



SHORT REPORT

Axenfeld-Rieger syndrome resulting from mutation of the *FKHL7* gene on chromosome 6p25

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Mutations in the forkhead-like 7 (*FKHL7*) gene have been recently shown to cause juvenile glaucoma and anterior segment anomalies. We report on a three-generation family with Axenfeld-Rieger syndrome (ARS), harboring an alteration in the *FKHL7* gene. Genetic linkage analyses excluded the ARS phenotype from chromosomes 4q25 and 13q14, the locations of the *PITX2* and *RIEG2* loci, respectively. Evidence of linkage was observed with markers at 6p25, near the *FKHL7* gene. Direct sequencing of *FKHL7* detected a C67T mutation that segregated with the ARS phenotype in this family, but was not detected in over 80 control chromosomes. This mutation is predicted to cause a nonsense mutation of the *FKHL7* protein (Gln23Stop) upstream of the forkhead DNA-binding domain, and thus to generate a truncated *FKHL7* protein product. This discovery broadly implicates *FKHL7* in ocular, craniofacial, dental, and umbilical development. *European Journal of Human Genetics* (2000) 8, 71–74.

Keywords: Axenfeld-Rieger syndrome; forkhead-like 7; *FKHL7*; *PITX2*; Axenfeld-Rieger anomaly; chromosome 6p25; mutation

Introduction

Axenfeld-Rieger syndrome (ARS; OMIM No 180500) is an autosomal dominantly inherited developmental disorder in which patients present with ocular and non-ocular clinical findings.¹ Ocular characteristics include hypoplasia of the anterior iris stroma, microcornea, anterior chamber synchia, corneal opacity, and juvenile onset glaucoma. Non-ocular features typically include maxillary hypoplasia, hypodontia, and failure of involution of the periumbilical skin. Patients with the autosomal dominantly-inherited Axenfeld-Rieger anomaly (ARA) present with the characteristic ocular features alone.

Various chromosomal aberrations have been found in association with ARS, including the involvement of chromosomes 4, 6, 9, 13, 18, and 21 (reviewed by Nielsen and colleagues²). Two genes underlying the ARS phenotype have been localized to chromosomal regions, the first to 4q25³ and

the second to 13q14,⁴ indicating that ARS is genetically heterogeneous.

Mutations of the *PITX2* gene have been shown to underlie the ARS phenotype in ARS families in which the disease phenotype is linked to 4q25.⁵ *PITX2* is a developmental control gene of the *bicoid* homeobox gene family. Interestingly, the phenotype in two families with iridogoniodysgenesis syndrome (IRID2; OMIM No 137600), an autosomal dominant condition in which patients present with iris stromal hypoplasia, goniodysgenesis, abnormal angle vascularity, and ARS-like non-ocular findings, have been shown to result from different missense mutations of the *PITX2* gene.^{6,7} To date, 10–30% of ARS patients have been found to have mutations of the *PITX2* gene located at chromosome 4q25⁵ (also Kulak and Walter, unpublished data, 1999).

Genetic linkage analysis localized a gene underlying ARA to chromosome 6p25.⁸ Independent results from our laboratory⁹ and that of Sheffield,¹⁰ demonstrated that ARA mapping to 6p25 results from mutations of the forkhead-like 7 (*FKHL7*) gene. *FKHL7* is a member of the *forkhead* transcription factor gene family shown to be involved in embryonic development, tissue-specific gene expression, and oncogenesis.^{11–15} Homozygous null mutations of *Mfl*, the

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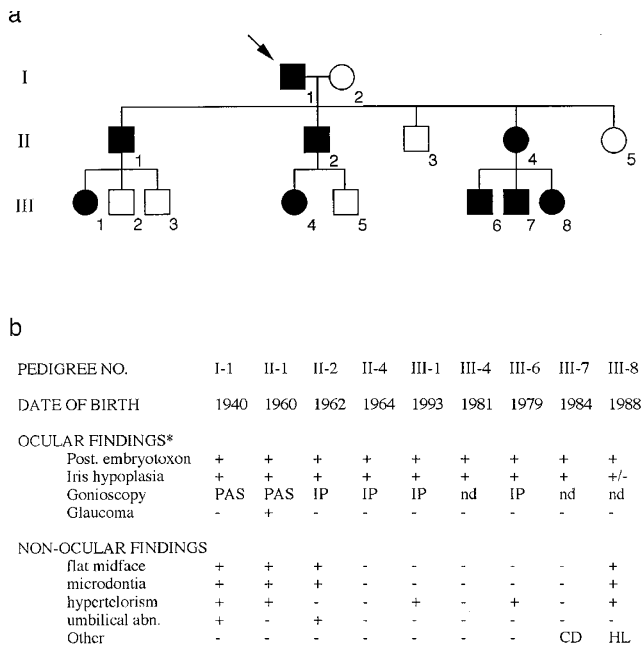


Figure 1 Three-generation family with nine members affected with Axenfeld-Rieger syndrome (ARS). **a** Pedigree of the family with ARS. Affected individuals are indicated by filled symbols, unaffected by unfilled symbols. The proband, affected individual I-1, is indicated by an arrow. **b** Clinical findings in *family members affected with ARS; post; posterior; PAS: peripheral anterior synchia; IP: iris processes; nd: not done; abn: abnormalities including failure of involution; CD: cardiac defect; HL: hearing loss.

murine homologue of *FKHL7*, have been shown to be responsible for the *congenital hydrocephalus (ch)* phenotype in mice.¹⁶ These results suggest that *FKHL7* has a key role in the development of the mammalian eye and brain.

We report here on a three-generation family with ARS, harboring a novel nonsense mutation in the *FKHL7* coding sequence, upstream of the *FKHL7* DNA-binding domain. This discovery not only indicates that mutations of *FKHL7* can result in both ARA and ARS, but also broadly implicates *FKHL7* in ocular, craniofacial, dental, and umbilical development.

Materials and methods

Subjects

The family with ARS studied in this report had nine affected individuals in three generations (Figure 1). Affected individuals presented with a variable degree of iris hypoplasia, displaced pupils (corectopia), and a prominent, anteriorly-displaced Schwalbe's line (posterior embryotoxon) to which peripheral iris strands were attached bridging the irido-corneal angle (Figure 2). Glaucoma was observed in individual II-1. A summary of the clinical findings in all affected



Figure 2 Photograph of the left eye of the proband (I-1) affected with ARS, displaying diffuse iris hypoplasia and posterior embryotoxon.

individuals is presented in Figure 1b. The study and collection of blood samples from all individuals included in this report were approved by the Toronto Hospital Committee for Research on Human Subjects.

Genotypic and molecular studies

Blood samples were collected in EDTA tubes and DNA prepared from isolated white blood cells as described.¹⁷ Oligonucleotide primers for microsatellite markers were obtained from Research Genetics (Huntsville, AL, USA) and ACGT (Toronto, ON, Canada). PCR primers and conditions for the amplification of the *FKHL7* gene were as described.⁹ PCR amplification for linkage and SSCP analyses were performed as described.¹⁸ PCR products for linkage analysis were separated upon 6% denaturing polyacrylamide gels. SSCP analysis was conducted on 6% acrylamide non-denaturing gels containing 7% glycerol, with electrophoresis at 60 W at 4°C. After electrophoresis, gels were dried and used for autoradiography. DNA sequencing was done manually using a ³³P-radiolabelled terminator thermosequenase cycle sequencing kit from Amersham-Pharmacia (Baie d'Urfé, PQ, Canada). All PCR products were purified using Qiagen columns (Mississauga, ON, Canada), prior to sequencing.

Linkage analysis

Linkage analysis was conducted with a DOS-compatible Power Macintosh 6100/66 computer. Lod score values were calculated using the MLINK option of the LINKAGE program. Analysis was conducted under the assumption that ARA is a fully penetrant autosomal dominant disease with a mutation rate of 10⁻⁶, and a gene frequency in the population of 0.0001. The allele frequencies were assumed to be equal for the markers used in this study.

Results

Linkage studies

Linkage of the ARS phenotype in this family to the *PITX2* locus at 4q25 was excluded by examination of three polymorphic markers in the 4q25 region, D4S3240, D4S2623, and D4S406 (data not shown). Examination of six polymorphic markers (D13S894, D13S1288, D13S1253, D13S1248, D13S263, and D13S325) spanning the *RIEG2* critical region at 13q14⁴ was consistent with exclusion of the *RIEG2* gene (data not shown). In contrast, complete linkage of ARS in this family to polymorphic loci flanking the *FKHL7* gene at chromosome 6p25 was found. A maximum lod score (Z_{\max}) of 2.71 at a recombination fraction (θ) of 0.00 was obtained with marker D6S344 located at the distal short arm of chromosome 6 (6p25). D6S344 is located approximately 10 kb from the *FKHL7* gene (Gould and Walter, unpublished data). Positive lod scores of 2.41 at θ of 0.00 were also found with D6S1600 and D6S967 also located at 6p25. Whilst the small size of the ARS family together with only partially informative markers precluded the Z_{\max} lod scores from reaching the critical value of 3.00 required to indicate significant linkage, this suggestion of possible linkage to polymorphic markers flanking the *FKHL7* gene was sufficient to initiate screening of the *FKHL7* gene for an ARS-associated mutation.

Mutation of the *FKHL7* gene associated with ARS

FKHL7 primer sets were used to PCR amplify the single exon *FKHL7* gene in affected individual (I-1) to identify the mutation of the *FKHL7* gene. Direct DNA sequencing of the *FKHL7* gene in DNA from I-1 revealed a C67T alteration within one allele of *FKHL7* (Figure 3). SSCP analysis indicated that this nucleotide alteration segregated with the disease phenotype in this family, but was not found in over 80 normal chromosomes (data not shown). This mutation is predicted to result in a Gln23Stop nonsense mutation prior to the *FKHL7* forkhead domain.

Discussion

To date, over 80 forkhead family genes have been cloned and characterized. There is increasing evidence that members of the forkhead/winged helix gene family play key roles in embryonic development, cell fate determination, tumorigenesis, and tissue-specific gene expression in both vertebrates.¹⁵ Recently, mutations of the thyroid transcription factor 2/forkhead-like 15 (*FKHL15*) and forkhead-like 7 (*FKHL7*) genes have been found to cause diseases – thyroid agenesis, cleft palate, and choanal atresia,¹⁹ and Axenfeld-Rieger anomaly,^{9,10} respectively.

We studied a family with nine members affected with ARS, exhibiting varying degrees of iris hypoplasia, posterior embryotoxon, and iris strands, in addition to non-ocular findings including umbilical anomalies, flat midfaces, and small teeth. Linkage analyses excluded the region that

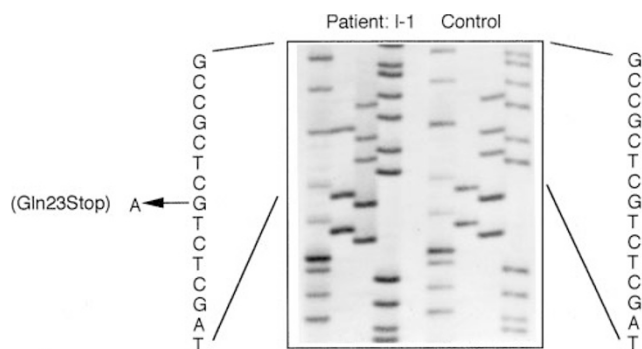


Figure 3 DNA sequence analysis indicating an ARS-associated mutation in the *FKHL7* gene. Autoradiographs showing sequence analysis of the mutation identified in the ARS family. PCR products were amplified using primer set C³ from DNA samples of individual I-1 and by a non-affected control individual, and were directly sequenced. Normal control sequence is shown on the right; sequence from affected individual I-1 is shown on the left. The DNA sequence from the reverse primer is shown in each case, with the lanes representing bases GATC from left to right. Position of the mutation is shown to the left. The predicted effect of the *FKHL7* mutation is indicated to the far left. Pedigree number is as in Figure 1.

contains the *PITX2* gene at 4q25 and the *RIEG2* minimal critical interval at 13q14 from underlying ARS in this family. Linkage analysis, however, indicated possible linkage of ARS in this family to markers flanking the 6p25 region containing the *FKHL7* gene. Direct DNA sequencing of the *FKHL7* gene in DNA from this family revealed a C67T alteration that segregated with the disease phenotype. This mutation, predicted to result in a Gln23Stop nonsense mutation upstream of the *FKHL7* forkhead domain (Figure 3), was not found in over 80 normal chromosomes.

This report is the first description of a *FKHL7* mutation associated with ARS. This work supports the increasing evidence that *FKHL7* has a significant role in the development of the anterior segment of the eye and brain. Mice with homozygous null alleles of *Mf1*, the murine homologue of *FKHL7*, die at birth with hydrocephalus, heart defects, eye anomalies, and multiple skeletal defects.¹⁶ Mutations have been identified in the *FKHL7* coding region in several small families affected with ARA.^{9,10} All of the mutations reported to date in ARA patients either have been upstream of the *FKHL7* forkhead DNA-binding domain and are predicted to result in premature protein truncation prior to the forkhead domain, or have resulted in substitutions of conserved amino acids within the forkhead DNA-binding domain. The *FKHL7* mutation described here (Gln23Stop), and the *Mf1* mutation underlying the *ch* phenotype in the mouse (Gln123Stop), are the only nonsense mutations described to date in the *FKHL7*/*Mf1* gene. Both the ARS and *ch* phenotypes involve non-ocular tissues, unlike ARA. It is possible that the *FKHL7* mutations associated with the clinically less severe ARA

phenotype could result from retention of residual *FKHL7* activity in the cases of *FKHL7* missense mutations, or from low level reinitiation of translation of the *FKHL7* mRNA from downstream inframe ATG codons in the two cases of *FKHL7* frameshift mutations. Interestingly, a 4.0 kb alternative transcript, smaller than the 4.5 kb *FKHL7* transcript observed in all other tissues examined, has been observed in fetal kidney, indicating that alternative *FKHL7* transcripts can also be produced in some tissues.^{9,10,12} It remains to be determined if such alternative *FKHL7* transcripts have a role in the development of the non-ocular dental, maxillary, and umbilical systems affected in patients with ARS. The nonsense mutation of *FKHL7* found in the family reported here indicates that haploinsufficiency of *FKHL7* could underlie the more clinically severe phenotype present in ARS patients.

To date, only 10–30% of ARS patients have been found to have mutations of the *PITX2* gene located at chromosome 4q25⁵ (also Kulak and Walter, unpublished data). Our finding of an ARS-associated *FKHL7* mutation raises the possibility that a significant proportion of ARS patients could have *FKHL7* mutations. In addition, the finding of ARS-associated *FKHL7* mutations expands the role of *FKHL7* in mammalian embryogenesis, implicating *FKHL7* in ocular, craniofacial, dental, and umbilical differentiation, as well as in developmental glaucoma.

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