ARTICLE

A novel, non-stop mutation in *FOXE3* causes an autosomal dominant form of variable anterior segment dysgenesis including Peters anomaly

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Anterior segment dysgenesis (ASD) is a spectrum of disorders that affect the anterior ocular chamber. Clinical studies on a Newfoundland family over the past 30 years show that 11 relatives have a variable ocular phenotype ranging from microcornea to Peters anomaly, segregating as an autosomal dominant trait. To determine the molecular etiology of the variable ASD in this family, we sequenced nine functional candidate genes and identified 44 variants. A point mutation in *FOXE3*, which codes for a transcription factor involved in the formation of the lens and surrounding structures, co-segregated with the variable ocular phenotype. This novel mutation (c.959G>T) substitutes the stop codon for a leucine residue, predicting the addition of 72 amino acids to the C-terminus of *FOXE3*. Two recent reports have also identified non-stop mutations in *FOXE3* in patients with variable ocular phenotypes and predict an extended protein. Although *FOXE3* is a lens-specific gene, we successfully isolated complementary DNA from lymphoblasts of an affected family member, and our sequencing results show that the c.959T allele is absent, suggesting that it may be degraded at the RNA level. Though preliminary, our results challenge the notion that an extended *FOXE3* protein causes ASD, and instead suggests a mechanism of haploinsufficiency in the case of non-stop mutations. This study adds to several reports that suggest that autosomal-dominant mutations within *FOXE3* cause ASD and has important clinical utility, especially for the diagnosis of mildly affected patients.

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INTRODUCTION

Peters anomaly (OMIM 604229) is a rare congenital disorder characterized by corneal opacities, adhesions (iris lens and/or lens cornea) due to malformations of the posterior corneal stroma and absence of Descemet's membrane and the corneal endothelium.¹ It is sometimes regarded as a sporadic condition with a low risk of recurrence and is often attributed to aneuploidy or fetal alcohol syndrome,^{2,3} though reports of inherited cases associated with other ocular anomalies, such as congenital cataracts,⁴ dysgenesis of the irido-corneal angle,⁵ and cataract-microcornea syndrome (OMIM 116150) are common. Peters anomaly can also be associated with other malformations, including Krause–Kivlin syndrome (Peters plus syndrome; OMIM 261540) and Axenfeld–Rieger syndrome (OMIM 602482). Peters anomaly and other ocular malformations that affect the anterior chamber of the eye (including the cornea, iris, lens, and iridocorneal angle), belong to a spectrum of disorders collectively known as anterior segment dysgenesis (ASD).

Primarily, mutations in genes that encode transcription factors that show expression in the eye or other structural components have been implicated in ASD. For example, genes encoding transcription factors expressed during the migration of neural crest cells in eye development, such as *PAX6*,⁶ *PITX2*,^{7,8} *FOXC1*,⁹ *FOXE3*,^{10,11} and *PITX3*,^{12,13}

have been associated with disorders such as aniridia, Axenfeld–Rieger syndrome, Peters anomaly, and congenital cataracts. Genes that encode structural components of the eye, such as the crystallins (ie, *CRYAA*)¹⁴ or the gap junction gene *GJA8*,¹⁵ have also been associated with various forms of ASD, such as congenital cataracts and microcornea-cataract syndrome, respectively.

We previously reported a proband diagnosed with congenital Peters anomaly and a family history of a variable form of autosomal dominant ASD, including microcornea, congenital cataracts, and scleralization of the iris.¹⁶ In this paper, we present the progression of the ocular phenotype in this family observed over a 30-year period and describe the functional candidate approach used to determine the causative gene.

MATERIALS AND METHODS

Clinical investigation of proband and relatives

We studied a four-generation family from the island of Newfoundland with mild-to-severe forms of ASD segregating as an autosomal dominant trait (Figure 1). The proband was diagnosed at birth in 1979 with Peters anomaly.¹⁶ Long-term clinical and ophthalmological examination on family members has since been carried out (Table 1), and has included visual acuity measurements, refraction, direct and indirect ophthalmoscopy, slit lamp examination, tonometry, gonioscopy, corneal diameter measurements, axial length measurements

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Figure 1 A four generation Newfoundland family segregating an autosomal dominant form of anterior segment dysgenesis. The proband (PID III-6: arrow) has congenital Peters anomaly.

(ultrasound), and external eye photographs as previously described.¹⁶ In all, 31 of 66 known family members have participated in this study, and of these, we have identified 11 cases of ASD across six sibships (Figure 1). Because of the variability of ocular phenotypes seen in this family, we conducted repeated, complete slit lamp examination of the cornea, iris, angle and lens, and measurement of the corneal diameter on a number of individuals. These examinations provided evidence for minimal diagnostic criteria, which included either corneal scleralization, lens opacities, strands in the angle, or small corneal diameter (<11 mm). This study was approved by the Human Investigations Committee, Memorial University, St John's, Newfoundland, Canada (HIC no. 02.116).

Selection and screening of functional candidate genes

Genes involved in the structural development of the eye or with previous association with ASD, such as Peters anomaly and related disorders (ie, Axenfeld–Rieger syndrome, Krause–Kivlin syndrome), were sequentially screened for mutations. *PAX6* encodes a transcription factor, which has been implicated in aniridia and Peters anomaly.^{6,17} *PITX2* (*RIEGL*) is primarily associated with Axenfeld–Rieger syndrome, a syndromic form of ASD with similar ocular phenotypes,⁸ though some mutations have been identified as causing non-syndromic Peters anomaly.⁷ *PITX2* interacts with the transcription factor *FOXC1*,^{9,18} and mutations in *FOXC1* produce a similar phenotype as seen with *PITX2* mutations. *PITX3* has been associated with autosomal-dominant congenital cataracts and Peters anomaly.^{12,13,19} Mutations in *B3GALT*, a galactosyltransferase gene, cause Peters plus syndrome, which consists of Peters anomaly, short stature, cleft lip, cleft palate, and mental retardation.²⁰ Though there have been no reports of incomplete or atypical forms of Krause–Kivlin syndrome showing only the ocular phenotype, *B3GALT* was screened in this family to check for possible hypomorphic alleles, which may cause the non-syndromic phenotype seen in this family. *CRYAA*, a member of the crystallin family, which constitutes the major structural protein in lens fiber cells has been associated with autosomal-dominant cataracts, microcornea, and corneal opacities.^{14,21} *GJA8* encodes a gap junction protein, which regulates ion and crystallin concentrations within the lens fiber cells.¹⁵ This gene has been associated with cataract-microcornea syndrome, and various other forms of cataracts. *CYP11B1* is a member of the cytochrome P450 superfamily, and mutations in this gene have been attributed to primary congenital glaucoma, and Peters anomaly.^{22–24} Finally, *FOXE3* is a lens-specific transcription factor which has been implicated in cases of ASD, including autosomal-dominant Peters anomaly,¹⁰ congenital cataracts,¹¹ and autosomal-recessive congenital primary aphakia.²⁵

DNA isolation and bi-directional sequencing

Genomic DNA was isolated from peripheral leukocytes²⁶ and screened for mutations in the proband and four relatives with ocular phenotypes (Figure 1:

pedigree identifier (PID) II-5; II-14; III-2; III-6; IV-1), representing the full range of phenotypes found in this family, and two unaffected individuals (PIDs: II-10; II-15). Oligonucleotide primers were designed using Primer 3 software (<http://frodo.wi.mit.edu/>) to amplify all coding regions, and UTRs of the following genes: *PAX6* (NM_001604), *PITX2* (NM_153426), *PITX3* (NM_005029), *CYP11B1* (NM_000104), *FOXC1* (NM_001453), *B3GALT* (NM_194318), *CRYAA* (NM_000394), *GJA8* (NM_005267), and *FOXE3* (NM_012186). Amplicons were run on a 1% agarose gel stained with SYBR Safe (Invitrogen by Life Technologies, Carlsbad, CA, USA) for size verification. Primer sequences and PCR conditions are available upon request.

Amplicons were purified using 50% S-300HR sephacryl (Amersham Biosciences, Uppsala, Sweden) and Multiscreen HTS filter plates (Millipore Corporation, Billerica, MA, USA). Bi-directional sequencing was carried out with BigDye Terminator Kit v 3.1 (Applied Biosystems, Foster City, CA, USA), and samples were run on an ABI 3130x1 DNA Analyzer. Electropherograms were inspected manually for quality and imported into Mutation Surveyor software v 3.2 (Transition Technologies, Toronto, ON, Canada) to detect sequence variants. All sequencing variants were checked for co-segregation with the ASD phenotype in the family. Only one allele (*FOXE3* c. 959G>T) co-segregated with the disease phenotype. Population frequency of this variant was determined using ethnically matched controls from a previous study.²⁷

Total RNA was extracted from Epstein–Barr virus-transformed B lymphocytes from one affected individual (PID IV-1) with Trizol (Invitrogen by Life Technologies, Carlsbad, CA, USA); this was followed by DNaseI treatment (Ambion, Austin, TX, USA). Complementary DNA (cDNA) synthesis was carried out; PCR was carried out using primers surrounding the stop codon of *FOXE3*, and the PCR products were analyzed by size fractionation using agarose gel electrophoresis and direct sequencing. A set of primers designed to amplify the intron between exons 5 and 6 of *GAPDH* was used as an internal control to check for the presence of genomic DNA contamination. These primers amplify a 170 bp fragment with a cDNA template, and a 278 bp fragment using a genomic template (Figure 3a). Primers and experimental conditions are available upon request.

RESULTS

Long-term clinical follow-up

The proband (PID III-6) is a 30-year-old male born at 35 weeks gestation (Figure 1). Bilateral dense corneal opacities were noted at birth and were initially attributed to maternal prenatal infection. Examination under anesthetic showed corneal opacification of the proband's right eye from the temporal side past the midline and hazy cornea nasally (Table 1; Figure 2a). Fine adhesions from the collarette of the iris to the cornea obscured the angle. The left cornea of the proband

Table 1 Review of ocular findings in family members

PID	Age at last examination	Cornea	Corneal diameter (mm)	Lens	Axial length (mm)	Visual acuity
III-6	30	Right – Peters anomaly Left – Peters anomaly	R – 9.25 L – 9.5	Cataract and adhesion from lens to cornea	R – 20.1 L – phthisical	R – 6/30 L – NLP
II-14	57	Moderate scleralization	R – 10 L – 10	PSC and nuclear cataracts. Extractions at 39 and 40 years	R – 21.2 L – 20.9	R – 6/6 L – 6/9
II-5	68	Moderate scleralization	R – 10 L – 10	Cataract extractions at 36 and 38 years	R – 21.5 L – 22.2	NA NA
II-7	65	Moderate scleralization	R – 8.5 L – 8.5	Cataract extractions at 32 and 36 years	R – 21.3 L – enucleated	HM NA
II-16	59	NA	R – MC L – MC	Cataract extractions at 27 and 29 years	NA	NA
III-7	20	NA	R – NA L – NA	AC, PSC, diffuse SC cataracts. Extractions at 16 years	NA	NA
III-2	41	Mild scleralization	R – 10.5 L – 10.5	AC, PSC. Cataract extractions at 40 years	R – 21.8 L – 22.3	R – 6/9 L – 6/6
III-3	39	Moderate scleralization	R – 9.5 L – 9.75	AP, nuclear, PSC cataracts. Cataract extractions at 21 years	R – 21.8 L – 22.8	R – 6/9 L – 6/9
IV-2	10	Left corneal opacity	NA	AP cataracts	NA	R – 6/6 L – 6/9
IV-1	6	Central corneal opacities	R – NA L – 9	Cataract extractions at 8 months	NA	R – 6/18 L – 6/18

Abbreviations: AC, anterior cortical; AP, anterior polar; HM, hand movement; MC, microcornea; NA, not available; NLP, no light perception; PID, pedigree identifier; PSC, posterior subcapsular; SC, subcapsular.

was more densely opaque with a large central adhesion from the cornea to the lens and peripheral adhesions from the iris to the cornea (Figure 2b). Both globes were of normal size, but corneal measurements were 10 mm horizontally and 9 mm vertically ($N \geq 11$ mm). At 6 months of age, the proband had a corneal transplant of the more severely affected eye (left) and optical iridectomy of the right eye. Pathology review of the left corneal button indicated absent Descemet's membrane, partial absence of Bowman's membrane, and thinning of the central cornea consistent with Peters anomaly. The corneal graft was rejected 6 weeks post-operatively and the eye became phthisical.

The proband was re-examined at 30 years of age by a clinical geneticist (BF; Figure 2c and d) and found to be of normal intelligence. His height was 172 cm (25th percentile); ratio of upper to lower segment 0.98; arm span 170 cm; weight 107 kg (>95 th percentile); and head circumference was 62 cm (this information was not available for other relatives). There was no facial dysmorphism apart from features related to a phthisical left eye due to post-operative complications: the left eyebrow was lower and the left palpebral fissure was shorter than that of the right eye. His palate was intact with a single uvula, and the philtrum was well developed (philtrum length 2 cm, 50–75th percentile). Ears were large (length of 7.3 cm), but were normal in position and contour. The only other minor physical anomaly was clinodactyly of the right second and third fingers and of the left second finger. The lack of extra-ocular phenotypes seen in this patient allowed us to rule out Krause–Kivlin syndrome or other related syndromes as a possible diagnosis for this individual.

The father of the proband (PID II-14) was examined at 27 years of age (the year the proband was born), and shown to have small posterior subcapsular and central nuclear cataracts, first noted at age seven (Table 1). His visual acuity was 6/7.5⁻³ in the right eye and 6/9 in left eye. He had bilateral microcornea (corneal diameter of 10 mm) and scleralization and vascularization of the cornea, particularly superiorly. On gonioscopy, fine iris processes were noted extend-

ing over the trabecular meshwork. Subsequently, he had cataract extractions at ages 39 and 40 because of decreasing visual acuity.

Three paternal aunts (PIDs II-5; II-7; II-16), who had lens opacities documented in childhood, had cataract extractions before 1979 (in their 20s or 30s) when mature cataracts developed. All three individuals presented with microcornea (corneal diameters of 8.5–10.5 mm), mild-to-moderate scleralization of the cornea with varying degrees of vascularization, particularly superiorly and inferiorly (Table 1). Three paternal cousins (PIDs III-2; III-3; III-7) had cataract extractions at ages 16–40 years. The cataracts were originally described as anterior polar, anterior cortical, nuclear, and posterior subcapsular cataracts. Six of these seven affected family members had favorable results post-operatively, although one had vitreous hemorrhage, choroidal detachment, and temporary hypotony of one eye. Individual PID II-7 had serious post-operative complications, including retinal detachments with failed repair, failed corneal grafting, and enucleation of a painful blind eye with hand movement vision only in the remaining eye (Table 1).

The youngest affected family members are PID IV-1 (6 years old) and PID IV-2 (10 years old; Figure 1). Individual PID IV-2 has anterior polar cataracts detected at birth, but has not yet required cataract extraction. Individual PID IV-1 had a complicated pre- and postnatal course with extreme premature birth at 24 weeks gestation. He was hospitalized for 4½ months during which time he had necrotizing enterocolitis with perforation of the bowel, and successful treatment of retinopathy of prematurity. Because of the size and central location of his anterior polar cataracts, he had cataract extractions at 8 months of age (Table 1). He also had bilateral iridectomies because of the central corneal opacities. His central acuity is recorded as 6/18, but his course continues to be complicated with a recent diagnosis of autism.

A paternal uncle who died of pneumonia at 6 months of age in the 1940s was likely affected, as he had 'white eyes' and was registered

Figure 2 Eye phenotype of the proband (PID III-6) over time. (a) Picture of the proband's right eye at 7 weeks showing corneal opacities consistent with Peters anomaly. (b) Surgical drawing of the proband's left eye showing keratolenticular adhesion, and corneal leukoma (pictures a and b are taken from Green and Johnson¹⁶ with permission). (c) Proband's right eye at 30 years of age. (d) Proband's phthisical left eye at 30 years of age.

blind with the Canadian National Institute for the Blind (PID II-9). Eight other paternal aunts and uncles and 15 paternal cousins had normal corneal diameters and clear corneas and lenses upon examination (although some had a prominent arcus suggesting elevated cholesterol levels).

Interestingly, there is no evidence that either of the grandparents had any form of ASD (DNA not available). For instance, the paternal grandfather of the proband (PID I-1) was examined and had corneal diameters of 11 mm, mild corneal scleralization, inferior arcus, and faint lens opacities, including anterior polar specks, and anterior and posterior cortical spokes but retained 6/7.5 and 6/6 visual acuity at 77 years of age. Two of his brothers had clear corneas and lenses in their 60s or 70s. The paternal grandmother of the proband (PID I-2) had corneal diameter of 11.5 mm, mild central endothelial corneal changes (Fuch's dystrophy), arcus senilis, and faint dot and spoke lens opacities when examined at 71 years. She subsequently had mature cataracts extracted at ages 78 and 81. On examination, her brother, sister, niece, and nephew all had clear corneas and lenses, and were considered unaffected. The phenotypes seen in the grandparents can all be attributed to the aging process. It is possible that one had a subclinical phenotype or that gonadal mosaicism was present.

Candidate gene screen reveals causative variant in *FOXE3*

Screening of the nine functional candidate genes in seven individuals (five affected; two unaffected PIDs: III-6, II-14; II-5; III-2; IV-1; II-10; II-15) revealed 45 sequence variants (Table 2). Segregation analysis showed co-segregation between the ASD trait and a non-stop mutation within the transcription factor gene *FOXE3* (c.959 G>T: p.X320L; Figure 3b) causing the elimination of the functional opal stop codon (UGA) at the 3' end of *FOXE3*. The next available stop codon is 213 bps downstream (in the 3' UTR) predict-

Figure 3 (a) Agarose gel (1%) stained with SYBR Safe showing PCR amplification of *FOXE3* from cDNA from a lymphoblastoid cell line of PID IV-1 (in quadruplicate). The internal control of GAPDH amplifying a 170 bp fragment to check for presence of cDNA (and absence of gDNA) is shown below. A genomic control is shown to the right, and the 278 bp fragment amplified from GAPDH exclusively in gDNA. (b, top) Electropherogram of the novel, non-stop mutation in *FOXE3* (c.959 G>T: p.X320L) identified in all affected family members. (Bottom) Electropherogram of the region surrounding the stop codon of *FOXE3* from the cDNA of an affected patient (PID IV-1) isolated from a lymphoblast cell line.

ing the addition of 72 amino acid residues to the C-terminus of *FOXE3*.

We then genotyped all available members of the extended family and found that the variant co-segregated with the disease phenotype (Table 2). This variant was not detected in 141 ethnically matched controls. To test whether this mutation would be present in the mRNA of *FOXE3*, RNA was isolated from a lymphoblast cell line of individual PID IV-1 and converted to cDNA. *FOXE3* is a lens-specific gene expressed only in the lens epithelium,¹⁰ as a result this approach

Table 2 Summary of 45 sequencing variants identified in nine functional candidate genes sequenced in five affected members with a range of anterior segment dysgenesis from mild phenotype to Peters anomaly, and two unaffected members

Gene	Sequence variants	Location of variant	Protein effect	dnSNP ID	Disease segregation
<i>PAX6</i> (11p13)	c.-4393 G<A	5' UTR	None	Not listed	No
	c.1074+107 C<T	Intronic	None	rs3026384	No
	c.1075-174 G<A	Intronic	None	rs2071754	No
	c.1225+44 T<G	Intronic	None	rs3026393	No
	c.1746+21 delA	Intronic	None	Not listed	No
<i>PITX2</i> (4q25)	c.-485 C<G	5' UTR	None	rs2739200	No
	c.629-105 C>A	Intronic	None	Not listed	No
	c.767-81 A>C	Intronic	None	Not listed	No
<i>PITX3</i> (10q24.32)	c.285 C<T	Coding	I95I	rs2281983	No
<i>FOXC1</i> (6p25.3)	c.1123_1124 ins CGG	Coding	ins 375 G	rs71807729	No
	c.1361 ins CGG	Coding	ins 456 G	Not listed	No
<i>CYP11B1</i> (2p22.2)	c.-12 C<T	5' UTR	None	rs2617266	No
	c.142 C<G	Coding	R48G	rs10012	No
	c.355 G<T	Coding	A119S	rs1056827	No
	c.1294 G<C	Coding	V432 L	rs1056836	No
	c.1347 T<C	Coding	D449D	rs1056837	No
	c.1358 A<G	Coding	N453S	rs1800440	No
	c.1633+ 210ins T	3' UTR	None	Not listed	No
	c.1633+350 C>A	3' UTR	None	Not listed	No
	c.1633+693 G>T	3' UTR	None	Not listed	No
	c.1633+809 del A	3' UTR	None	Not listed	No
	c.1633+875 A>G	3' UTR	None	Not listed	No
	c.1633+2408 A<T	3' UTR	None	Not listed	No
<i>B3GALT1</i> (13q12.3)	c.71-5 del T	Intronic	None	Not listed	No
	c.271-58 delT	Intronic	None	Not listed	No
	c.271-67 T>C	Intronic	None	Not listed	No
	c.347+20 C<G	Intronic	None	Not listed	No
	c.348 T<C	Coding	H116H	Not listed	No
	c.596+20 delC	Intronic	None	Not listed	No
	c.597-168 delA	Intronic	None	Not listed	No
	c.780+58 A<G	Intronic	None	Not listed	No
	c.781-124 G<C	Intronic	None	Not listed	No
	c.781-31 ins CATA	Intronic	None	Not listed	No
	c.1065-142 T<C	Intronic	None	rs1041073	No
	c.1108 G<A	Coding	E370K	Not listed	No
	c.1497+31 G>T	3' UTR	None	Not listed	No
	c.1497+37 A>G	3' UTR	None	Not listed	No
	c.1497+127 G>A	3' UTR	None	Not listed	No
	c.1497+938 delT	3' UTR	None	Not listed	No
	c.1497+1548 C>T	3' UTR	None	Not listed	No
<i>CRYAA</i> (21q22.3)	c.6 C<T	Coding	D2D	Not listed	No
<i>GJA8</i> (1q21.1)	c.1365+26 delA	Intronic	None	Not listed	No
<i>FOXE3</i> (1p33)	c.959 G>T	Coding	X320L	Not listed	Yes
	c.960+72 T>C	3' UTR	None	rs2820969	No
	c.960+77 A>G	3' UTR	None	rs6666370	No

The non-stop mutation identified in *FOXE3* indicated in bold.

was not guaranteed to succeed. However, we were able to amplify a 458bp cDNA product from *FOXE3* surrounding the c.959 G>T mutation (Figure 3a). Direct sequencing revealed that the c.959 G>T mutation was absent in the cDNA (Figure 3b), suggesting that the mRNA transcribed from the 'non-stop' allele may be degraded before being translated, or it is possible that the RNA is not transcribed at all.

DISCUSSION

Peters anomaly is a rare congenital disorder often regarded as a sporadic condition with a low risk of recurrence and attributed to aneuploidy or

fetal alcohol syndrome. Inherited cases associated with malformations of the anterior chamber of the eye have also been described. In 1986, we first reported a family with an autosomal dominant form of ASD with the proband exhibiting Peters anomaly.¹⁶ This four generation family from Newfoundland has had extensive clinical assessment over the course of 30 years, revealing a progression over time of the ASD phenotype in affected family members. For example, the father of the proband (PID-II-14) had mild cataracts at the age of 7 and required cataract extractions at the ages of 39 and 40 because of decreasing visual acuity. Other affected family members (PIDs II-5; II-7; II-16) have also shown a non-static phenotype with mild cataracts noted at young ages

progressing to mature cataracts causing decreased visual acuity, making extractions necessary at a later age.

The anterior chamber is formed by three successive waves of embryonic cells derived from the neural crest. Aberration in the migration of these cells can cause anterior chamber defects, such as microcornea, corneal opacities, or congenital cataracts.⁵ Using a functional candidate gene approach, we looked for mutations in this family, in genes previously associated with ASD or Peters anomaly and identified a novel non-stop mutation in *FOXE3* (c.959 G>T; p.X320L; Figure 3b), which segregated with the variable ocular phenotype, and was absent from 282 ethnically matched chromosomes. Though rare, non-stop mutations are reported in a number of cases involving various diseases, such as in non-classic 3- β -HSD congenital adrenal hyperplasia,²⁸ and excessive hemorrhaging because of mutation of coagulation FX.²⁹

The normal interaction of mouse *Foxe3* with genes responsible for cellular differentiation and proliferation provides an explanation for the presence of small lenses and other eye malformations seen in humans. In mice, *Foxe3* encodes a DNA-binding transcription factor expressed during the formation of the lens placode and assists in the formation of the lens itself.³⁰ Mutations in *Foxe3* reduce the ability of the transcription factor to bind DNA, causing formation of a small lens.¹⁰ Sometimes the anterior lens epithelium does not separate from the cornea, resulting in keratolenticular adhesions,¹⁰ which we observed in the proband with Peters anomaly. One explanation is that this adhesion is caused by a dysregulation of genes responsible for apoptosis within the corneal stalk, which is thought to degrade during lens morphogenesis as a result of apoptosis.³¹ An alternative explanation is that mutation of *Foxe3* may cause a dysregulation of cadherin proteins, such as E-cadherin, which are present in the corneal stalk.³¹ *Foxe3* also controls the expression of *Cryax* within the developing lens, and dysfunction of *Cryax* expression reduces the solubility of the crystallin protein complex causing crystallization and potential cataract formation. *Foxe3* is responsible for the downregulation of *Prox1*, which controls a gene responsible for blocking cell cycle progression (cyclin dependant kinase inhibitor; *Cdkn1c*). As *Foxe3* expression becomes dysregulated, *Prox1* expression increases, subsequently increasing *Cdkn1c* expression causing a reduction of cellular proliferation in the anterior lens.^{30,32} *Foxe3* also controls expression of platelet-derived growth factor receptor- α (*Pdgfra*), which is responsible for lens fiber differentiation within the anterior lens epithelium.³⁰ Post-natally, *FOXE3* is expressed exclusively in the anterior lens epithelium which is the only site of cell proliferation within the lens. This restricted cellular expression is consistent with the non-syndromic phenotype observed in *FOXE3* mutation carriers.^{30,31}

Mutations in *FOXE3* and its involvement in human ASD and/or Peters anomaly were first identified by Semina *et al*,¹¹ who described two patients with apparent autosomal dominant posterior embryotoxon and congenital cataracts caused by a heterozygous single nucleotide insertion which altered the 5' terminal amino acids, and added an additional 111 amino acids to the *FOXE3* protein.¹¹ A second report of *FOXE3* and its involvement in ASD and/or Peters anomaly was published by Ormestad *et al*,¹⁰ who identified a heterozygous missense mutation (c.524 G>T; p.R90L) coding for the fork head DNA-binding domain of the *FOXE3* protein of a single affected individual with familial Peters anomaly, though no other DNA samples from the family were available to confirm segregation. Recently, Iseri *et al*³³ described four other families containing *FOXE3* mutations, c.21_24del:p.M71IfsX216, c.146G>C:p.G49A, c.244A>G: p.M82V, c.958T>C p.X320ArgextX72, all of which are hypothesized to be pathogenic. Homozygous mutations within *Foxe3/FOXE3* can also cause autosomal recessive congenital aphakia within

mice (*dyl* mice)³⁰ and humans respectively.²⁵ Also, mice containing heterozygous mutations within *Foxe3* showed histological ocular malformations upon investigation,¹⁰ further showing the ability of *FOXE3* to cause dominant and recessive ocular conditions.

Two recent reports (Iseri *et al*³³ and Brémond-Gignac³⁴) identified similar non-stop mutations within *FOXE3* (c.958T>C: p.X320ArgextX72 and c.959G>C: p.X320SerextX72 respectively) in patients with ocular diseases. Iseri *et al*³³ reported a family with autosomal dominant ASD in a proband diagnosed with unilateral Peters anomaly as well as in four family members segregating congenital cataracts, microphthalmia, and iris coloboma. Brémond-Gignac *et al*³⁴ reported a single patient with congenital cataract and no family history of ocular disease. Both of these studies predict a similar 72 amino acid extension of the *FOXE3* protein, though no functional analyses were performed to confirm this. It is clear that functional studies must be conducted to confirm that an extended protein is produced, as our results suggest that these non-stop RNA species may be degraded. The mutation identified in the Newfoundland family (c.959 G>T) occurs in the stop codon of *FOXE3* and according to our preliminary data from cDNA (lymphoblastoid cell line) of individual PID IV-1, the mRNA transcript containing the non-stop allele is degraded (or not transcribed) suggesting haploinsufficiency of *FOXE3* (Figure 3). An alternative interpretation of the data is that the RNA containing the c.959 T allele may be more unstable than that of the wild type, making it under-represented in total RNA. This could also explain why the mutation was not detected by direct sequencing of cDNA, thus quantitative and translation based studies are required to confirm the absence of both the mutant RNA and the extended protein.

Semina *et al*¹¹ also described an insertion which caused the addition of 111 amino acids to the *FOXE3* protein. It is possible that this mutation would also undergo the same type of mRNA degradation that we observe. We are currently conducting quantitative studies of the *FOXE3* c.959 G>T mutation within human cells to validate our hypothesis of RNA degradation.

In summary, our results implicate *FOXE3* in the pathology of ASD and corroborate other recent reports of ASD cases with *FOXE3* mutations. Therefore, mutations within *FOXE3* may explain currently unsolved ASD cases and should be screened. Discovery of genes and mutations causing ASD in families is of particular clinical relevance. A molecular diagnosis of ASD cases through mutation screening can provide accurate risks of recurrence, especially in light of mild or subclinical phenotypes, which may show progression over time such as those seen in the family seen in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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