Heterozygous and Homozygous Mutations in *PITX3* in a Large Lebanese Family with Posterior Polar Cataracts and Neurodevelopmental Abnormalities

Carla Bidinost,[1] *Masayuki Matsumoto,*[2] *Daniel Chung,*[2] *Nabiha Salem,*[3] *Kang Zhang,*[4] *David W. Stockton,*[5,6,7] *Antoine Khoury,*[3] *Andre Megarbane,*[3] *Bassem A. Bejjani,*[1,8] *and Elias I. Traboulsi*[2]

Purpose. The *PITX3* gene, which codes for a homeobox bicoidlike transcription factor is responsible for dominant cataract and anterior segment mesenchymal dysgenesis in humans. In the current study, a family with autosomal dominant posterior polar cataract (PPC) and a *PITX3* mutation that cosegregates with the disease was examined. Also studied were two siblings who were homozygous for the *PITX3* mutation who had microphthalmia and significant neurologic impairment.

Methods. A genome-wide screen, linkage analysis in the *PITX3* chromosomal region 10q25, haplotype analysis, and sequencing of the *PITX3* gene were performed on 28 affected and 14 unaffected member of a three-generation Lebanese family.

Results. Genome-wide linkage analysis showed a lod score of 3.56 at $\theta = 0.00$ on chromosome 10 at area q25. Analysis of the haplotypes and phenotypes confined the disease locus to a region on 10q25 between the markers *D10S1239* and *D10S1268*. A candidate gene, *PITX3*, maps to that region. Sequencing of the *PITX3* gene revealed a heterozygous G deletion mutation in 25 of the 42 family members. In addition, two siblings from a consanguineous marriage were found to be homozygous for the deletion.

Conclusions. This is the first report of homozygous *PITX3* mutations in humans. The phenotype in these individuals highlights the role of *PITX3* in ocular and central nervous system (CNS) development. (*Invest Ophthalmol Vis Sci.* 2006;47: 1274–1280) DOI:10.1167/iovs.05-1095

Congenital cataracts occur in 30 of each 100,000 births in developed countries. They are most commonly inherited as autosomal dominant traits and have a variety of phenotypic presentations.[1,2] Autosomal dominant congenital cataract (ADCC) is heterogeneous and has been assigned to a wide set of genes, including some that are responsible for the early development of the anterior segment of the eye, as well as others involved in the maturation of the lens.[1] Among these genes, mutations in *PITX3* (Mendelian Inheritance in Man [MIM] 602669; National Center for Biotechnology Information [NCBI], Bethesda, MD), a transcription factor containing a homeodomain (HD), have been demonstrated to cause cataracts and anterior segment mesenchymal dysgenesis (ASMD) in several families of distinct ethnic origins.[3,4] Three mutations have been reported in human *PITX3*: a G-to-A substitution in the N-terminal region, 38G→A(S13N)[4]; a deletion of a single nucleotide (650delG)[3]; and a 17-bp insertion (656ins17, described elsewhere as 657-673dup17).[3,4] the last two affecting the C-terminal portion of the protein. Also, two deletions have been described in the mouse, one of 652 bp in the 5′ untranslated region (UTR) and the other of 1423 bp in the proximal promoter and exon 1 of *Pitx3*, in the homozygous aphakia (*ak*) mouse in which they cause microphthalmia and arrest in lens development.[5,6]

Pitx3 is expressed in the developing lens, skeletal muscle, and dopaminergic neurons of the substantia nigra in the brain.[7] Neurons in the substantia nigra are responsible for fine movement control. It has recently been demonstrated that the *ak* mouse also has an abnormal profile in the output of some spatial movements.[8] To date, there have been no reports of humans homozygous or compound heterozygous for *PITX3* mutations.

Here we report a three-generation Lebanese family with posterior polar cataract (PPC) in 28 affected individuals and a mutation in *PITX3* (650delG) that cosegregates with the disease. In addition, two brothers from a consanguineous mating were found to be homozygous for the deletion and showed a more severe ocular and neurologic phenotype, with severe microphthalmia and neurologic deficits. This is the first report of human homozygosity for a mutation in *PITX3* and the first evidence of the involvement of this gene in human neurologic development.

Methods

Subjects

Forty-two members of a PPC family have been enrolled in a genetic research program through the Universit é St. Joseph Hôtel-Dieu de France (Beirut, Lebanon). The ophthalmic information for each individual was scored as affected or unaffected by two of the authors (FIT, AK), both ophthalmologists. The information on each patient was obtained from medical records when available, or from personal examination. Some of the examinations were limited and were performed during a field trip to the remote village where many members of this family resided. By history and by review of the medical records, all study subjects had had clinically suspected cataract since birth or

From the [1]Health Research and Education Center, Washington State University Spokane, Spokane, Washington; the [2]Center for Genetic Eye Diseases, Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, Ohio; the [3]Medical Genetics Unit, Faculty of Medicine, Saint Joseph University, Beirut, Lebanon; the [4]Department of Ophthalmology and Visual Sciences, University of Utah Health Sciences Center, Salt Lake City, Utah; the Departments of [5]Molecular and Human Genetics, [6]Medicine, and [7]Ophthalmology, Baylor College of Medicine, Houston, Texas; and the [8]Sacred Heart Medical Center, Spokane, Washington.

Supported in part by a grant from the Blind Children's Center, Los Angeles, California.

Submitted for publication August 18, 2005; revised November 15, 2005; accepted February 9, 2006.

Disclosure: **C. Bidinost**, None; **M. Matsumoto**, None; **D. Chung**, None; **N. Salem**, None; **K. Zhang**, None; **D.W. Stockton**, None; **A. Khoury**, None; **A. Megarbane**, None; **B.A. Bejjani**, None; **E.I. Traboulsi**, None

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

Corresponding author: Elias Traboulsi, Cole Eye Institute, Cleveland Clinic Foundation, i32, 9500 Euclid Avenue, Cleveland, OH 44195; trabouc@ccf.org.

shortly thereafter. Twenty-eight affected and 14 unaffected family members were examined and enrolled in the study. Other recognized systemic associations or combinations of infantile cataract were excluded whenever possible. Each subject, or the responsible adult on behalf of minors, signed a consent form for participation in these investigations, which was approved by the Review Board for Human Subject Research at the Cleveland Clinic Foundation and the parallel committees at the Universit é St. Joseph Hôtel-Dieu de France. The study research followed the tenets of the Declaration of Helsinki.

Markers and Genotyping

Genotyping was performed (PRISM Linkage Mapping Set ver. 2.5, MD10; Applied Biosystems, Inc. [ABI], Foster City, CA) for the whole genome according to the manufacturer's recommendations. Selected markers were chosen from chromosome 10, area q25, according to the Ensembl Genome Browser (http://www.ensembl.org[9]) and their chromosomal positions were confirmed on the sequence of the SuperContig NT_030059 (accession number ENSG00000107859). Marker information and primers were obtained from UniSTS (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists/ provided in the public domain the National Center for Biotechnology Information, Bethesda, MD). We designed a new polymorphic marker, *Ch10cat2*, positioned between markers *D10S1266* and *D10S1265*. The primers for *Ch10cat2* are: forward 5′-CACAGAGACACAGGCAAAGGC-3′; reverse 5′-GCTGTGTGTACGCATCTCTGTTTTG-3′. The observed PCR product size range was 113 to 129 bp. The amplification reactions were performed in a thermal cycler (GeneAmp PCR system 9700; Perkin Elmer, Wellesley, MA). The amplified products were visualized on a sequencer (3100 Sequencer; ABI) according to the manufacturer's recommendations. The data were analyzed on computer (Genotyper 3.7; ABI). The Mendelian inheritance was confirmed with PedCheck ver. 1.1 software (http://watson.hgen.pitt.edu/register/docs/pedcheck.html/ developed by Jeff O'Connell, University of Pittsburgh, Pittsburgh, PA).

Linkage Analysis

Linkage analysis was performed on the genome-wide genotype data and identified a positive lod score of 3.56 (θ = 0.00) to the marker *D10S2470* on chromosome 10q25. This region was investigated with a more detailed analysis. The genotypes from 22 published and custom-designed markers in the critical region were analyzed with conventional parametric two-point linkage analysis using the FASTLINK program suite (http://softlib.cs.rice.edu/ provided in the public domain by Rice University, Houston, TX). A dominant model of inheritance with 90% penetrance in both heterozygous and homozygous individuals was used with a disease allele frequency of 0.001, followed by multipoint analysis using Allegro.[10] To accomplish this with Allegro's constraints on family size, the large pedigree was broken down into seven subpedigrees. Tabular and graphic two-point and multipoint linkage data were inspected to establish the most likely region with the gene.

Mutation Analysis

PCR primers were designed to amplify and sequence the exons and intron–exon junctions of all four coding exons of *PITX3*, according to GenBank (http://www.ncbi.nlm.nih.gov/GenBank/ provided in the public domain by NCBI). Their sequences are in Table 1. Also, PCR primers were designed to amplify and to sequence a 1-kb region, including the 5′-UTR upstream of the exon 1 translation start site and the 3′-UTR of exon 4. Their sequences are in Table 1. All amplification reactions were performed with standard PCR conditions in the thermal cycler (GeneAmp PCR system 9700; Perkin Elmer). The amplified products were purified (QIAquick PCR Purification Kit; Qiagen, Valencia, CA) and sequenced (BigDye Terminator Cycle Sequencing Ready Reaction; DNA sequencing kit; ABI) in the forward and reverse directions, according to the manufacturer's recommendations. Sequencing results were visualized (3100 Sequencer; ABI), according to the manufacturer's recommendations. The data were analyzed on computer (Sequencher ver. 3.1.1 software; Gene Codes, Ann Arbor, MI).

TABLE 1. Primers for *PITX3*

Primer	Sequence
Exon 1-F	5′-CCCTGGTCTGCCATAAAGTG-3′
Exon 1-R	5′-TTTAGGGATTCCAAGGTCCA-3′
Exon 2-F	5′-GGCTGGGGTTGAGAAAGGCG-3′
Exon 2-R	5′-CCACTCGCTGGCTCCCACC-3′
Exon 3-F	5′-GCAGCCCCGGTGGGAGC-3′
Exon 3-R	5′-GGGAGGGGGCAGGTGGG-3′
Exon 4-F	5′-CCGTCTCTAGCCACCTCATC-3′
Exon 4-R	5′-CCAGTCAAAATGACCCCAGT-3′
Upstream distal-F	5′-AAGTCAGAGAGGGCCGAAGT-3′
Upstream distal-R	5′-CCAAGTGGGCGAGAGTAGAG-3′
Upstream proximal-F	5′-ATCCACTTTCCTCGGGGTAG-3′
Upstream proximal-R	5′-ACAGGCAGACTCCCAGTAGC-3′
3′UTR-F	5′-CAACCTTAGTCCGTGCCAGT-3′
3′UTR-R	5′-GAAGAGGACTCAAGCGCAAC-3′

Amplified *PITX3* exon 4 products were digested with the *Stu*I (New England BioLabs, Beverly, MA) restriction enzyme to test for the 650delG variant allele. PCR products from the wild-type samples do not have a *Stu*I restriction site and remain undigested (793 bp). Deletion of the guanine residue creates a *Stu*I restriction site, resulting in fragments of 456 and 337 bp. To ensure full digestion, samples were incubated overnight at 37°C. Undigested and digested products were analyzed on 3% agarose gels.

RESULTS

Linkage Analysis

A three-generation Lebanese family was screened for dominant PPC. The genome-wide genotyping and linkage analysis performed in 21 affected family members indicated a significant positive lod score of 3.56 (θ = 0.00) with marker *D10S2470* on chromosome 10, region q25 (data not shown). Follow-up linkage analysis with 28 affected and 14 unaffected family members in the 10q25 region increased the lod score to 6.56 (θ = 0.00) with marker *D10S1268* (Table 2).

Haplotype Analysis

We performed haplotype analysis in the family with five informative polymorphic markers from the positive lod score region on 10q25. We found appropriate co-segregation of an "affected haplotype" (Fig. 1, shown in green) with the PPC phenotype in all family members except one, (2567), who had the affected haplotype but was phenotypically unaffected. We also found two recombinant individuals (2575 and 2581) in the interval of the five markers shown. The analysis of the haplotypes and phenotypes confines the locus to the region bounded by markers *D10S1239* and *D10S1268* (Fig. 1), which are the two markers with the highest lod scores in the linkage analysis.

PITX3 Mutation Analysis

Because *PITX3* maps between *D10S1239* and *D10S1268* and is responsible for ADCC and ASMD, the coding sequences of the gene were analyzed. A heterozygous G-deletion mutation at position 650 of exon 4 (Fig. 2) was found in 25 of the 42 members of the family. The mutation was confirmed by restriction enzyme digestion of *PITX3* exon 4 PCR products with *Stu*I. The heterozygous samples with deleted sequences showed a digested profile of three bands, whereas the wild-type were not digested and the homozygous were fully digested with only two bands (Fig. 3). The digestion profiles confirmed the sequence data for all individuals in the family

TABLE 2. Two-Point Lod Score for Markers on 10q25

Marker	Location (cM)	Lod Score for θ						
		.00	.01	.05	.10	.20	.30	.40
D10S1667	100.92	−6.132	−3.944	−1.721	−0.737	0.109	0.342	0.252
D10S1777	101.76	−3.684	−1.902	0.118	0.859	1.243	1.053	0.555
D10S2475	103.43	−3.383	−1.593	0.386	1.084	1.407	1.166	0.621
D10S1786	103.53	−5.146	−3.844	−1.330	−0.340	0.317	0.361	0.136
D10S1761	106.11	0.963	0.965	0.931	0.826	0.599	0.464	0.300
D10S1658	106.25	−3.627	−1.480	1.166	2.192	2.570	2.098	1.141
D10S579	109.33	0.609	0.598	0.549	0.493	0.408	0.319	0.181
D10S2470	112.58	−0.631	1.279	2.442	2.687	2.419	1.742	0.833
D10S536	114.73	−0.115	1.796	2.948	3.164	2.810	2.023	0.984
D10S200	117.42	−3.702	−0.877	1.107	1.769	1.952	1.524	0.743
D10S198	120.09	1.608	1.599	1.545	1.447	1.179	0.839	0.445
D10S603	120.37	1.533	2.780	3.768	3.966	3.550	2.620	1.339
D10S1266	120.65	0.439	1.688	2.683	2.924	2.674	1.987	1.016
Cb10Cat2	120.89	0.945	2.201	3.226	3.479	3.183	2.387	1.251
D10S1265	120.96	−0.037	0.863	1.594	1.825	1.747	1.337	0.728
D10S1710	121.07	1.100	1.075	0.964	0.824	0.549	0.302	0.106
D10S1239	121.81	6.567	6.502	6.169	5.673	4.473	3.022	1.359
D10S1268	122.82	6.568	6.533	6.305	5.902	4.806	3.384	1.662
D10S1760	128.19	−0.797	1.379	2.891	3.234	2.924	2.078	0.961
D10S554	130.9	−4.261	−2.157	−0.061	0.728	1.165	1.010	0.559
D10S187	135.24	−3.803	−0.968	0.978	1.607	1.756	1.313	0.587

(data not shown). This mutation has been described in a Hispanic family with PPC (Addison PKF et al. *IOVS* 2004;45: ARVO E-Abstract 4749). The mutation cosegregates with the disease except for one nonpenetrant individual (number 2567) who carries the heterozygous deletion but has a normal phenotype (Figs. 1, 3). Two family members reported as affected (2553 and 2558) were found not to have the mutation. Although the clinical reports indicated that these two people had the familial disease, they had had cataract extraction before examination by the authors, and so they possibly had some other form of cataract. Also, two siblings from a consanguineous marriage (2594 and 2595) were found to be homozygous for the deletion (Figs. 2, 3).

Clinical Features

Reduced vision and cataracts were reportedly present since birth in all patients in whom this information was available. No signs of anterior segment mesenchymal dysgenesis were observed in any patient who had a slit lamp evaluation, and none had had glaucoma before or after the extraction of cataracts. The cataracts were of the posterior polar type and appeared as 2- to 3-mm disks of dense whitish material in the center of the posterior lens capsule (for example, see Fig. 4). Some patients also had cortical and nuclear lens opacities of variable severity and density. Some had undergone cataract extraction early in life or in middle age, whereas others did not for a variety of social reasons and lack of access to care. Visual acuity was preserved, even in those who did not undergo cataract extraction. One such patient had a visual acuity of 20/60, despite the presence of a central dense posterior capsular opacity.

The severely affected siblings (2594 and 2595) had bilateral microphthalmia with corneal opacification and extremely poor vision. They had always been wheelchair bound and were severely developmentally delayed. They were 23 and 19 years of age, respectively, and were born to consanguineous parents. They had one developmentally normal 18-year-old sister with cataract and heterozygous deletion in *PITX3*. Both boys were born at term by normal vaginal delivery and had no neonatal distress.

Their ocular abnormalities and blindness were noted in the first 3 months of life, but significant physical disability became apparent only by the age of 15 to 18 months when they failed to walk. They also had a significant delay in speech development. By the age of 4 to 6 years they started having abnormal movements of their head and upper limbs and contractures in flexion of the knees. They acquired sphincter control at approximately age 10. Their encephalopathies were described as static, and they showed no signs of neurologic deterioration over the past two decades. On neurologic examination, they showed major disequilibrium when forced to stand upright. The knees were flexed with severe contractures of the hamstring muscles. They could not understand orders of medium complexity. There were choreiform movements of the head and upper limbs and to a lesser extent, of the trunk. The upper body and limb tone was normal, but increased with contractures in the lower limbs. Reflexes were present but weak. There was no clear-cut response of plantar reflex (withdrawing of the foot, lack of cooperation). No sensory abnormalities were detectable, except blindness. Cerebellar functions were not assessable because of lack of cooperation. Vital signs were normal. Their clinical course had not shown any significant progressive deterioration over the past two decades, according to their parents. Some stereotypic behavior, such as eye-rubbing in the older brother and hitting of the face in the younger, was observed. The older brother had bilateral valgus deformities of the ankle joints. The younger brother was slightly less severely affected. Magnetic resonance imaging (MRI) of the brain in both brothers was normal except for the presence of optic nerve thinning and small globes.

DISCUSSION

Inherited PPCs are some of the least common types of congenital cataracts. Three distinct *PITX3* mutations have been reported to date that cause ADCC in families of different ethnic origins.[3,4,10] Most of these families were described to have PPC, defining the CPP4 locus.[3] Anterior segment dysgenesis has also been associated with these mutations[4,11] and has been attributed to interference with the normal function of *PITX3* in early anterior eye development.[12] The mutation we found in the reported family cosegregates with the disease except for

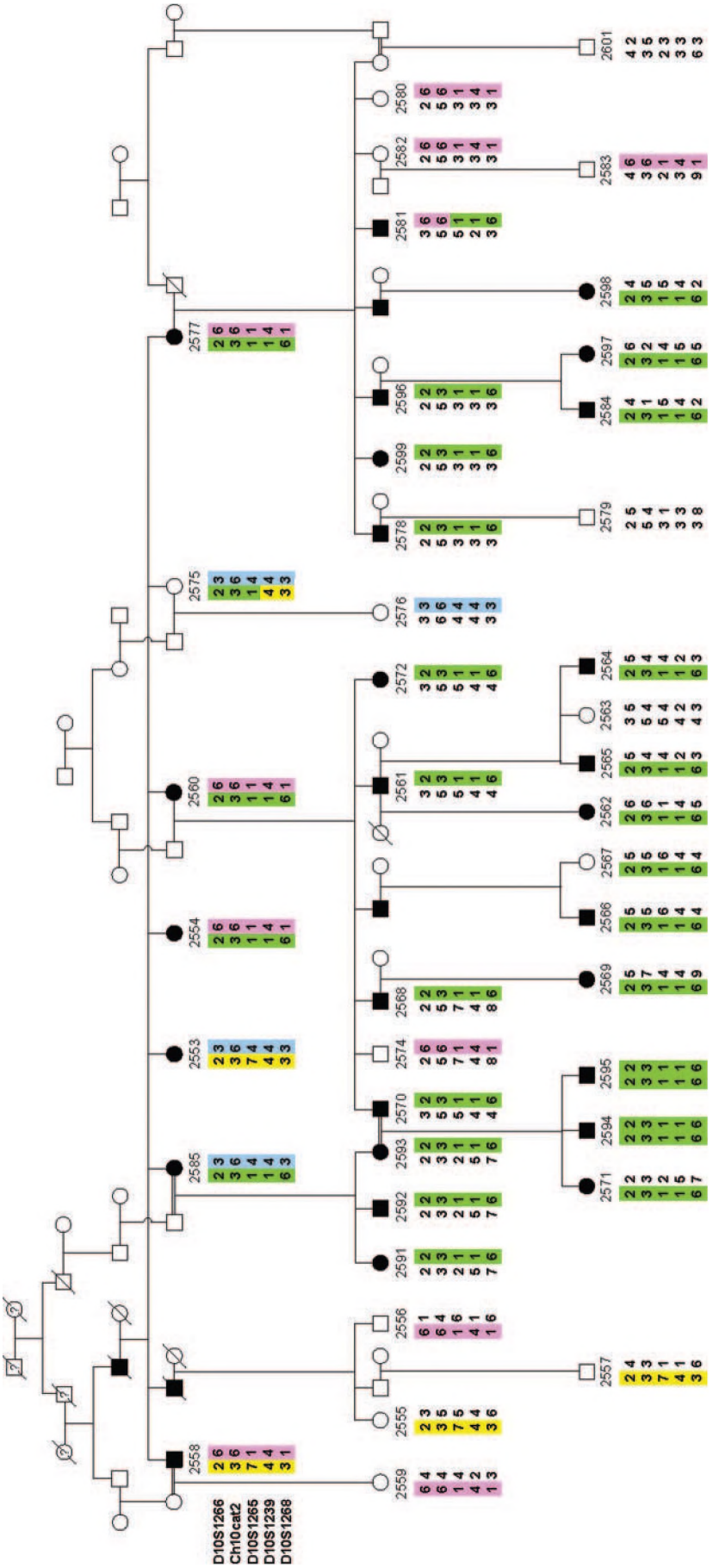

FIGURE 1. Pedigree of the family and haplotypes for markers on chromosome 10 around *PITX3*. *Black symbols*: affected individuals. The original haplotypes for the first generation are colored; *green*: segregating haplotype.

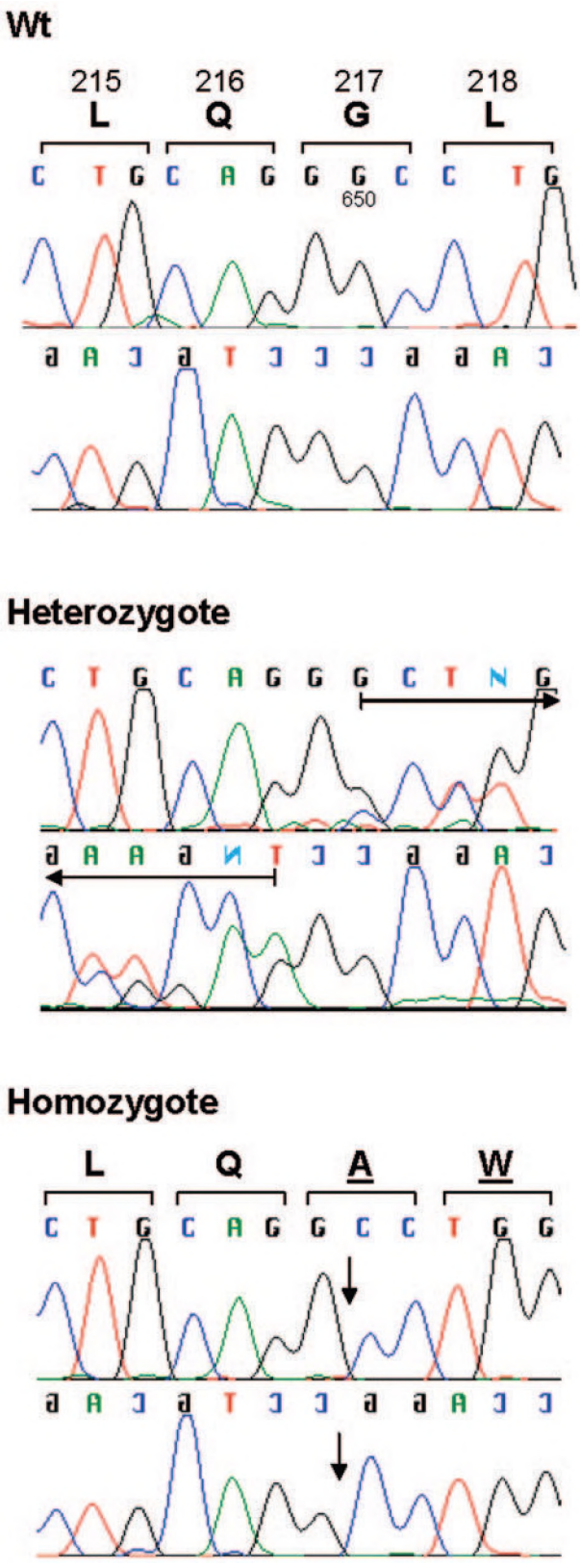

FIGURE 2. Deletion mutation in *PITX3* (650delG). Forward (*top*) and reverse (*bottom*) sequencing chromatograms are shown for each example. The G 650-base and the amino acid sequence and codon number are indicated in the wild-type panel. The amino acid changes are shown *underscored* in the homozygote panel. *Lateral arrows*: frameshift; *vertical arrows*: G deletion in the heterozygote and homozygote panels.

individual 2567, who appeared to be nonpenetrant for the PPC phenotype. Variable expressivity of the cataract and anterior segment dysgenesis phenotype has been observed in families with the *PITX3* insertion mutation (656ins17).[11,13]

PITX3 is a paired-like homeodomain transcription factor that belongs to the RIEG/PITX homeobox gene family.[12] The 650delG and the 656ins17 mutations in *PITX3* are close to one another and affect the C-terminal region of the gene. The 650delG mutation is 86-amino-acid residues upstream of the C terminus of the PITX3 protein. Although this region does not include the homeodomain, it is predicted that the resultant peptide lacks a 14-amino-acid motif identified within the C-terminal region of at least 40 paired class homeobox transcription factors and named the OAR domain.[14] Although the function of the domain is not well understood, mutations in the OAR domain of *Pitx2*, a gene associated with Rieger syndrome in humans, suggest a transactivation regulatory role by inhibiting DNA binding and promoting interaction with other transcription factors.[15–17] Also, it was demonstrated for the Prx1a homeoprotein, that the OAR domain inhibits transactivation,[18] and, in the Cart1 homeoprotein, the domain serves as an intramolecular switch of its transactivation activity.[19] However, RNA microinjection experiments with other bicoidclass homeobox genes like *Xrx1* (the *Xenopus* homologue to *RAX*) have demonstrated that the absence of the OAR domain can cause a passive repression of the downstream target genes.[20]

Because the human conditions described to date for *PITX3* mutations are autosomal dominant, the phenotypes have been attributed to haploinsufficiency.[4] Nevertheless, as deletion of the OAR domain leads to transcriptional activation in *Pitx2*,[15] we cannot rule out the possibility that the mutation described herein induces transactivation instead of downregulation of target genes. Also, we cannot exclude the possibility that the mutation could result in a passive repression mechanism caused by the OAR-deleted PITX3 protein. Further experiments are needed to determine the specific role of the OAR domain in the function of PITX3.

The phenotype of the two siblings who were homozygous for the C-terminal deletion of PITX3 was much more severe than that of the heterozygous family members. In addition to having PPCs as in heterozygotes, the homozygous individuals had microphthalmia, blindness, and a neurologic disorder characterized by mental retardation, choreiform movements, and increased muscle tone and decreased deep tendon reflexes of the lower extremities. Imaging studies of the two homozygous siblings did not reveal any gross abnormalities of brain development; hence, it is likely that their neurologic syndrome is the result of cellular or interneuronal connection abnormalities. This phenotype, however, implies an essential role for *PITX3* in normal ocular and CNS development.

Another paired box gene involved in eye and brain development, *Pax6*, has been found in homozygous state to produce phenotypic ocular and extraocular defects. Small eye (*Sey*) mouse is a homozygous mutant of *Pax6* that lacks eyes, has craniofacial abnormalities, and dies soon after birth.[21] Heterozygous mutations in *PAX6* have been described in humans with aniridia and other ocular anomalies such as cataract and Peters anomaly.[1] Compound heterozygous *PAX6* mutations in humans have also been described with severe craniofacial and central CNS defects and no eyes.[22] *PAX6* appears to be a master control gene that synchronizes the events during the formation of the anterior eye and other central nervous structures.[23] The target genes regulated by *PITX3* are not yet established, though it seems to be an important key gene in the development of the eye and other tissues. Further studies are needed to elucidate the molecular role of this transcription factor in early development.

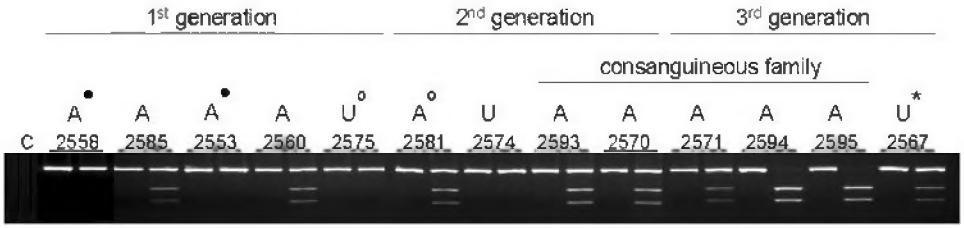

FIGURE 3. *Stu*I restriction enzyme digestion of amplified *PITX3* exon 4 DNA for representative individuals. For each sample, undigested, and digested DNA was loaded in the *left* and *right lanes*, respectively. A, affected; U, unaffected. The parents and siblings of the consanguineous family having two homozygote individuals are indicated. *Filled circles*: phenocopies; *open circles*: recombinants; *asterisk*: nonpenetrant individual.

It was recently demonstrated in mice that *Pitx3* is expressed in the mesencephalic dopaminergic (DA) neurons that are located in the ventral midbrain and that form the substantia nigra compacta (SNc) and the ventral tegmental area.[8] The role of DA neurons is related to movement and behavior, and so they are a target for the understanding of the molecular mechanisms that underlie schizophrenia, addictive behavioral disorders, and Parkinson's disease.[7] Moreover, *Pitx3* defines the neuronal population required for spontaneous locomotor activity, and is involved in the specification of properties of DA neurons during terminal differentiation and maintenance.[24,25] It has been demonstrated that the *ak* mice have no *Pitx3* expression in the SNc and that in the absence of *Pitx3*, the DA neurons do not survive.[26] Surprisingly, these mice have no motor defects, altered posture, waking pattern, or tremor, although they show aberrant behavior in a climbing test and lower overall motor activity levels.[8] Both homozygous brothers in this report showed severe disorders of motor function, including inability to walk, chorea, and diplegia with flexion contractures of the lower limbs, in accordance with the role of *PITX3* in movement skills and motor output capability, suggesting a role for *PITX3*, not only in anterior eye development and SNc differentiation and maintenance, but also in the CNS. In Parkinson's disease, there is a loss-of-function of adult SNc DA neurons, and the reason for this selective effect is unknown.[7] The two patients with homozygous deletion in *PITX3* did not show classic symptoms of early Parkinson's disease, but their clinical picture may be compatible with advanced Parkinson's disease.

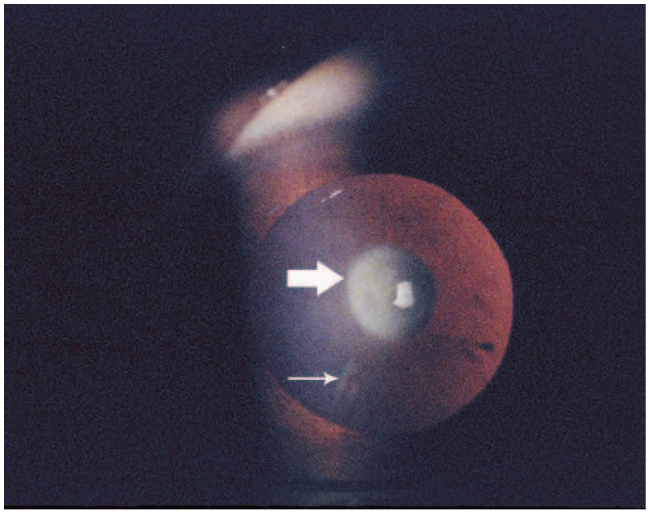

FIGURE 4. Retroilluminated view of pupillary space in an affected individual shows central posterior capsular round opacity (*large arrow*) and a few radial cortical opacities (*small arrow*).

The human ocular abnormalities found in the siblings with homozygous *PITX3* mutations resembled those of the *ak* mouse. It is conceivable that the severe neurologic phenotype in these siblings is due to homozygous or heterozygous mutations at a locus unlinked to *PITX3*. This however is unlikely, however, because the four consanguineous marriages in the family produced eight offspring. Of these, only two individuals had neurologic abnormalities, and these were the two individuals who were homozygous for the *PITX3* mutation. It is still possible that a locus that is closely linked to *PITX3* is responsible for the neurologic phenotype in a recessive model, since these two children were the only ones who had a homozygous genotype at that putative locus. Although this is impossible to investigate at this point, it is an unlikely possibility, given the suspected role of PITX3 in brain development. Furthermore, the ocular and CNS phenotypes can be readily explained by the *PITX3* expression pattern, which supports the contention that the phenotype is caused by the *PITX3* mutation.

The neurologic phenotype of the two homozygous patients reveals a potential new role of *PITX3* in the development of the nervous system that has not been described before in humans.

References

1. Graw J. Congenital hereditary cataracts. *Int J Dev Biol.* 2004;48:1031–1044.
2. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. *Surv Ophthalmol.* 2004;49:300–315.
3. Berry V, Yang Z, Addison PK, et al. Recurrent 17 bp duplication in PITX3 is primarily associated with posterior polar cataract (CPP4). *J Med Genet.* 2004;41:e109.
4. Semina EV, Ferrell RE, Mintz-Hittner HA, et al. A novel homeobox gene PITX3 is mutated in families with autosomal-dominant cataracts and ASMD. *Nat Genet.* 1998;19:167–170.
5. Rieger DK, Reichenberger E, McLean W, Sidow A, Olsen BR. A double-deletion mutation in the Pitx3 gene causes arrested lens development in aphakia mice. *Genomics.* 2001;72:61–72.
6. Semina EV, Murray JC, Reiter R, Hrstka RF, Graw J. Deletion in the promoter region and altered expression of Pitx3 homeobox gene in aphakia mice. *Hum Mol Genet.* 2000;9:1575–1585.
7. Smidt MP, Smits SM, Burbach JP. Homeobox gene Pitx3 and its role in the development of dopamine neurons of the substantia nigra. *Cell Tissue Res.* 2004;318:35–43.
8. Smidt MP, Smits SM, Bouwmeester H, et al. Early developmental failure of substantia nigra dopamine neurons in mice lacking the homeodomain gene Pitx3. *Development.* 2004;131:1145–1155.
9. Hubbard T, Barker D, Birney E, et al. The Ensembl genome database project. *Nucleic Acids Res.* 2002;30:38–41
10. Gudbjartsson DF, Jonasson K, Frigge ML, Kong A. Allegro, a new computer program for multipoint linkage analysis. *Nat Genet.* 2000;25:12–13.

11. Addison PK, Berry V, Ionides AC, Francis PJ, Bhattacharya SS, Moore AT. Posterior polar cataract is the predominant consequence of a recurrent mutation in the PITX3 gene. *Br J Ophthalmol.* 2005;89:138–141.
12. Semina EV, Reiter RS, Murray JC. Isolation of a new homeobox gene belonging to the Pitx/Rieg family: expression during lens development and mapping to the aphakia region on mouse chromosome 19. *Hum Mol Genet.* 1997;6:2109–2116.
13. Hittner HM, Kretzer FL, Antoszyk JH, Ferrell RE, Mehta RS. Variable expressivity of autosomal dominant anterior segment mesenchymal dysgenesis in six generations. *Am J Ophthalmol.* 1982;93:57–70.
14. Galliot B, de Vargas C, Miller D. Evolution of homeobox genes: Q50 Paired-like genes founded the Paired class. *Dev Genes Evol.* 1999;209:186–197.
15. Amendt BA, Sutherland LB, Russo AF. Multifunctional role of the Pitx2 homeodomain protein C-terminal tail. *Mol Cell Biol.* 1999;19:7001–7010.
16. Cox CJ, Espinoza HM, McWilliams B, et al. Differential regulation of gene expression by PITX2 isoforms. *J Biol Chem.* 2002;277:25001–25010.
17. Vadlamudi U, Espinoza HM, Ganga M, et al. PITX2, beta-catenin and LEF-1 interact to synergistically regulate the LEF-1 promoter. *J Cell Sci.* 2005;118:1129–1137.
18. Norris RA, Kern MJ. The identification of Prx1 transcription regulatory domains provides a mechanism for unequal compensation by the Prx1 and Prx2 loci. *J Biol Chem.* 2001;276:26829–26837.
19. Brouwer A, ten Berge D, Wiegerinck R, Meijlink F. The OAR/aristaless domain of the homeodomain protein Cart1 has an attenuating role in vivo. *Mech Dev.* 2003;120:241–252.
20. Andreazzoli M, Gestri G, Angeloni D, Menna E, Barsacchi G. Role of Xrx1 in Xenopus eye and anterior brain development. *Development.* 1999;126:2451–2460.
21. Hill RE, Favor J, Hogan BL, et al. Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature.* 1991;354:522–525.
22. Glaser T, Jepeal L, Edwards JG, Young SR, Favor J, Maas RL. PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. *Nat Genet.* 1994;7:463–471.
23. Cvekl A, Tamm ER. Anterior eye development and ocular mesenchyme: new insights from mouse models and human diseases. *Bioessays.* 2004;26:374–386.
24. Chung S, Hedlund E, Hwang M, et al. The homeodomain transcription factor Pitx3 facilitates differentiation of mouse embryonic stem cells into AHD2-expressing dopaminergic neurons. *Mol Cell Neurosci.* 2005;28:241–252.
25. Nunes I, Tovmasian LT, Silva RM, Burke RE, Goff SP. Pitx3 is required for development of substantia nigra dopaminergic neurons. *Proc Natl Acad Sci USA.* 2003;100:4245–4250.
26. van den Munckhof P, Luk KC, Ste-Marie L, et al. Pitx3 is required for motor activity and for survival of a subset of midbrain dopaminergic neurons. *Development.* 2003;130:2535–2542.