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FOXE3 plays a significant role in autosomal recessive microphthalmia

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Abstract

FOXE3 forkhead transcription factor is essential to lens development in vertebrates. The eyes of *Foxe3/foxe3*-deficient mice and zebrafish fail to develop normally. In humans, autosomal dominant and recessive mutations in *FOXE3* have been associated with variable phenotypes including anterior segment anomalies, cataract and microphthalmia. We undertook sequencing of *FOXE3* in 116 probands with a spectrum of ocular defects ranging from anterior segment dysgenesis and cataract to anophthalmia/microphthalmia. Recessive mutations in *FOXE3* were found in four of 26 probands affected with bilateral microphthalmia (15% of all bilateral microphthalmia and 100% of consanguineous families with this phenotype). *FOXE3*-positive microphthalmia was accompanied by aphakia and/or corneal defects; no other associated systemic anomalies were observed in *FOXE3*-positive families. The previously reported c.720C>A (p.C240X) nonsense mutation was identified in two additional families in our sample and therefore appears to be recurrent, now reported in three independent microphthalmia families of varied ethnic backgrounds. Several missense variants were identified at varying frequencies in patient and control groups with some apparently being race-specific, which underscores the importance of utilizing race/ethnicity-matched control populations in evaluating the relevance of genetic screening results. In conclusion, *FOXE3* mutations represent an important cause of nonsyndromic autosomal recessive bilateral microphthalmia.

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Web Resources

The URLs for data presented herein are as follows:
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim
NCBI Protein Database, http://www.ncbi.nlm.nih.gov/protein/
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/

Keywords

FOXE3; aphakia; sclerocornea; microphthalmia; isolated; nonsyndromic; recessive; consanguinity

INTRODUCTION

The *Foxe3* gene was initially found to underlie the mouse *dysgenetic lens* (*dyl*) phenotype that is limited to the eye and consists of variable degrees of microphthalmia, corneal opacity, small and irregular lenses, and lenticular-corneal adhesions [Sanyal and Hawkins, 1979]. The phenotype was shown to be caused by two missense mutations in the forkhead domain of *Foxe3* [Blixt et al., 2000; Brownell et al., 2000]. Ormestad et al. [2002] demonstrated that the *dyl* mutant Foxe3 is defective in DNA binding and thus represents a null allele. While *dyl+/-* heterozygotes were initially reported to be unaffected, closer examination revealed that approximately 40% of heterozygous mutant mice show an ocular phenotype resembling Peters' anomaly, consisting of a stalk-like connection between the cornea and lens, central leukoma, cataract, or other corneal abnormalities [Ormestad et al., 2002].

The expression and function of *Foxe3/foxe3/FOXE3* was found to be conserved in vertebrates and to correspond well with the reported phenotypes. During development, *Foxe3* transcripts are present in the developing lens and presumptive midbrain of mouse embryos. In adult mice, expression is limited to the anterior lens epithelium [Blixt et al., 2000; Brownell et al., 2000]. Similarly, *foxe3* expression was noted in the eye and brain of zebrafish embryos, with the highest levels in the lens epithelial cells, and *foxe3* transcripts were also detected in the adult lens and brain [Shi et al., 2006]. Similar to *Foxe3*-deficient mice, zebrafish *foxe3* morphants demonstrate small eyes [Shi et al., 2006]. Finally, expression studies in human embryo coronal head sections (Carnegie stage 16 and 17) found *FOXE3* expressed in the developing lens only, with the strongest expression in the anterior lens epithelium [Iseri et al., 2009]; the authors note that brain expression of *Foxe3* in mice is absent by the equivalent embryonic stage, thus brain expression may be present in earlier stages of human development.

Human mutations in *FOXE3* (OMIM 601094) were first identified by Semina et al. [2001] in a patient with posterior embryotoxon, cataracts, and myopia (OMIM 107250). The patient and her mother, also affected with posterior embryotoxon and cataracts, both carried an autosomal dominant c.943InsG (p.L315RfsX117) mutation. In a second family, a different dominant mutation in *FOXE3* was detected in a patient with Peters' anomaly, eccentric corneal opacities, and glaucoma, but no cataract [Ormestad et al. 2002]. The c.524G>T (p.R90L) mutation resulted in an amino acid substitution in the DNA-binding domain; however, the mutant protein was able to bind DNA *in vitro*. Recently, Iseri and colleagues [2009] reported two additional dominant mutations in families with variable phenotypes. The first mutation, c.958T>C (p.X320RextX72), was seen in a proband with bilateral microphthalmia, right aphakia, sclerocornea, and coloboma, and left Peters' anomaly with congenital cataract as well as in other family members affected with cataract and coloboma; the second change, c.146G>C (p.G49A, reported as p.G48A by the authors), was seen in a proband with unilateral microphthalmia/bilateral coloboma and other family members affected with cerulean or late-onset cataracts [Iseri et al., 2009].

The first case of autosomal recessive disease caused by mutations in *FOXE3* was reported by Valleix et al. [2006] in a pair of siblings from a consanguineous union (coefficient of inbreeding (F) =1/8, double first cousins) affected with congenital primary aphakia (aphakia, microphthalmia, and anterior segment aplasia; OMIM 610256). Both siblings were homozygous for a c.720C>A (p.C240X) nonsense mutation. The parents were found to be

heterozygous carriers of the same mutation; both had normal eye examinations [Valleix et al., 2006]. Iseri and coauthors [2009] described two additional consanguineous families affected with recessive *FOXE3* mutations. The first mutation, c.244A>G (p.M82V), was found in a family with bilateral microphthalmia, aphakia, and sclerocornea in some members and anterior segment dysgenesis with glaucoma and normal eye size in others. The second homozygous mutation, c.21_24del (p.M7IfsX216), was identified in a family affected with bilateral microphthalmia, sclerocornea, and aphakia. All obligate carriers in both pedigrees were unaffected.

In order to further define the spectrum of phenotypes associated with mutations in *FOXE3*, we undertook screening of its coding region in patients affected with various ocular disorders.

METHODS

Human subjects

The study was approved by the Institutional Review Boards of the Children's Hospital of Wisconsin and Albert Einstein Healthcare Network with informed consent obtained from every subject. The screening included 116 probands with the following phenotypes: 26 patients with bilateral microphthalmia (13 isolated and 13 with non-ocular anomalies), 14 with unilateral microphthalmia (nine isolated and five with non-ocular anomalies), 19 with anophthalmia in at least one eye (four bilateral anophthalmia/microphthalmia (A/M) isolated, 14 bilateral A/M with non-ocular anomalies, and one unilateral anophthalmia with non-ocular anomalies), 29 with anterior segment dysgenesis without microphthalmia including 15 with Peters' anomaly (12 isolated and 17 with extra-ocular anomalies), four with glaucoma with normal anterior segment and no extra-ocular anomalies (one congenital and three adult-onset), five with coloboma with no extra-ocular anomlies, eight with isolated cataract with no extra-ocular anomalies (nine congenital/juvenile and one adult-onset), and 11 with other ocular defects. Extraocular defects were highly variable and included developmental delay and craniofacial anomalies (abnormal ear shape or position, microcephaly, or dysmorphic facial features) in half of such cases. Other frequently associated features included genitourinary defects, short stature, structural brain abnormalities, and heart defects.

Eight patients with previously identified *SOX2* mutations were excluded from this screening (one patient with bilateral microphthalmia, two with unilateral microphthalmia, and five with anophthalmia). Of the 116 probands, 78 were Caucasian, 15 were Hispanic, five were African American, 13 were Asian, two were Caucasian/Asian, and three were unreported.

FOXE3 mutation screen

The *FOXE3* coding region was examined by direct DNA sequencing of PCR products, as previously described [Reis et al., 2008] and using Mutation Surveyor (SoftGenetics, State College, PA) to analyze sequences; the following primers were used to generate PCR products: set 1 forward, 5'-TGGGAGAGGAAATTAGAGGG-3', and reverse, 5'-ACCTTGACGAAGCAGTCGTT-3', and set 2 forward, 5'-CGCAAGTGGCAGAACAGCAT-3', and reverse, 5'-TAGCAGGAGTTTGAGTCCAG-3'. The Reference sequence NM_012186.2 was used.

To obtain control data for normal variation in *FOXE3* in these ethnic populations, we determined exact gene sequence from 127/161 Caucasian, 80/84 Hispanic, 79/83 African American, and 55/46 Asian control individuals for *FOXE3* sets 1/2, correspondingly, using the above approach. The Caucasian control DNA samples were received from the European Collection of Cell Cultures (Salisbury, UK) while the African American, Hispanic, and

Asian control panels were received from the Coriell Institute for Medical Research (Camden, NJ). All control individuals were reported as unaffected at the time of sample collection; however, it should be noted that since detailed phenotypic information for control persons is not available and age at sample collection is variable, it is possible that some individuals with mild or late-onset ocular conditions are included in these control populations.

RESULTS

A number of variants were identified in patients and controls (Table I). Homozygous or compound heterozygote variants which were not seen in control populations were identified in four families. All heterozygous variants identified in patients were also seen in control populations, thus no dominant disease-causing mutations in *FOXE3* were identified in probands.

Patient 1 is a 2½-year-old Caucasian female with severe bilateral microphthalmia and sclerocornea (Fig 1, Table II). The iris, lens, and optic disc could not be visualized. MRI of the head showed small optic nerves and globes, but normal brain structures. Renal ultrasound at 2 months showed mild right pelvic dilation but voiding cystourethrogram was normal. Development was normal. This patient was the only child born to these parents, who were reported to be unaffected but were not available for detailed ophthalmological examination. There was no history of consanguinity. The patient was found to be a compound heterozygote with a c.244A>G (p.M82V) missense mutation, previously reported [Iseri et al., 2009], and a c.705delC (p.E236SfsX71) frameshift mutation (Supplemental Fig 1). The c.244A>G mutation results in a predicted substitution of a highly conserved residue located in the DNA-binding forkhead domain (Figure 2), while the c.705delC (p.E236SfsX71) is predicted to cause C-terminal truncation of normal protein sequence and frameshift with 70 additional amino acids in the new reading frame. Examination of parental samples identified the presence of the c.244A>G (p.M82V) mutation in the reportedly unaffected mother and the c.705delC (p.E236SfsX71) mutation in the reportedly unaffected father.

Patient 2 is an 11-month-old Caucasian male (United Arab Emirates) with bilateral microphthalmia, aphakia, and abnormal anterior segment with sclerocornea and dysplastic irides (Table II). He also had elevated intraocular pressure with probable optic nerve head cupping. Brain imaging studies were not available. He had no extraocular anomalies and his development was appropriate for age. The patient had two brothers with no ocular defects. The parents were first cousins (F=1/16) and the father was reported to be unaffected. The mother was reported to have decreased visual acuity and unilateral corneal ‘cloudiness’ with no other details available. Neither parent was available for detailed ophthalmological examination. This patient was found to have a homozygous c.557delT (p.F186SfsX38) mutation predicted to result in frameshift with 37 erroneous amino acids after the frameshift (Supplemental Figure 1). Parental testing showed that both parents carry a single copy of the same mutation seen in their child. No other mutations in *FOXE3* were seen in either parent.

Patient 3 is a seven-year-old Asian male (Bangladesh) with bilateral asymmetric microphthalmia (the left eye is more severely affected than the right), aphakia, corneal opacity, and glaucoma. Cranial CT also demonstrated possible optic disc drusen and coloboma of the right optic nerve along with normal brain structures. In addition, the patient had autism and global developmental delay (Table II). There was termination of a sibling pregnancy after sonographic detection of holoprosencephaly. A chromosomal anomaly, 46,XX,del(7)(q34), was found in the aborted fetus. There was an additional younger sibling who was unaffected. The parents were first cousins (F=1/16) and were reported to be

unaffected but were not available for detailed ophthalmological examination. The patient was found to have an apparently homozygous c.720C>A (p.C240X) mutation (Supplemental Figure 1), previously reported [Valleix et al., 2006]. Parental samples were not available for testing. A deletion of the second allele could not be ruled out, but is unlikely given the history of consanguinity.

Patient 4 is a Caucasian female (Kuwait) with bilateral microphthalmia with glaucoma, coloboma, and opaque corneas with no view of the anterior segment (Table II). Severe central ectasia was noted with central scarring and complete corneal neovascularization of the right and almost no central perforation and staphylomatous malformation of the left cornea. MRI of the head showed Chiari 1 malformation as well as attenuation of the optic nerves, chiasm, and optic tract. She also had nystagmus and an umbilical hernia. Development was normal. There was an affected brother, Patient 5, with bilateral microphthalmia, glaucoma, and corneal ectasia with central scarring and complete corneal neovascularization in both eyes (Table II). The anterior segment was not visible. He also had nystagmus and hypertrichosis on his back. Development was normal; brain imaging studies were not available. There were two unaffected siblings. The parents were first cousins (F=1/16) and were also reported to be unaffected but were not available for detailed ophthalmological examination. The patient and her affected brother were found to have a homozygous c.720C>A (p.C240X) mutation (Supplemental Fig 1), as seen in Patient 3 and previously reported [Valleix et al., 2006]. Testing of parental samples showed both parents carry a single copy of this mutation.

Screening of different control groups did not identify the above described variants in either homozygous or heterozygous state in normal populations (with a total of 341 and 374 control individual sequences obtained for *FOXE3* set 1 and set 2, respectively).

In addition to these apparently disease-causing mutations, we identified several variants in our patient and control populations at varying frequencies (Table I). Even though some variants seem to show enrichment in patient versus control populations (c.146G>C (p.G49A), c.601G>A (p.V201M) and c.898A>G(p.S300G)), the differences are likely to be attributed to size/local population variations between the groups rather than disease associations. Of special interest is the c.146G>C (p.G49A) change that was previously reported as a disease-causing mutation [Iseri et al. 2009]. We observed this variant in one African American patient and four control individuals of African American descent. Our patient carrying the c.146G>C variant has been previously described [Patient 8; Reis et al., 2008]. Briefly, his phenotype shows overlap with Peters-plus syndrome, consisting of unilateral Peters' anomaly and microphthalmia, bilateral brachydactyly of the hands, bilateral cleft lip and palate, bilateral ear tags, hydrocephaly, and dysmorphic facial features but normal growth. The patient's brother, who is unaffected, was also found to carry this variant; his unaffected mother does not carry the variant, and the father was not available for testing. The c.146G>C variant was not seen in cases or controls of Caucasian, Hispanic, or Asian descent, suggesting it is a variant specific to the African American population.

DISCUSSION

Based on our results, *FOXE3* coding region mutations appear to be a significant cause of bilateral microphthalmia (15%: 4/26 in the examined *SOX2*-negative population or 4/27 of all collected bilateral microphthalmia cases). *FOXE3*-associated microphthalmia demonstrates an autosomal recessive mode of inheritance and, therefore, the mutations were primarily observed in consanguineous unions, with one compound heterozygote also identified. The previously reported c.720C>A (p.C240X) nonsense mutation [Valleix et al. 2006] was identified in two additional unrelated families of Asian and Caucasian descent in

our sample and therefore seems to be a recurrent mutation (Figure 2). Also, the c.244A>G (p.M82V) mutation that was previously reported in one consanguineous pedigree [Iseri et al. 2009] was identified in one proband in our study (Patient 1; Table II) together with a second *FOXE3* mutation.

Mutations in *FOXE3* appear to result in nonsyndromic ocular disease since the majority of affected individuals demonstrate no extraocular defects (Table II). Of the 16 cases with recessive mutations and 12 cases with dominant mutations, three were reported with developmental delay\behavioral difficulties (one possibly related to pregnancy complications) and four with other mild extra-ocular anomalies; the observed systemic anomalies reveal no consistent patterns and, for consanguineous families, may be related to the increased likelihood of homozygozity at other loci. In terms of ocular features, the majority of patients carrying two recessive mutations in *FOXE3* are affected with microphthalmia and significant corneal defects (Table II); eight patients were diagnosed with aphakia, but the anterior segment could not be visualized in five of the remaining probands due to the corneal opacity or lack of availability for examination; interestingly, affected relatives in two families were reported with aphakia and normal eye size along with homozygous *FOXE3* mutations [Iseri et al., 2009]. *FOXE3* does not appear to contribute to microphthalmia with normal anterior segment structures nor to anophthalmia.

Since *FOXE3* mutations are associated with the recessive form of microphthalmia, we analyzed our population for pedigrees suggestive of recessive inheritance and identified eight families. Four families demonstrated consanguinity, each with a coefficient of inbreeding (F) of 1/16 (= first cousins) or greater; three of these families were found to have *FOXE3* mutations (Families 2-4) and the only consanguineous family in which mutations in *FOXE3* were not identified was a family affected with clinical anophthalmia, rather than microphthalmia. Therefore *FOXE3* mutations were found in 100% (3/3) of consanguineous pedigrees with bilateral microphthalmia. In addition to the consanguineous families, there were four non-consanguineous pedigrees with healthy parents and affected sibling pairs who did not carry mutations in *FOXE3*; one sibling pair was affected with microphthalmia, aphakia, and anterior segment dysgenesis, two pairs with microphthalmia and cataract, and the final pair with microphthalmia (and glaucoma in one sibling). Since these are small pedigrees, it is impossible to definitively establish the mode of inheritance without identification of causative mutations. The occurrence of multiple affected siblings can be indicative of recessive inheritance or may be due to gonosomal mosaicism of a dominant mutation in one of the parents, as was shown for other anophthalmia/microphthalmia genes [Faivre et al., 2006; Ragge et al., 2005; Schneider et al., 2008]. Mutations in other regions of *FOXE3* (regulatory regions that have yet to be identified) or other genetic factors ought to be responsible for the phenotype in the remaining cases.

While multiple genes have been linked to syndromic forms of autosomal recessive microphthalmia, only three other loci have been reported for isolated autosomal recessive microphthalmia (OMIM: 251600, 610093, 611038), each accounting for a small proportion of patients with anophthalmia/microphthalmia. Homozygous mutations in *VSX2* (formerly *CHX10*, OMIM: 142993) have been identified in several consanguineous families of Middle-Eastern origin affected with nonsyndromic anophthalmia/microphthalmia, sometimes associated with cataract, anterior segment anomalies, or coloboma (2% of initial cohort of patients with anophthalmia/microphthalmia) [Ferda Percin et al., 2000; Bar-Yosef et al., 2004; Faiyaz-Ul-Haque et al., 2007], but no mutations were identified in a cohort of 198 children with anophthalmia/ microphthalmia or coloboma from Scotland [Morrison et al., 2002]. Two probands with nonsyndromic clinical anophthalmia (one unilateral, one bilateral) were reported with compound heterozygous mutations in RAX (OMIM: 601881) (1% of initial cohort of patients with anophthalmia/ microphthalmia) [Voronina et al., 2004;

Lequeux et al., 2008]. In addition to these known genetic factors, the 14q32 region (OMIM: 251600) has also been identified as a locus for recessive microphthalmia in a large consanguineous family from Pakistan [Bessant et al., 1999]; the phenotype, consisting of nonsyndromic bilateral microphthalmia with severe corneal defects but no evidence of lenticular anomalies, overlaps that seen in patients with *FOXE3* mutations.

No autosomal dominant mutations in *FOXE3* were observed within our proband population that included 26 patients with Peters' anomaly and/or cataract, consistent with the previously reported dominant *FOXE3* phenotypes. It is interesting that both heterozygous and homozygous mutations in *FOXE3* can result in ocular disease, yet heterozygous carriers of the homozygous mutations described in this paper, by Valleix et al. [2006], and by Iseri et al. [2009] are unaffected, with the exception of the mother of Patient 2, who carries a single copy of the c.557delT mutation and is reported to have unilateral corneal 'cloudiness' and decreased visual acuity. The heterozygous *FOXE3* disease-causing mutations may operate through a dominant-negative mechanism; the previously reported mutations which cause dominant disease are missense or late truncating mutations, while the recessive *FOXE3* mutations are mainly nonsense/frameshift mutations earlier in the coding region (Figure 2). However, the mother of Patient 1 carries a missense mutation (M82V) that changes a highly conserved amino acid in the FOXE3 DNA-binding forkhead domain, similar to the previously reported dominantly inherited Peters' anomaly variant (R90L) (Figure 2) and is unaffected. Another possible explanation for the phenotypic variability of *FOXE3* mutations is the presence of modifying mutations at other genetic loci that can vary between families/individuals. Further research is needed to determine why some heterozygous mutations result in disease while others only manifest symptoms when present in homozygous (or compound heterozygote) form.

Interestingly, several *FOXE3* variants appear to be race-specific, underscoring the importance of utilizing race/ethnicity-matched control populations in evaluating the relevance of genetic screening results. The c.601G>A (p.V201M) variant was observed in patients and controls of Hispanic background and one patient of Asian background, but not in other groups; the c.898A>G (p.S300G) substitution was seen in patients and controls of Caucasian origin only. Finally, the c.146G>C (p.G49A) variant was only found in African American patients and controls (Table I). The c.146G>C change has been previously described by Iseri and colleagues [2009] in a family with a highly variable phenotype ranging from unilateral microphthalmia and coloboma in the proband to late-onset cataract in the maternal grandmother. The race/ethnicity of this family and control samples included in the study was not reported. In our study, the c.146G>C change was detected in an African-American patient affected with a Peters plus-like condition (involving multiple extraocular features), his unaffected brother, and four control individuals of African American descent. Although it is possible that this variant results in a highly variable phenotype which includes some very mild and/or late-onset conditions, therefore explaining the presence of this variant in an unaffected brother and race-matched control individuals, overall it appears to be more plausible that this change represents a normal race-specific variation in *FOXE3* gene. Further functional studies and mutation analysis of affected and unaffected individuals of varied racial/ethnic backgrounds is needed to define what role, if any, this and other *FOXE3* variants play in ocular disease.

In conclusion, our results demonstrate an important role for *FOXE3* in nonsyndromic bilateral microphthalmia. *FOXE3*-associated microphthalmia is accompanied by aphakia and/or corneal defects and demonstrates an autosomal recessive mode of inheritance. Mutations in *FOXE3* do not appear to be involved in anophthalmia, microphthalmia with normal anterior segment structures, or syndromic eye anomalies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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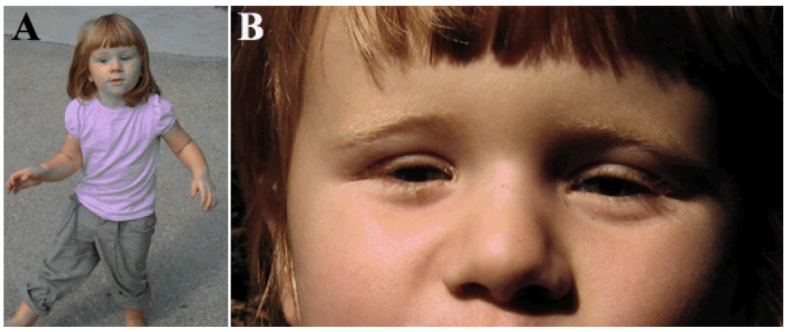

Figure 1. Patient photographs

A) Patient 1 at 2-½-years-old with ocular prosthetics. Note absence of facial dysmorphology and other defects. B) Patient 1 with prosthetics removed demonstrating bilateral severe microphthalmia.

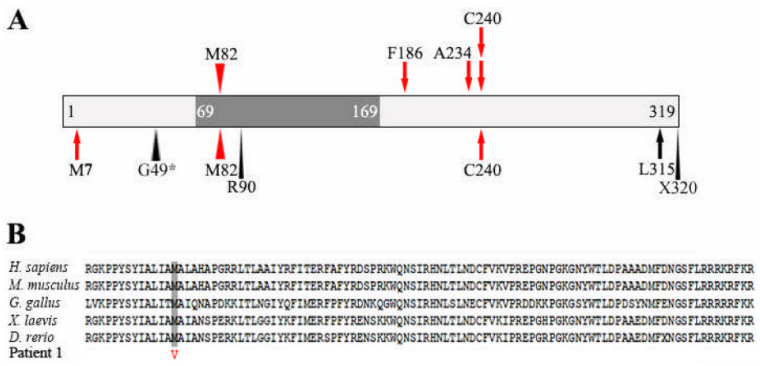

Figure 2. *FOXE3* mutations

A) Schematic drawing demonstrating the relative positions of *FOXE3* mutations identified in our study (top) and previously reported (bottom). The FOXE3 forkhead domain is shown as a dark grey box. Missense mutations are denoted by arrowheads while stop codon/ frameshift mutations are indicated with arrows; recessive mutations are shown in red. B) Alignment of FOXE3 forkhead domain sequences showing conservation of the M82 amino acid in vertebrates. Sequences were obtained from NCBI Protein Database: *Homo sapiens* (NP_036318.1), *Mus musculus* (NP_056573.1), *Gallus gallus* (NP_990337.1), *Xenopus laevis* (NP_001079202.1), and *Danio rerio* (NP_001073150.1).

Table I

cing results in patient and control samples (this study).

n effect	Caucasian patients	Caucasian controls	Hispanic patients	Hispanic controls	African American patients	African American controls	Asian patients	Asian controls	Total patients**	Total controls
I82V	0.6% (1/156)	0	0	0	0	0	0	0	0.4% (1/232)	0
SfsX38	1% (2/156)	0	0	0	0	0	0	0	0.9% (2/232)	0
SfsX71	0.6% (1/156))	0	0	0	0	0	0	0	0.4% (1/232)	0
240X	1% (2/156)	0	0	0	0	0	8% (2/26)	0	1.7% (4/232)	0
D6N	0	0	0	0	0	3% (5/158)	0	0	0	0.7% (5/682)
45A	0	0	0	0	0	0	0	1% (1/110)	0	0.1% (1/682)
49A	0	0	0	0	10% (1/10)	2.5% (4/158)	0	0	0.4% (1/232)	0.6% (4/682)
53P	0	0	0	0	0	0	0	2.7% (3/110)	0	0.4% (3/682)
92L	0	0	0	0	0	0.6% (1/158)	0	0	0	0.1% (1/682)
141K	0	0	0	0	0	3.6% (6/166)	0	0	0	0.8% (6/748)
149S	0	0	0	0.6% (1/168)	0	0	0	0	0	0.1% (1/748)
170A	38% (60/156)	42% (135/322)	37% (11/30)	18% (30/168)	40% (4/10)	22% (37/166)	31% (8/26)	34% (31/92)	37% (86/232)	31% (233/748)
176V	0	0.3% (1/322)	0	0	0	0	0	0	0	0.1% (1/748)
201M	0	0	10% (3/30)	3% (5/168)	0	0	4% (1/26)	0	1.7% (4/232)	0.7% (5/748)
206A	2% (3/156)	0.6% (2/322)	0	1% (2/168)	0	0.6% (1/166)	8% (2/26)	3% (3/92)	2.6% (6/232)	1.6% (12/748)
261L	0	0	0	0	0	0	0	1% (1/92)	0	0.1% (1/748)
277G	0	0	0	0	0	0.6% (1/166)	0	0	0	0.1% (1/748)
300G	2% (3/156)	0.3% (1/322)	0	0	0	0	0	0	1.3% (3/232)	0.1% (1/748)
310D	0	0	0	0.6% (1/168)	0	0.6% (1/166)	0	0	0	0.3% (2/748)

ll calculations.

n for all variants included in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/)

emina et al., 2000; Valliex et al., 2006; Iseri et al., 2009)

ing mutation (Iseri et al., 2009)

** = Includes three patients with unreported race/ethnicity and two with mixed racial background

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Table II

Summary of *FOXE3* mutations reported to date and associated phenotypes.

Reference	Mutation	Protein Change	Ocular Features Bilateral unless indicated	Other Features	Family History	Consanguinity
HOMOZYGOUS/ COMPOUND HETEROZYGOUS MUTATIONS						
Patient 1, this study	heterozygous c.244A>G; c.705delC	p.M82V; p.E236SfsX71	Severe microphthalmia, sclerocornea (anterior segment not visualized)	Mild pelvic dilation with normal VCUG	Unaffected parents both heterozygotes	No
Patient 2, this study	homozygous c.557delT	p.F186SfsX38	Microphthalmia, aphakia, sclerocornea,	None	Parents both heterozygotes, mother with unilateral corneal ‘cloudiness’	Yes
Patient 3, this study	homozygous c.720C>A	p.C240X	Microphthalmia, aphakia, corneal opacity, glaucoma	Autism, developmental delay	Parents unaffected, DNA not available	Yes
Patient 4, this study	homozygous c.720C>A	p.C240X	Microphthalmia, coloboma, glaucoma, corneal opacity (anterior segment not visualized	Chair 1 malformation, umbilical hernia	Affected brother (below), Unaffected parents both heterozygotes	Yes
Patient 5, this study	homozygous c.720C>A	p.C240X	Microphthalmia, glaucoma, corneal opacity (anterior segment not visualized)	Hypertrichosis	Sibling of above	Yes
Case 1, Iseri et al., 2009	Homozygous c.244A>G	p.M82V	Microphthalmia, aphakia, sclerocornea	None	Large consanguineous pedigree, multiple affected	Yes
Case 2, Iseri et al., 2009	Homozygous c.244A>G	p.M82V	Anterior segment dysgenesis and congenital glaucoma	None	Member of large pedigree (above)	Yes
Case 3, Iseri et al., 2009	Homozygous c.244A>G	p.M82V	Microphthalmia, sclerocornea	None	Member of large pedigree (above)	Yes
Case 4, Iseri et al., 2009	Homozygous c.244A>G	p.M82V	Anterior segment dysgenesis, congenital glaucoma	None	Member of large pedigree (above)	Yes
Case 5, Iseri et al., 2009	Homozygous c.244A>G	p.M82V	Microphthalmia, sclerocornea	None	Member of large pedigree (above)	Yes
Case 6, Iseri et al., 2009	Homozygous c.244A>G	p.M82V	Aphakia, sclerocornea, absent anterior chamber, central nodular corneal degeneration	None	Member of large pedigree (above)	Yes
Case 7, Iseri et al., 2009	Homozygous c.21_24del	p.M7IfsX216	Microphthalmia, aphakia, sclerocornea, aniridia	None	Two affected cousins	Yes

Reference	Mutation	Protein Change	Ocular Features Bilateral unless indicated	Other Features	Family History	Consanguinity
Case 8, Iseri et al., 2009	Homozygous c.21_24del	p.M7IfsX216	Aphakia, sclerocornea	None	Cousin of above	Yes
Case 9, Iseri et al., 2009	Homozygous c.21_24del	p.M7IfsX216	Congenital blindness	Developmental delay, pregnancy complications	Cousin of above	Yes
Valleix et al., 2006	homozygous c.720C>A	p.C240X	Aphakia, asymmetric microphthalmia, right megalocornea, elevated IOP	None	Two affected siblings; unaffected parents both carry mutation	Yes
Valleix et al., 2006	homozygous c.720C>A	p.C240X	Microphthalmia, aphakia, sclerocornea	None	Sibling of above	Yes
HETEROZYGOUS MUTATIONS						
Semina et al., 2001	heterozygous c.943InsG	p.L315RfsX117	Posterior embryotoxon, cataract, myopia	None	Affected mother also carries mutation	No
Semina et al., 2001	heterozygous c.943InsG	p.L315RfsX117	Posterior embryotoxon, cataract	None	Mother of above	No
Ormestad et al., 2002	heterozygous c.524G>T	p.R90L	Peters' anomaly, glaucoma	None	Large autosomal dominant pedigree; no other family members tested	No
Case 10, Iseri et al., 2009	Heterozygous c.958T>C	p.X320RextX72	R microphthalmia, aphakia, sclerocornea, coloboma; L Peters' anomaly, microphthalmia, congenital cataract	Prominent incisors	Family members with coloboma, cataract	No
Iseri et al., 2009	Heterozygous c.958T>C	p.X320RextX72	Nuclear sclerosis, cataracts	None	Mother of Case 10	No
Iseri et al., 2009	Heterozygous c.958T>C	p.X320RextX72	Partial iris coloboma, cataracts	None	Maternal uncle of Case 10	No
Iseri et al., 2009	Heterozygous c.958T>C	p.X320RextX72	Early adult onset cataracts (20s)	None	Maternal aunt of Case 10	No
Iseri et al., 2009	Heterozygous c.958T>C	p.X320RextX72	Cataract extraction age 40	None	Maternal grandmother of Case 10	No
Case 11, Iseri et al., 2009	Heterozygous c.146G>C*	p.G49A*	R microphthalmia, microcornea, coloboma; L coloboma	Mild behavioral difficulties	Family members with cataract	No
Iseri et al., 2009	Heterozygous c.146G>C*	p.G49A*	Cerulean cataract	None	Mother of Case 11	No
Iseri et al., 2009	Heterozygous c.146G>C*	p.G49A*	Cerulean cataract	None	Brother of Case 11	No

Reference	Mutation	Protein Change	Ocular Features Bilateral unless indicated	Other Features	Family History	Consanguinity
Iseri et al., 2009	Heterozygous c.146G>C*	p.G49A*	Cataract extraction age 70	None	Maternal grandmother of Case 11	No

Highlighted rows indicate probands/unrelated affected individuals

* indicates probable race-specific variant (please see Table I)