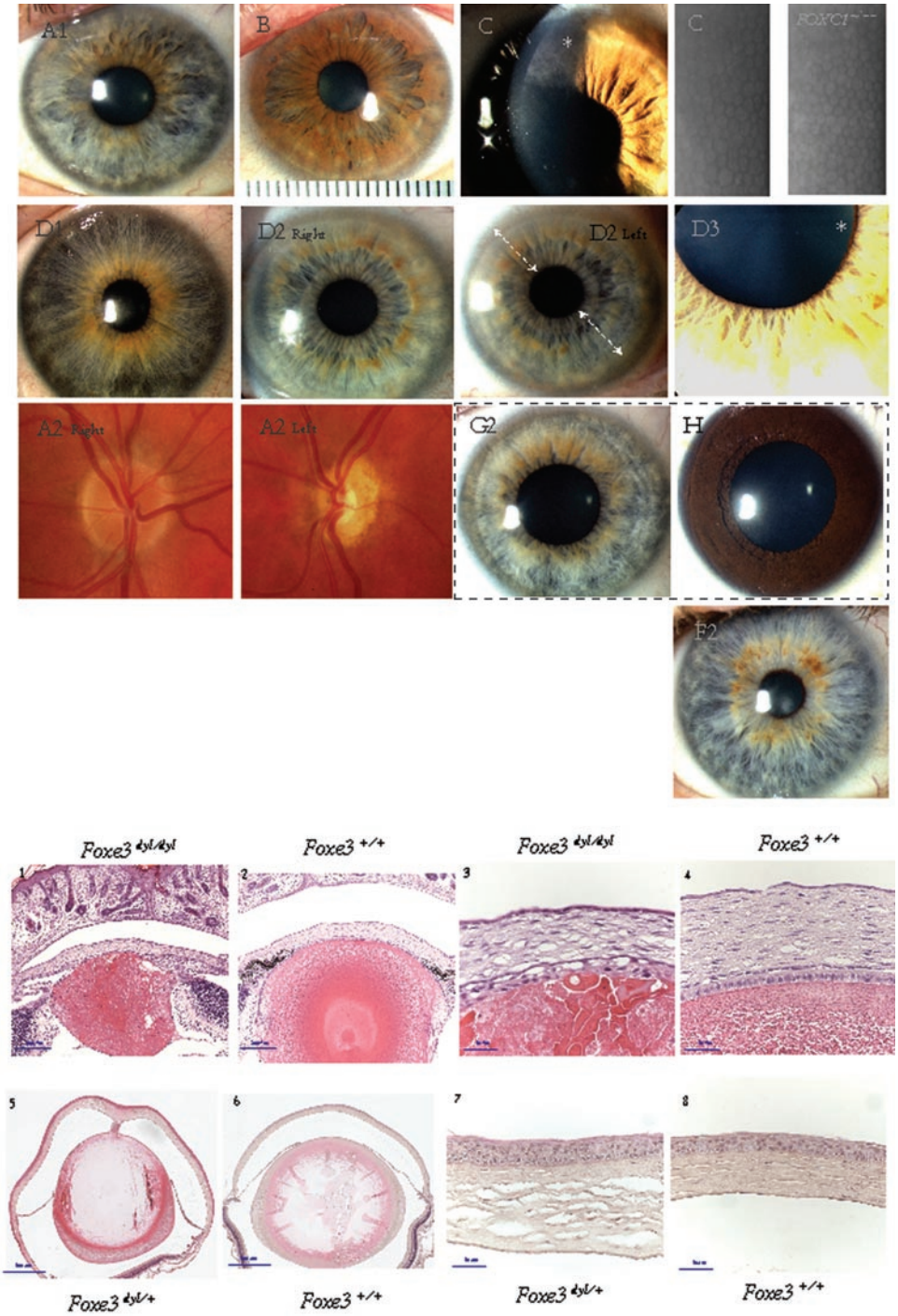
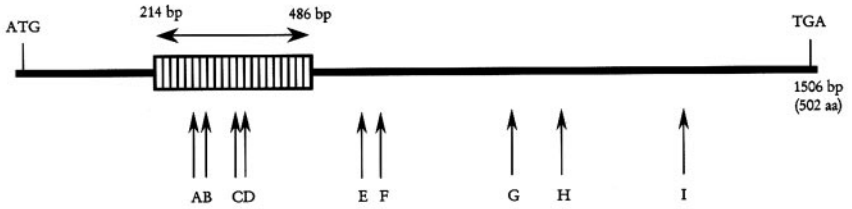


FIGURE 2. Photographs illustrating *FOXC2* mutation ocular phenotypes (labeled as in Fig. 3B). (**A**) Partially absent anterior iris stroma, most prominent in the *upper right* quadrant. (**B**) More extensive stromal hypoplasia with reduced corneal diameter less than 10 mm (normal 11.0–12.5 mm, half-millimeter scale *inset*). (**C**) Localized unilateral corneal opacification (✻) at the level of the endothelium with normal central corneal endothelial cell morphology (specular micrograph from 6p25 segmental duplication for comparison). (**D**) Abnormal iris architecture; note iris hypoplasia phenotype (**D2**) exposing iris sphincter (visible as a *pale ring* around the pupil) and the unilateral pupillary displacement (corectopia) in the left eye (*arrows*). (**D3**) The iris ruff (✻), a frill of *brown* tissue that surrounds the pupil, is absent inferiorly in association with iris atrophy (not shown). (**F2**) Mild iris thinning compared with normal irides (*boxed*) observed in individuals (**G2**, **H**) with mutations downstream of the forkhead domain. (**A2**) Unilateral optic nerve hypoplasia. (**A1**, **A2**, and so forth, represent different affected individuals from pedigree A.) Histologic sections from homozygous (**1**, **3**), heterozygous (**5**, **7**), *dyl* mice and wild-type litter mates (**2**, **4**, **6**, **8**), newborn (**1**–**4**), and 9-week-old adults (**5**-**8**). (**1**) *Dyl* eye illustrating the small and abnormal lens. (**3**) Higher-magnification view showing irregular anterior lens epithelium, grossly abnormal corneal endothelium and stroma, and reduced corneal thickness compared with the wild-type. (**5**) *Dyl* heterozygote showing typical corneolenticular adhesion with increased corneal thickness extending peripherally. (**7**) Higher magnification view of swollen corneal stroma.

passing *FOXC1*, and mutations in *FOXC2*, and *Foxe3* indicate the potential of this method.

The 6p25 segmental duplications increase dosage of gene(s) expressed in the neural crest cell–derived periocular mesenchyme, the cellular precursors of the corneal stroma.[1,23] This effect provides one explanation for the increased corneal thickness—namely, that increased dosage of *FOXC1* (and or *FOXF2*) results in cellular hyperplasia or increased recruitment of cells into the developing cornea. Although the relative contributions of *FOXC1* and *FOXF2* cannot be readily determined without transgenic models, a considerable body of evidence exists that the precise dosage of *Foxc1/FOXC1* alone is critical for normal ocular development.[1–4,8,12,24] The view that increased *FOXC1* gene dosage is responsible for increased corneal thickness is supported by the profound corneal changes in the null mutant $Foxc1^{cb/cb}$.[1] However the dose-dependent function of forkhead genes[1,3,4,24,25] precludes exclusion of a role for *FOXF2*. The altered corneal thickness observed with $Foxe3^{dyl/dyl}$ and $Foxe3^{dyl/+}$ mice supports the human data, especially in that *dyl* mutations are believed to generate a null *Foxe3* allele.[16] Taken together, the *dysgenetic lens* and 6p25 duplication data indicate that normal corneal development is dependent on the precise dose and levels of activity of these transcription factors. *Foxc1* and *Foxe3* share considerable forkhead domain nucleotide homology (82% vs. 74% for *FOXC1/FOXF2*) and have related roles in the develop-

A



B

	Mutation	Ocular abnormalities			Ocular asymmetry	Systemic abnormalities	
		Iris	Angle	Retina / Optic nerve		Lymphedema	Distichiasis
A1	323delT	+	-	-	-	+	+
A2	323delT	+	+	+	+	-	+
B	333delG	+	+	-	-	+	+
C	362G→A	-	-	-	+	+	+
D1	374 C→T	+	+	+	-	+	-
D2	374 C→T	+	-	-	+	+	+
D3	374 C→T	+	+	-	+	+	+
D4	374 C→T	+	-	-	+	-	+
D5	374 C→T	+	+	-	-	+	+
D6	374 C→T	+	-	-	-	-	+
	Forkhead domain mutations	9/10	5/10	2/10	5/10	7/10	9/10
E1	595-596insC	-	*	*	-	+	+
E2	595-596insC	+	*	-	-	+	+
F1	638-639insGT	-	-	-	-	+	+
F2	638-639insGT	+	-	-	-	+	+
G1	922-929del7	+	-	-	-	+	+
G2	922-929del7	-	-	-	-	+	+
H	1142-1143insC	-	-	-	-	-	+
I	1302-1308del/insGGA	+	-	-	-	+	+
	Non Forkhead domain mutations	4/8	0/6	0/7	0/8	7/8	8/8

FIGURE 3. (**A**) Representation of the *FOXC2* coding sequence illustrating position of each mutation relative to forkhead domain (*hatched box*). (**B**) Summary of phenotypes observed with each mutation (+ present, − absent, * patient declined gonioscopy or fundus examination). The mutations correspond with the families previously reported.[19,20]

ment of the cornea and anterior chamber.[1,8,13] These roles appear to be evolutionarily conserved, because similar phenotypes occur with *FOXC1* encompassing duplications and *Foxe3* mutations, in two species descended from a common ancestor approximately 112 million years ago. Increased CCT in nonglaucomatous/non–surgically treated eyes of affected individuals from the duplication pedigrees excludes the possibility of confounding due to the presence of glaucoma or its treatment sequelae. The absence of similar changes in the deletion pedigree, *Foxc1*$^{+/-}$ or *Foxc1*$^{cb/+}$ mice, suggests two possible interpretations. Either increased gene dosage has a more profound effect on CCT than reduced dosage or the challenges inherent in cutting axial histologic sections in 2-mm murine globes, reflected in the wide range of in vitro measurements (up to 36%), may mask any alteration in CCT of comparable magnitude to that observed in humans (mean 13%). In view of the swelling, shrinking, and mechanical distortion that occurs during dehydration, embedding, and cutting of histologic sections, availability of a corneal pachymeter capable of in vivo murine measurements may offer better accuracy. This would contribute to the rapid advances being made in murine ocular phenotyping[26] and help determine whether the size of effect in *dyl* mice exceeds that of other mutants.

The increased corneal thickness in the duplication pedigrees has clinical implications, because it leads to overestimation of IOP, independent of the tonometric method used.[27] The magnitude of this effect remains imprecisely defined, although correction factors have been calculated, by extrapolating the relationship between normal corneal thickness and IOP to thicker corneas or determining the effect induced increases in corneal thickness have on IOP. A correction factor of 2.5 ± 1.1 mm Hg for each 10% increase in CCT, derived from a meta-analysis of studies in chronic conditions including glaucoma,[21] indicates that IOP would be overestimated by 3 to 9 mm Hg in individuals with CCT between 600 and 725 μm. Such increases in measured IOP would be expected to lead to excessive treatment to lower a pressure that may remain falsely elevated. This may partially explain the increased rate of glaucoma diagnosis in the duplication pedigrees (~100%) compared with *FOXC1* mutations (~50%) (Walter MA, Kulak KC, Héon E, Ritch R, Pearce WG, Damji KF, Allingham RR, Shields MB, ARVO Abstract 2809, 2000). The increased CCT observed in iris hypoplasia makes corneal pachymetry advisable in this subset of patients with glaucoma and potentially represents a diagnostic marker for 6p25 segmental duplications (21/27 affected and 1/20 unaffected individuals had CCT ≥ 580 μm).

The ocular hypertension treatment study, which demonstrated that CCT was a powerful predictor for the development of primary open-angle glaucoma,[28] has emphasized the importance of measuring corneal thickness. The *dyl* data raise the possibility that alterations in CCT may be a common feature of mutations in genes regulating anterior segment development. This merits investigation in patients with conditions including Peters anomaly, Axenfeld-Rieger syndrome, aniridia, and microphthalmia—developmental phenotypes in which measurement of IOP remains the cornerstone of clinical management. Should similar changes in CCT be observed, there would be broad implications for the management of certain pediatric glaucoma subtypes.

We also provide the first evidence that most patients with *FOXC2* mutations have anterior segment ocular anomalies. These were milder than those caused by *FOXC1* mutations and were unassociated with glaucoma, recapitulating the murine *Foxc2* haploinsufficiency phenotype.[2] Mutations within the *FOXC2* forkhead domain were associated with more severe iris anomalies and with iridocorneal angle anomalies, ocular asymmetry, and occasionally abnormalities of the ocular posterior segment (Figs. 2, 3). In contrast, mutations downstream of the forkhead domain either resulted solely in subtle iris anomalies or caused no discernible ocular phenotype. The more severe phenotypes associated with forkhead domain mutations is consistent with the interpretation that alterations to this highly conserved DNA-binding motif have a greater effect on *FOXC2* function than mutations elsewhere. This genotype–phenotype correlation, albeit based on examination of 18 patients with nine mutations, concurs with that observed with *FOXL2* in which the position of the mutation relative to the forkhead domain and or the size of the predicted protein correlates with the blepharophimosis syndrome phenotype observed.[29,30] Of interest, the three *Foxc1/FOXC1* mutations that cause extraocular phenotypes lie upstream of or within the forkhead domain.[7,8,31] Studies of the functional effects of *FOXC1* mutations have demonstrated that these generate hypomorphic or null alleles by altering DNA binding or transactivation domains,[32,33] and similar biochemical characterization should be undertaken with *FOXC2*.

The eye is ideally suited to the study of phenotypic differences between orthologues, because of its accessibility to detailed phenotyping and its composition from an interface of embryologically distinct tissues. The novel features associated with 6p25 segmental duplications and *FOXC2* mutations demonstrate that some discrepancies between the reported ocular phenotypes of *Foxc1/FOXC1* and *Foxc2/FOXC2* are attributable to unrecognized phenotypes, reiterating the importance of murine phenotypes as a guide to the human. The close relationship between animal and human orthologues can be used either to guide gene identification, or alternatively with the approach adopted in this study, to identify novel phenotypic features. As genetic research progresses increasingly toward understanding gene function, and away from gene identification, the value of this strategy may increase.

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References

1. Kidson SH, Kume T, Deng K, Winfrey V, Hogan BL. The forkhead/winged-helix gene, Mf1, is necessary for the normal development of the cornea and formation of the anterior chamber in the mouse eye. *Dev Biol.* 1999;211:306–322.
2. Smith RS, Zabaleta A, Kume T, et al. Haploinsufficiency of the transcription factors FOXC1 and FOXC2 results in aberrant ocular development. *Hum Mol Genet.* 2000;9:1021–1032.
3. Kume T, Deng K, Hogan BL. Murine forkhead/winged helix genes Foxc1 (Mf1) and Foxc2 (Mfh1) are required for the early organogenesis of the kidney and urinary tract. *Development.* 2000;127:1387–1395.
4. Kume T, Jiang H, Topczewska JM, Hogan BL. The murine winged helix transcription factors, *Foxc1* and *Foxc2*, are both required for cardiovascular development and somatogenesis. *Gen Dev.* 2001;15:2470–2482.
5. Nishimura D, Swiderski R, Alward W, et al. The forkhead transcription factor gene FKHL7 is responsible for glaucoma phenotypes which map to 6p25. *Nat Genet.* 1998;19:140–147.
6. 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.
7. Swiderski RE, Reiter RS, Nishimura DY, et al. Expression of the *Mf1* gene in developing mouse hearts: implication in the development of human congenital heart defects. *Dev Dyn.* 1999;216:16–27.
8. Kume T, Deng K, Winfrey V, Gould DB, Walter MA, Hogan BL. The forkhead/winged helix gene *Mf1* is disrupted in the mouse mutation *congenital hydrocephalus. Cell.* 1998;93:985–996.
9. Hong H, Lass JH, Chakravarti A. Pleiotropic skeletal and ocular phenotypes of the mouse mutation congenital hydrocephalus (*ch/Mf1*) arise from a winged helix/forkhead transcription factor gene. *Hum Mol Genet.* 1999;8:625–637.
10. 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.
11. 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.
12. Lehmann OJ, Ebenezer ND, Ekong R, et al. Interstitial 6p25 duplications and deletions cause ocular developmental abnormalities and glaucoma. *Invest Ophthalmol Vis Sci.* 2002;43:1843–1849.
13. Blixt Å, Mahlapuu M, Aitola M, Pelto-Huikko M, Enerback S, Carlsson P. A forkhead gene, *FoxE3*, is essential for lens epithelial proliferation and closure of the lens vesicle. *Genes Dev.* 2000;14:245–254.
14. 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.
15. Semina EV, Brownell I, Mintz-Hittner HA, et al. Mutations in the human forkhead transcription factor *FOXE3* associated with anterior segment ocular dysgenesis and cataracts. *Hum Mol Genet.* 2001;10:231–236.
16. Ormestad M, Blixt Å, Churchill A, Martinsson T, Enerback S, Carlsson P. *Foxe3* haploinsufficiency in mice: a model for Peters' anomaly. *Invest Ophthalmol Vis Sci.* 2002;43:1350–1357.
17. Wang Wh, McNatt LG, Shepard AR. Optimal procedure for extracting RNA from human ocular tissues and expression profiling of the congenital glaucoma gene *FOXC1* using quantitative RT-PCR. *Mol Vis.* 2001;7:89–94.
18. Fang J, Dagenais SL, Erickson RP, et al. Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. *Am J Hum Genet.* 2000;67:1382–1388.
19. Bell R, Brice G, Child AH, et al. Analysis of lymphoedema-distichiasis families for *FOXC2* mutations reveals small insertions and deletions throughout the gene. *Hum Genet.* 2001;108:546–551.
20. Brice G, Mansour S, Bell R, et al. Analysis of the phenotypic abnormalities in lymphoedema-distichiasis syndrome in 74 patients with *FOXC2* mutations or linkage to 16q24. *J Med Genet.* 2002;39:478–483.
21. 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.

22. Sanyal S, Hawkins RK. Dysgenetic lens (dyl): a new gene in the mouse. *Invest Ophthalmol Vis Sci.* 1979;18:642–645.
23. Aitola M, Carlsson P, Mahlapuu M, Enerback S, Pelto-Huikko M. Forkhead transcription factor *Foxf2* is expressed in mesodermal tissues involved in epithelio-mesenchymal interactions. *Dev Dyn.* 2000;218:136–149.
24. Winnier GE, Kume T, Deng K, et al. Roles for the winged helix transcription factors *Mf1* and *Mfb1* in cardiovascular development revealed by nonallelic noncomplementation of null alleles. *Dev Biol.* 1999;213:418–431.
25. Topczewskaa JM, Topczewskib J, Solnica-Krezelb L, Hogan BL. Sequence and expression of zebrafish *foxc1a* and *foxc1b*, encoding conserved forkhead/winged helix transcription factors. *Mech Dev.* 2001;100:343–347.
26. Smith RS, Korb D, John SWM. A goniolens for clinical monitoring of the mouse iridocorneal angle and optic nerve. *Mol Vision.* 2002;8:26–31.
27. Bhan A, Browning AC, Shah S, Hamilton R, Dave D, Dua HS. Effect of corneal thickness on intraocular pressure measurements with the pneumotonometer, Goldmann applanation tonometer, and Tono-Pen. *Invest Ophthalmol Vis Sci.* 2002;43:1389–1392.
28. 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.
29. Crisponi L, Deiana M, Loi A, et al. The putative forkhead transcription factor *FOXL2* is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. *Nat Genet.* 2001;27:159–166.
30. De Baere E, Dixon MJ, Small K. Spectrum of *FOXL2* mutations in blepharophimosis-ptosis-epicanthus inversus (BPES) families demonstrate a genotype-phenotype correlation. *Hum Mol Genet.* 2001;10:1591–1600.
31. Mirzayans F, Gould DB, Heon E, et al. Axenfeld-Rieger syndrome resulting from mutation of the FKHL7 gene on chromosome 6p25. *Eur J Hum Genet.* 2000;8:71–74.
32. Saleem RA, Banerjee-Basu S, Berry FB, Baxevanis AD, Walter MA. Analyses of the effects that disease-causing missense mutations have on the structure and function of the winged-helix protein *FOXC1. Am J Hum Genet.* 2001;68:627–641.
33. Berry FB, Saleem RA, Walter MA. FOXC1 transcriptional regulation is mediated by N- and C-terminal activation domains and contains a phosphorylated transcriptional inhibitory domain. *J Biol Chem.* 2002;277:10292–10297.