Heliyon 8 (2022) e12543

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The clinical and genetic findings in a Chinese family with Axenfeld-Rieger syndrome

Lingyan Cheng [a,1], Yinong Zhang [a,1], Yuzhi Ding [b], Zhilan Yuan [c], Xiao Han [d,*]

[a] Department of Ophthalmology, The Affiliated Wuxi No. 2 People's Hospital of Nanjing Medical University, No. 68 Zhongshan Road, Wuxi, Jiangsu, 214000, People's republic of China
[b] Department of Ophthalmology, Zhongda Hospital Southeast University, No. 87 Dingjiaqiao, Nanjing, Jiangsu, 210000, People's republic of China
[c] Department of Ophthalmology, Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital, No. 300 Guangzhou Road, Nanjing, Jiangsu, 210029, People's republic of China
[d] Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, No. 818 Tianyuan Road, Nanjing, Jiangsu, 211166, People's republic of China

ARTICLE INFO

Keywords:
Axenfeld-rieger syndrome
Exome sequencing
PITX2 splicing mutation

ABSTRACT

Objecive: To describe the clinical and genetic findings of an Axenfeld-Rieger syndrome (ARS) family with a new *PITX2* splicing mutation.

Methods: A Chinese ARS family with five affected individuals was recruited. Exome sequencing was performed on the proband and the variant (C.253-9C > A) in *PITX2* gene was detected as a pathogenic mutation. Sanger sequencing was performed for verification and cosegregation analysis. Real-time polymerase chain reaction (RT-PCR) and Western blotting were performed to verify the expression of the pathogenic gene.

Results: All the patients showed abnormalities in the anterior segment of both eyes including posterior embryotoxon, corectopia, iris dysplasia, and iridocorneal tissue adhesions. In addition, they all presented systemic features, including maxillary hypoplasia, underbite, hypodontia, conical teeth. Only III-7 showed obvious umbilical skin. In the *PITX2* family, we identified a novel heterozygous splicing mutation (C.253-9C > A) which was confirmed by Sanger sequencing to be completely cosegregated with the ARS phenotype. Real-time quantitative PCR and Western results showed that *PITX2* mRNA and protein expression were significantly lower in patients compared with unrelated normal controls.

Conclusion: In the ARS pedigree, we summarized the variable phenotype, described a novel *PITX2* splicing mutation which expand the genetic spectrum of ARS. We further confirmed the possibility of development of ARS induced by this *PITX2* gene deficiency.

1. Introduction

Axenfeld-rieger syndrome (ARS) is a clinically rare autosomal dominant genetic disorder that can also be sporadic, with a global incidence of 1:200,000 [1]. A similar case was first described in 1934 by German ophthalmologists Theodor Axenfeld and Rieger. It has since become known as a series of diseases characterized by ocular, craniofacial, dental, and periumbilical abnormalities. According to the clinical characteristics, Axenfeld-Rieger syndrome is generally divided into three subtypes: Axenfeld abnormality, which is manifested by obvious thickening and advanced Schwalbe line (posterior embryotoxon), anterior iris adhesion to Schwalbe line and iris stroma dysplasia, often binocular onset; Rieger's abnormality, combined with polypupil or pupil ectopic on the basis of Axenfeld's abnormality; Rieger syndrome refers to the presence of Rieger abnormality accompanied by systemic dysplasia, such as small teeth, sparse or absent teeth, zygomatic dysplasia, underbite, umbilical skin, hearing abnormality, cardiovascular, neurological and skeletal abnormalities.

It is known that important structures of the eye, such as the ciliary body, cornea and iris stroma, depend on adequate migration of neural crest (NC) cells during early embryonic development, and AR syndrome is characterized by abnormal migration of NC cells. These abnormalities are regulated by transcription factors in different genes [2, 3]. In the third trimester of pregnancy, the original endothelium covering the cornea

* Corresponding author.
E-mail address: hanxiao@njmu.edu.cn (X. Han).
[1] These two authors contribute equally to this work.

https://doi.org/10.1016/j.heliyon.2022.e12543
Received 13 July 2022; Received in revised form 5 November 2022; Accepted 14 December 2022

undergoes reabsorption, and disruption of this process may lead to the appearance of posterior corneal embryonic ring and abnormal iris insertion, resulting in polypupil or uveal ectropy. At the same time, abnormal anterior chamber development may affect schlemm tube development, leading to abnormal outflow of aqueous outflow and increasing the risk of glaucoma. It is reported that glaucoma occurs in about 50% of AR patients, and these patients can become completely blind within a few years [4]. Studies have found that abnormal migration of NC associated with ARS often affects vestibular function, which may lead to hearing loss in some ARS patients.

Clinically, the phenotype of ARS varies greatly among cases and even between two eyes of the same patient [5, 6, 7]. Hence, AR syndrome has significant atypism and clinical heterogeneity, which often makes diagnosis more difficult. In recent years, with the development of exome sequencing technology, genetic testing has gradually become a powerful tool for clinical diagnosis of this disease. Currently, five chromosomal loci associated with ARS have been identified, which are 4q25, 6p25, 11p13, 13q14, and 16q24, including *PITX2*, *FOXC1, PAX6, and FOXF1* genes [8]. About 40% of ARS cases have mutations in *PITX2* and *FOXC1* genes [9, 10]. In general, mutations in the *PITX2* gene appear to be more likely to be associated with eye, tooth and umbilical abnormalities, while *FOXC1* mutations appear to be associated with isolated eye or ocular, heart and/or hearing defects [11, 12, 13]. These mutations in *FOXC1* include intragenic mutations, microscopic and submicroscopic deletions, and duplications, which plays an important roles in embryogenesis, tissue-specific gene expression, and tumor development [14]. P*ITX2* mutations, which include intragenetic mutations, microscopic and submicroscopic deletions, and chromosomal rearrangements such as translocations, play an important role in the genetic control of development, particularly in pattern formation and cell fate determination [15]. Most mutations in *PITX2* are loss-of-function mutations, resulting in defective DNA binding or/and reduced transactivation ability of downstream genes. Both *PITX2* and *FOXC1* are dose sensitive; changes in the levels of functional proteins (elevated or decreased) are one of the mechanisms of disease [5]. Meanwhile, *PITX2a* and *FOXC1* co-localize in the same nuclear subregion, and in the same conduction pathway, *PITX2* binds to *FOXC1* as a negative regulator and inhibits the activity of *FOXC1* target genes. Once *PITX2* mutation can activate the functional activity of *FOXC1*. This may explain why *FOXC1* replication and *PITX2* deletion cause the same phenotype [16].

Despite extensive work, only a preliminary understanding of the molecular pathogenesis of ARS has been achieved. Given the cost and acceptance of gene detection and the level of pathogenic gene analysis, the potential genetic defects in about 60% of ARS cases are still unknown. The database of mutated genes discovered and established through research is limited, which needs to be improved. In our study, we identified a new gene mutation in a Chinese ARS family (C.253-9C > A) and described the clinical characteristics of these patients.

2. Materials and methods

2.1. Subjects and clinical evaluation

The present study included a Chinese ARS family who presented to the clinic in 2022. All subjects received standard ophthalmic examination, including assessment of best corrected visual acuity (BCVA), Goldmann tonometry, slit-lamp biomicroscopy and gonioscopy, and indirect ophthalmoscope fundus examination. Other clinical examinations including optical coherence tomography (OCT, Clinico, RTVUE XR, USA), anterior segment photography (7S-1, Topcan, Japan), ultrasonic biological microscopy (UBM, AVISO, Echograph Class 1-Type B, France), and Carl Zeiss Humphrey visual field analyzer (HFA 750I, USA) were assessed. All patients were examined by the same physician for eye, dental, abdomen and hearing abnormalities. Color echocardiography was performed in all patients to determine the presence of cardiovascular outflow tract malformations. None of these patients had pituitary abnormalities with developmental delay. The diagnosis of ARS is based on history, clinical features and genetics. In addition, 150 normal controls were recruited for DNA extracts in the study. This study was performed after obtaining approval from the Bioethics Committee of The Affiliated Wuxi No. 2 People's Hospital of Nanjing Medical University and in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All subjects provided written informed consent for the examination (including extraction of their DNA) and use of their clinical data for publication.

2.2. Gene amplification, capture and sequencing

The main process of target region acquisition and sequencing was to use the capture chip to enrich the DNA fragments of the target region and then sequence with the help of high-throughput second-generation sequencing platform. Target sequence capture chips (MyGenostics, GenCap) was used to capture all exons of 23,000 genes. Whole exome sequencing was performed on the proband (IV-2). The specific process of capture was as follows: the genomic DNA was randomly broken into fragments, linked with the mixture of illumina PE junction oligonucleotides, the product was amplified and purified by link-mediated polymerase chain reaction (ligation-mediated Polymerase chain reaction, LM-PCR), and the quality of the DNA library was detected. The above PCR products were hybridized with the target region capture chip to enrich the target region sequence. With the help of Illumina Next 500 sequencing platform, the captured sequences were sequenced, and the original data was processed preliminarily, including image recognition and sample discrimination.

2.3. Bioinformatics analysis

After removing the contamination and joint sequences from the original sequencing data, we compared the filtered sequences to the human genomic reference sequence (hg19) in NCBI database using BWA software (http://bio-bwa.sourceforge.net/), and used GATK software (https://software.broadinstitute.org/gatk/) to analyze single nucleotide variation (single nucleotide variation, SNV) and insertion deletion mutation (inserts and deletions). INDEL). Then all SNP and INDEL were annotated by ANNOVAR software (http://annovar.openbioinformatics.org/en/latest/). The mutation sites with frequency less than 0.05 were screened out in the normal human database, including the Thousand Human Genome Project (http://www.1000genomes.or/), Exome Variant Server (http://evs.gs.washington.edu/EVS/) and EXAC (http://exac.broadinstitute.org/). Missense mutations SIFT (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/) and GERP++ (http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html) were used to predict the pathogenicity and conservatism, and the changes of shear sites were analyzed by SPIDEX (http://www.deepgenomics.com/spidex) software. The variant (C.253-9C > A) in *PITX2* was ultimately detected as the pathogenic gene. Then we used sanger sequencing to performed the verification and cosegregation analysis.

2.4. RNA expression analysis by real-time quantitative PCR

Total RNA was extracted from peripheral blood lymphocytes of three patients and three unrelated healthy controls using TRIzol reagent (Thermo Scientific, Inc., Waltham, MA, USA). CDNA was synthesized from 0.1 μg total RNA using RevertAid First Strand cDNA Synthesis Kit (Vazyme Biotech Co., Ltd., China) and Oligo (dT) primers. Applied Biosystems®StepOne™Plus REAL-TIME PCR system (Thermo Scientific, Inc., Waltham, MA, USA) Real-time quantitative PCR was performed using Maxima SYBR Green qPCR Master Mixes (Thermo Scientific, Inc., Waltham, MA, USA). Data was normalized to β-actin and analyzed by contrast CT.

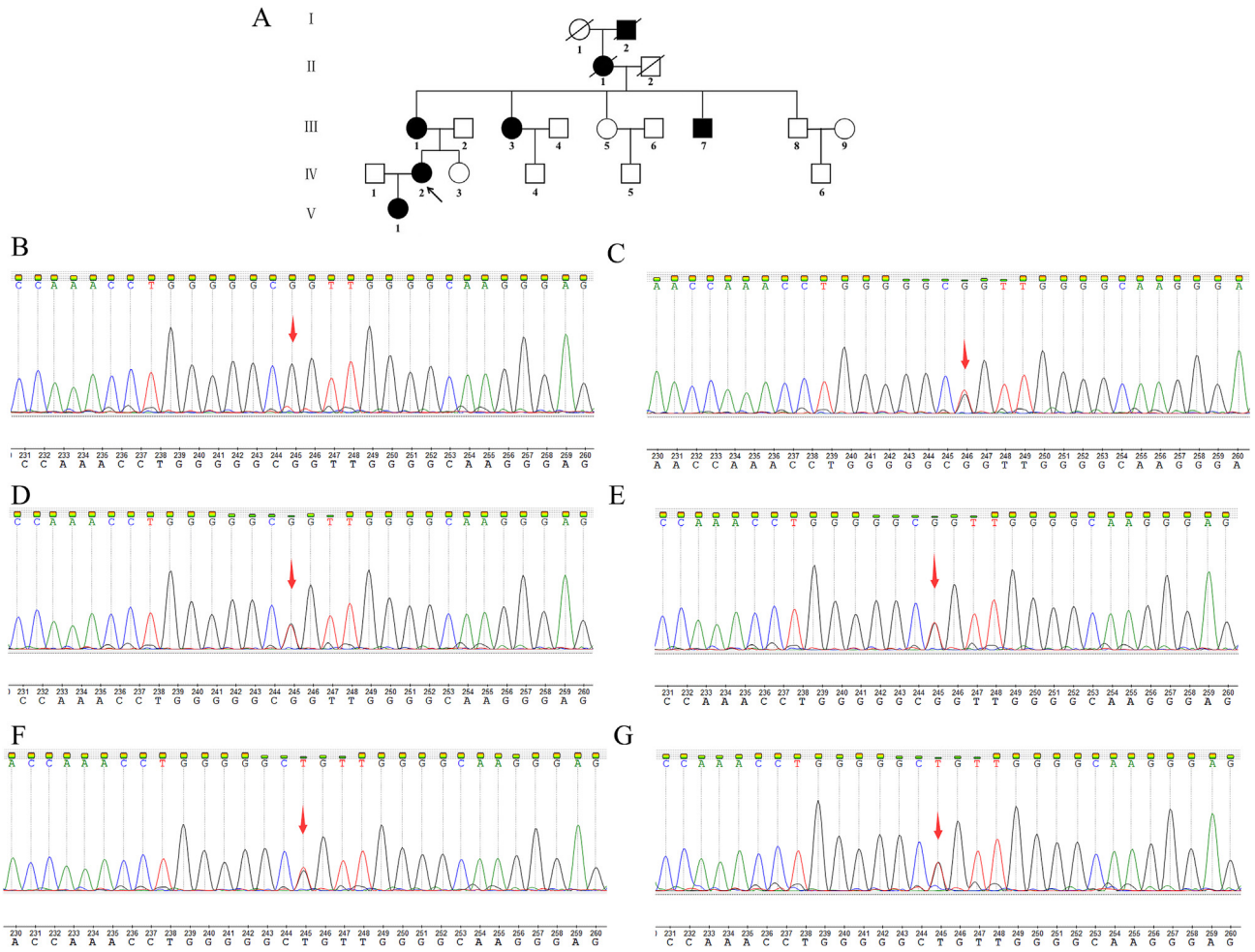

Figure 1. (A) Pedigrees of the Chinese Axenfeld-Rieger syndrome family. Proband IV-2 is noted with an arrow. (B–G) The DNA sequence on chr4:111539853 (NM_153427 exon5 c. 253-9C > A) in normal control (B) and the patients (C, III-1; D, III-2; E, III-7; F, IV-2; G, V-1).

2.5. Western Blotting and antibodies

The buffy coat of the blood was harvested and lysed in RIPA buffer. The protein concentration was determined by the bicinchoninic acid (BCA) protein reagent (Tiangen, Beijing, China). Equal amounts protein samples were run on 10% SDS-PAGE gels, and subsequently transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membranes were probed with primary antibodies. After incubated with the secondary antibodies (HRP-conjugated goat anti-rabbit IgG, Abcam), the signal bands were visualized by enhanced chemiluminescence (ECL) Western blotting detection reagent. GAPDH was served as the loading control.

3. Results

3.1. Clinical characterization

Five affected members were confirmed in the family: the proband and her daughter, mother, uncle and aunt (Figure 1A). The proband (IV-2) was a 29-year-old female who came to the outpatient department of our hospital due to binocular distension for one year and was diagnosed as binocular hypertension by other hospitals. Case 2 (III-1), a 49-year-old female, the mother of the proband, complained of binocular distension and blurred vision. She underwent binocular trabeculectomy because of uncontrollable high intraocular pressure (≥50 mmHg) in local hospital twenty years ago, and had been receiving prostaglandins eye drops for intraocular pressure control in last eight years. Case 3 (III-3), female, 48-years-old, the aunt of the proband, had poor vision in both eyes with nystagmus and had never received any treatment. The sclera of her both eyes had become obviously thin and the eyeball was significantly enlarged. Case 4 (III-7) was the uncle of the proband, who was blind with his left eye due to glaucoma, and had undergone ophthalmectomy of this eye in local hospital. Two months ago, he received glaucoma surgery in his right eye. Case 5 (V-1), female, 6 -years-old, daughter of the proband, was found characteristic ocular abnormalities without symptoms when accompanied with the proband to our hospital.

All the patients presented the anterior segment abnormalities of both eyes (Figure 2), including varying degrees of posterior embryotoxon, corectopia, iris dysplasia, and iridocorneal tissue adhesions. III-3 was the most serious, with long-term high intraocular pressure resulting in corneal edema, scleral thinning and eyeball dilation, which made it impossible to perform UBM, OCT and visual field examinations. III-3 and III-7 were observed with extensive iris stroma dysplasia accompanied by iridocorneal adhesion and corectopia. III-1 showed slight uveal ectropy in addition to iridocorneal adhesion and iris hypoplasia. Though there seemed no abnormal iris and pupil observed in IV-2 under the slit lamp, UBM and gonioscope showed the presence of iridocorneal adhesion which resulted in her high intraocular pressure. V-1, as the youngest patient in the family, presented obvious corectopia in her left eye, iris hypoplasia in both eyes and mild iridocorneal adhesion by gonioscope. Due to the lack of cooperation, the information provided by the anterior segment OCT examination was limited. There was no abnormal intraocular pressure in her day visit yet. The results of other ocular examinations including BCVA, IOP, cup-to-disc (CD) ratio, humphrey visual

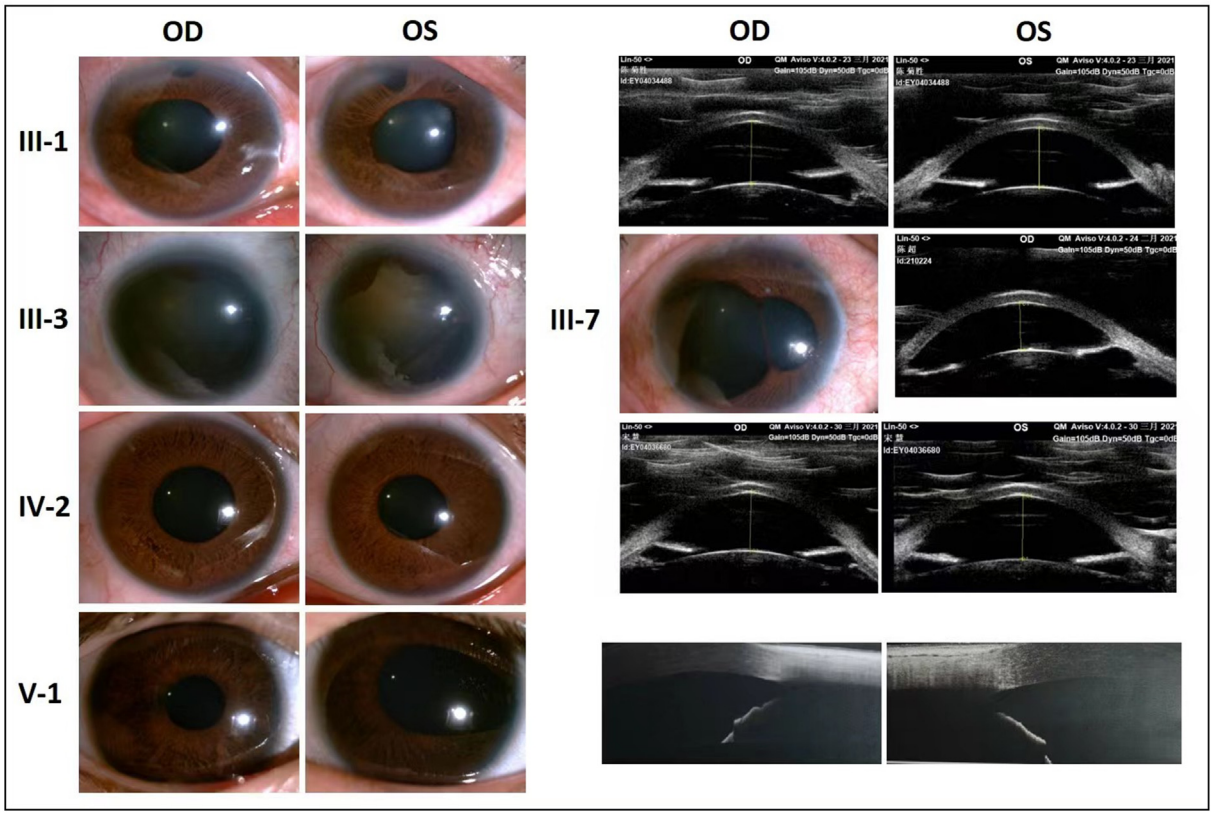

Figure 2. Ocular clinical features of affected family members with Axenfeld-Rieger syndrome.

Table 1. Ocular examination of affected family members with Axenfeld-Reiger Syndrome.

Ocular examination	III-1		III-3		III-7		IV-2		V-1	
	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
BCVA	20/200	FC/10 cm	LP	NLP	0.3	NLP	20/20	20/20	20/20	20/20
IOP (Goldmann tonometry, mmHg)	24	29	40	43	46	N/A	26	24	10	12
C/D	1.0	0.9	—	—	1.0	N/A	0.7	0.7	0.4	0.5
FHA: MD (dB)	-27.47	-29.38	—	—	-19.78	N/A	-9.39	-6.21	—	—
Average RNFL	59	63	—	—	52	N/A	87	94	115	106

BCVA best corrected visual acuity, IOP introcular pressure, C/D cup-to-disc ratio, FHA Humphrey Visual Field Analyzer, MD Mean Deviation, dB decibel, RNFL retinal nerve fiber layer, NLP No light perception; N/A: the patient can not finish the examination because of absence of the eyeball.

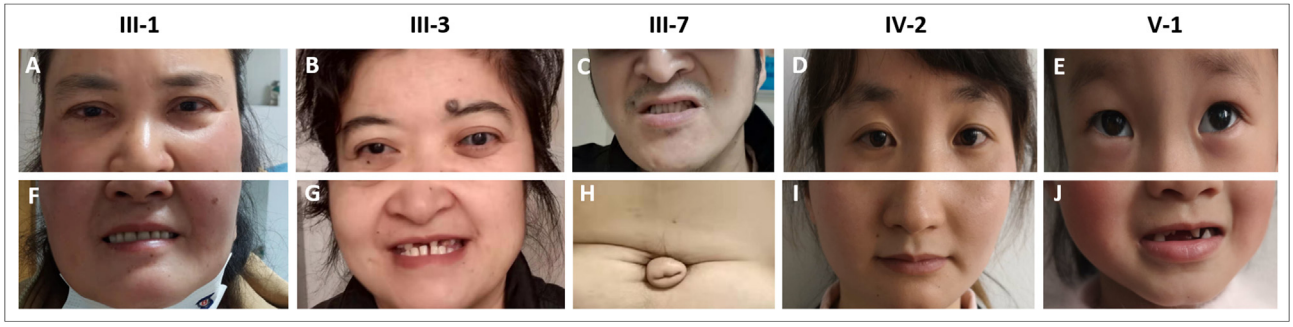

Figure 3. Systemic photographs of affected family members with Axenfeld-Rieger syndrome.

field analyzer (HFA) and the average RNFL are shown in Table 1 in details.

All the patients had maxillary hypoplasia, underbite, hypodontia, conical teeth (Figure 3A–G, I, J). Some of them had dental implants in adolescence. Only III-7 showed obvious umbilical skin (Figure 3H). These patients showed no abnormalities in hearing, cardiovascular, neurological, or skeletal conditions. Table 2 summarized the clinical characteristics of the ocular and systemic features of the patients in this pedigree.

Table 2. Summary of clinical features of affected family members with Axenfeld-Rieger syndrome.

Affected family members		III-1	III-3	III-7	IV-2	V-1
Current age (years old)		49	48	38	29	6
Onset age (years old)		29	7	35	28	6
Gender		F	F	M	F	F
Clinical Features						
Eye	Iris hypoplasia	+	+	+	+	+
	Polycoria	−	+	+	−	−
	Corectopia	+	+	+	−	+
	Shallow anterior chamber	−	−	−	−	−
	Iridocorneal tissue adhesions	+	+	+	+	−
	Posterior embryotoxon	+	+	+	+	+
	Secondary Glaucoma	+	+	+	+	−
Craniofacial Region	Flattened midface	+	+	+	+	+
Teeth	Hypodontia, conical teeth	+	+	+	+	+
Abdomen	Redundant periumbilical skin	−	−	+	−	−
Hearing	Hearing loss	−	−	−	−	−
Cardiovascular Abnormal	Cardiovascular outflow tract malformation	−	−	−	−	−
Pituitary abnormalities	Developmental delays	−	−	−	−	−

3.2. Targeted exome sequencing and sanger validation

After screening according to the standard pedigree, we found that five of the eight subjects had a heterozygous variation on chr4:111539853 (NM_153427 exon5 c. 253-9C > A (spling)) which was a low frequency mutation in the *PITX2* gene and the pedigree. The frequency in the normal person database was negative. According to literatures, there was no correlation of this locus in the database. There was no pathogenic analysis of this locus in Clivar database either. By consulting the literature, the nearby variant sites IVS5-11A > G which affected the splicing and gene expression was once reported in a Chinese family with Axenfeld-Rienger syndrome. The selected variant sites were then verified by PCR and Sanger sequencing. PCR primer pairs were designed by Primer 3.0 online software (http://primer3.ut.ee/). The PCR products were sequenced by Sanger and analyzed on ABI 3130 Genetic Analyzer (Applied Biosystems). By Sanger sequencing, we verified that the proband IV-2 and other family members (III-1, III-3, III-7 and IV-1) carried c. 253-9C > A heterozygous variation (Figure 1 C–G) which was absent in the 150 unrelated controls (Figure 1B). In summary, our results suggest that the new *PITX2* mutation C. 253-9C > A is the pathogenic mutation in this ARS family. All pathogenic mutations detected in relative genes were presented in Table 3, those benign variations are not listed.

3.3. PITX2 expression analysis by PCR and Western Blotting

We analyzed the expression of *PITX2* in peripheral blood lymphocytes of our three patients and three unrelated normal controls through real-time quantitative PCR. Two pairs of primers were designed according to the *PITX2* mRNA sequences before and after the mutant site C. 253 to detect the expression of the *PITX2* mRNA. We investigated the effect of the detected mutation on *PITX2* expression in the ARS family. Compared with normal controls, the protein expression level of *PITX2* in patients decreased by about 40% ($P < 0.05$; Figure 4A), while the mRNA

Table 3. Variants detected in relative genes.

Gene	Chromosomal location	Transcript exons	Nucleotide amino acid	Hom/het	Phenotype (genetic pattern)	Pedigree results
FUT 2	chr19:49206817	NM_000511;exon2	c.604C > T (p.R202X)	het	1. Vitamin B12 plasma level QTL1 (-) 2. Bombay Blood Type (AR)	III-1
AKA P9	chr7:91707050	NM_005751;exon30	c.6806A > T (p.D2269V)	het	Long QT syndrome type 11 (AD)	III-1 III-3 III-7
HSPA 9	chr5:137893619	NM_004134;exon13	c.1572T > G (p.I524M)	het	Even-Plus syndrome (AR)	III-1 III-3
WFS 1	chr4:6303278	NM_006005;exon8	c.1756G > A (p.A586T)	het	Wolfram syndrome type 1 (AR)	III-2
FREM1	chr9:148511285	NM_144966;exon7	c.1149T > C (p.D383=)	het	Manitoba + Oculotrichoanal syndrome (AR)	III-1 III-3 III-7
ROR 2	chr9:944933197	NM_004560;exon7	c.1178C > T (p.S393L)	het	1. Robinow syndrome (AR) 2. Brachydactyl (toe) deformity B1 (AD)	III-2
SOX6	chr11:16256119	NM_033326;exon4	c.535 + 9T>A (splicing)	het	Tolchin-Le Caignec syndrome (AD)	III-2
HMC N1	chr1:185966661	NM_031935;exon25	c.3874 + 5G>A (splicing)	het	Age-related macular degeneration type 1 (AD)	III-1 III-7
SRC AP	chr16:30745856	NM_006662;exon31	c.6649C > A (p.P2217T)	het	Floating-Harbor syndrome (AD)	III-2
PIKF YVE	chr2:209204275	NM_015040;exon30	c.4806C > T (p.S1602=)	het	Corneal spotty dystrophy (AD)	III-2

AD Autosomal dominant inheritance; AR Autosomal recessive inheritance. Hom homozygous; het heterozygosis.

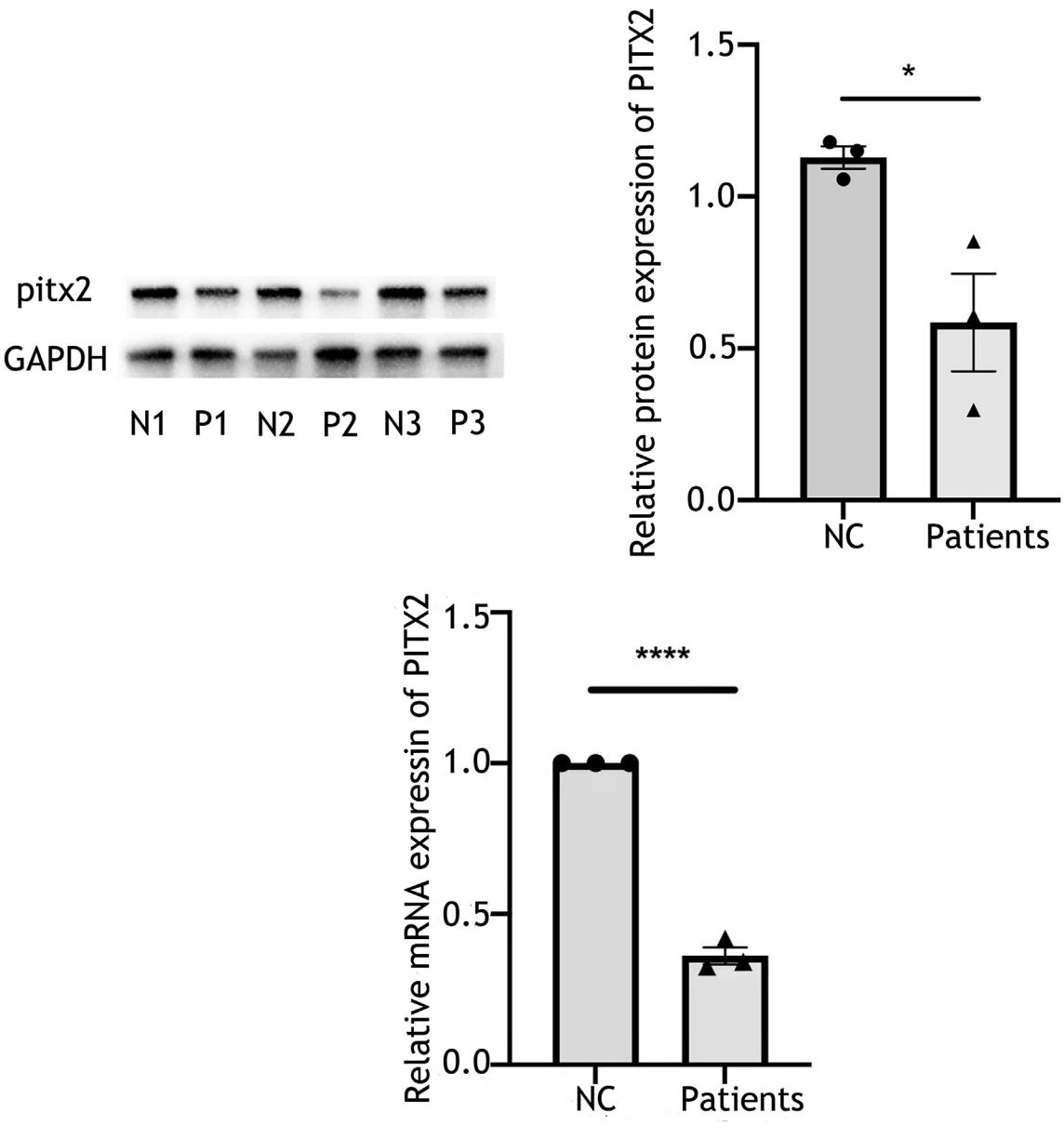

Figure 4. The protein and mRNA expression of *PITX2* in lymphocytes of affected family members and normal controls. P1, P2 and P3 represent III-1,V-1 and IV-2 respectively; N1, N2 and N3 represent normal controls.

expression level of *PITX2* in patients was reduced by about 60% ($P < 0.01$; Figure 4B).

4. Discussion

Anterior segment dysplasia (ASD) includes a range of conditions associated with abnormal structural development of the anterior segment and an increased risk of glaucoma. A common form of ASD is Axenfeld-Rieger syndrome (ARS), which is characterized by specific ocular abnormalities with or without systemic abnormalities. Ocular abnormalities include posterior embryotoxon, polycoria or corectopia, iridocorneal tissue adhesions and iris hypoplasia which is rare autosomal dominant disorder characterized by a poorly developed iris stroma. Common systemic abnormalities include craniofacial malformations with telecanthus, prominent forehead and flattened midface. Hearing loss, heart abnormalities, developmental delays, and other variable characteristics have also been reported. The phenotypes associated with tooth abnormalities usually include hypoplasia and/or abnormal tooth shape, such as small teeth and conical teeth [17]. About 50–85% of ARS patients develop secondary glaucoma [18], which is often tricky to treat and can lead to severe optic nerve damage and visual field defects.

In our study, there was no gender diversity in this disease, and the onset age of the patients was adolescents. The younger the onset age, the more obvious the clinical phenotype and the more serious the secondary glaucoma. All patients had different levels of posterior embryotoxon and iris hypoplasia which was consistent with previous studies [19]. Those with severe iris hypoplasia presented obvious pupil abnormalities. The depth of the central anterior chamber was normal in all of these patients, but the angle closure due to iridocorneal adhesions resulted in secondary glaucoma. 80% of patients in our study had glaucoma, most of whom were diagnosed with both ARS and glaucoma. V-1, the youngest patient in the family, had pupil ectopia with her left eye. Though we could not observe the anterior chamber angle by UBM, the gonioscope suggested that the patient had mild iridocorneal adhesions and closure angle, which was also consistent with her current normal intraocular pressure. Notably, of all the patients in the pedigree, only III-7 appears redundant

periumbilical skin. In addition, all the patients had extremely similar features, such as maxillary hypoplasia with flattening of the midface and abnormal teeth, while those normal individuals in this pedigree didn't have these features. We speculated that ocular abnormalities and facial dysplasia in patients with AR syndrome are regulated by the same mutant gene.

Studies have shown that AR syndrome is associated with mutations on multiple chromosomes 4, 6, 9, 13, 18, 21 and commonly correlates with mutations in *FOXC1* on 6p25 distal microdeletion [20]. In addition, many other genes including *PITX2*, *FOXC2* and *FKHL7* are also involved in the pathogenesis of ARS [21, 22]. Approximately 10%–30% of patients with ARS present *PITX2* mutations [20]. 40–70% of ARS cases are associated with mutations in *FOXC1* and *PITX2*, located on chromosomes 6p25 and 4q25, respectively [23]. Heterozygous mutations in the *FOXC1* and *PITX2* cause 16% and 10% of cases respectively [24]. *PITX2*, expressed in neural crest cells, is essential for the normal development of the optic stalk and the formation of anterior segment structure [25]. It has at least four different transcription forms, *PITX2a*, *PITX2b*, *PITX2c*, and *PITX2d*, which have different biological characteristics. In normal vertebrates, these subtypes regulate the development of the heart, lungs, brain, teeth, pituitary gland, and intestine alone or in overlapping regions and in different doses [26]. It is reported that *FOXC1* is more likely to be associated with congenital heart disease, whereas *PITX2* is always associated with dental abnormalities and/or umbilical anomalies [27]. Research has characterized *PITX2* mutations as splice-site mutations, deletions and chromosomal translocations in patients with ARS [23].

In our study, we identified a novel *PITX2* heterozygous mutation which had not yet been reported. It was a splicing mutation on chr4:111539853 (NM_153427 exon5 c. 253-9C > A) that was co-isolated with the ARS phenotype in our pedigree. *PITX2* belongs to the *RIEG/PITX* homeobox family and encodes a 33 kDa bicoid-related homeodomain transcription factor. The protein contains a 60-amino acid homeodomain at the N-terminal and a 14-amino acid C-terminal OAR domain, which mediates protein-protein interactions and self-inhibitory interactions with the N-termina [28]. Mutations in the *PITX2* protein, which is involved in regulating the development of the anterior segment, as well as in several non-ocular tissues, including heart, and pituitary gland, are associated with ARS and other anterior segment abnormalities, including Peter's like abnormalities, iris dysplasia syndrome (IGDS), and iris hypoplasia (IH) [29, 30, 31]. Our real-time quantitative PCR and Western Blot results revealed the effect of this detected mutation on *PITX2* expression in the ARS pedigree. Compared with normal individuals in the pedigree, the expression level of *PITX2* was significantly decreased in patients. This is consistent with previous reports, although the *PITX2* mutation sites reported are different [32, 33]. It revealed that the *PITX2* mutation resulted in significant and extremely low *PITX2* full-length mRNA expression in afected individuals, suggesting that the mutant protein had a dominant negative inhibitory effect on wild-type *PITX2* expression in addition to haploidy deficiency. Since normal eye development and function require strict control of *PITX2*, altered levels of functional proteins are responsible for the ARS phenotype in pedigrees [33].

Although genetic mutations associated with AR syndrome are gradually being identified, the molecular mechanisms of the mutated sites remain to be further studied. The mechanisms referring diversities in clinical phenotypes among individuals carrying the same mutant gene in this study are worthy of further investigation. Furthermore, prospective studies are needed to determine ocular progression in this pedigree, especially in V-1.

5. Conclusion

In the study, we summarized the variable phenotype in the ARS family, described a novel *PITX2* splicing mutation in the ARS family by combining exome sequencing with segregation analysis. The analysis of the expression level of *PITX2* further confirmed the possibility of development of ARS induced by this *PITX2* gene abnormality.

Declarations

Author contribution statement

Lingyan Cheng: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yinong Zhang: Contributed reagents, materials, analysis tools or data.

Yuzhi Ding: Analyzed and interpreted the data.

Zhilan Yuan; Xiao Han: Conceived and designed the experiments.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e12543.

References

[1] X. Wu, H. Xie, T. Wu, et al., A novel mutation of FOXC1 in a Chinese family with Axenfeld-Rieger syndrome, Exp. Ther. Med. 18 (2019) 2255–2261.
[2] F. Idrees, D. Vaideanu, S.G. Fraser, et al., A review of anterior segment dysgeneses, Surv. Ophthalmol. 51 (2006) 213–231.
[3] M.B. Shields, E. Buckly, G.K. Klintworth, et al., Axenfeld-Rieger syndrome. A spectrum of developmental disorders, Surv. Ophthalmol. 29 (1985) 387–409.
[4] K. Li, M. Tang, M. Xu, et al., A novel missense mutation of FOXC1 in an Axenfeld-Rieger syndrome patient with a congenital atrial septal defect and sublingual cyst: a case report and literature review, Exp. Ther. Med. 18 (2019) 2255–2261.
[5] G.N. Kim, C.S. Ki, S.W. Seo, et al., A novel forkhead box C1 gene mutation in a Korean family with Axenfeld–Rieger syndrome, Mol. Vis. 19 (2013) 935–943.
[6] R.A. Honkanen, D.Y. Nishimura, R.E. Swiderski, et al., A family with Axenfeld–Rieger syndrome and Peters Anomaly caused by a point mutation (Phe112Ser) in the FOXC1 gene, Am. J. Ophthalmol. 135 (3) (2003) 368–375.
[7] M. Seifi, T. Footz, S.A. Taylor, et al., Comparison of bioinformatics prediction, molecular modeling, and functional analyses of FOXC1 mutations in patients with Axenfeld–Rieger syndrome, Hum. Mutat. 38 (2) (2017) 169–179.
[8] K. Li, L. Yang, Y. Liu, et al., Novel genetic findings in a Chinese family with Axenfeld-rieger syndrome, J. Ophthalmol. 2017 (2017), 5078079.
[9] L. Huang, Y. Meng, X. Guo, Novel PITX2 mutations including a mutation causing an unusual ophthalmic phenotype of Axenfeld-rieger syndrome, J. Ophthalmol. 2019 (2019), 5642126.
[10] M. Chekhchar, A. Charadi, A. Achibane, et al., Axenfeld- Rieger syndrome: a case report, J. Fr. Ophtalmol. 42 (2019) e157–158.
[11] Z. Tumer, D. Bach-Holm, Axenfeld-Rieger syndrome and spectrum of PITX2 and FOXC1 mutations, Eur. J. Hum. Genet. 17 (2009) 1527–1539.
[12] B. D'haene, F. Meire, I. Claerhout, Expanding the spectrum of FOXC1 and PITX2 mutations and copy number changes in patients with anterior segment malformations, Invest. Ophthalmol. Vis. Sci. 52 (2011) 324–333.
[13] M.H. Strungaru, I. Dinu, M.A. Walter, Genotype-phenotype correlations in Axenfeld-rieger, Invest. Ophthalmol. Vis. Sci. 48 (2007) 228–237.
[14] N. Weisschuh, E. De Baere, B. Wissinger, et al., Clinical utility gene card for: Axenfeld-Rieger syndrome, Eur. J. Hum. Genet. 19 (3) (2011).
[15] B. D'Haene, F. Meire, I. Claerhout, et al., Expanding the spectrum of FOXC1 and PITX2 mutations and copy number changes in patients with anterior segment malformations, Investig. Ophthalmol. Vis. Sci. 52 (1) (2011) 324–333.
[16] C.R. French, Mechanistic insights into Axenfeld-rieger syndrome from zebrafish foxc1 and pitx2 mutants, Int. J. Mol. Sci. 22 (18) (2021) 10001.
[17] N. Weisschuh, P. Dressler, F. Schuettauf, et al., Novel mutations of FOXC1 and PITX2 in patients with Axenfeld-Rieger malfor- mations, Invest. Ophthalmol. Vis. Sci. 47 (2006) 3846–3852.

[18] M. Seifi, M.A. Walter, Axenfeld-Rieger syndrome, Clin. Genet. 93 (2018) 1123–1130.
[19] J.R. Corona-Rivera, A. Corona-Rivera, L.C. Zepeda-Romero, et al., Ring chromosome 6 in a child with anterior segment dysgenesis and review of its overlap with other FOXC1 deletion phenotypes, Congenital. Anom. 59 (2019) 174–178.
[20] F. Mirzayans, D.B. Gould, E. Héon, et al., Axenfeld-Rieger syndrome resulting from mutation of the FKHL7 gene on chromosome 6p25, Eur. J. Hum. Genet. 8 (2000) 71–74.
[21] R.S. Smith, A. Zabaleta, T. Kume, et al., Haploinsufficiency of the trans- cription factors FOXC1 and FOXC2 results in aberrant ocular development, Hum. Mol. Genet. 9 (2000) 1021–1032.
[22] D.Y. Nishimura, R.E. Swiderski, W.L. Alward, et al., The forkhead transcription factor gene FKHL7 is responsible for glaucoma phenotypes which map to 6p25, Nat. Genet. 19 (1998) 140–147.
[23] W.L. Alward, Axenfeld-Rieger syndrome in the age of molecular genetics, Am. J. Ophthalmol. 130 (2000) 107–115.
[24] I.J. de Vos, A.P. Stegmann, C.A. Webers, et al., The 6p25 deletion syndrome: an update on a rare neurocristopathy, Ophthalmic Genet. 38 (2017) 101–107.
[25] A.L. Evans, P.J. Gage, Expression of the homeobox gene Pitx2 in neural crest is required for optic stalk and ocular anterior segment development, Hum. Mol. Genet. 14 (2005) 3347–3359.
[26] C.J. Cox, H.M. Espinoza, B. McWilliams, et al., Differential regulation of gene expression by PITX2 isoforms, J. Biol. Chem. 277 (2002) 25001–25010.
[27] Y. Zhang, X. Chen, L. Wang, et al., Heterogeneity of Axenfeld-rieger syndrome: molecular and clinical findings in Chinese patients, Front. Genet. 20 (2021) 732170.
[28] B.A. Amendt, E.V. Semina, W.L. Alward, Rieger syndrome: a clinical, molecular, and biochemical analysis, Cell. Mol. Life Sci. 57 (2000) 1652–1666.
[29] D. Ai, J. Wang, M. Amen, et al., Nuclear factor 1 and T-cell factor/LEF recognition elements regulate Pitx2 transcription in pituitary development, Mol. Cell Biol. 27 (2007) 5765–5775.
[30] W. Doward, R. Perveen, I.C. Lloyd, et al., A mutation in the RIEG1 gene associated with Peters' anomaly, J. Med. Genet. 36 (1999) 152–155.
[31] S.C. Kulak, K. Kozlowski, E.V. Semina, et al., Mutation in the RIEG1 gene in patients with iridogoniodysgenesis syndrome, Hum. Mol. Genet. 7 (1998) 1113–1117.
[32] D.P. Sun, Y.H. Dai, X.J. Pan, et al., A Chinese family with Axenfeld-Rieger syndrome: report of the clinical and genetic findings, Int. J. Ophthalmol. 10 (2017) 847–853.
[33] L. Zhang, Y. Peng, P. Ouyang, et al., A novel frameshift mutation in the PITX2 gene in a family with Axenfeld-Rieger syndrome using targeted exome sequencing, BMC Med. Genet. 20 (2019) 105.