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A Spectrum of *FOXC1* Mutations Suggests Gene Dosage as a Mechanism for Developmental Defects of the Anterior Chamber of the Eye

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Mutations in the forkhead transcription–factor gene (*FOXC1*), have been shown to cause defects of the anterior chamber of the eye that are associated with developmental forms of glaucoma. Discovery of these mutations was greatly facilitated by the cloning and characterization of the 6p25 breakpoint in a patient with both congenital glaucoma and a balanced-translocation event involving chromosomes 6 and 13. Here we describe the identification of novel mutations in the *FOXC1* gene in patients with anterior-chamber defects of the eye. We have detected nine new mutations (eight of which are novel) in the *FOXC1* gene in patients with anterior-chamber eye defects. Of these mutations, five frameshift mutations predict loss of the forkhead domain, as a result of premature termination of translation. Of particular interest is the fact that two families have a duplication of 6p25, involving the *FOXC1* gene. These data suggest that both *FOXC1* haploinsufficiency and increased gene dosage can cause anterior-chamber defects of the eye.

Introduction

Glaucoma is the second leading cause of vision loss worldwide, with an estimated 6.7 million people blinded by this disorder (Quigley 1996). Much progress has been made during the past few years in the identification of the genes and loci responsible for this heterogeneous group of disorders. This recent progress includes the discovery that mutations in the *MYOC* (MIM 601652) gene are responsible for many cases of the relatively rare juvenile-onset form of primary open-angle glaucoma, as well as for a small subset of the more common, adult-onset form of the disease (Stone et al. 1997; Fingert et al. 1999; Alward 2000). In addition, the cytochrome P450, subfamily I, polypeptide 1 gene (*CYP1B1* [MIM 601771]) has been shown to be one of the genes involved in primary congenital glaucoma (Stoilov et al. 1997). Progress has also been made in the identification of genes responsible for developmental forms of glaucoma. To date, the genes responsible for developmental forms of glaucoma have proved to be transcription factors.

One developmental anomaly associated with congenital glaucoma is the Axenfeld-Rieger anomaly (ARA). ARA consists of a spectrum of developmental defects of the anterior chamber of the eye, with wide variability in expression. When these defects are associated with extraocular findings of maxillary hypoplasia, hypodontia, microdontia, and umbilical abnormalities, the disorder is known as "Axenfeld-Rieger syndrome" (ARS). The *PITX2* (MIM 601542) gene at 4q25 has been identified as the gene involved in the development of Rieger syndrome, type 1 (MIM 180500) (Semina et al. 1996). A number of phenotypically similar disorders associated with glaucoma and involving development of the anterior chamber of the eye have also been localized to the distal short arm of chromosome 6 (Mears et al. 1996; Gould et al. 1997; Graff et al. 1997; Jordan et al. 1997). Mutations in the forkhead/winged-helix transcription-factor gene *FOXC1* (previously referred to as "*FKHL7*" [MIM 601090]) have been identified in numerous families with ARA (Mears et al. 1998; Nishimura et al. 1998). However, mutations in *FOXC1* were not found in all families with goniodysgenesis or with ARA linked to 6p; this has prompted speculation that a second locus involved in the development of the anterior chamber of the eye may exist within 6p25 (Mears et al. 1998). Another locus for ARS, RIEG2 (MIM 601499), has been mapped genetically to 13q14 in a single large family (Phillips et al. 1996). In addition, there is evidence, from the *FOXC2*-knockout mouse (Smith et al. 2000), that defects in the *FOXC2* gene

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(MIM 602402) at 16q24 may also be responsible for a subset of ARA. However, screening of a cohort of patients with ARA did not demonstrate any mutations in this gene (Smith et al. 2000).

In this study we have screened for *FOXC1* mutations in a cohort of patients who exhibit abnormalities of the anterior chamber of the eye. Nine mutations were detected, eight of which had been unreported previously. Among these mutations are two partial 6p25 duplications, both of which include the *FOXC1* gene, found in two families. The spectrum of mutations detected in *FOXC1* suggests that precise regulation of expression of this gene is needed for normal eye development.

Patients/Families and Methods

Patients and Families

Patients were identified either through the Department of Ophthalmology at the University of Iowa or by investigators collaborating at other institutions. A signed informed-consent form was obtained from each patient prior to the collection of a sample of whole blood (5–10 ml) by protocols approved by the Institutional Review Board at the University of Iowa. Genomic DNA was isolated through use of methods that have been published elsewhere (Nishimura et al. 1998).

Mutation Screening

Screening of the *FOXC1* gene was performed by a combination of sequencing and SSCP analysis. Direct sequencing of the coding region was carried out both for the 5′ portion of the gene, including the forkhead DNA-binding domain, and for the 3′ region of the gene. Mutation detection in the remainder of the gene proved to be difficult to accomplish by sequence analysis, because of the presence of two polymorphic polyglycine tracts. Thus, this region was screened by SSCP analysis. Mutation screening for the *PITX2* gene was performed by sequence analysis with previously published primers, as well as with some new primers designed to cover the proximal and distal portions of the gene. These new primer pairs are RG3-forward (CCTGTCATCACAAGAGCATG) and reverse (CTGGCCAGGCTCGAGTTACA); and RG4-forward (GCCGCCGACTCCTCCGTATG) and reverse (GGTCCTAGGATCCCGGCGCT). Sequence analysis was carried out by the direct sequencing of PCR products, by previously published protocols, on ABI 373 and 377 sequencers. Variants detected by the SSCP analysis were also characterized by direct sequencing.

Marker Development and Genotyping

Sequence data for selected plasmid artificial chromosomes (PACs) in the 6p25 region were downloaded from The Sanger Centre Web site and, for analysis, were imported into SEQUENCHER, version 3.1.1 (GeneCodes). Each sequence was analyzed manually for the presence of short tandem repeats (STRs), with a requirement that there be $\geqslant$28 consecutive base pairs of repetitive DNA. A variety of such repetitive sequences was detected with 2-, 3-, and 4-bp repeat units. Primers were selected manually to PCR-amplify these repeats such

Table 1

Summary of All Published *FOXC1* Mutations.

Diagnosis	Position[a]	Mutation	Reference
Axenfeld anomaly	26–47	22-bp insertion	Present study
ARS	67	Gln23Stop	Mears et al. (1998)
ARA	93–102	10-bp deletion	Mears et al. (1998)
Axenfeld anomaly	99–108	10-bp deletion	Present study
Rieger anomaly	116–122	8-bp deletion	Present study
Axenfeld anomaly	153–162	11-bp deletion	Nishimura et al. (1998)
Rieger anomaly	210	1-bp deletion	Swiderski et al. (1999)
Rieger anomaly	236	Pro79Leu	Present study
IRID1	245	Ser82Thr	Mears et al. (1998)
ARA	261	Ile87Met	Mears et al. (1998)
ARA	262–265	1-bp insertion	Present study
Rieger anomaly	335	Phe112Ser	Nishimura et al. (1998)
Axenfeld anomaly	378	Ile126Met	Nishimura et al. (1998)
Axenfeld anomaly	392	Ser131Leu	Nishimura et al. (1998)
Rieger anomaly	392	Ser131Leu	Present study
Axenfeld anomaly	1512	1-bp deletion	Present study
Iris hypoplasia	NA	6p25 duplication	Present study
Peter anomaly	NA	6p25 duplication	Present study
Peter anomaly	NA	2;6 unbal trans	Nishimura et al. (1998)
Congenital glaucoma	NA	6;13 bal trans	Nishimura et al. (1998)

[a] NA = not applicable.

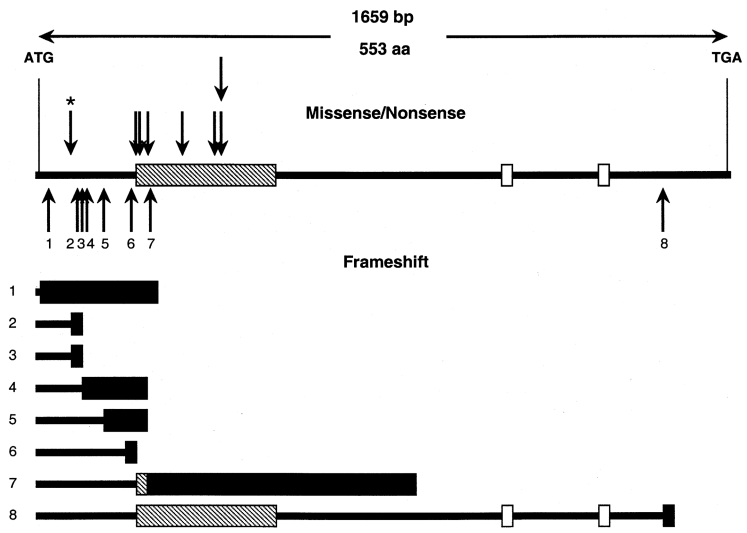

Figure 1 Summary of the mutations detected in this study and of those that have been published elsewhere (Mears et al. 1998; Nishimura et al. 1998; Swiderski et al. 1999; Mirzayans et al. 2000). The hatched boxes indicate the location of the forkhead domain within the *FOXC1* coding sequence. The two white boxes represent the locations of two polymorphic poly-glycine tracts (Mears et al. 1998) that are labeled "Fkh8" and "Fkh10" in figure 4. The distribution of missense, nonsense (indicated by *), and frameshift mutations are illustrated. The predicted protein translations are shown below the gene diagram for the frameshift mutations. The blackened boxes represent those areas of the protein that, because of the frameshift mutations, are translated differently than the normal FOXC1 protein.

that each amplified fragment was <250 bp. The primers were synthesized commercially (Research Genetics) and were tested both for PCR amplification and for polymorphic content. The PCR conditions have been published elsewhere (Nishimura et al. 1998). The heterozygosity of each STRP was determined by the genotyping of 14 unrelated white individuals. A subset of these STRPs was used for the analysis of samples of DNA from the patients.

Physical Mapping

Construction of the physical map through use of YACs has been described elsewhere (Nishimura et al. 1998).

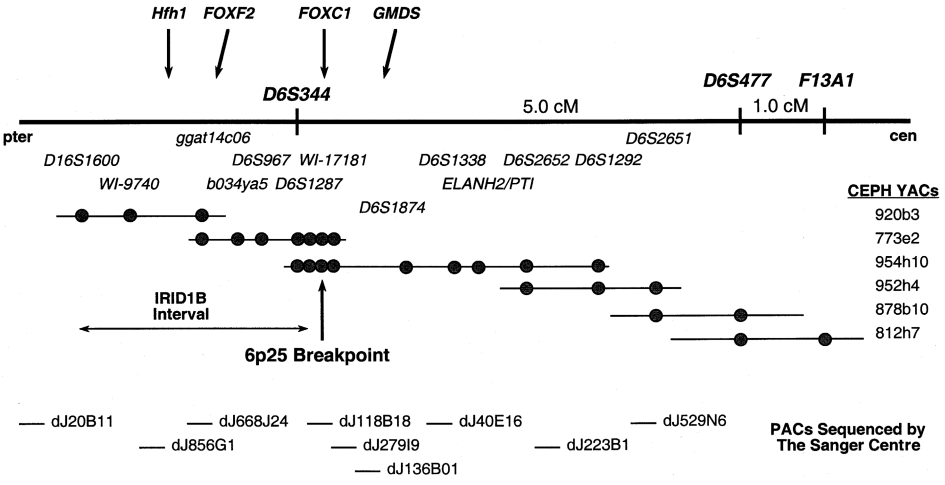

Figure 2 Physical map of the 6p25 region that surrounds the *FOXC1* gene. The approximate location of the proposed IRID1B locus (Mears et al. 1998) is shown. The approximate location of PACs sequenced by The Sanger Centre is shown at the bottom of the figure. Genetic markers derived from these sequences are listed in table 2.

Table 2

Genetic Markers Developed from the 6p25 Region

Marker Name	Repeat Class	Repeat Number	Expected Size (bp)	Temperature (°C)	Status of Polymorphism	Heterozygosity (%)	Sequence	
							Forward	Reverse
20B11-GT	GT	14	143	53	Present	50.00	TAGAGGTTTGAAAAGTATATG	CACTGCAGTGGATCAGTAGCT
20B11-AC	AC	13	141	51	Present	71.43	TTTGAGCACAGCCTCAAATAG	TCTCCTTAAATACATGTATAC
856G1-GT	GT	16	147	55	Present	15.38	AAAACGTAGGTTGGTGACATG	ACTTACTGCAAATGAGCCAGT
856G1-TG	TG	15	121	65	Present	38.46	GACTCCAGCCTTCTAAGGCGA	CCTCCAGCACTGTGCCAGGCA
668J24-GT	GT	17	168	55	Present	85.71	ATCTTAAAGGAGCTCTACTGA	CTGAATGTTTCCAAAGCCCGT
668J24-GATA	GATA	12	144	52	Present	71.43	GGATAGATGGATGGACAGATG	CATCCATGTATCTATCATCTA
668J24-CA1	AC	12	150	57	Present	35.71	CCAGCCTGAGCAACAAAGCGA	CCTAACCGTCTCTTCTGTGCT
668J24-CA2	CA	21	182	42	Present	85.71	ATTTCAACAAGTAACCAATAC	GGGAGGGCAGCTGGCAGGCTG
668J24 -GCC	GCC	9	149				CGCCGCGCGTGCAGCCCGGTC	GAGGACGAGGACGAGGCGCAG
118B18-GT	GT	24	134	55	Present	85.71	AAGCAAGATCCATGTTGCTGA	CTCAACATCTACAGTCCTAC
118B18-AC	AC	15	116	55	Present	64.29	AGAATATGGGTCAGAGCTGCA	TAAAGAGCTATAGCACTTGAT
279I9-GT	GT	16	132	55	Present	85.71	AGCCTACAGACTCGGGTCTCA	GGATGGGTGCTCTATCAACTG
279I9-TG	TG	23	225	53	Present	85.71	ATATGAAAATCATACCACAGT	GCACTCATTCCAAAGTTCTAG
136B1-GAAA	GAAA	12	187	55	Present	78.57	CCACCATTCCCAGCCAGATGC	AGTAGGCTTTCCTCTGCTCAG
136B1-CA	CA	14	163	55	Present	64.29	AAAGGTGGAAACAACTCACGT	CCATGTTGTAGCATGTGTCAG
136B1-DG	DG	Many	182	55	Present	57.14	CTGTGATTGCACCACTCCACT	GTGTGCCACCATGCCCAGCTA
40E16-TTA	TTA	13	208	53	Present	64.29	TATTTTGCCCAGATCCACCAG	GGGAGGCGGACGTTGCAGTGA
40E16-AT1	AT	10	182				TCTGCGGCATTAATTTATGCT	ATGCACTTTGCAAAGGAGTAC
40E16-AT2	AT	13	196				CTGGTTGCATTCTGAAGCTCA	CCACTGCACTCCAGCCTGACT
40E16-CATA	CATA	7	212				GAGCTTGCAGTGAGCTGAGAT	GCAAGGTGAAGGCAGTGTGAC
40E16-CA	CA	18	172	60	Present	50.00	AGGGCCATCAGCTGACACATC	GGGCTGGATTTGTCACATGTC
223B1-CA1	CA	18	129	55	Present	84.62	TCTCTGCATCATGCACCATGC	GGAACAGGTTAACAGGTTTAG
223B1-CA2	AC	18	154	55	Present	78.57	GAAGTAGGCTAATAATTAGAT	ACCCTATGTGCTAAACGGTGA
223B1-AC	AC	20	139	51	Present	92.86	CTACACAACTATAACTTTGCA	ACCCAGAAAATTCACCTACAT
223B1-CATA	CATA	8	137	51	Present	57.14	ACTGCACTCCAGCCTGGCGAC	AAAGTGTCATTATCGAAGATG
223B1-DG	CT,CA	20,15	141	53	Present	71.43	TCAAGATGAGCCTCAATCTCA	AGCCCTGTGTTTCCTAACTGC
1013A10-TG	TG	22	152		Present	71.43	ATTTATAGGTTGGTGTTCCTA	GGCCTTCATAAGAGGAGGTAG
232P20-GA	GA	19	142		Present	78.57	AACATTTTCTGTCCTTGTGAC	TCACTGCATTCCCTCTTCTAG
529N6-GT	GT	17	160		Present	85.71	CTTCTAACAATCGCAGTCTGC	CCCACTGCCAGACTGGGTCAT
72E17-GT	GT	23	176		Present	38.46	GGTGGTGTGGGTTATGAGATC	ACCAGGAGTGGGGCTCCTGAT

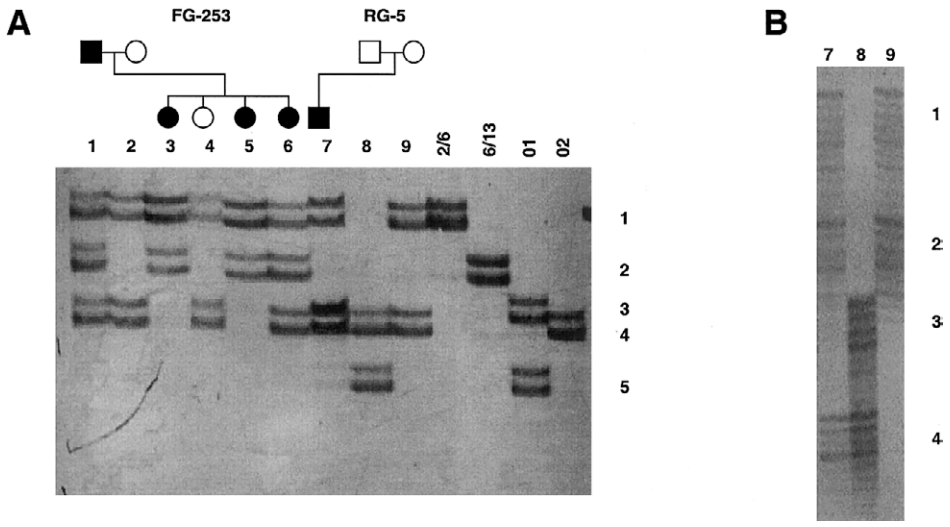

Figure 3 Genotyping results showing evidence for partial 6p25 duplication in families RG-5 and FG-253. *A*, Results for the marker *668J24-GATA*. Sample identification is given at the top of the gel image. Families FG-253 (sample numbers 1–6) and RG-5 (sample numbers 7–9) have a partial duplication of 6p25. The "2/6" label denotes a sample with an unbalanced translocation involving chromosomes 2 and 6 (partial deletion of 6p25). The "6/13" label denotes a sample with a balanced translocation of chromosomes 6 and 13. The 01 and 02 samples are CEPH control samples (1331-01 and 1331-02). Samples 1 and 6 have three alleles, whereas samples 3, 5, and 7 demonstrate allele-intensity differences. *B*, Example of three alleles (sample 7) for the marker *118B18-GT* in family RG-5.

PACs were identified by BLASTN analysis, with sequences that were derived from a variety of sequence-tagged sites (STSs) within the 6p25 region surrounding the *FOXC1* gene and that were compared against the high throughput genomic sequence database. Other physical-mapping information (FISH analysis by The Sanger Centre) was also used to confirm the localization of the PACs to the distal region of 6p. STSs from this region were tested for amplification from the PACs, to confirm the results of the BLASTN analysis.

Duplication Analysis

Genomic DNA samples from families RG-5 and RG-253 were amplified under conditions that have been described elsewhere (Nishimura et al. 1998). Results of genotyping were examined visually for either the presence of three alleles or for intensity differences between alleles, for each genotyping reaction. The difference in allele intensity was confirmed by densitometry for two polymorphisms that are located within the *FOXC1* gene. Charge-coupled–device images of the silver-stained gels were analyzed with the NIH Image, version 1.62 software package, which is available from the NIH Image Web site. Visual inspection and densitometry of PCR products also confirmed linear amplification of the PCR reactions for the *FOXC1* polymorphisms. A range of amplification cycles (25, 30, 35, 40, and 45) was used to demonstrate that the PCR conditions used for amplification of the *FOXC1* polymorphisms was within the linear portion of the amplification profile.

Results

Mutations in Patients with Anterior-Chamber Defects

A total of 70 probands with congenital anterior-chamber defects of the eye were screened for mutations within the coding region of *FOXC1*, through use of a combination of SSCP and sequence-based screening assays. These patients had been screened previously for mutations in the *PITX2* gene and had not been found to harbor any pathological *PITX2* variants. Nine *FOXC1* mutations were detected, eight of which were novel. Five of these mutations cause a translational-reading-frame alteration that results in premature termination of the *FOXC1* transcript. Table 1 presents a summary of all *FOXC1* mutations described to date. The location of each mutation within the *FOXC1* gene is illustrated in figure 1.

Three frameshift mutations were detected upstream of the forkhead domain: a 22-bp insertion (position 26–47) and a 10-bp deletion (position 99–108) were each found in a single proband diagnosed with Axenfeld anomaly, and an 8-bp deletion (position 116–123) was detected in a proband diagnosed with Rieger anomaly. This mutation was also detected in the similarly affected sibling of the proband. All three of these mutations pre-

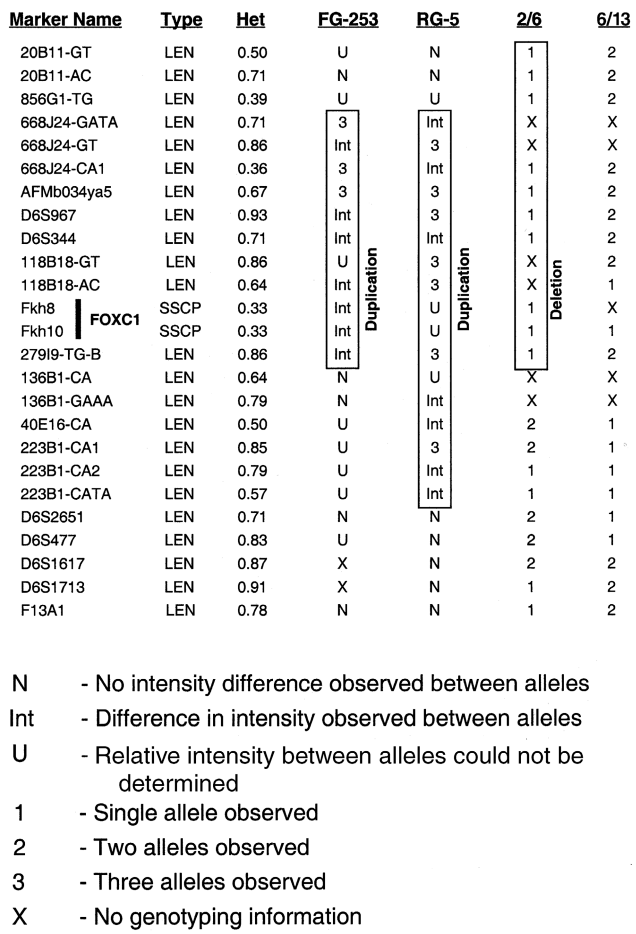

Figure 4 Genetic analysis of the two families with partial duplication (FG-253 and RG-5), as well as of the sample with partial deletion (2/6). The markers are listed in order, according to their placement on the physical map. Markers derived from the same PAC are listed in their correct order as derived from the sequence data, but the orientation of the cluster could not be determined accurately. The boxes indicate the proposed extent of each duplication or deletion.

dict premature termination of translation and production of proteins (comprising 88, 40, and 78 amino acids, respectively) that lack a forkhead domain.

A single-base insertion (C at a location between positions 262 and 265) was detected within the forkhead domain in a single proband diagnosed with ARA. This mutation results in a truncated *FOXC1* protein of 304 amino acids that contains the first 16 amino acids of the forkhead domain. In one proband with Rieger anomaly, a novel C236T mutation was detected that results in a proline-to-leucine change at the seventh amino acid of the forkhead domain. In addition, the Ser131Leu mutation that had been seen previously in a family with Axenfeld anomaly was again detected in a single proband diagnosed with Rieger anomaly. This is the first reported instance of a *FOXC1* mutation being found in apparently unrelated patients.

Finally, a single mutation was found downstream of the forkhead domain. This mutation involves a single-nucleotide deletion that was found in two siblings who were diagnosed with Axenfeld anomaly. The deletion occurred at position 1512 and is predicted to produce a truncated protein product of 517 amino acids. This mutant protein contains the entire forkhead domain but is missing the last 49 amino acids of the normal FOXC1 protein. None of the changes reported in this study have been detected in 96 control individuals from an ethnically similar population.

Physical Map of 6p25

Physical mapping information, along with sequence data from The Sanger Centre, was used to identify PACs that were likely to map to 6p25. DNA sequences from the PAC inserts were screened by BLASTN analysis, with STSs that had previously been placed on a YAC-based physical map (Nishimura et al. 1998). The locations of the PACs are shown in figure 2. The sequences of these PACs were then screened for the presence of STRs of $\geq$30 consecutive base pairs. A total of 30 STRPs were developed that were good candidates for having a high level of polymorphism in the human population (table 2). Two additional forkhead genes, *FOXF2* [MIM 603250] and *HFH1*, were also placed within this physical map.

Partial Duplication of 6p25 as a Cause of Disease

Cytogenetic studies demonstrated a de novo abnormal chromosome 6 that contained additional material of unknown origin at 6p25.1 in all studied cells from the proband from family RG-5. A whole-chromosome stain of chromosome 6 showed that the additional material originated in chromosome 6. A partial duplication of 6p25 was discovered by the genotyping of DNA, from the proband and parents, with highly polymorphic STRP markers from the 6p25 region. The molecular-analysis results also suggested that the duplication event was maternally derived. The genotyping of marker *668J24-GATA* is presented in figure 3, illustrating the presence of three alleles in some samples, and, in other samples, intensity differences between alleles in the heterozygous state. The extent of the partial 6p25 duplication is shown in figure 4.

The discovery of the patient with a partial 6p25 duplication prompted us to use a subset of the tightly linked genetic markers to genotype the entire cohort of probands with anterior chamber defect, to screen for the presence of small deletions or duplications. In another proband, three alleles were detected for the marker *AFMb034ya5*, suggesting a possible duplication of 6p25. This result was confirmed by additional markers in the region. This du-

plication was passed on to three of the four children of the proband. All four of the individuals who harbored the partial 6p25 duplication were diagnosed with iris hypoplasia (MIM 308500). Neither the unaffected spouse nor the sole unaffected offspring was found to have this duplication. The extent of the partial 6p25 duplication in this family is shown in figure 4.

Genetic markers flanking the *FOXC1* gene were found to have three alleles in the proband from family RG-5. This result strongly suggests that the *FOXC1* gene is duplicated in this patient. In family FG-253, markers that demonstrated three alleles could be detected only distal to the *FOXC1* gene. Thus, the hypothesis of *FOXC1* duplication in this family rests solely on the detection of intensity differences between alleles. Two *FOXC1* intragenic polymorphisms were genotyped in family FG-253. Experiments were performed to determine the PCR cycling conditions that produced products that were within the linear range of amplification. Between the control samples and the affected individuals in family FG-253, visual evidence of consistent and reproducible differences in allele intensities of the *FOXC1* polymorphism was found. This difference in the intensity of the alleles was confirmed by densitometry of PCR bands amplified from affected individuals in family FG-253 and from control samples. This result shows direct evidence for duplication of the *FOXC1* gene in this family.

Discussion

This study extends the catalog of *FOXC1*-gene mutations that can disrupt the normal development of the anterior chamber of the eye. Glaucoma occurs in ~50% of patients with such angle defects. Interestingly, the majority of the mutations can be grouped into two types. The first type represents missense mutations within the forkhead domain that presumably alter or abolish recognition of the target sequence of *FOXC1*. The precise delineation of the mechanistic effects of these mutations must await binding studies involving these types of mutations. The second type is composed of frameshift mutations that occur upstream of the forkhead domain. These mutations result in a mutant protein that is truncated prior to the forkhead domain. Such a protein would presumably lack the ability to recognize the *FOXC1* target sequence, and it would not have any functionally important elements distal to the site of premature termination. These two groups constitute the majority (15/18) of mutations reported to date.

We have detected a single frameshift mutation that is located very near the end of the coding region. The protein produced by this mutant sequence should have a fully functional forkhead domain as well as any functionally important elements prior to the site of premature termination. The mutant protein lacks the last 49 amino acids present in the wild-type *FOXC1* protein. The distal location of this change suggests that functionally important elements may be present at the end of the FOXC1 protein. Alternatively, the carboxy-terminus may play a role in proper protein folding. *FOXF2*, which is a distant family member of *FOXC1* and is also located at 6p25, has been shown to have activation domains downstream of the forkhead domain (Blixt et al. 1998). The DNA sequence near the end of the *FOXC1* gene is conserved between human, mouse, rat, *Xenopus*, and chicken, consistent with the presence of a functional element in the region.

The physical map of the region is useful for the detailed localization of sequenced large-insert clones and of candidate genes. Two other forkhead genes, *FOXF2* and *HFH1* are located distal to *FOXC1* within 6p25. This region has been postulated to contain a second locus (IRID1B), for iridogoniodysgenesis, type 1 (IRID1 [MIM 601631]), important for the development of the anterior chamber of the eye (Mears et al. 1998). *FOXF2* and *HFH1* are located within the critical interval that has been proposed for IRID1, subtype B, and can be considered candidate genes. However, no mutations have been detected in either of these genes (D.Y.N., C.C.S., V.C.S., unpublished data). Genetic markers developed from STRs contained within the sequence of mapped large-insert clones have proved to be very useful for the characterization of DNA rearrangements within 6p25. Such markers will also be useful for linkage-mapping studies within the 6p25 region.

The identification of two unrelated families who have partial duplication of 6p25 and defects of the anterior chamber of the eye suggests that the proper dosage of a gene in this region is required for normal eye development. All three of the forkhead genes (*FOXC1*, *FOXF2*, and *HFH1*) are contained within each of the duplicated regions in the two families. There are likely to be other genes within this relatively large area of 6p25. Thus, the creation of a mouse model with a *FOXC1* transgene would aid in the determination of whether the eye defects observed in the two families in this study are due solely to the extra copy of *FOXC1* or to an extra copy of a different gene(s) within the duplicated regions.

The diagnosis of isolated anomalies in the anterior chamber of the eye in patients with gross structural rearrangements involving *FOXC1* raises the possibility that anterior-chamber defects in other families in which there is evidence of linkage to 6p25 may result from mechanisms other than changes within the *FOXC1* coding sequence. Sequence alterations or structural rearrangements located outside of the *FOXC1* coding region that result in gene up-regulation or down-regulation could explain the lack of *FOXC1* coding-sequence mutations in 6p25 linked families. Thus, a second 6p25 gene involved in anterior-chamber defects might not exist. Leh-

mann et al. (2000) have recently reported a large duplication, also in the 6p25 region, that results in an anterior-chamber defect, supporting this hypothesis.

Dosage effects have been observed for the *PAX6* (MIM 106210) gene in human and mouse studies. Duplication of the 11p12-p13 region containing the *PAX6* gene often results in abnormalities of the eye (Glaser et al. 1994; Aalfs et al. 1997). Similar results have been observed for 6p25 duplications, although it is difficult to discern a consistent pattern to the type of defect observed. This is likely to be due to differences in the clinical evaluations performed by different groups, as has been noted in similar studies involving 6p25 deletions. In the case of *PAX6*, the observations in humans have been supported by experimental evidence from transgenic-mouse studies (Schedl et al. 1996). Mice that carry multiple copies of *PAX6* on a wild-type background were found to have developmental abnormalities of the eye. Thus, similar studies with *FOXC1* may prove to be useful.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

NIH Image Home Page, http://rsb.info.nih.gov/nih-image/ (for NIH Image, version 1.62)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *MYOC* [MIM 601652], *CYP1B1* [MIM 601771], *PITX2* [MIM 601542], RIEG1 [MIM 180500], *FOXC1* [MIM 601090], RIEG2 [MIM 601499], *FOXC2* [MIM 602402], *FOXF2* [MIM 603250], iris hypoplasia [MIM 308500], IRID1 [MIM 601631], and *PAX6* [MIM 106210])

Sanger Centre, The, http://www.sanger.ac.uk/ (for FISH analysis) and ftp://ftp.sanger.ac.uk/pub/human/sequences/ (for sequence data)

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