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Axenfeld–Rieger syndrome and spectrum of *PITX2* and *FOXC1* mutations

Axenfeld–Rieger syndrome (ARS) is a rare autosomal dominant disorder, which encompasses a range of congential malformations affecting the anterior segment of the eye. ARS shows genetic heterogeneity and mutations of the two genes, *PITX2* and *FOXC1*, are known to be associated with the pathogenesis. There are several excellent reviews dealing with the complexity of the phenotype and genotype of ARS. In this study, we will attempt to give a brief review of the clinical features and the relevant diagnostic approaches, together with a detailed review of published *PITX2* and *FOXC1* mutations.

In brief

- Axenfeld–Rieger syndrome (ARS) is an umbrella term used to describe a variety of overlapping phenotypes, in which the major physical condition is the anterior segment dysgenesis of the eye.
- Patients with ARS may also present systemic malformations with incomplete penetrance and variable expressivity. The major systemic features are mild tooth abnormalities (microdontia, hypodontia, oligodontia and adontia) and redundant periumbilical skin. Craniofacial dysmorphism such as maxillary hypoplasia, sensory hearing loss, hypertelorism and congenital heart defects may also be part of the clinical spectrum.
- ARS is a dominantly inherited condition with genetic heterogeneity.
- Mutations in the transcription factors, *PITX2* and *FOXC1*, lead to ARS.
- The mutations show great diversity from intragenic mutations (*PITX2* and *FOXC1*) to submicroscopic deletions (*PITX2* and *FOXC1*) or duplications (*FOXC1*), to chromosome rearrangements (*PITX2*).
- There is no clear genotype–phenotype relationship but ARS patients with systemic changes usually have *PITX2* mutations.
- The underlying genetic defect is unknown in 60% of the cases and there are at least two more loci associated with ARS, but the genes involved are yet to be identified.
- The major clinical concern is the risk of developing sight-threatening glaucoma, which is observed in 50% of the patients.

Introduction

Axenfeld–Rieger syndrome (ARS) is mainly characterised by anterior segment abnormalities (anterior segment dysgenesis, ASD) of the eye, and comprises a clinically and genetically heterogeneous group of conditions with a varying degree of developmental abnormalities involving both ocular and extraocular structures.[1] Several classifications of anterior segment disorders have been suggested and the terminology used is quite complex. In this review, the general term ARS will be used for the conditions, Axenfeld anomaly, Axenfeld syndrome, Rieger anomaly and Rieger syndrome.[2,3] As our understanding of the embryology and genetics of the eye development increases, these terminologies and classifications may take new shapes. The other disorders of the anterior segment, such as Peters anomaly and iris hypoplasia/iridogoniodysgenesis anomaly/syndrome will be mentioned only briefly and the focus of the review will be mainly on the ARS.

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Clinical features

The clinical features of ARS can be roughly divided into ocular and non-ocular (systemic) changes.

Ocular changes

The ocular abnormalities observed in ARS affect mainly the iris, cornea and the chamber angle (Figure 1a–d).

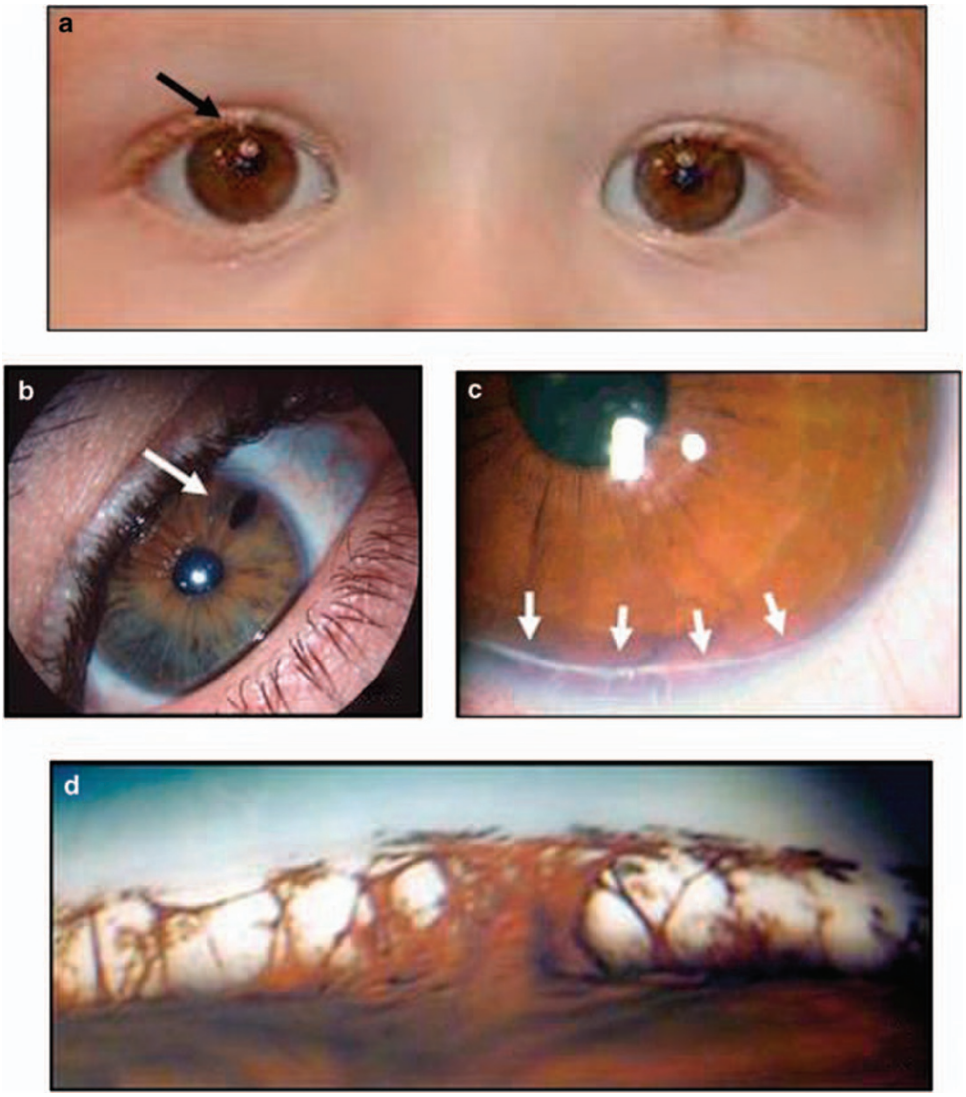

Figure 1 Ocular changes observed in Axenfeld–Rieger syndrome. (**a**) Corectopia on the right eye (displaced pupil is shown by an arrow); (**b**) Polycoria (the extra hole on the iris is shown by an arrow); (**c**) Posterior embryotoxon, the prominent Schwalbe's line is indicated with arrows; (**d**) Iris strands bridging the chamber angle as seen on gonioscopy.

Iris Observation of iris changes is important in establishing the ARS diagnosis and these include thinning of the iris (hypoplasia), displacement of the pupil (corectopia) (Figure 1a) or hole formation in the iris mimicking multiple pupils (polycoria) (Figure 1b). The iris changes may be very subtle (only slight peaking of the pupil) and may seem to be normal without examination of the iridocorneal angle (gonioscopy). Dependent on the placement of the pupil corectopia and polycoria may cause photophobia and cosmetic problems.

Cornea The Schwalbe's line (the peripheral termination of Descemet's membrane and the anterior limit of the trabeculum) is prominent and displaced anteriorly (posterior embryotoxon) (Figure 1c). It appears as a white line on the posterior cornea, near limbus and can be observed in slit lamp examination and it is easily diagnosed on gonioscopy. Posterior embryotoxon is found in most ARS patients, but is not required for diagnosis.[4] Approximately 15% of the general population has posterior embryotoxon, without increased risk of developing glaucoma.[5] When posterior embryotoxon is identified in a patient with an anterior segment disorder, the first consideration should be ARS. Absence of other corneal abnormalities, such as megalocornea, sclerocornea and corneal opacity are the useful criteria in distinguishing ARS from other anterior segment disorders.

Chamber angle In ARS, a characteristic chamber angle appearance is observed and the iris strands bridge the

iridocorneal angle to the trabecular meshwork (Figure 1d). The iris processes/strands may be attached to the Schwalbe's line and may have variable thickness. Presence of these changes should be examined with gonioscopy, whenever ARS is suspected.

Increased ocular pressure (IOP) leading to glaucoma is the major consequence of the eye dysgenesis observed in ARS, where approximately half of the patients develop secondary glaucoma.[1] Glaucoma can develop in infancy, but usually occurs in adolescence or early adulthood. In some cases it can be observed after middle age. As ARS patients are at risk of glaucoma development throughout their lives, they should be examined annually for the changes in IOP and the optic nerve head.

Systemic findings

Axenfeld–Rieger syndrome patients may have accompanying systemic features. The most characteristic features are mild craniofacial dysmorphism (Figure 2), dental anomalies and redundant periumblical skin. The midface abnormalities include hypertelorism, telecanthus, maxillary hypoplasia with flattening of the mid-face, prominent forehead, and broad, flat nasal bridge. Dental abnormalities may be small teeth (microdontia) or fewer teeth than normal. In the abdominal region, a failure of involution of the skin resulting in redundant periumbilical skin can be seen and this may be mistaken for an umbilical hernia. Hypospadia in males, anal stenosis, pituitary abnormalities and growth retardation may also be found. Systemic changes other than these are usually not considered as the classical features of ARS.

Other disorders with ASD

Idrees *et al*[3] have suggested a very useful classification of anterior segment dysgeneses based on the underlying embryological abnormality. In this classification, diseases caused by abnormal neural crest migration and differentiation are termed anterior segment dysgeneses-neural crest and these include ARS, infantile/primary congenital glaucoma (PCG), iris hypoplasia/iridogoniodysgenesis anomaly/syndrome (IH, suggested as a single disorder[2,3]), Peters anomaly, congenital hereditary endothelial dystrophy, sclerocornea and megalocornea. Anterior segment disorders of primarily non-neural crest origin include aniridia, which mainly affects the posterior iris.

Disorders of ASD, which should be considered in differential diagnosis of ARS, are IH, Peters anomaly and PCG. In IH iris hypoplasia and goniodysgenesis is present, but posterior embryotoxon or iris adhesions are not found.[3] Peters anomaly is an ASD with central absence of the corneal endothelium, Descemet's membrane and posterior corneal stroma, leading to central corneal opacity, which is not present in ARS. Cataract may also be present in Peters anomaly.[6] Patients with PCG have buphthalmos, goniodysgenesis and a high IOP causing corneal edema, photophobia and tearing, and embryotoxon or iris adhesions are normally not observed.

Embryology of the anterior segment

The anterior segment of the eye includes all the structures (cornea, iris and chamber angle) lying between the front surface of the cornea and the front surface of the vitreous. A developmental defect of these structures, which are

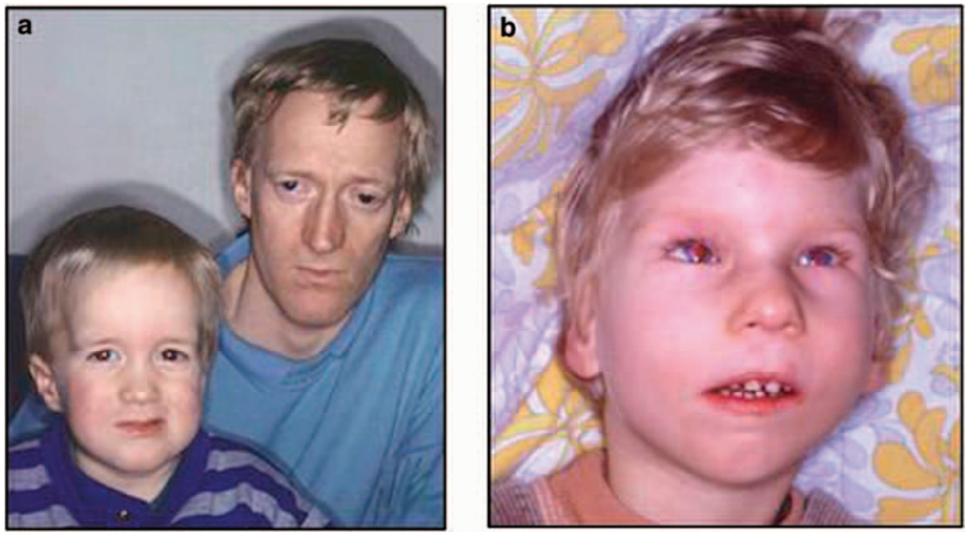

Figure 2 (**a**) Clinical photographs of father (33 years) and son (3 years) affected with Axenfeld–Rieger syndrome. (**b**) Female patient (two and half years). All the patients show similar morphological features, including telecanthus, broad nasal bridges and prominent lower lips. The father and the female patient have maxillary hypoplasia and additionally the female patient has dental anomalies with small crowns.

derived from the neural crest cells, will lead to ASD. Neural crest cells are neuroectodermal cells, which migrate from the crest of the neural tube to several sites in the developing embryo. In the eye, neural crest cells migrate to the developing anterior chamber of the eye forming the keratocytes and corneal endothelium, iris stroma cells, melanocytes, trabecular meshwork and juxtacanalicular tissue.[3,7,8] Developmental arrest that occurs late in gestation results in the neural crest cells being retained over parts of the iris and anterior chamber angle.[3] This affects the anatomy of the anterior chamber angle, eventually effecting aqueous drainage and causing glaucoma as observed in ARS. In addition, contraction of the primordial layer causes iris stromal thinning, corectopia and hole formation, all of which are malformations observed in ARS. Retention of the neural crest cells may also result in iris hypoplasia, Peters anomaly, anirida, sclerocornea, megalocornea and primary congenital glaucoma.

Neural crest cells play an important role not only in the development of the anterior segment, but they also give rise to many structures, such as bone and cartilage of the skull, teeth and dermis, which may explain involvement of other organs in ARS.

Genetic basis of ARS

The conditions that comprise ARS are inherited in autosomal dominant manner with high penetrance. ARS is genetically heterogeneous and mutations in different genes lead to similar clinical conditions. ARS has been associated with mutations of two known genes: *PITX2* (the pituitary homeobox 2 gene) at 4q25[9] and, *FOXC1* (the forkhead box C1 gene, *FKHL7*) at 6p25.[10,11] A third locus was suggested by deletion of 13q14, supported by linkage analyses, but a disease-causing gene has not been identified yet.[12,13] In two isolated cases, deletion of the 16q23-q24 region[14] and deletion of the *PAX6* gene at 11p13,[15] respectively, were related to ARS, but these findings were not supported by other studies. In ~60% of patients the genetic defect in ARS is not known (not caused by mutation of an already identified gene).[16] However, accurate estimation of the prevalence of mutations, is hampered by the fact that this is a rare condition and screening of large cohorts is not possible.

PITX2 and *FOXC1*

The two known ARS-associated genes, *PITX2* and *FOXC1*, both encode developmental transcription factors (Table 1). Transcription factors are proteins, which regulate expression of downstream target genes through binding to specific DNA sequences and activating transcription. Transcription factors play an important role in orchestrating normal embryonic development and their expression is regulated precisely in temporal and spatial patterns.

The *PITX2* gene encodes a homeodomain-containing transcription factor, which recognizes and binds to specific

Table 1 Genes associated with ARS phenotype: summary data

	PITX2	*FOXC1*
Full name	Paired-like homeodomain transcription factor 2	Forkhead box C1
Alternative symbols	*RIEG1*	*FKHL7*
Chromosomal location	4q25	6p25.3
Genomic size	20 kb	3.5 kb
Number of exons	6 (alternatively spliced)	1 (no introns)
Transcript size	2125 bp	1659 bp
mRNA transcripts	4 alternatively spliced transcripts: *PITX2A-D*	1
Tissue expression (as studied in mouse tissues)	Periocular mesenchyme, dental epithelium, first branchial arch, umbilicus, pituitary primordium and limb buds in mouse embryos	Periocular mesenchyme, prechondrogenic mesenchyme, meninges, endothelial cells and kidney in mouse embryos
Protein size	PITX2A: 271 amino acids PITX2B: 317 amino acids PITX2C: 324 amino acids	553 amino acids
Important protein domains	DNA-binding homeodomain OAR domain (*otp, aristaless, rax*)	DNA-binding forkhead domain Two activation domains Inhibitory domain
Most common mutation types	Intragenic mutations Microscopic and submicroscopic deletions Chromosome rearrangements	Intragenic mutations Microscopic and submicroscopic duplications and deletions
Associated phenotypes	Most commonly ARS without systemic changes Rare cases with iris hypoplasia/ iridogoniodysgenesis syndrome; Peters anomaly; ring dermoid of the cornea	Most commonly ARS with systemic changes Rare cases with iris hypoplasia/ iridogoniodysgenesis syndrome; Peters anomaly; primary congenital glaucoma; aniridia

DNA sequences through the homeodomain, and functions as a transcription regulator during embryogenesis and development of different tissues of the anterior segment. The gene produces four mRNA transcripts (PITX2A-D), but a translation product of PITX2D has never been detected. The three other isoforms (A–C) differ at the N terminus, but they all include the 60-amino-acid homeodomain. They all have identical C termini with a conserved 14-amino-acid OAR domain (*otp, aristaless* and *rax*), which is predicted to mediate protein–protein interactions and self-inhibitory interactions with the N terminus.[17] The smallest isoform, PITX2A (32 kDa), is the best studied with regards to ARS malformations and the *PITX2* mutations reviewed in this study are described with regards to this isoform (Supplementary Figure 1).[18]

The *FOXC1* gene is a member of the forkhead family of transcription factors that play important roles in embryogenesis, tissue-specific gene expression and tumor development. *FOXC1* recognizes and binds to specific DNA sequences through the conserved 110-amino-acid forkhead domain (FH), and thereby activates the target genes. Transactivaton function requires two activation domains, AD-1 and AD-2, and the activity of these domains is attenuated by the inhibitory domain (ID). FOXC1 is suggested to have a key role in cardiac, renal, ocular and cerebral morphogenesis.[19]

During mouse embryogenesis, both *Pitx2* and *Foxc1* are expressed in the organs and tissues affected in ARS. In the developing mouse eye, Pitx2 and Foxc1 are co-localized in periocular mesenchyme and they interact physically through crucial functional domains.[20] Furthermore, PITX2 can act as a negative regulator of FOXC1 activity, by binding to FOXC1 and repressing activation of putative FOXC1 target genes.[20] This interaction has importance in understanding the effects of the different mutations of these genes as explained below.

PITX2 defects and ARS

The most common *PITX2* defects leading to ARS are point mutations (Table 2), but several cases of chromosomal aberrations, such as interstitial deletions and/or translocations involving chromosome 4q25, have been described in patients with overlapping ARS phenotypes. Chromosome rearrangements involving *PITX2* or its surrounding genomic landscape have been observed in 10 ARS patients,[22–29] and only eight of these cases have been investigated further.[23–26,28,29] Three of the cases had deletions at the 4q25 breakpoints directly affecting *PITX2,*[25,28,29] and in five cases the translocation breakpoints were located up to 90 kb upstream of the gene.[23,24,26] In about 14 ARS patients, the genetic defect was a microscopic or submicroscopic deletion of the 4q25 region including *PITX2*.[24,28,30–39] In only few cases, the extent of the deletions was characterized by molecular means[25,28,38] and clinical features of these patients do not differ substantially from the phenotypes caused by point mutations of *PITX2*.[28] Submicroscopic duplications of *PITX2* have not been reported in ARS patients, but duplication of the distal region of 4q (including 4q25 and *PITX2*) has been described in one patient.[40]

To our knowledge, intragenic mutations of *PITX2* have been described in 41 patients and these include, missense ($n=18$), nonsense ($n=4$) and splice-site mutations ($n=5$), and deletions/insertions/duplications ($n=14$). All these mutations are described in Table 2 and shown in Supplementary Figure 1. Most of the missense mutations (15 out of 18) are within the homeodomain of the gene, which plays a major role in target DNA motif recognition and binding.

PITX2 mutations have also been associated with Peters anomaly,[41] iris hypoplasia/iridogoniodysgenesis syndrome,[42,43] and ring dermoid of the cornea,[44] but these are single cases and *PITX2* mutations are mainly detected in ARS patients with systemic changes.

FOXC1 defects and ARS

The most common *FOXC1* defects leading to ARS are point mutations (Table 3), but segmental and telomeric chromosome rearrangements of the chromosome region 6p25, including the *FOXC1* gene, occur almost at a similar prevalence.[45,46] Balanced chromosome rearrangements involving *FOXC1* or its surrounding landscape are rare, but a balanced t(6;13) translocation in an ARS patient lead to identification of *FOXC1*, as a causative gene for this disorder.[11] On the other hand, there are more than 40 deletions, either interstitial or telomeric, involving 6p25[45,47,48] and the patients frequently present with ocular, craniofacial, skeletal, cardiac, and renal malformations, hearing loss, and hydrocephalus.[47] The phenotypic variation seen in these patients are largely because of the size of the deletions and the genes involved. Ocular anomalies of the anterior segment, such as posterior embryotoxon and iris hypoplasia, are commonly observed in these patients, and these are attributed to the deletion of the *FOXC1* gene or its regulatory elements. Furthermore, interstitial duplications of *FOXC1* have also been observed in patients with ASD.[49–51]

To our knowledge intragenic mutations of *FOXC1* have been described in 46 patients and these include missense ($n=23$) and nonsense mutations ($n=6$), and deletions/insertions/duplications ($n=17$). These mutations are described in Table 3 and shown in Supplementary Figure 2. All the missense mutations, except one, are within the forkhead domain and many of these mutations were investigated functionally (Table 3). *FOXC1* mutations are mainly detected in ARS patients without extraocular changes, but *FOXC1* mutations have also been detected in patients with systemic abnormalities (Table 3). Furthermore, *FOXC1* mutations are also detected in rare cases of Peters anomaly,[52,53] iris hypoplasia/iridogoniodys-

Table 2 *PITX2* intragenic mutations, their effects on protein function and associated phenotype

Mutation	*Exon*	*Domain*	*Phenotype*[a]	*Effect on protein function*	*References*[b]
Missense mutations					
p.Arg43Trp	5	HD	ARS		Idrees *et al*[3]
p.Leu54Gln	5	HD	ARS	Unable to bind DNA and deficient transactivation.	Semina *et al*[9] Amendt *et al*, 1998 Amendt *et al*[17] Kozlowski and Walter[68]
p.Phe58Leu	5	HD	ARS		Vieira *et al*, 2006
p.Arg62His	5	HD	ARS (1) Ring dermoid of the cornea (1)		Amendt *et al*[17] Xia *et al*[44]
p.Pro64Leu	5	HD	ARS (2) ARS +Sella turcica anomaly (1)		Phillips *et al*, 2002 Weisschuh *et al*, 2006 Meyer-Marcotty *et al*, 2008
p.Pro64Arg	5	HD	ARS		Weisschuh *et al*, 2006
p.Thr68Pro	5	HD	ARS	Unable to bind DNA and deficient transactivation. *Pitx2* cannot transactivate *Dlx2* promoter leading to abnormal tooth development.	Semina *et al*[9] Amendt *et al*, 1998 Amendt *et al*[17] Kozlowski and Walter[68] Espinoza *et al*, 2002 Saadi *et al*, 2001
p.Arg69His	5	HD	Iridognio-dysgenesis syndrome	Reduced DNA-binding activity.	Kulak *et al*[43] Amendt *et al*[17] Strungaru *et al*[69] Kozlowski and Walter[68]
p.Val83Leu	5	HD	ARS	Gain-of-function mutation: decreased DNA binding, but increased transactivation.	Priston *et al*[67]
p.Arg84Trp	5	HD	Iris hypoplasia	Reduced DNA binding and transactivation. *Dlx2* promoter can be transactivated.	Alward *et al*[42] Amendt *et al*[17] Kozlowski and Walter[68] Espinoza *et al*, 2002
p.Trp86Cys	6	HD	ARS		Li *et al*, 2008
p.Lys88Glu	6	HD	ARS (2)	Defective DNA binding and transactivation, but has a dominant negative effect on wild-type protein.	Amendt *et al*[17] Perveen *et al*, 2000 Saadi *et al*, 2001
p.Arg90Cys	6	HD	ARS		Perveen *et al*, 2000
p.Arg90Pro	6	HD	ARS		Phillips *et al*, 2002
p.Arg91Pro	6	HD	ARS (2)	Unable to bind DNA and deficient transactivation	Semina *et al*[9] Amendt *et al*, 1998 Amendt *et al*[17]Priston *et al*[67] Kozlowski and Walter[68]
p.Leu105Val	6		ARS		Phillips *et al*, 2002
p.Asn108Thr	6		ARS		Phillips *et al*, 2002
p.Gly137Val	6		ARS+Fuchs' endothelial dystrophy		Kniestedt *et al*, 2006
Nonsense mutations					
p.Glu55X	5	HD	ARS		Vieira *et al*, 2006
p.Trp94X	6	HD	ARS		Amendt *et al*[17]
p.Tyr121X	6		ARS		Vieira *et al*, 2006
p.Trp133X	6		ARS	Gain-of-function: increased DNA binding, transactivation and dimerization.	Semina *et al*[9] Amendt *et al*[17] Saadi *et al*, 2006
Splice-site mutations					
c.47-1G>T	IVS4		ARS (2)	Severly truncated, poorly expressed protein	Lines *et al*, 2004 Maciolek *et al*, 2006 Strungaru *et al*[69]
c.47-1G>C	IVS4		ARS		Perveen *et al*, 2000
c.252+5G>C	IVS5		ARS (1) Only ocular changes (1)	Poorly expressed truncated protein	Semina *et al*[9] Amendt *et al*[17] Maciolek *et al*, 2006
c.253-2A>T	IVS5		ARS		Doward *et al*[41] Perveen *et al*, 2000

Table 2 (*Continued*)

Mutation	*Exon*	*Domain*	*Phenotype*[a]	*Effect on protein function*	*References*[b]
c.253-11A>G	IVS5		ARS (2)	Protein with truncated HD is expressed at same levels as wild-type protein	Semina *et al*[9] Amendt *et al*[17] Borges *et al*, 2002 Maciolek *et al*, 2006
Deletions/insertions					
c.114delG	5		Only ocular changes		Lines *et al*, 2004 Strungaru *et al*[69]
c.134_137delACTT	5	HD	ARS		Wang *et al*, 2003
c.151_171dup21	5	HD	ARS	Reduced DNA-binding activity; no detectable transactivation	Priston *et al*[67]
c.160-252_253-734del575	5-6	HD and OAR	ARS		De la Houssaye[70]
c.285_286delAA	6	HD	ARS		Perveen *et al*, 2000
c.356delA	6		ARS		Perveen *et al*, 2000
c.366delC	6		ARS	Gain-of-function: increased DNA binding, transactivation and dimerization.	Saadi *et al*, 2006
c.416delC	6		ARS		Lines *et al*, 2004 Strungaru *et al*[69]
c.500_501insC	6		ARS		Perveen *et al*, 2000
c.del652_653insAAG	6		ARS		Perveen *et al*, 2000
c.669_670insCGACTCCT	6		ARS		Vieira *et al*, 2006
c.679delT	6		ARS	Disrupted phosphorylation and protein interaction	Brooks *et al*, 2004 Espinoza *et al*, 2005
c.690delG	6		ARS		Borges *et al*, 2002

The mutations described in this table are shown on the *PITX2* cDNA sequence in Supplementary Figure 1. The nucleotide numbering is relative to the coding DNA sequence of *PITX2A*, wherein nucleotide +1 is the A of the ATG translation initiation codon.
The mutations are described as suggested by den Dunnen and Antonarakis.[21] Some of the published mutations are therefore re-described accordingly.
HD, homeodomain; OAR, OAR domain; ND, not determined.
[a]Number of unrelated patients/families are given in parenthesis.
[b]Complete reference list for mutations and functional studies can be found in Supplementary list 1.

genesis syndrome,[10,49,50] primary congenital glaucoma[54] and aniridia.[55]

Disease causing mechanisms of *PITX2* and *FOXC1* mutations

The mutations observed for *PITX2* and *FOXC1* show a broad spectrum suggesting that different disease-causing mechanisms lead to ASD.

Both PITX2 and FOXC1 are dosage sensitive and alteration in the level of functional protein (either increased or decreased) is a disease-causative mechanism. Microscopic or submicroscopic deletions of both *PITX2* and *FOXC1* are known to result in ARS through haploinsufficiency (presence of a single copy of the gene, in which the other copy is inactivated because of mutation). Furthermore, several studies have shown that duplication of *FOXC1* may also lead to ARS phenotype. Berry *et al*[20] have shown that FOXC1 transcriptional activity was negatively regulated by PITX2 and this explains how contrasting mutations (duplication of *FOXC1* and deletion of *PITX2*) lead to similar phenotypes: PITX2 binds to FOXC1 and this represses activation of putative FOXC1 target genes. When *PITX2* is mutated, the FOXC1 target genes are activated leading to ARS. Duplication of *FOXC1* may also have a similar effect, through overcoming the repression activity of PITX2. Identification of target genes regulated by PITX2 and FOXC1 will enable further understanding of the underlying ARS pathologies.

Chromosomal rearrangements some 90 kb upstream of *PITX2* may also be pathological and this is likely to be because of removal of some regulatory elements from the gene, leading to the reduction of gene expression.[56] Such long-range position effects have previously been suggested for other disorders[57] and interestingly *FOXC1* has also been associated with long-range position effects.[58]

Some of the missense mutations leading to amino acid substitutions and other types of mutations have been investigated by functional studies and these mutations alter the protein function in varying degrees (Tables 2–3 and Supplementary Figures 1–2). Most of the intragenic *PITX2* mutations are loss-of-function mutations, which result in defective DNA binding, decreased transactivation capability of downstream genes, or both (Table 2). However gain-of-function mutations of *PITX2* were also detected (Table 2) and this suggests that excess PITX2 may also lead to ARS. A single mutation with a dominant negative effect on the wild-type protein has also been described (Table 2). Similarly, missense mutations of *FOXC1* were shown to effect the nuclear localization, DNA-binding ability and

Table 3 *FOXC1* intragenic mutations, their effects on protein function and associated phenotype

Mutation	*Domain*	*Phenotype*[a]	*Effect on protein function*[b]	*References*[c]
Missense mutations				
p.Pro79Arg	FH	Eye+micrognathia		Weisschuh *et al*, 2006
p.Pro79Leu	FH	Eye	Nuclear localization ++ DNA-binding capacity + Transactivation +	Nishimura *et al*[50] Saleem *et al*, 2003a Saleem *et al*, 2004
p.Pro79Thr	FH	ARS	Nuclear localization ++ DNA-binding capacity + Transactivation +/−	Suzuki *et al*, 2001 Saleem *et al*, 2003a Saleem *et al*, 2004
p.Ser82Thr	FH	Eye+hearing loss+heart defect	Nuclear localization +++ DNA-binding capacity + Transactivation +	Mears *et al*[10] Strungaru *et al*[69] Saleem *et al*, 2001 Saleem *et al*, 2003a Saleem *et al*, 2004
p.Ala85Pro	FH	Eye+heart defect		Fuse *et al*, 2007
p.Leu86Phe	FH	Eye (2) Eye+obesity +short stature +myocardial infarction +dental abnormality	Nuclear localization +++ DNA-binding capacity ++ Transactivation−	Strungaru *et al*[69] Saleem *et al*, 2003a Saleem *et al*, 2003b Saleem *et al*, 2004
p.Ile87Met	FH	Eye	Reduced protein stability	Mears *et al*[10] Strungaru *et al*[69] Saleem *et al*, 2001 Saleem *et al*, 2003a Saleem *et al*, 2004
p.Ile91Ser	FH	Eye	Nuclear localization – DNA-binding capacity + Transactivation ––	Kawase *et al*, 2001 Saleem *et al*, 2003a Saleem *et al*, 2004
p.Ile91Thr	FH	Eye	Nuclear localization + DNA-binding capacity + Transactivation ––	Mortemousque *et al*, 2004 Saleem *et al*, 2003a Saleem *et al*, 2004
p.Phe112Ser	FH	Eye+heart defect ARS and Peters anomaly	Nuclear localization+++ DNA-binding capacity+++ Transactivation −	Nishimura *et al*[11] Swiderski *et al*[65] Saleem *et al*, 2001 Saleem *et al*, 2003a Saleem *et al*, 2004 Honkanen *et al*[52]
p.Tyr115Ser	FH	Eye+hearing loss		Weisschuh *et al*, 2006
p.Ile126Met	FH	Eye	Nuclear localization +++ DNA-binding capacity +++ Transactivation −	Nishimura *et al*[11] Saleem *et al*, 2001 Saleem *et al*, 2003a Saleem *et al*, 2004
p.Arg127His	FH	Eye+hypertelorism	Nuclear localization – DNA-binding capacity – Transactivation –	Kawase *et al*, 2001 Saleem *et al*, 2003a Saleem *et al*, 2004
p.His128Arg	FH	Primary congenital glaucoma		Chakrabarti *et al*[54]
p.Leu130Phe	FH	Eye Eye+umbilicus +hypertelorism	Significant impairment of nuclear localization, DNA-binding capacity and transactivation	Strungaru *et al*[69] Ito *et al*, 2007
p.Ser131Leu	FH	Eye (2)	Nuclear localization ++ DNA-binding capacity− Transactivation –	Nishimura *et al*[11] Nishimura *et al*, 2001 Saleem *et al*2001 Saleem *et al*, 2003a Saleem *et al*, 2004
p.Cys135Tyr	FH	Primary congenital glaucoma		Chakrabarti *et al*[54]
p.Gly149Asp	FH	Eye+hypospadia+heart defect		Weisschuh *et al*, 2006
p.Met161Val	FH	Eye+umbilicus+hearing loss		Weisschuh *et al*, 2006
p.Met161Lys	FH	Eye (2) Iridogoniodysgenesis anomaly	Nuclear localization +++ DNA-binding capacity ++ Transactivation +/−	Panicker *et al*, 2002 Komatireddy *et al*, 2003 Saleem *et al*, 2003a Saleem *et al*, 2004 Murphy *et al*, 2004 Khan *et al*, 2008
p.Gly165Arg	FH	Eye +dental abnormality	Normal DNA-binding Nuclear localization +++ DNA-binding capacity +++ Transactivation +/−	Murphy *et al*, 2004 Strungaru *et al*[69] Saleem *et al*, 2004

Table 3 (*Continued*)

Mutation	*Domain*	*Phenotype*[a]	*Effect on protein function*[b]	*References*[c]
p.Arg169Pro	FH	Eye +hearing loss	Nuclear localization +++ DNA-binding capacity + Transactivation +/−	Murphy *et al*, 2004 Strungaru *et al*[69] Saleem *et al*, 2004
p.Pro297Ser	ID	Eye		Strungaru *et al*[69]
Nonsense mutations				
p.Gln2X	AD-1	Eye		Komatireddy *et al*, 2003
p.Gln23X	AD-1	ARS		Mirzayans *et al*, 2000 Strungaru *et al*[69]
p.Ser48X	AD-1	Eye		Weisschuh *et al*, 2006
p.Gln120X	FH	ARS and Peters anomaly		Weisschuh *et al*[53]
p.Gln123X	FH	Eye		Komatireddy *et al*, 2003
p.Trp152X	FH	ARS		Cella *et al*, 2006
Deletions/insertions				
c.26_47ins22	AD-1	Eye		Nishimura *et al*, 2001[50] Kawase *et al*, 2001
c.81_89del9	FH	Primary congenital glaucoma (found also in unaffected) +CYP1B1 mutation (R368H)		Chakrabarti *et al*[54]
c.93_102del10	FH	Eye		Mears *et al*[10] Strungaru *et al*[69]
c.99_108del10	FH	Eye		Nishimura *et al*[50]
c.116_123del8	FH	Eye		Nishimura *et al*[50]
c.153_163del11	FH	Eye		Nishimura *et al*[11]
c.210delG		Eye+hearing loss or heart defect		Swiderski *et al*, 1999
c.264_265insC	ID	Eye		Nisihmura *et al*, 2001
c.286_287insG	ID	Eye+hypertelorism		Kawase *et al*, 2001
c.363delC	ID	Eye		Strungaru *et al*[69]
c.437_453del17	AD-2	Eye+hypertelorism+ telecantus+hearing loss		Fuse *et al*, 2007
c.718_719delCT		Eye		Cella *et al*, 2006
c.738delG		Eye+hypertelorismus+umblicus		Weisschuh *et al*, 2006
c.848_871dup25		Primary congenital glaucoma		Chakrabarti *et al*[54]
c.1086delC		Primary congenital glaucoma (found also in unaffected) +CYP1B1 mutation (R368H)		Chakrabarti *et al*[54]
c.1511delT		Eye		Weisschuh *et al*, 2009
c.1512delC		Eye		Weisschuh *et al*, 2006

The mutations described in this table are shown on the *FOXC1* cDNA sequence in Supplementary Figure 2. The nucleotide numbering is relative to the coding DNA sequence of *FOXC1*, wherein nucleotide +1 is the A of the ATG translation initiation codon.
The mutations are described as suggested by den Dunnen and Antonarakis.[21] FD, forkhead domain; ID, inhibitory domain.
[a]Number of unrelated patients/families are given in parenthesis; Eye, only ocular symptoms; unrelated patients with different phenotypes are listed separately.
[b]Functional studies are summarized as described by Saleem *et al*, 2004. Nuclear localization is compared with wild-type FOXC1 with +++ indicating 81–100% of the cells showing exclusive nuclear localization; ++, 61–80%; +, 41–60%; +/−, 21–40%; and −,0–20%. DNA binding is compared to wild-type FOXC1 with +++indicating wild-type or near wild-type levels; ++, 2–4-fold reduction; +, 5–9-fold reduction; +/−;10-fold reduction; -, >10-fold. Scales for transactivation are the same as those used for nuclear localization.
[c]Complete reference list for mutations and functional studies can be found in Supplementary list 2.

transactivation function of the protein in varying degrees (Table 3).

Phenotypic features of Foxc1 and Pitx2 knockout mice

Homozygous null mutants of *Pitx2* generated from a targeted null allele (*Pitx2*$^{-/-}$) exhibit septal and valve defects, single atrium, abnormal cardiac positioning, pulmonary isomerism, omphalocele, early arrest in pituitary development, defect in tooth organogenesis, defective development of the mandibular and maxillary facial prominences and multiple eye defects.[59–61] Heterozygote *Pitx2*$^{+/-}$ mice have thinning of the ventral body, small body size, eye and tooth defects as described in some studies,[59,60] whereas in other studies heterozygote alleles do not show an obvious haplosufficiency phenotype.[61] Overexpression of Pitx2 in mouse corneal mesenchyme

and iris results in corneal opacification, corneal hypertrophy, irido-corneal adhesions and severely degenerated retina.[62]

Most homozygous null mutants of *Foxc1*, generated either from a targeted allele (*Foxc1*$^{-/-}$) or the spontaneous mutation congenital hydrocephalus (*Foxc1*$^{ch/ch}$) die pre- and perinatally with hemorrhagic hydrocephalus and severe skeletal, cardiovascular and ocular defects.[63] The heterozygote *Foxc1*$^{+/-}$ and *Foxc1*$^{ch/+}$ mice have anterior segment abnormalities similar to those observed in patients. These include eccentric and irregularly shaped pupils, iris hypoplasia, displaced Schwalbe's line and abnormal aqueous humor drainage structures, but IOP is not elevated. Interestingly, the penetrance of clinical abnormalities in mice depends on their genetic background, which also would explain the clinical variation observed in ARS patients with similar mutations.[64] Even though Foxc1 is suggested to play a critical role in the formation of the atrial septum and cardiac valves during embryogenesis,[65] *Foxc1*$^{+/-}$ mutants do not show cardiovascular abnormalities.[66]

Genotype–phenotype correlations

Axenfeld–Rieger syndrome is characterized by complete penetrance, but disease severity shows variability. The same mutation may result in different manifestations not only in unrelated patients, but also within the same family. In general, ARS patients who display defects in other organ systems, such as teeth or umbilicus, have mutations of the *PITX2* gene; meanwhile, in patients with isolated ASD, mainly *FOXC1* mutations are detected.

There is no obvious correlation between the localization of the mutation in the *PITX2* gene and the severity of the phenotype (Table 2). For example, the Arg62His mutation within the homeodomain may lead to both ARS and ring dermoid of cornea (Table 2). However, functional studies give clues to the effect of the mutation on the phenotypic outcome (Table 2). An illustrative example is the two consecutive missense mutations within the homeodomain (Val83Leu and Arg84Trp), which lead to ARS and iris hypoplasia, respectively. Functional studies showed that the Val83Leu mutation was a gain-of-function mutation,[67] whereas the Arg84Trp mutation resulted in reduced DNA-binding and transactivation,[68] giving a plausible explanation to the differences in the clinical severities. The characterized *PITX2* missense mutations imply that the severity of the clinical phenotype is proportional to the residual function of the mutant PITX2 proteins.[68] In heterozygotes, 50% of the function will result in severe ARS form, but additional activity contributed by mutant allele may result in milder forms.[68]

FOXC1 mutations are mainly detected in patients with isolated ASD, but systemic abnormalities have also been described (Table 3). The most common extraocular changes observed are heart abnormalities and hearing loss. As for the *PITX2* gene, the same *FOXC1* mutation may lead to different clinical manifestations. For example, the Leu86Phe mutation leads to ocular changes in one patient; meanwhile, another patient has several systemic abnormalities, including obesity, short stature, myocardial infarction and dental abnormality (Table 3). Functional studies of the *FOXC1* missense mutations do not imply a strong correlation between the protein function and the phenotype (Table 3). Strungaru *et al*[69] suggest that patients with *FOXC1* duplication have a more severe prognosis in glaucoma development compared to patients with intragenic *FOXC1* mutations.

Diagnostic approaches

Clinical diagnosis

In the clinic, ARS patients are mainly diagnosed by the ophthalmologists and the diagnosis remains primarily clinical. As systemic changes are occasional findings of ARS, it will be useful to examine the patient for further changes, such as face and tooth abnormalities (microdontia, hypodontia, oligodontia and adontia), redundant periumbilical skin and facial dysmorphism to facilitate the diagnosis. In case of an ASD, it is important to examine the patient annually with slit lamp examination, including gonioscopy, IOP measurements and funduscopy to assess the retinal nerve fiber layer and optic nerve head, evaluating if the patient develops glaucoma. Autoperimetry (automated measurements of the visual fields) is necessary whenever glaucoma is suspected. As ARS is a congenital disorder, the patient may be very young raising problems in ophthalmological examinations and may necessitate application of general anesthesia.

Genetic diagnosis

A patient with ARS may have a mutation in either of the genes, *PITX2* or *FOXC1* (Figure 3). Finding of a systemic change is suggestive of a *PITX2* mutation, but *FOXC1* mutations cannot be excluded from patients with systemic symptoms.

As the mutation spectrum for both *PITX2* and *FOXC1* is very broad, several different methods should be applied for the genetic diagnosis of ARS. The most frequent mutations of *PITX2* or *FOXC1* are intragenic mutations, which can normally be detected by direct sequencing using patient DNA. Microscopic or submicroscopic deletions (for *PITX2* and *FOXC1*) or duplications (for *FOXC1*) can be investigated using FISH, quantitative PCR or MLPA (multiplex ligation-dependent probe amplification). High-resolution array-CGH (microarray-based comparative genomic hybridization) may be an advantageous alternative as it is possible to investigate both *PITX2* and *FOXC1* at the same time with this technology and this may also give the

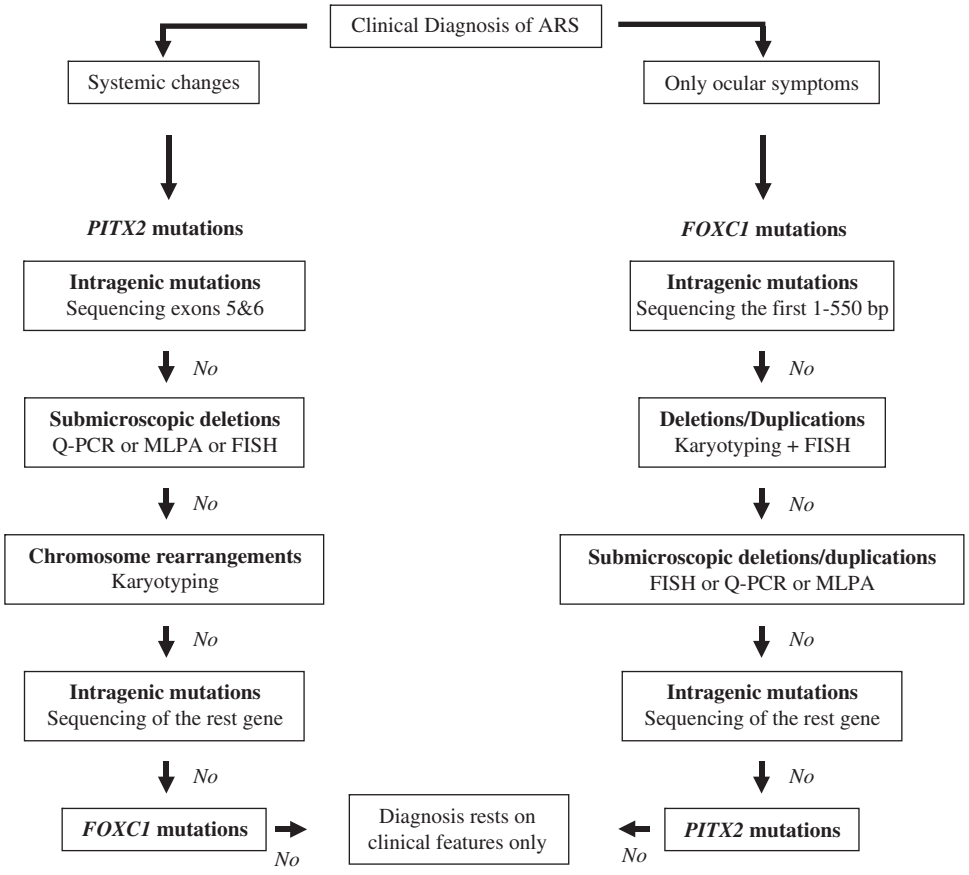

Figure 3 Diagnostic strategy for ARS. A mutation involving PITX2 or FOXC1 can be found in about 40%, leaving the diagnosis in 60% of the patients to rest on clinical features only. Mutation screening of exons 5 and 6 of *PITX2*; and the first 550 bp of *FOXC1* is recommended initially as these regions code for the DNA-binding domains of the respective genes and they are the hot spots for mutations.

opportunity to isolate a yet unidentified gene associated with ARS. As a substantial number of *PITX2* mutations are balanced chromosome rearrangements, classical chromosome analyses should also be carried out in cases, wherein a mutation cannot be detected by the above mentioned methods.

The underlying genetic defect is unknown in ~60% of ARS patients and partial gene deletions of *PITX2* or *FOXC1* may be the causative defect in a number of cases. This is illustrated by the finding of an intragenic deletion of *PITX2* in an ARS patient using quantitative genomic PCR analysis.[70] This deletion can neither be detected by mutation screening because of the presence of the wild-type allele, nor by FISH analysis and array-CGH because of the resolution limit. MLPA may be a suitable method, as it enables to perform multiplex PCR reactions in which several specific sequences are simultaneously quantified. Using this method, it is possible to detect both the submicroscopic chromosome rearrangements and intragenic deletions/duplications of *PITX2* and/or *FOXC1* simultaneously in ARS patients.

Treatment

The most important symptom that necessitates treatment in ARS is glaucoma. In case of glaucoma, medical therapy is recommended before the initiation of surgical intervention. Medications that decrease aqueous output (β-blockers, α-agonists and carbonic anhydrase inhibitors) are more beneficial than those affecting outflow. However, α-agonists should be used with caution in young children because of the potential for CNS depression.[71] If medical therapy is not enough, procedure of choice is trabeculectomy with the adjunctive use of antimetabolites. In case of congenital glaucoma, surgery is preferred. In addition, if photophobia is present in patients with corectopia and polycoria, they may use contact lenses to cover the holes in the iris.

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