REPORT

Homozygous Nonsense Mutation in the *FOXE3* Gene as a Cause of Congenital Primary Aphakia in Humans

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Congenital primary aphakia (CPA) is a rare developmental disorder characterized by the absence of lens, the development of which is normally induced during the 4th–5th wk of human embryogenesis. This original failure leads, in turn, to complete aplasia of the anterior segment of the eye, which is the diagnostic histological criterion for CPA. So far, the genetic basis for this human condition has remained unclear. Here, we present the analysis of a consanguineous family with three siblings who had bilateral aphakia, microphthalmia, and complete agenesis of the ocular anterior segment. We show that a null mutation in the *FOXE3* gene segregates and, in the homozygous state, produces the mutant phenotype in this family. Therefore, this study identifies—to our knowledge, for the first time—a causative gene for CPA in humans. Furthermore, it indicates a possible critical role for *FOXE3* very early in the lens developmental program, perhaps earlier than any role recognized elsewhere for this gene.

Human aphakia is a rare congenital eye disorder in which the lens is missing. It has been histologically subdivided into primary and secondary forms, in accordance with the severity of defects of the ocular tissues, whose development requires the initial presence of a lens.[1–4] Congenital primary aphakia (CPA) results from an early developmental arrest, around the 4th–5th wk of gestation in humans, that prevents the formation of any lens structure and leads to severe secondary ocular defects, including a complete aplasia of the anterior segment of the eye.[4,5] In contrast, in secondary aphakic eyes, lens induction has occurred, and the lens vesicle has developed to some degree but finally has progressively resorbed perinatally, leading, therefore, to less-severe ocular defects. Few cases fulfill the diagnostic criteria for CPA, and the most famous cases histologically documented are those described by Manschot in 1963.[4]

So far, the underlying genetic causes of CPA have remained obscure, but they undoubtedly are diverse, since lens formation is dependent upon complex interactions of numerous tissues deriving from surface ectoderm, neural ectoderm, and neural crest mesenchyme.[6] However, a major critical step in lens development is lens induction, a multistep process that leads to the formation of the lens placode.[7] Many genes, including transcription factors, homeobox genes, and signaling molecules, have been shown to be involved in the control of each stage of lens development. Although many of these components have been well defined and assembled in a provisional genetic pathway, numerous questions remain concerning the temporal and spatial coordination of their expression.[7–12]

Here, we have investigated a consanguineous family with three children (IV.1, IV.3, and IV.4) who have bilateral aphakia in association with microphthalmia, complete aplasia of the anterior segment of the eye, absence of iris, and retinal dysplasia (figs. 1*A,* 2*B,* and 3*A*). All the family members underwent a complete ophthalmologic examination that did not reveal ocular abnormalities in either parent. In addition, all three affected children were referred to a pediatrician for an examination of associated systemic anomalies, but none were detected, in particular cerebral malformations or dysmorphic features (figs. 2*C,* 2*D,* 3*C,* and 3*D*). In this pedigree, there was no relevant family history of ocular phenotype, and, since the parents were consanguineous, this aphakia was most probably inherited in a recessive manner. The first proband (IV.1) of this kindred was born with bilateral aphakia and microphthalmia and exhibited sclerocornea (data not shown). This child died of infant sudden death syndrome at age 3 mo, for unknown reasons. Infectious and toxic causes were ruled out, and chromosomal analysis showed a normal karyotype. An autopsy was performed and did not reveal systemic malformation or neurological distortion, and both eyes were removed for histological examination. Histological examination of all sections of both eyes from proband IV.1 indicated absence of lens or any residual lens structures associated with a complete aplasia of the anterior segment, absence of iris, no ciliary body, no trabecular meshwork or Schlemm's canal, and dysplasia of the retina (fig. 4*A*–4*C*). All these severe anterior ocular defects fulfill the diagnostic criteria of CPA, rather than that of secondary congenital aphakia.[4] The corneal epithelium

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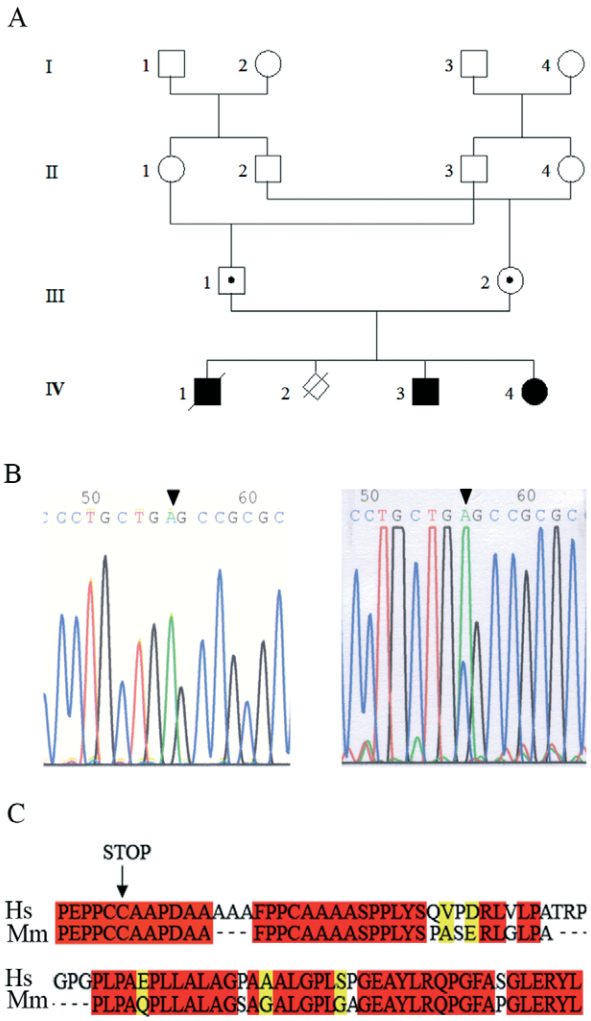

Figure 1. Identification of the *FOXE3* nonsense mutation in the family with CPA. *A,* Pedigree of the family with CPA, showing double consanguinity and recessive inheritance pattern of the ocular phenotype. The affected individuals are identified by a blackened symbol. Phenotypically normal carriers of the mutation are indicated by a dot within the unblackened symbol. *B,* Sequence electrophoregrams of the novel C240X mutation. *Left,* Partial sequence data from affected individual IV.3, indicating homozygosity (one peak: the mutated nucleotide A) for the C240X mutation. *Right,* Sequence data from his clinically asymptomatic mother (III.2), showing heterozygosity (two peaks: the normal nucleotide C and the mutated nucleotide A). Arrowheads indicate the position of the mutated nucleotide. *C,* Comparison of human (Hs) and mouse (Mm) *FOXE3* C-terminal amino acid sequences. *Red background,* Identical amino acids. *Yellow background,* Conservative substitutions. The arrow points to the residue substituted in our patient.

appeared thinner and consisted of three cell layers, rather than four or five. The corneal stroma contained cells with fibroblastic configuration and was abnormally vascularized (fig. 5*A*). The corneal endothelium was not observed in either eye, and, after periodic acid schiff staining, neither Bowman's layer nor Descemet's membranes were detected (fig. 5*A*). Instead of a corneal endothelium at the posterior surface of the corneal stroma, there was a mass of a highly vascularized fibromuscular tissue, which was covered by a pigmented epithelium that occasionally formed some rudimentary pseudofolds, evoking the aspect of ciliary processes (fig. 5*A* and 5*B*). By immunochemistry, this pigmented epithelium stained positively for vimentin antibody, but it was unstained with desmin, α-smooth muscle actin, and CK7/CK20 antibodies, for a cytoskeletal profile suggestive of a ciliary epithelium (fig. 5*C* and 5*D*). The layer of smooth muscular cells at the posterior face of the corneal stroma was of variable thickness, and these cells were found to be positive for anti–α-smooth muscle actin and antidesmin antibodies and negative for antivimentin antibody (figs. 4*B* and 5*D*). Immunohistochemical analysis with HMB45 antibody, which marks melanocyte cells, was performed, and the choroid and a small number of cells within the vascular fibromuscular tissue adherent to the posterior face of the corneal stroma stained positive (fig. 5*E* and 5*F*). In comparison with human fetal eyes at 7, 13.5, and 17.5 wk of development, we found that ciliary muscle and choroid were the only ocular structures positive for HMB45 antibody (data not shown). The retina appeared dysplastic, with rosette-like structures suggesting that the absence of lens most probably disturbed but did not prevent the invagination of the optic vesicule (fig. 4*A* and 4*C*).[13] However, outside the rosettes, the retina showed an apparently normal architecture, in which all neuronal nuclear layers were recognizable, reinforcing the idea that retinogenesis is relatively normal in the absence of lens.[14] This observation also indicates that the lens is required for proper optic cup morphogenesis, therefore supporting the view that contact between the optic vesicle and the prelens ectoderm, but not the lens per se, triggers the optic vesicle to form a cup.[14]

In the same family, two other children were born with bilateral aphakia, which was confirmed by magnetic resonance imaging (MRI) (figs. 2*C,* 2*D,* 3*C,* and 3*D*). Ophthalmologic examination of proband IV.3 revealed asymmetric microphthalmia, a right megalocornea, and normal intraocular pressure (IOP) (fig. 2*B*). However, at the age of 3 years, IOP progressed in the patient's right eye, which led to severe buphthalmos requiring trabeculectomy. In contrast to her brother, proband IV.4 presented at birth with symmetric microphthalmia with aphakia and sclerocornea, with an IOP of 10 mm Hg in both eyes (fig. 3*A* and 3*B*). At the present time, these children, aged 10 and 9 years, are in good health and have no intellectual or neurologic impairment. During the third and the fourth pregnancies, prenatal ultrasonography and MRIs successively performed at 16, 19, and 28.5 wk of gestation revealed absence of lens or capsular remnants in both eyes for probands IV.3 and IV.4, indicating that either the lens had never been formed or was resorbed very early during embryogenesis (fig. 2*A*). The probability that both lenses had formed and were bilaterally resorbed completely and

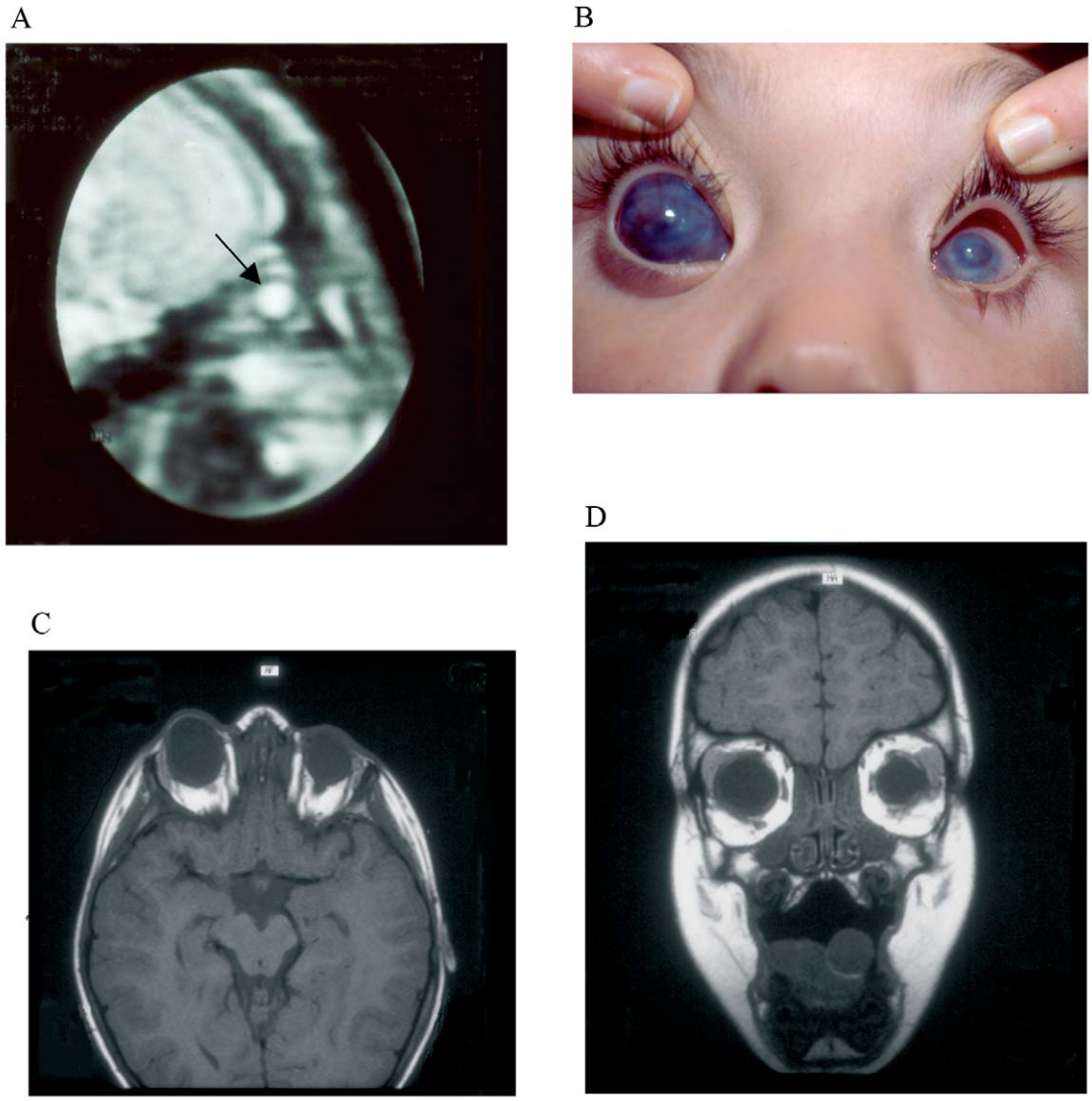

Figure 2. Clinical phenotypes of patient IV.3. *A,* MRI (T2-weighted image) sagittal section at 28.5 wk of gestation. The fetal eyeball appears as a white sphere (*arrow*), and the lens, normally visible inside as a black and well-circumscribed sphere, is absent. *B,* Aspect of eyes of proband IV.3 at the age of 1 mo, revealing bilateral microphthalmia with aphakia in the right eye and extreme microphthalmia of the left eye, preventing correct examination. *C* and *D,* Axial and sagittal sections of cerebral MRI (T1-weighted images) at the age of 4 years, showing absence of lens, asymmetric eyeballs, and normal appearance of brain structures.

symmetrically in both affected children is highly unlikely. Furthermore, the complete aplasia of the anterior segment of the eye, documented histologically in both aphakic eyes of proband IV.1, argues in favor of an absence of lens formation during embryogenesis.

Since the karyotypes of the three infants were normal and the size of the family did not allow linkage analysis, we attempted the strategy of the candidate gene to identify the genetic cause of this congenital malformation. Therefore, on the basis of either animal models or spatial and temporal pattern of expression, we studied several genes, including *PAX6,*[15] *PITX3* and its promoter,[16,17] *SIX3,*[18] *BMP7,*[19] *SOX2,*[12] *FOXC1,*[20] and *FOXE3*[21–24] (MIM 601094). After receiving informed consent from the family, we analyzed the entire coding region and intron-exon boundaries of these genes by bidirectional direct sequencing, and all these genes were excluded as being causative, except for *FOXE3*. In both living affected children (IV.3 and IV.4), we identified a homozygous nucleotide transversion, C→A, at nucleotide position 720, according to the sequence published by Semina et al.[24] (GenBank accession number AF275722). This nucleotide variation predicts the substitution of the cytosine (TGC) at position 240 in the resulting amino acid sequence by a nonsense codon (TGA; C240X) (fig. 1*B*). This nucleotide variation was not found in 100 chromosomes from control individuals, which sug-

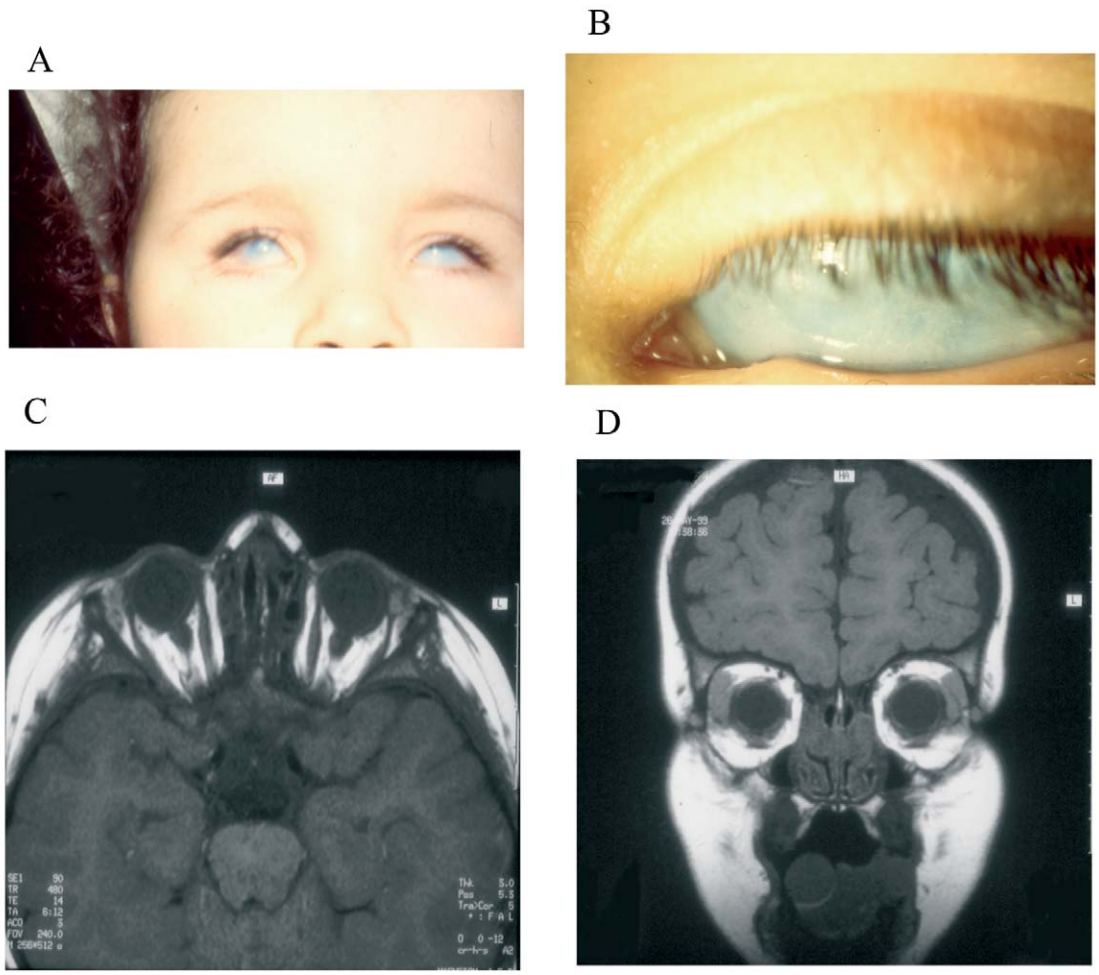

Figure 3. Clinical phenotypes of patient IV.4, the affected daughter. *A,* Ocular appearance showing bilateral and symmetric microphthalmia. *B,* Higher magnification of her left orbit, illustrating the sclerocornea. *C* and *D,* Axial and sagittal sections of cerebral MRI (T1-weighted images) at the age of 3 years, showing absence of lens, symmetric eyeballs, and normal appearance of brain structures.

gests that it is not a common SNP. Sequence analysis confirmed that both parents carried this mutation in the heterozygous state, which supports the hypothesis that the transmission of the phenotype is compatible with autosomal recessive inheritance (fig. 1*A* and 1*B*). This mutation results in the truncation of the 80 C-terminal aa that represent more than one-quarter of the total protein length of 319 aa. The functional consequences of premature termination codon (PTC) mutations are commonly expected to be null alleles, because these mutant mRNA are likely to be detected by RNA surveillance and to be degraded by nonsense-mediated decay (NMD).[25] This mechanism prevents the accumulation of truncated proteins that could interfere with the function of the normal protein or that could have toxic effects in the cell, thereby protecting many heterozygous carriers of genes with PTC mutations from manifesting dominantly inherited disease phenotypes. Here, both carrier parents had normal ocular examination, which supports the hypotheses that mRNA containing C240X has most probably been subjected to NMD and that the congenital aphakia in the children results from *FOXE3* haploinsufficiency.

Elsewhere, two human *FOXE3* missense mutations were identified in the heterozygous state in two families independently presenting anterior segment dysgenesis (ASD) with cataract or Peters' anomaly.[23,24] Of these, one was a single-nucleotide frameshift insertion, 15 bp upstream of the stop codon in *FOXE3,* that replaced the last 5 aa of the predicted protein with 116 novel residues.[24] The second missense mutation reported in *FOXE3* gave rise to a nonconservative amino acid substitution (Arg90Leu) located in the DNA-binding domain, without impairing the fixation of *FOXE3*.[23] These two *FOXE3* missense mutations were found in the heterozygous state and caused a dominantly inherited lens phenotype milder than that observed in our family, which had a recessively inherited CPA. The fact that currently reported *FOXE3* mutations result in variable ocular anterior segment phenotypes and distinct inheritance patterns highlights the complexity of this disease condition. Ocular ASD is a phenotypic and

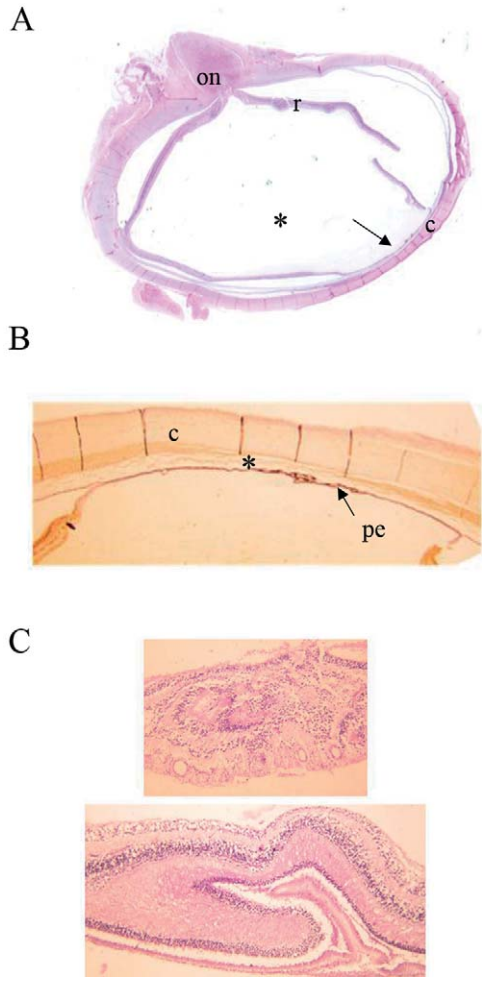

Figure 4. Histologic aspect of eyes from proband IV.1. *A,* Photograph representing section through the entire eye (hematoxylin-eosin staining [HES]). Note the absence of lens, with a empty cavity most probably corresponding to the vitreous (*), the aplasia of the anterior chamber (*arrow*), the dysplastic retina, and the presence of the optic nerve (magnification 4 ×). c = Cornea; on = optic nerve; r = retina. *B,* Histological photograph of the anterior segment of the eye, stained with anti–α-smooth muscular actin antibody. The anterior chamber space, including iris and ciliary body, is not formed, and the posterior face of the corneal stroma is lined by a layer of muscular smooth cells, a fibroconnective tissue, and a continuous layer of pigmented cells (*). pe = Pigmented epithelium. *C,* Abnormal folds of the retina with rosette-like structures in which the retinal lamination is disturbed (HES; magnification 20 ×).

genetically heterogeneous condition that has also been associated with mutations in *PAX6, FOXC1, PITX3,* or *PITX2*.[15,16,20] All these genes have not been exhaustively screened in the patients with *FOXE3* mutations reported by Semina et al.[24] or Ormestad et al.[23] It is, therefore, not excluded that the presence of additional variants in genes having related functions in the development of the anterior segment of the eye could likely influence the penetrance and expressivity of ocular phenotypes observed in patients with *FOXE3*. At the present time, the small number of patients reported with *FOXE3* mutations limits an accurate genotype-phenotype correlation, which will await the complete understanding of the pathogenic mechanism of *FOXE3* mutations.

The involvement of *FOXE3* in this family is interesting, in different regards. First, it is a gene whose expression is restricted to prelens and lenticular tissues, which provides the opportunity to better define the direct role of the lens on the formation of the diverse structures of the eye.[21,22] In this family, the disease phenotype is perfectly restricted to ocular tissues, as is expected for this gene. Similarly to *Foxe3*$^{-/-}$ mice, the brain structures of our patients have not shown any malformations, although *Foxe3* shows transient expression in discrete regions of the developing midbrain between E8.5 and E12.0 of mouse embryogenesis.[21,22] Secondly, our data emphasize the role of *FOXE3* as essential for early developmental stages of the lens—an unexpected finding—while the molecular cascades that result in the formation of a lens are being elucidated.[6,7,26] In the mouse, two spontaneous *Foxe3* missense mutations predicted to be null alleles cause the recessive dysgenetic lens (*dyl*) mouse phenotype.[21,22] The *dyl* mice, which are viable and appear normal, have defects in lens vesicle closure and separation, which implies that *Foxe3* is an essential regulator of this event. Finally, mice deficient in *Foxe3* through targeted gene disruption show lens abnormalities similar to those described for the *dyl* mice.[27] Despite these two mouse *Foxe3* models, the murine lens phenotype remains milder than that observed in our aphakic children carrying the C240X nonsense mutation in the human gene counterpart. Usually, haploinsufficiency mutations in murine and in human orthologous genes result in similar phenotypes, and a large number of animal models have contributed to the understanding of human diseases. However, interspecies differences exist, and it is accepted that mice are often more tolerant of haploinsufficiency for developmental genes than are humans.[10] One possibility to explain these phenotypic differences may be the existence in the mouse of an additional *Foxe* gene family member, which may be functioning partly redundantly to *Foxe3*. Indeed, appreciable differences have been noted elsewhere between ocular phenotypes of certain human and murine forkhead orthologues, whose mechanisms have not been clarified.[28] Alternatively, the possibility remains that our *FOXE3* nonsense mutation does not result in total loss of function, and we cannot formally exclude the possibility that this mutation could result, in fact, in partial loss of function. The human *FOXE3* gene contains a single coding-exon genomic structure, and some intronless genes have been reported to be insensitive to NMD.[25] Consequently, it remains possible that PTC mutations in *FOXE3* could escape RNA surveillance and could produce truncated mutant proteins. In this scenario, absence of lens in our patients could result in the production of a truncated protein that

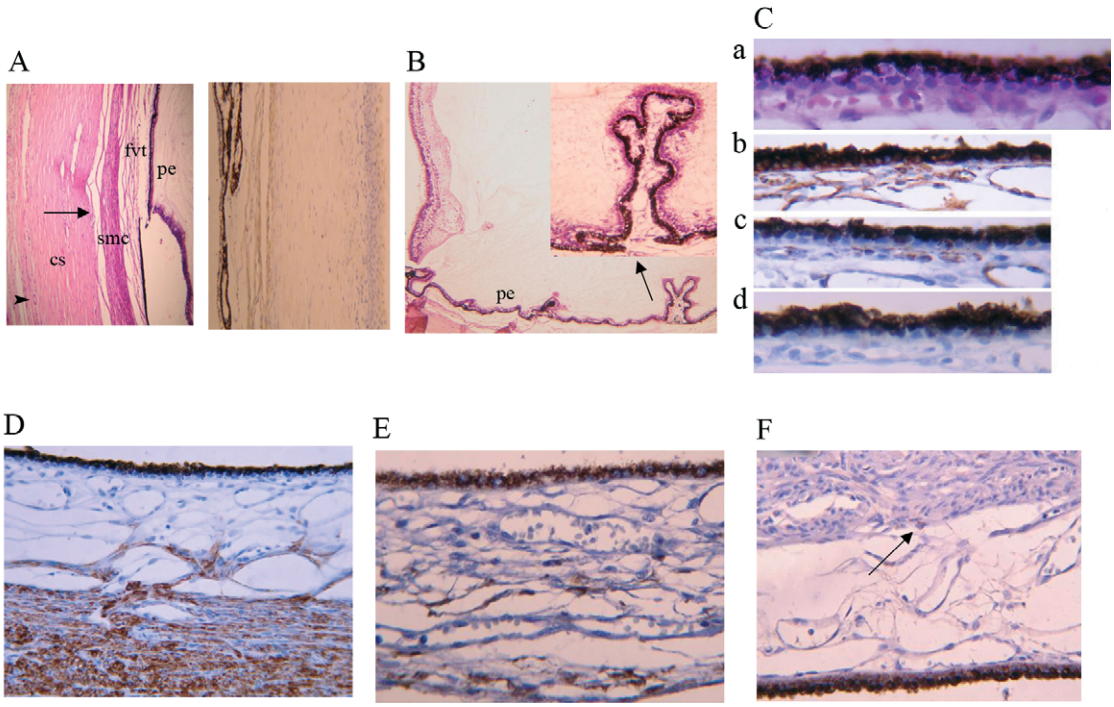

Figure 5. Histologic and immunohistochemical analyses of eyes from proband IV.1. *A,* HES (*left panel*) and immunohistochemical staining with anti–α-smooth muscular actin (*right panel*) of the posterior corneal stroma, showing absence of corneal endothelium and Descemet's membrane, vascularized corneal stroma (*arrowhead*), and the different abnormal tissues that adhered to the posterior face of the cornea (magnification 10 × and 20 ×, respectively). The arrow points to the site where corneal endothelium and Descemet's membrane normally take place in the cornea. cs = Corneal stroma; fvt = fibrovascular tissue; pe = pigmented epithelium; smc = smooth muscular cells. *B,* HES showing the pigmented epithelium that occasionally forms some folds resembling ciliary processes, with higher magnification (20 ×) shown in the box. *C,* HES (*panel a*) and immunohistochemical staining with antivimentin (*panel b*), anti–α-smooth actin (*panel c*), and anti-CK7 (*panel d*) antibodies of the pigmented ciliary epithelium. Only vimentin immunohistochemical staining is positive (magnification 40 ×). *D,* Immunohistochemical staining with antidesmin antibody (magnification 40 ×). Only smooth muscular cells lining the posterior face of the corneal stroma are positive. *E* and *F,* Immunohistochemical positive staining with anti-HMB45 antibody in the choroid and in some cells (*arrow*) within the ectopic fibromuscular tissue adherent to the cornea.

is still capable of binding DNA but is devoid of the 80 C-terminal aa. *FOXE3* is a transcription factor that acts by switching on and off expression of other genes and that must be correctly activated or repressed at the appropriate time during lens development. However, the targets of *FOXE3* are not known, and it is not known whether *FOXE3* could act synergistically with other partner(s), as *Pax6* and *Sox2* do, both of which form a co–DNA-binding complex that regulates initiation of lens development.[12] The C240X mutation lies outside the forkhead domain of *FOXE3,* but it is included in a 26-aa motif (PEPPCCAAP-DAAAAAFPPCAAAASPPLYS) that is highly conserved between mouse and human, which suggests that this motif is, most probably, important for the normal function of *FOXE3* (fig. 1*C*). We could conceive that these residues might be essential, for example, to exert the transactivation effects of *FOXE3* in its interactions with some unidentified partners, even though DNA binding still occurred. Thus, a full understanding of the potential role of *FOXE3* in early stages of lens development would require the identification of genes that may be directly controlled by *FOXE3* at this specific developmental stage and the elucidation of the mechanisms by which *FOXE3* exerts its actions.

However, in the developing eye of the mouse, the onset of *Foxe3* expression begins very early in the presumptive lens ectoderm, and its expression is first detected as early as E.8.75, a period when the *Pax6* ectoderm enhancer becomes active and directs *Pax6* expression in the surface ectoderm (SE).[7,22,29] During lens placode formation at E.9.5, *Foxe3* expression is upregulated in the SE, in conjunction with transcription factors *Six3, Sox2/Sox3,* and *Prox1*.[8,11,12] Then, *Foxe3* expression becomes confined to the lens vesicle as this structure detaches from the SE (E10.5), a process that fails in both *dyl* and *Foxe3*$^{-/-}$ mice. Therefore, the temporal expression pattern of *Foxe3* highly supports a possible role for *Foxe3* in earlier stages of lens development upstream of events such as lens vesicle separation or lens lineage proliferation. A unique demonstration in support of this hypothesis is the identification here—to our knowledge, for the first time—of a nonsense mutation in *FOXE3* that cosegregates with familial CPA.

Although identifications of human disease-causing genes have been often guided by study of their murine orthologues, the family in this article illustrates that the converse approach might also provide a means of identifying a novel phenotype that has not yet been revealed in mice.

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Web Resources

The accession number and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nih.gov/Genbank/ (for *FOXE3* [accession number AF275722])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *FOXE3*)

References

1. Beebe DC, Coats JM (2000) The lens organizes the anterior segment: specification of neural crest cell differentiation in the avian eye. Dev Biol 220:424–431
2. Gould DB, Smith RS, John SW (2004) Anterior segment development relevant to glaucoma. Int J Dev Biol 48:1015–1029
3. Graw J (1999) Cataract mutations and lens development. Prog Retin Eye Res 18:235–267
4. Manschot W (1963) Primary congenital aphakia. Arch Ophthalmol 69:551–577
5. Barishak YR (1992) Embryology of the eye and its adnexae. Dev Ophthalmol 24:1–142
6. Graw J (2003) The genetic and molecular basis of congenital eye defects. Nat Rev Genet 4:876–888
7. Lang RA (2004) Pathways regulating lens induction in the mouse. Int J Dev Biol 48:783–791
8. Ashery-Padan R, Gruss P (2001) Pax6 lights-up the way for eye development. Curr Opin Cell Biol 13:706–714
9. Dimanlig PV, Faber SC, Auerbach W, Makarenkova HP, Lang RA (2001) The upstream ectoderm enhancer in *Pax6* has an important role in lens induction. Development 128:4415–4424
10. Fitzpatrick DR, van Heyningen V (2005) Developmental eye disorders. Curr Opin Genet Dev 15:348–353
11. Goudreau G, Petrou P, Reneker LW, Graw J, Loster J, Gruss P (2002) Mutually regulated expression of *Pax6* and *Six3* and its implications for the Pax6 haploinsufficient lens phenotype. Proc Natl Acad Sci USA 99:8719–8724
12. Kamachi Y, Uchikawa M, Tanouchi A, Sekido R, Kondoh H (2001) Pax6 and SOX2 form a co-DNA-binding partner complex that regulates initiation of lens development. Genes Dev 15:1272–1286
13. Ashery-Padan R, Marquardt T, Zhou X, Gruss P (2000) Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. Genes Dev 14:2701–2711
14. Hyer J, Kuhlman J, Afif E, Mikawa T (2003) Optic cup morphogenesis requires pre-lens ectoderm but not lens differentiation. Dev Biol 259:351–363
15. Hanson IM, Fletcher JM, Jordan T, Brown A, Taylor D, Adams RJ, Punnett HH, Van Heyningen V (1994) Mutations at the *PAX6* locus are found in heterogeneous anterior segment malformations including Peters' anomaly. Nat Genet 6:168–173
16. Semina EV, Ferrell RE, Mintz-Hittner HA, Bitoun P, Alward WL, Reiter RS, Funkhauser C, Daack-Hirsch S, Murray JC (1998) A novel homeobox gene *PITX3* is mutated in families with autosomal-dominant cataracts and ASMD. Nat Genet 19:167–170
17. Semina EV, Murray JC, Reiter R, Hrstka RF, Graw J (2000) Deletion in the promoter region and altered expression of *Pitx3* homeobox gene in aphakia mice. Hum Mol Genet 9: 1575–1585
18. Hsieh YW, Zhang XM, Lin E, Oliver G, Yang XJ (2002) The homeobox gene Six3 is a potential regulator of anterior segment formation in the chick eye. Dev Biol 248:265–280
19. Wawersik S, Purcell P, Rauchman M, Dudley AT, Robertson EJ, Maas R (1999) BMP7 acts in murine lens placode development. Dev Biol 207:176–188
20. Honkanen RA, Nishimura DY, Swiderski RE, Bennett SR, Hong S, Kwon YH, Stone EM, Sheffield VC, Alward WL (2003) A family with Axenfeld-Rieger syndrome and Peters anomaly caused by a point mutation (Phe112Ser) in the *FOXC1* gene. Am J Ophthalmol 135:368–375
21. Blixt A, Mahlapuu M, Aitola M, Pelto-Huikko M, Enerback S, Carlsson P (2000) A forkhead gene, *FoxE3,* is essential for lens epithelial proliferation and closure of the lens vesicle. Genes Dev 14:245–254
22. Brownell I, Dirksen M, Jamrich M (2000) Forkhead *Foxe3* maps to the dysgenetic lens locus and is critical in lens development and differentiation. Genesis 27:81–93
23. Ormestad M, Blixt A, Churchill A, Martinsson T, Enerback S, Carlsson P (2002) *Foxe3* haploinsufficiency in mice: a model for Peters' anomaly. Invest Ophthalmol Vis Sci 43:1350–1357
24. Semina EV, Brownell I, Mintz-Hittner HA, Murray JC, Jamrich M (2001) Mutations in the human forkhead transcription factor *FOXE3* associated with anterior segment ocular dysgenesis and cataracts. Hum Mol Genet 10:231–236
25. Holbrook JA, Neu-Yilik G, Hentze MW, Kulozik AE (2004) Nonsense-mediated decay approaches the clinic. Nat Genet 36:801–808
26. Grainger RM (1992) Embryonic lens induction: shedding light on vertebrate tissue determination. Trends Genet 8:349–355
27. Medina-Martinez O, Brownell I, Amaya-Manzanares F, Hu Q, Behringer RR, Jamrich M (2005) Severe defects in proliferation and differentiation of lens cells in *Foxe3* null mice. Mol Cell Biol 25:8854–8863
28. Lehmann OJ, Tuft S, Brice G, Smith R, Blixt A, Bell R, Johansson B, Jordan T, Hitchings RA, Khaw PT, John SW, Carlsson P, Bhattacharya SS (2003) Novel anterior segment phenotypes resulting from forkhead gene alterations: evidence for cross-species conservation of function. Invest Ophthalmol Vis Sci 44:2627–2633
29. Williams SC, Altmann CR, Chow RL, Hemmati-Brivanlou A, Lang RA (1998) A highly conserved lens transcriptional control element from the Pax-6 gene. Mech Dev 73:225–229