Axenfeld-Rieger Anomaly

A Novel Mutation in the Forkhead Box C1 (FOXC1) Gene in a 4-Generation Family

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Objective: To characterize DNA mutations in a pedigree of Axenfeld-Rieger anomaly (ARA) (Online Mendelian Inheritance of Man 601631), a clinically and genetically heterogeneous, autosomal dominantly inherited disorder associated with anterior chamber abnormalities and glaucoma.

Design: Observational case-control and DNA linkage and screening studies.

Participants: Affected (10 cases) and unaffected (5 controls) members of a family with ARA.

Methods: Clinical characteristics of ARA were documented by history or physical examination of symptomatic individuals. With their informed consent, a blood sample was collected from each of 10 affected and 5 unaffected family members. DNA was tested for linkage to the locus at chromosome 6p25, a known locus for ARA/Rieger syndrome. A candidate gene previously implicated by history or physical examination of symptoms and other anterior segment disorders. DNA from family members with ARA further characterized the molecular origin of developmental glaucoma and other anterior segment disorders.

Results: Direct sequencing of FOXC1 detected a new mutation, T272C, that segregated with the ARA phenotype in this family and was not detected in DNA from family members without ARA. This mutation, a T→C transition, is predicted to result in a change of isoleucine to threonine (Ile91Thr) in a highly conserved location within the first helix of the forkhead domain.

Conclusion: Characterization of the FOXC1 mutation in family members with ARA furthers our understanding of the molecular origin of developmental glaucoma and other anterior segment disorders.


Axenfeld-Rieger anomaly is genetically as well as clinically heterogeneous. Many chromosomal aberrations involving chromosomes 4, 6, 9, 13, 18, and 21 have been identified in patients affected with ARA, and linkage studies have identified at least 3 loci for the abnormalities in ARA. The first locus, at chromosome 4q25, which displayed the ARA phenotype, was mapped in 1992 by Murray et al and named RIEG1. Subsequently, the RIEG1 abnormality was identified as a mutation in the PITX2 gene in a patient with RS. PITX2 is a bicoid homeobox gene that is expressed in the anterior structures of the eye and regulates the expression of other genes during embryonic development. To date, 9 mutations of the PITX2 gene have been reported, all resulting in various anterior segment disorders such as RS, iridocorneal endothelial syndrome type 2, and Peters anomaly (or anterior chamber cleavage syndrome).
A second locus, for the RS phenotype labeled RIEG2 (Online Mendelian Inheritance of Man 601499), was linked at chromosome 13q14 by Phillips et al in 1996 using a large 4-generation family. The gene responsible for RS at this locus has not yet been identified.

A third locus for ARA has been mapped to chromosome 6p25 at the IRDI locus. Subsequently, mutations in the forkhead box C1 (FOXC1) gene were identified in some patients with ARA by Mears et al and Nishimura et al. FOXC1 (previously called FKHL7 or FREAC3) is a member of the forkhead wing/helix transcription-factor family and is a monomeric DNA-binding protein consisting of 553 amino acids that is encoded by a single exon of 1659 base pair. More than 100 proteins encoding this evolutionarily conserved domain have been identified so far in species ranging from yeast to man. Including the present study, 15 mutations of the FOXC1 gene have been reported to date, all resulting in a variety of anterior segment disorders (Table 1).

Table 1. Summary of FOXC1 Mutations Reported to Date

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Change</th>
<th>Disorders</th>
<th>Localization</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-bp INS</td>
<td>Glu23Ter</td>
<td>AINS 26-47</td>
<td>Axenfeld anomaly</td>
<td>5' of the forkhead domain</td>
</tr>
<tr>
<td>10-bp DEL</td>
<td>DEL 93-102</td>
<td>ARA and glaucoma</td>
<td>5' of the forkhead domain</td>
<td>Mears et al</td>
</tr>
<tr>
<td>8-bp DEL</td>
<td>DEL 99-108</td>
<td>Axenfeld anomaly</td>
<td>5' of the forkhead domain</td>
<td>Nishimura et al</td>
</tr>
<tr>
<td>11-bp DEL</td>
<td>DEL 132-143</td>
<td>Axenfeld anomaly, iris hypoplasia, and glaucoma</td>
<td>5' of the forkhead domain</td>
<td>Nishimura et al</td>
</tr>
<tr>
<td>Pro70Leu</td>
<td>C236T</td>
<td>Rieger anomaly</td>
<td>Into the forkhead domain, 5' of helix 1</td>
<td>Nishimura et al</td>
</tr>
<tr>
<td>Ser82Thr</td>
<td>G245C</td>
<td>Anterior segment dysgenesis and glaucoma</td>
<td>Into the forkhead domain, 5' of helix 1</td>
<td>Mears et al</td>
</tr>
<tr>
<td>Ile87Met</td>
<td>C261G</td>
<td>ARA, variable degree of iris hypoplasia, displaced pupils</td>
<td>3' of the forkhead domain</td>
<td>Nishimura et al</td>
</tr>
</tbody>
</table>

Abbreviations: ARA, Axenfeld-Rieger anomaly; bp, base pair; DEL, deletion; Glu, glutamic acid; Ile, isoleucine; INS, insertion; Leu, leucine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Ter, terpin; Thr, threonine.

Methods

PATIENT MATERIAL

Clinical evaluations included history, examination of the anterior and posterior chambers (funduscopy), testing of visual acuity and the visual fields, and measurement of IOP. For genotype analysis, a 20-mL sample of blood was collected in an EDTA-coated tube from each family member who was available for testing and who gave informed consent. DNA was isolated and analyzed using standard techniques, as follows. Oligonucleotide primers were obtained from the Centre de Recherches du Centre Hospitalier et Universitaire Laval (CHUL), Quebec, Quebec.

DNA SEGMENT ISOLATION, AMPLIFICATION, AND GENOTYPING

Six (CA), microsatellite markers, including 4 markers (Genethon, Paris, France), identified by Dib et al—AFMa350zc9 at D6S1600, AFM092xb7 at D6S344, AFM205xh4m at D6S1617, and AFM08ysh3 at D6S1685, and 2 new markers, AM01 and CA43, developed by searching the Human Genome Working Draft sequence for (CA), and (TG), repeats—were tested for linkage at 6p25.

Genethon Markers

Polymerase chain reaction (PCR) amplification was performed with 100-ng DNA to which was added 200mM each of primer; 200mM each of [\(\text{dATP}\)], deoxyadenosine triphosphate ([\(\text{dCTP}\)], deoxyguanosine triphosphate ([\(\text{dTTP}\)], deoxythymidine triphosphates ([\(\text{dGTP}\)], and dideoxynucleotide triphosphates ([\(\text{ddUTP}\)])]. 1×PCR buffer: 10mM Tris (hydroxymethyl) aminomethane (pH 9.0 at room temperature); 50mM potassium chloride; 1.5mM magnesium chloride; 0.1% Triton X-100; and 0.001% gelatin in water to achieve a total sample volume of 40 µL. Each reaction was overlaid with 25 µL of light mineral oil to prevent evaporation.

Polymerase chain reaction specificity was increased by including a “hot-start” step in which samples were denatured for 5 minutes at 95°C. After the hot-start step, 1U of Thermus aquaticus DNA polymerase in 1×PCR buffer was added to achieve a final PCR sample volume of 50 µL. Each sample was put through 35 cycles of denaturing at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extending at 72°C for 30 seconds.

Polymerase chain reaction products were separated on 6% denaturing polyacrylamide gels. After electrophoresis, the gel was transferred to a positively charged nylon membrane (Boehringer-Mannheim, Quebec, Canada) and hybridized with a digoxigenin–11-2',3'-dideoxy-uridine-5'-triphosphate (DIG-11-ddUTP) 3' end-labeling CA probe, according to the instructions.
in the DIG System User’s Guide for filter hybridization (Roche Diagnostic, Basel, Switzerland). The probe was made chromo-luminescent with Lumigen PPD (Roche, Montreal, Quebec), which is the 4-methoxy-4-(3-phosphatephenyl)spiro-(1,2-dioxetane-3,2’-adamantane) substrate for alkaline phosphatase, before being exposed to the single emulsion film, BioMax MR-1 (Eastman Kodak Co, Rochester, NY) that provides maximum resolution and sensitivity for 12 to 24 hours.

**AMO1 and CA43 Markers**

For the AMO1 (forward, 5’-CTGTTAAGGAGGTTGAGG-3’; reverse, 5’-AGTTCAAAAATGCACTTGCC-3’) and CA43 (forward, 5’-AGTGGAAACAACTCAGC-3’; reverse, 5’-AGTGCTAAAAAGGTGAC-3’) markers, PCR amplification was performed with 50 ng of DNA to which was added 200 µM of each primer, 200 µM each of dCTP, dGTP, and dTTP, 10 µM of dATP, 1.5 µCi of deoxyadenosine 5’-[α-35S] thiotriphosphate, triethylammonium salt–dATP, and 1×PCR buffer to achieve a total sample volume of 15 µL. Each reaction was overlaid with 25 µL of light mineral oil to prevent evaporation.

Polymerase chain reaction specificity was increased through a hot-start step (denaturing for 5 minutes at 95°C) before 1 U of *T. aquaticus* DNA polymerase, in 5 mL of 1×PCR buffer was added to reach a final volume of 20 µL. As a control for the determination of allele size, the Centre de l’Etude du Polymorphisme Humain, Paris, France, control DNA 134702 sequence was amplified and loaded on the gel together with the patient’s DNA sequences.

**SEQUENCING STUDIES**

Betaine (Sigma-Aldrich Co, St Louis, Mo) was used for amplification of DNA sequences. Oligonucleotide primers used for DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2. DNA sequencing were obtained from the Centre de Recherches du CHUL and from Synovis Life Technologies Inc, St Paul, Minn, and are given in Table 2.

## Table 2. Oligonucleotides for PCR Amplification of FOXC1 Gene

<table>
<thead>
<tr>
<th>Set</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Gene Region</th>
<th>PCR Products, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AGGAGCGACCCCGAACGA</td>
<td>CGGTTACTGCGTCTGGTG</td>
<td>N-terminal</td>
<td>346</td>
</tr>
<tr>
<td>2</td>
<td>ATGGAGCTTACTGCAAC</td>
<td>GGAAGCTTCGTTCTGGAAC</td>
<td>Forkhead box</td>
<td>372</td>
</tr>
<tr>
<td>3</td>
<td>TGGACCAGACTCTACAC</td>
<td>CGCGAGCGACTCATGTGTT</td>
<td>3’ Region of the forkhead domain</td>
<td>492</td>
</tr>
<tr>
<td>4</td>
<td>ACCATAGCCAGGGCTTCCAG</td>
<td>CAGGTACCAGAGGTTAGG</td>
<td>3’ Region of the forkhead domain</td>
<td>517</td>
</tr>
<tr>
<td>5</td>
<td>CAAGCCATGACGCTTGACG</td>
<td>AGAAGAAAAGACTGTTATCTGG</td>
<td>C-terminal</td>
<td>798</td>
</tr>
</tbody>
</table>

**RESULTS**

Based on clinical information, 13 members of this pedigree were considered to have ARA (Table 3). None of them displayed nonocular symptoms associated with RS. DNA analysis was completed for 15 members (10 with and 5 without ARA) (Figure). As depicted on the Figure, segregation of the disorder was clearly autosomal dominant with 5 affected males and 8 symptomatic females as well as presence of male-to-male transmission.

**PHENOTYPES**

In the proband (IV:1), a posterior embryotoxon (PE) was visible in the clear zone of each cornea (Table 3) and both irises were abnormal (1A in Table 3). On echography, the axial length measured 20 mm and the corneal diameter measured 12 mm in both eyes. The IOP averaged 13 mm Hg in both eyes and reached maximal values of 26 and 27 mm Hg (Table 3).

The proband’s 27-year-old father (III:4) was seen with Rieger-type anomalies. Although his visual acuity was 20/20 in both eyes, both eyes had severe PE and corneal opacities and the iris of the right eye consisted only of a temporal atrophic zone. However, there was no polycoria and the IOP and fundus were normal in both eyes.

The proband’s 24-year-old aunt (III:3) had a moderate PE in each eye but normal IOP.

The proband’s 30-year-old grandfather (II:3) had first been examined by an ophthalmologist at the age of 27 mm Hg in both eyes and reached maximal values of 26 and 27 mm Hg (Table 3).

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Slitlamp examination at that time revealed severe bilateral temporal PE, anterior synechiae extending over a 200° area, and iris atrophy. Funduscopy showed glaucomatous atrophy of the optic nerve in the right eye and glaucomatous papillar excavation in the left eye. Visual field testing showed a Bjerrum scotoma in the left eye and a small nasal islet scotoma in the right eye. This individual underwent bilateral trabeculectomy in 1973. At the time of the current study, his visual acuity was 20/20 OD and 20/25 OS. The right eye had tubular retraction and a temporal islet scotoma as well as the nasal islet scotoma present before surgery. Intraocular pressure was 15 mm Hg bilaterally.

Individual II:2, a 58-year-old man, has congenital ARA. He underwent surgery twice (at ages 30 and 42 years) for bilateral glaucoma. His visual acuity is light perception OD and 20/20 OS with alteration in the visual field. Both eyes have transparent cornea, PE, corectopia (more pronounced in the right eye), and corneo-iridal adhesions that may be responsible for the corectopia. Intraocular pressure remains difficult to control medically, even after 2 operations on each eye for glaucoma.

Individual II:6 is a 45-year-old woman who has been followed up since her childhood for trabeculo-irido-corneal dysgenesis with corectopia. She has polycoria and a history of retinal detachment in the right eye. The left eye does not have polycoria but does have areas of iris atrophy associated with a PE and posterior synechiae of the Rieger type. Her best-corrected visual acuity is 20/25 OD and light perception OS. Although IOP reached maxima of 30 and 26 mm Hg, respectively, it has been normal in both eyes since surgery for glaucoma.

Individual III:7 has congenital bilateral glaucoma for which he underwent surgery at the age of 18 months. His visual acuity is 20/20 OD and 20/30 OS. He has bilateral irido-corneal dysgenesis with PE, goniodysgenesis, and corectopia aggravated by filtering procedures performed during his childhood. His IOP is being maintained in the normal range by topical β-adrenergic blocking medication and funduscopy shows no excavation of the papillae.

Individuals II:8, II:9, III:10, and III:11 all have clear corneas with PE and goniodysgenesis without glaucoma. Individuals I:1 and I:3 were dead at the time of the study. They both were reported to be blind owing to glaucoma.

### LINKAGE ANALYSIS
Examination of 6 microsatellite markers (D6S1600, AMO1, D6S344, CA43, D6S1617, and D6S1685) flanking the FOXC1 gene at locus 6p25 showed linkage of this locus with ARA in this family. Nine of the 10 affected individuals shared a complete haplotype for markers spanning the region when a recombination event occurred between markers D6S344 and CA43 in affected individual FR021. A maximum lod score (Zmax) of 2.82 at a recombination fraction (θ) of 0.00 was observed with marker D6S344. With reference to BAC 118B18 of the working draft sequence, D6S344 is located approximately 10 kb from FOXC1. D6S1685 also had a positive lod score of 2.39 at a θ of 0.00.

The maximum lod score of 3.00 required to indicate significant linkage was not reached in this study because the family size was small and some of the markers were not very polymorphic. Nevertheless, we judged the lod scores to be high enough to look for an ARA-associated mutation in FOXC1.
MUTATION OF THE FOXC1 GENE ASSOCIATED WITH ARA

The FOXC1 genes of 2 affected and 2 unaffected family members were sequenced manually. This direct DNA sequencing of FOXC1 showed a T272C alteration in 1 allele of FOXC1 in both of the affected patients. The association between this mutation and the ARA phenotype was confirmed by the results of automatic sequencing of the FOXC1 gene for all 15 family members from whom
blood samples had been obtained. Indeed, the T272C alter-
tation was present in all 10 family members with ARA and
absent in the 5 unaffected members. No sequence
variation was observed at this position in FOXC1 in 54
healthy French-Canadian individuals representing a total
of 108 chromosomes sequenced. The FOXC1T272C mu-
tation is predicted to result in a change of an isoleucine
to threonine at codon 91 of the polypeptide, a highly con-
served amino acid of the first helix of the FOXC1 fork-
head domain.

The forkhead/winged helix transcription factors are char-
acterized by a 100–amino acid, monomeric DNA-
binding domain and play critical roles during early
embryonic development, cell differentiation and special-
ization, tumorigenesis, and tissue-specific gene expres-
sion in both vertebrates and invertebrates. To date, more
than 100 forkhead family genes have been cloned and
characterized in species ranging from yeast to man.

Table 4. Amino Acid Alignments of Forkhead Domain Proteins *

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Amino Acid Position in the Forehead Domain Protein</th>
<th>*</th>
</tr>
</thead>
<tbody>
<tr>
<td>h FOXC1</td>
<td>82-SYIALITMA</td>
<td>QNAPDKKIQ-100</td>
</tr>
<tr>
<td>h FOXC1</td>
<td>129-SYIALITMA</td>
<td>LGSPKKIRL-147</td>
</tr>
<tr>
<td>h FOXC1</td>
<td>27-SYIALITMA</td>
<td>QSPPTKIRL-45</td>
</tr>
<tr>
<td>h FOXC2</td>
<td>104-SYIALITMA</td>
<td>QSSPSKRIL-122</td>
</tr>
<tr>
<td>h FOXC1c</td>
<td>15-SYNALITMA</td>
<td>RQSPEKRLR-33</td>
</tr>
<tr>
<td>h FOXC1</td>
<td>10-SYNALITMA</td>
<td>HGAPDPKRL-28</td>
</tr>
<tr>
<td>h FOXXoA</td>
<td>189-SYIALITMA</td>
<td>TMDPKQIL-207</td>
</tr>
<tr>
<td>h FOXL1</td>
<td>53-SYIALITMA</td>
<td>GDAPEGRVL-71</td>
</tr>
<tr>
<td>h FOXL1</td>
<td>65-SYALITMA</td>
<td>ESSAEKRLT-83</td>
</tr>
<tr>
<td>r ihm-3B</td>
<td>163-SYIALITMA</td>
<td>QGSPNKMLT-181</td>
</tr>
<tr>
<td>r lth-1</td>
<td>104-SYIALITMA</td>
<td>RDSAGGRVL-116</td>
</tr>
<tr>
<td>Consensus</td>
<td>SYIALITMA</td>
<td>QQ/SPPK/ERL</td>
</tr>
</tbody>
</table>

Abbreviations: h, human; r, rat.
*Residue corresponding to isoleucine at codon 91 in FOXC1 (boxed) is conserved.

The forkhead/winged helix transcription factors are char-
acterized by a 100–amino acid, monomeric DNA-
binding domain and play critical roles during early
embryonic development, cell differentiation and special-
ization, tumorigenesis, and tissue-specific gene expres-
sion in both vertebrates and invertebrates. To date, more
than 100 forkhead family genes have been cloned and
characterized in species ranging from yeast to man.

To date only 1 mutation causing anomalies of the ante-
crior chamber has been found outside the forkhead box.
This mutation, a 1–base pair deletion of the nucleo-
tide 1512, is located in the C-terminal region. In addi-
tion to these mutations, distinct duplications encom-
passing FOXC1 has recently been reported in 3 different
families with iris hypoplasia and glaucoma. Most of the
FOXC1 mutations described so far em-
phasized the critical role of the forkhead domain for DNA
binding and nuclear localization. Nishimura et al also re-
ported a mutation 3’ of the forkhead box, which sug-
gested the presence of functionally important elements at
the end of the FOXC1 protein. This assumption was en-
forced by the fact that the DNA sequence near the end of
the FOXC1 gene is conserved in man, mouse, rat, Xene-
opus species, and chicken, and by in vitro studies show-
ing that the C-terminus of the FOXC1 protein contains
an activation domain. In addition to missense and frame-
shift mutations affecting FOXC1, it seems that underex-
pression or overexpression (as with duplication of the gene)
of FOXC1 can cause defects in the anterior chamber of the
eye. For example, Smith et al demonstrated that haplo-
insufficiency of the transcription factors FOXC1 in the
mouse (Foxc1+/−) resulted in histologically evident ante-
rior segment abnormalities in every affected mouse. Mor-
over, Fox1 homozygous mutants (Fox1−/−) died during
the perinatal period, indicating that this gene plays a
role in embryonic development.

In the 10 patients with ARA we sequenced, we found
a missense mutation (T272C) in the first helix of the fork-
head domain of FOXC1. The alignment of amino acids
in forkhead domain proteins (Table 4) indicates that
the isoleucine at codon 91 in the forkhead domain is
highly conserved. The mutation we report herein oc-
urs in this conserved motif. This suggests that the
Ile91Thr mutation we describe occurs in a nuclear local-
ization sequence of FOXC1 and contributes to anterior
chamber abnormalities by hindering nuclear localiza-
tion. Saleem et al recently studied the effect of 5 mis-
sense mutations of the winged/helix domain found in pa-
ients with AR malformations. Although these authors
did not investigate the Ile91Thr variation, they dem-
strated that mutations in the FOXC1 forkhead domain
reduced stability, DNA binding, or transactivation, all
causing a decrease in the ability of the polypeptide to trans-
activate genes. Further experimentation should reveal the
exact mechanism(s) by which the Ile91Thr mutation alter
FOXC1 transactivation.

The most important feature of ARA is the high risk of
developing glaucoma, which causes progressive nar-
rowing of the visual field and, when uncontrolled, blind-
lessness. It was estimated, for 2000, that almost 6 million
people worldwide have developed glaucoma. Glau-
coma is often insidious and rarely hurts, and it is be-
cause its severe consequences may be minimized if it is
diagnosed early, it becomes important to understand the
genetic bases of disorders of the anterior chamber of the
eye. Our results serve to improve the understanding of the
role FOXC1 plays in developmental glaucoma and ex-
pands the knowledge of the genetic causes of anterior seg-
ment disorders.

Submitted for publication September 5, 2002; final revi-
sion received March 25, 2004; accepted May 10, 2004.
This study was supported by grants MOP-13428 from
the Canadian Institutes for Health Research Ottawa, On-
tario, and 548 from the Canada Foundation for Innova-
tion, Ottawa.

Dr Raymond is a Fonds de la Recherche en Santé du Québec National Investigator.

We thank all the families and patients who partici-
pated in this study.

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REFERENCES