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Original article

## Clinical presentation of a variant of Axenfeld–Rieger syndrome associated with subtelomeric 6p deletion

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### Abstract

We report a 22-year-old female with a variant of the Axenfeld–Rieger Syndrome (ARS) and discuss its relation with the subtelomeric 6p deletion. An ARS variant has been described in two familial cases of Axenfeld–Rieger Anomaly (ARA) featuring specific extra ocular manifestations—hypertelorism, midface hypoplasia, mild sensorial deafness, hydrocephaly, psychomotor delay and flattened femoral epiphyses. We proposed that this set of characteristics represents a separate syndrome within the ARS. On the other hand, there have been reported four cases with cryptic de novo pure 6pter microdeletions detected by specific subtelomeric probes in patients with ARS characteristics. We describe a 6pter deletion detected by SNP genotyping and confirmed by FISH and MLPA involving the *FOXC1* gene in a patient with ocular and systemic findings that fit perfectly with the variant mentioned above. We conclude that the ARS variant belongs to the ARS phenotypic spectrum, which includes flattened femoral epiphyses as a feature. © 2006 Elsevier Masson SAS. All rights reserved.

**Keywords:** Axenfeld–Rieger; Subtelomeric deletion; 6p; *FOXC1*

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## 1. Introduction

The Axenfeld–Rieger Syndrome (ARS) is characterized by a wide phenotypic spectrum of defects in the anterior segment of the eye (Axenfeld–Rieger Anomaly), accompanied by systemic signs and mental retardation (Rieger Syndrome). Inheritance is autosomal-dominant with incomplete penetrance and variable expressivity for the ocular and extra ocular signs. [1,7,12,16,27]. ARS owes its name to Axenfeld, who in 1920 described a patient with posterior embryotoxon and iris strands attached to Schwalbe's line—Axenfeld Anomaly. Rieger described additional changes in the iris, such as hypoplastic iridic stroma, corectopia and polycoria—Rieger Anomaly. The combination of these anomalies is known as Axenfeld–Rieger Anomaly (ARA) [29]. The ARS is used to describe patients showing ARA together with systemic signs, especially dental, umbilical and cardiac alterations [7,9,22].

At present, two different genes encoding transcription factors (*PITX2* [19,22,26] and *FOXC1*) are known to cause the alterations observed in the ARS. In addition, at least two genetic loci involve genes that have not yet been characterized (13q14 [23] and 16q24 [30]). Furthermore, two other putative genes have been implicated (*PAX6* [24] and *MAF* [10]). *FOXC1* (6p25) [21], a forkhead transcription factor, is a regulator of morphogenesis. Haploinsufficiency and reduced transactivation [25,28] of *FOXC1* cause ARA [8,21], ARS [20] and Iridogoniodysgenesis Anomaly [15,18]. No phenotype/genotype correlation exists.

Table 1

Comparison of the clinical findings between our case (ARS variant, OMIM: 109120) and five others with the 6p subtelomeric deletion syndrome

	Patient	Variant	Le Caignec et al. (2005), # 1	Le Caignec et al. (2005), # 2	Anderlid et al. (2003)	de Vries et al. (2003)	Maclean et al. (2005)
Estimated deletion size (Mb)	2.7	–	5.5	4.8	2.1	–	2–7
Deafness	+	+	+	+	+		
Developmental delay	+	+	+	+	+	+	+
Posterior embryotoxon	+	+	+	+			+
Anterior chamber eye anomaly	+	+	+	+	+	+	+
Iris hypoplasia	+	+	+	+			+
Polycoria/corectopia	+	+					+
Hypertelorism/telecanthus	+	+	+	+	+	+	+
Proptosis	+	+	+				+
Abnormal skull shape/hydrocephalus	+	+	+	+			+
Prominent forehead	+	+	+	+			+
Flat midface/hypoplasia	+	+	+				
Downslanting palpebral fissures				+	+		+
Broad nose/nasal bridge			+	+	+		
Philtrum anomalies			+	+	+		+
Dental anomalies	+			+	+		
Ear anomalies	+	+	+	+	+		+
Heart anomalies					+	+	+
Flat femoral epiphyses	+	+					

Several cases with an ARS phenotype and microscopically visible 6p deletions have been reported and two distinctive clinical phenotypes have been established according to the location of the defect, interstitial deletions (6p22–6p24) and terminal deletions (6p24–pter) [4]. However, to our knowledge there have been reported only four cases with cryptic de novo pure 6pter microdeletions detected by specific subtelomeric probes [2,6,14] and one case with a 6p25 microdeletion due to a de novo 6;18 translocation [17]. There is only one report of an interstitial 6pter deletion where the *FOXC1* gene was not deleted [4]. The size of the deletions have been estimated to range between 2.1 and ~7 Mb with the exception of one case for which no precise data was available. The ARS phenotype found in individuals with distal chromosome 6p deletions is characterized by mental retardation, abnormal skull shape with a prominent forehead and midface hypoplasia, downslanting palpebral fissures, broad nose, anomalies of the philtrum, and ocular anomalies —ARA and hypertelorism— deafness, cardiac defects and clinodactyly [2,4,6,13] (Table 1).

A variant form of ARS has been reported in two unrelated families (OMIM 109120). The first is a family with ARA and psychomotor retardation, communicating hydrocephaly, proptosis, hypertelorism, prominent forehead, flat midface, mild sensorineural deafness, hypotonia and joint laxity with dislocation of the hips [5]. The second family has the same features and partial absence of eye muscles [3]. The authors hypothesized that this group of characteristics represented a separate syndrome within ARS, an idea also supported by Kelly in 1982 [11].

We report the third case of the aforementioned ARS variant—in a patient whose clinical findings are highly similar to those described by De Hauwere et al. and Chitty et al.— who presented, in addition a subtelomeric deletion at 6p25.

## 2. Materials and methods

### 2.1. Clinical presentation

The index patient is a 22-year-old female, born at the 40th week of gestation without complications and from non-consanguineous parents. At birth, she presented a clubfoot on the right side and dysmorphic facial features (hypertelorism). At the age of 4 years, she was diagnosed with strabismus, bilateral glaucoma and femoral osteochondritis with flattened femoral epiphyses (Fig. 1b). In addition, hydrocephaly was diagnosed when she was 5 years old. The patient also had a long history of dental complications needed orthodontic treatment. The patient was referred by an ophthalmologist to the Department of Genetics with a diagnosis of ARA characterized by posterior embryotoxon, proptosis, epicanthic folds, peripheral displacement of the pupils, iris strands and upper scotoma in the visual field of both eyes (Fig. 2).

Physical examination showed a weight of 99 kg ( $p > 97$ ), height was 159 cm ( $p50$ ) and head circumference 60.5 cm ( $p > 97$ ). There was mild hirsutism, a prominent forehead, flat midface, epicanthic folds, hypertelorism, low-set ears, bulbous nose, short philtrum, high arched palate and micrognathia (Fig. 1a). The patient had mild mental retardation and mild sensorineural deafness.

Her father showed only glaucoma and epicanthus without mental retardation or anterior chamber anomalies. The mother was not available for physical examination or for genetic studies.



Fig. 1. (a) Frontal and lateral views showing the characteristic facies of the patient (prominent forehead, flat midface, epicanthus, hypertelorism, low-set ears, bulbous nose, short philtrum, high arched palate and micrognathia), (b) X-ray of the hip showing the flattened femoral epiphyses.

## 2.2. Cytogenetic study

Cytogenetic analysis was performed on blood from the proband and her father according to routine procedures using GTG banding (550 bands).

## 2.3. Molecular study (SNP analysis and MLPA)

Genomic DNA was extracted from peripheral blood samples from the proband and her father. DNA from the proband was used as template for a labeling reaction that was performed according to the manufacturer's specifications (Affymetrix, Santa Clara, CA, USA). Labeled DNA was then hybridized to an Affymetrix GeneChip Human Mapping 50 K Xba 240 Array (Affymetrix, Santa Clara, CA, USA) and processed according to the manufacturer's specifications at the University of Iowa DNA Core Facility. The raw data from the chip were processed

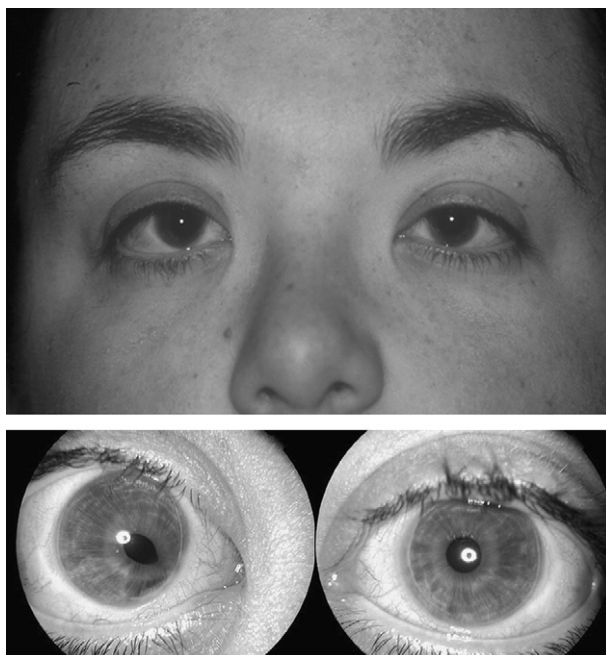


Fig. 2. Ocular examination of both eyes showed: proptosis, epicanthic folds, peripheral displacement of the pupils and iris strands.

with the Affymetrix GeneChip Chromosome Copy Number Analysis Tool (CCNAT). Detailed information on the genomic organization of the 6p25 region was obtained from the UCSC Genome Browser (<http://genome-test.cse.ucsc.edu/>).

Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA P036B and P070 subtelomere probe mixes (MRC-Holland, Amsterdam, The Netherlands) was performed on DNA samples from the proband and her father. The 6p probes detected the gene *IRF4*, localized between 300 and 400 kb from the end of the chromosome.

#### 2.4. FISH studies

FISH study with a panel of subtelomeric probes was carried out using the Chromoprobe Multiprobe-T system kit according to the supplier's instructions (Cytocell, Adderbury, England). The subtelomere specific probe for short arm of chromosome 6 was localized in clone RP1-62I11, located within the 300 kb from the end of the chromosome.

### 3. Results

Karyotypes from the proband and her father were apparently normal (46,XX and 46,XY, respectively) at the 550-band level of resolution. However, the presence of the additional clinical findings in addition to ARS in the proband suggested a cryptic deletion or duplication. SNP genotyping of the entire genome was performed to examine this possibility. Analysis of the SNP data with the Affymetrix CCNAT software package suggested that a region at 6p25

was likely to be deleted in the proband. Visual analysis of the SNP genotypes revealed a region in which 54 contiguous SNPs were homozygous. This region overlapped the deleted region predicted by the CCNAT software. The maximum size of the deletion was estimated to be 2.7 Mb as shown in Fig. 3. No other regions of deletion or duplication were detected by CCNAT.

FISH analysis with subtelomeric probes confirmed a pure deletion of chromosome 6p25 in the proband. The deletion was also confirmed using MLPA. In the unaffected father, no deletion was detected using MLPA and no rearrangement affecting the subtelomeric regions of chromosome 6 was observed. The probes used in both techniques to characterize the distal 6p25 deletion region are included within the segment 400 kb from the telomere of the chromosome.

#### 4. Discussion

We present a patient with features of the ARS and a phenotype suggestive for a subtelomeric 6p deletion. This was confirmed by whole genome SNP genotyping, FISH and MLPA. The ocular and systemic findings in the present patient fit perfectly with the cases reported before by De Hauwere [5] and Chitty [3], who described two patients with a variant ARS of unknown cause. The finding of a deletion at 6p25 involving the *FOXC1* gene in the present patient indicates that this particular subtype of ARS is caused by the deletion of distal 6p. The 2.7 Mb deletion found in this case contains the *FOXC1* gene, involved in ARS (Fig. 3). A number of other genes are also included within the deleted region: *C6ORF195*, *DUSP22*, *FOXF2*, *FOXQ1*, *GMDS*, *HUS1B*, *IRF4*, *SEC5L1* and *WRNIP1*. Additional five anonymous cDNA clones also map to this region: *AK091913*, *AK122581*, *AK124997*, *AK125751* and *AK128409*. It is likely that one or more of these genes are responsible for the additional clinical findings observed in this patient.

As described by Le Caignec et al., there is a well defined phenotype associated with 6pter deletions, which includes characteristic facial appearance, ocular anomalies (generally, defects of the anterior chamber of the eye), hearing loss and mental delay [14]. In our case, an accurate ophthalmologic assessment together with the finding of multiple congenital malformations led to the precise diagnosis of an ARS variant that later guided us to the cytogenetic and molecular analysis that established the underlying genetic defect of this pathology. Compared to the cases described by Le Caignec et al. [14] only one clear difference is observed in the present patient, i.e. flattened femoral epiphyses. In the previously reported patients [3,5], this feature is not mentioned.

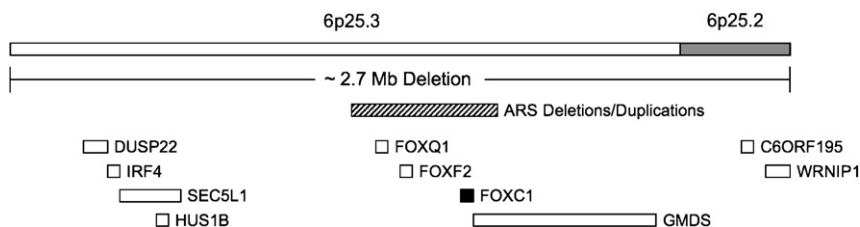


Fig. 3. Region of deletion within 6p25. The deletion encompasses all of 6p25.3 and part of 6p25.2 as depicted in the figure. The hatched box indicates the region containing published deletions and duplications that are associated with the ARS phenotype. The boxes at the bottom of the figure depict the known genes that are contained in this region with the black box indicating the *FOXC1* gene that is known to cause ARS.

In conclusion, in patients presenting with features of this type of ARS, an evaluation of the copy number of the *FOXC1* gene should be performed. Such studies, in addition to an exhaustive phenotypic analysis—including the presence of flattened femoral epiphyses—might improve our knowledge about mechanisms that underlie the wide range of expressivity of the Axenfeld–Rieger Syndrome and its variants.

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