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Complex genomic rearrangement with deletion of *PITX2* in a Chinese family with Axenfeld–Rieger syndrome: A case report and literature review

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Purpose: This study identified the genetic causes of Axenfeld–Rieger syndrome (ARS) in a Chinese family and evaluated their clinical phenotype and clinical treatment.
Methods: We recruited a Chinese family with ARS. The proband presented with bilateral ectopic pupils, periumbilical redundancy, craniofacial abnormalities, and dental abnormalities after birth and was diagnosed with ARS. The symptoms were the same for her younger brother. Blood samples were collected from four family members: the proband, her brother, and her parents. Whole-genome sequencing (WGS) was performed to identify probable genetic variants in the proband. To confirm the identified variants, samples from the other family members were subjected to quantitative polymerase chain reaction (qPCR) and Sanger sequencing.
Results: Based on the results of WGS, we suspected a deletion region and an inversion region around the *PITX2* gene. Through qPCR and Sanger sequencing, we identified a complex rearrangement involving a 6.15 Mb deletion on Chromosome 4, including the *PITX2* coding region (Hg38; chr4:110617776–116769011), a 45.71 Mb inversion (Hg38; chr4:116769011–162481408), and a 14-bp deletion (Hg38; chr4:162481409–162481422). Interestingly, the father's copy number was normal, but Sanger sequencing revealed the same breakpoints. This indicated that the father is a balanced rearrangement carrier, and the children are unbalanced rearrangement carriers. While similar deletions and many breakpoints in this region have been reported, this specific rearrangement is novel.
Conclusions: Using WGS, qPCR, and Sanger, we found a complex genomic rearrangement with the deletion of *PITX2* in a Chinese family with ARS. The clinical characteristics of the affected individuals were reported. The current findings broaden our understanding of the phenotype and variant spectrum associated with ARS caused by *PITX2* deletion.

Axenfeld–Rieger syndrome (ARS; OMIM 180500, OMIM 601499, OMIM 602482) is an uncommon autosomal dominant disorder with an incidence of approximately 1 in 50,000 to 100,000 newborns [1]. The typical clinical manifestations of ARS include ocular and systemic phenotypes, and the disease is divided into three types. In ARS Type 1, eye involvement is typically bilateral or rarely unilateral. Bilateral iris hypoplasia, polycoria, iridocorneal adhesion, corectopia, posterior embryotoxon caused by the anterior displacement of Schwalbe's line, and glaucoma are common ocular abnormalities [2]. Systemic manifestations include periumbilical redundancy and umbilical hernia [3–5]. Characteristic craniofacial features include maxillary hypoplasia, hypertelorism, telecanthus, a flattened midface with a broad, flat nasal bridge, a thin upper lip, and a prominent lower lip. Dental characteristics include microdontia, short roots, taurodontism, teeth with unusual shapes, and hypodontia/oligodontia of the primary and permanent dentition [6–8]. It is caused by variants in paired-like homeodomain transcription factor 2 (*PITX2*). At present, no single gene variant associated with ARS Type 2 has been found [9]. ARS Type 3 manifests as a variety of phenotypes, including eye defects, hearing loss, heart abnormalities, dental anomalies, and facial deformities. It is caused by variants in forkhead box C1 (*FOXC1*). The dental and facial malformations associated with Type 3 are distinct from those associated with Type 1. Facial deformities mostly include hypertelorism and ear anomalies, while tooth deformities mainly include enamel hypoplasia [1,5,6,9,10]. ARS has also been linked to two additional genes (*CYP1B1*, 2p22.2, OMIM 601771 [11] and *PRDM5*, 4q27, OMIM 614161 [12]) and one locus (13q14) [13].

Two key genes in ARS, *FOXC1* (6p25, OMIM 601090) and *PITX2* (4q25, OMIM 601542), have been identified using conventional genetic approaches. ARS Type 1 is caused by heterozygous variants of *PITX2*, while Type 3 is caused by heterozygous variants of *FOXC1*. Patients with ARS have a variety of *PITX2* and *FOXC1* variants, including point variants, insertion variants, deletion variants, and chromosomal deletions [1,14]. *PITX2* encodes a bicoid homeodomain

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protein belonging to the RIEG/PITX homeobox family. It is involved in the development of the eyes, teeth, and abdominal organs [1]. ARS, iridogoniodysgenesis syndrome, and Peters anomaly are all linked to variants in this gene [15]. In this report, we present a novel complex genomic rearrangement with the deletion of *PITX2* identified in two Chinese siblings with both ocular and systemic anomalies.

METHODS

Patients: This study investigated a Chinese family of four. Two siblings were diagnosed with ARS due to systemic and ocular abnormalities. Each participant completed an informed consent form and agreed to provide blood samples. They also consented for their medical information and examination materials to be used for scientific research and publication. This study was performed in adherence with the tenets of the Declaration of Helsinki. Approval was obtained from the Ethics and Medical Research Committee of the Army Medical Center of the People's Liberation Army of China (2023-3).

Genetic analysis: Genomic DNA was extracted from 3 ml of peripheral blood collected from the patients and their parents. Whole-genome capture was performed using an Illumina sequencer with 2 × 150 bp read lengths with an average coverage depth of 30×. FastQC, the Genome Analysis Toolkit (GATK), and ANNOtate VARiation (ANNOVAR) were used for quality control, mapping, variant calling, and variant annotation during whole-genome sequencing (WGS), which was completed using the GATK Best Practices workflow. Variants with an allele frequency greater than 0.01 were then removed from the discovered variants using 1000 Genome, EXAC03, and ESP6500 filters. We selected functional variants with missense, splicing, stop-gain/stop-losses, and insertion and deletion variants. RefGene, Gene Ontology, the Kyoto Encyclopedia of Genes and Genomes, Sorting Intolerant From Tolerant (SIFT), PolyPhen V2, and Mutation Taster were used to annotate the functions and conservativeness of genes. Combined annotation-dependent depletion (CADD), deleterious annotation of genetic variants using neural networks (DANN), Eigen, the Human Gene Mutation Database, Online Mendelian Inheritance in Man (OMIM), and ClinVar were used to predict whether gene variants were (possibly) damaging. Structural and copy number variants were analyzed using LUMPY, a new framework for structural variant discovery [16]. The copy number was detected by fluorescence quantitative analysis according to the primer sequence (Table 1), with *POLR2A* and *RPP14* set as internal parameters. Primers were designed according to the NCBI database GRCh38. The copy numbers of four patients and three control samples was determined via fluorescence quantitative polymerase chain reaction (qPCR). Primers were designed using the Primer3 software. Three repetitive qPCR tests were performed using SYBR Premix Ex Taq reagent on a 7300 Real-time PCR System. Finally, the exact sequence was further determined via Sanger sequencing.

Case reports:

Patient history—*Patient 1*: The proband was an 11-year-old Chinese girl who presented with severely impaired eyesight in her 10th year of life. She was a premature baby born at 34 weeks and 5 days of gestation, with a birthweight of 2.0 kg (24th percentile) and length of 46.0 cm (66th percentile), to unrelated Chinese parents who are both phenotypically normal. Her mother reported that the proband had "bilateral ectopic pupils" from birth, and she did not start walking until she was 1.5 years of age. Her parents denied that she had any intellectual problems. However, she exhibited unusual behaviors, including always keeping her head down, not looking at people, not answering when her name was called, talking to herself, playing with and looking at her hands, dozing off, and having a limited attention span.

A protuberant umbilicus, a flattened midface with a broad, flat nasal bridge, a thin upper lip, a prominent lower lip, dental abnormalities, shortening of the upper labial frenulum, electrocardiography abnormalities, proteinuria, esotropia, low vision, high intraocular pressure (IOP), iridocorneal adhesion, corneal degeneration, polycoria, and glaucoma were detected in the proband. The clinical data for the proband is presented in Table 2 and Figure 1. During her first visit at age 10, Goldmann tonometry showed IOPs of 43.5 mmHg (OD) and 44.0 mmHg (OS). Even after the administration of medication, IOP remained poorly controlled. After two micropulse cyclophotocoagulation surgeries when she was 11 years old, the proband's IOPs were 10.5 mmHg (OD) and 7.0 mmHg (OS) at the one-week follow-up after the second surgery.

Patient 2: The proband's 9-year-old younger brother was a full-term baby at 38 weeks gestation, with a birthweight of 3.4 kg (68th percentile) and a length of 48.0 cm (27th percentile). He had undergone corrective surgery for a funnel chest when he was 5 years old. At the age of 7, he was diagnosed with attention-deficit/hyperactivity disorder, autism spectrum disorder, and mental retardation due to hyperactivity and disruptiveness at school. At the same age, he scored 50 points on the Wechsler Intelligence Test for Children (the normal value is 90–110). Atomoxetine hydrochloride was used to control his hyperactivity, improving his symptoms.

Table 1. qPCR analysis for *PITX2* and neighboring genes.

ID	ENPEP-e12		PITX2-e4		FAM241A-e2		NDST4-e4		NDST4-e2	
	Forward primer chr4:110563420-110563440 TCTTTTCACTCCACAC-GCGAA		Forward primer chr4:110632981-110633000 GCGGCAGTTGGTCTC-CATTC		Forward primer chr4:112186789-112186810 AACATGGGCTTCA-CAAGGATGT		Forward primer chr4:114970370-114970392 CACTTCCACCACAAGATCA-CAAC		Forward primer chr4:115076717-115076737 TGGTACTGAAATCG-GCTGGAC	
	Reverse primer chr4:110563506-110563484 ACAGGTGTTGTTTGAGA-AGGACC		Reverse primer chr4:110633082-110633061 ACGTAGTCTCATCT-GAGCCCTG		Reverse primer chr4:112186879-112186859 GGCCAAGGAACCACAG-CATAA		Reverse primer chr4:114970462-114970439 GAGCAGATGATTCTCAA-CAAGGAA		Reverse primer chr4:115076823-115076803 GACACATCCAAAACG-GACCCT	
	ACN		ACN		ACN		ACN		ACN	
	ENPEP-e12/ POLR2A	ENPEP-e12/ RPP14	PITX2-e4/ POLR2A	PITX2-e4/ RPP14	FAM241A-e2/ POLR2A	FAM241A-e2/ RPP14	NDST4-e4/ POLR2A	NDST4-e4/ RPP14	NDST4-e2/ POLR2A	NDST4-e2/ RPP14
I-1	1.769	1.677	1.733	1.646	2.077	1.968	1.520	1.438	1.555	1.475
I-2	1.752	2.000	1.422	1.626	2.070	2.363	1.616	1.841	1.701	1.942
II-1	1.604	1.599	**0.722**	**0.721**	**0.987**	**0.984**	**0.791**	**0.787**	**0.782**	**0.780**
II-2	1.849	1.887	**0.596**	**0.609**	**1.056**	**1.078**	**0.869**	**0.885**	**0.871**	**0.889**
PC-1	2.041	2.162	1.906	2.024	2.034	2.155	2.167	2.291	1.987	2.106
PC-2	2.002	1.929	2.096	2.024	1.995	1.923	1.968	1.893	2.015	1.943
PC-3	1.958	1.909	1.997	1.952	1.971	1.923	1.865	1.815	1.998	1.951

ΔCt=Ct(Purpose segment)- Ct(Internal reference gene); Relative copy number=2^(-ΔCt); Absolute copy number (ACN)=(Test sample relative copy number / Relative copy number of control samples) *2; POLR2A and RPP14 genes are internal reference genes on autosomes, and we assumed their copy number to be 2 in all samples. PC-1, 2, and 3 are normal negative controls. Deleted sequences are in bold numbers. Primer design range according to NCBI database GRCh38.

Table 2. Clinical data of the patients with ARS recruited in our study.

ID	Age (years)	Gender	BCVA (OD/ OS)	IOP (OD/ OS)	C/D (OD/ OS)	AL (OD/ OS)	ACD (OD/ OS)	CCT (OD/ OS)	PAS	pupil	PE	CEC	Glu	Others ocular defect	CA	DA	PU	ECG abnormalities	Others
Patient 1	11	M	HM/CF	43.5/ 44	1.0/ 1.0	29.47/ 26.83	1.99/ 2.23	729/ 746	PAS	Corec-topia, multiple pupils	PE	1354/ -	Glu	esotropia	CA	DA	PU	None	ASD, short-ening of the upper labial frenulum, proteinuria
Patient 2	9	F	0.1/0.1	11.6/ 10.4	0.3/ 0.4	21.59/ 22.06	2.47/ 2.42	696/ 671	PAS	corec-topia	PE	-	None	None	CA	DA	PU	None	ASD, short-ening of the upper labial frenulum

Note: F, female; M, male; BCVA, best corrected visual acuity; OD, right eye; OS, left eye; HM, hand movement; CF, counting fingers; IOP, intraocular pressure; C/D, cup-disc ratio; AL, axial length; ACD, anterior chamber depth; CCT, central corneal thickness; PAS, peripheral anterior synechiae; PE, posterior embryotoxon; CEC, corneal endothelial count; Glu, Glaucoma; CA, craniofacial abnormalities; DA, dental abnormalities; PU, protuberant umbilicus; ASD, autism spectrum disorder; –, unknown.

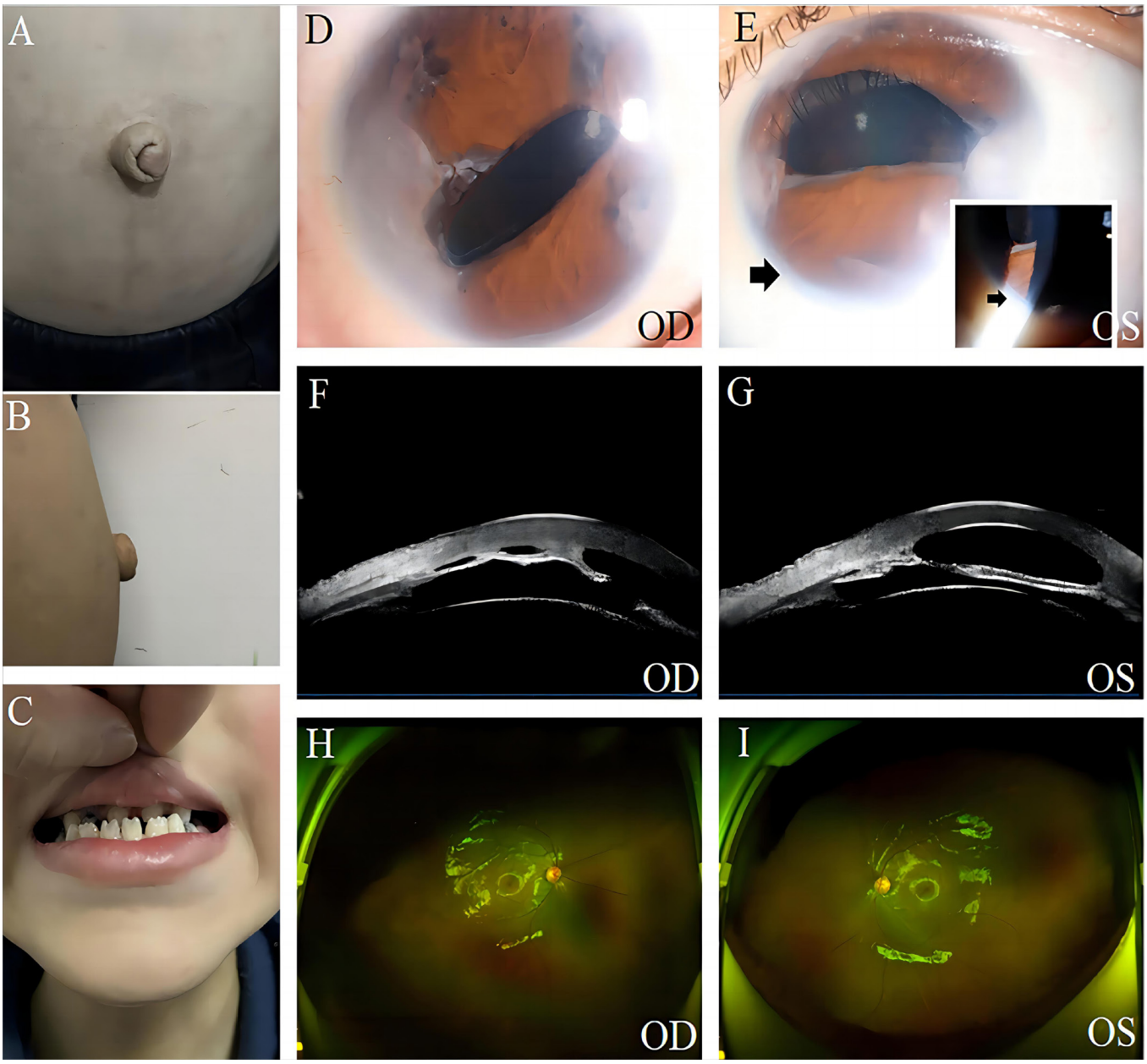

Figure 1. Clinical features of the proband. **A**, **B**: Physical examination showed a protuberant umbilicus. **C**: Dental examination revealed a missing upper incisor as well as some abnormally shaped teeth. **D**, **E**: Slit-lamp images revealed polycoria, corneal degeneration, and iris hypoplasia in both eyes. **F**, **G**: UBM revealed high insertion of the iris root and anterior synechia. **H**, **I**: Scanning laser ophthalmoscopey (SLO) indicated that the cup-to-disk ratio was 1.0 in both eyes.

He also had a protuberant umbilicus. His craniofacial findings were similar to those of the proband. Dental examination revealed widely spaced teeth and some abnormally shaped teeth. He had no missing teeth, with only 27 adult teeth due to age. The patient underwent upper labial frenulum resection at 5 years old. Ophthalmological examination revealed poor vision, iris atrophy, non-round pupils, and posterior embryotoxon. His clinical data are presented in Table 2 and Figure 2. A two-generational pedigree of the patients' family is shown in Figure 3A.

RESULTS

A large deletion spanning *PITX2* and *LOC107986306* (chr4:110617776–116769011) was found in the proband using WGS. The deletion is 6.15 Mb and contains 86 genes (Appendix 1), affecting cytogenic bands 4q25q26. The

results of the variant analysis are shown in Appendix 2, with no pathogenic/likely pathogenic variants found in other genes. To verify the deletion and determine the exact DNA breakpoint, qPCR analysis and Sanger sequencing were performed for the four family members. The copy numbers of five predetermined candidate regions were quantified via qPCR. These were located in exon 4 of *PITX2,* exon 12 of *ENPEP*, exon 2 of *FAM241A*, exon 4 of *NDST4*, and exon 2 of *NDST4*. Compared to normal negative controls and their parents, the siblings' expression levels of *PITX2-e4*, *FAM241A*, *NDST4-e4*, and *NDST4-e2* suggested copy number deletions. There were no significant abnormalities in the copy number of *ENPEP-e12* in both siblings. Neither parent had copy number deletions. In the experiment, we used two normal negative control genes, and the results were consistent (Table 1). Sanger sequencing revealed that the siblings

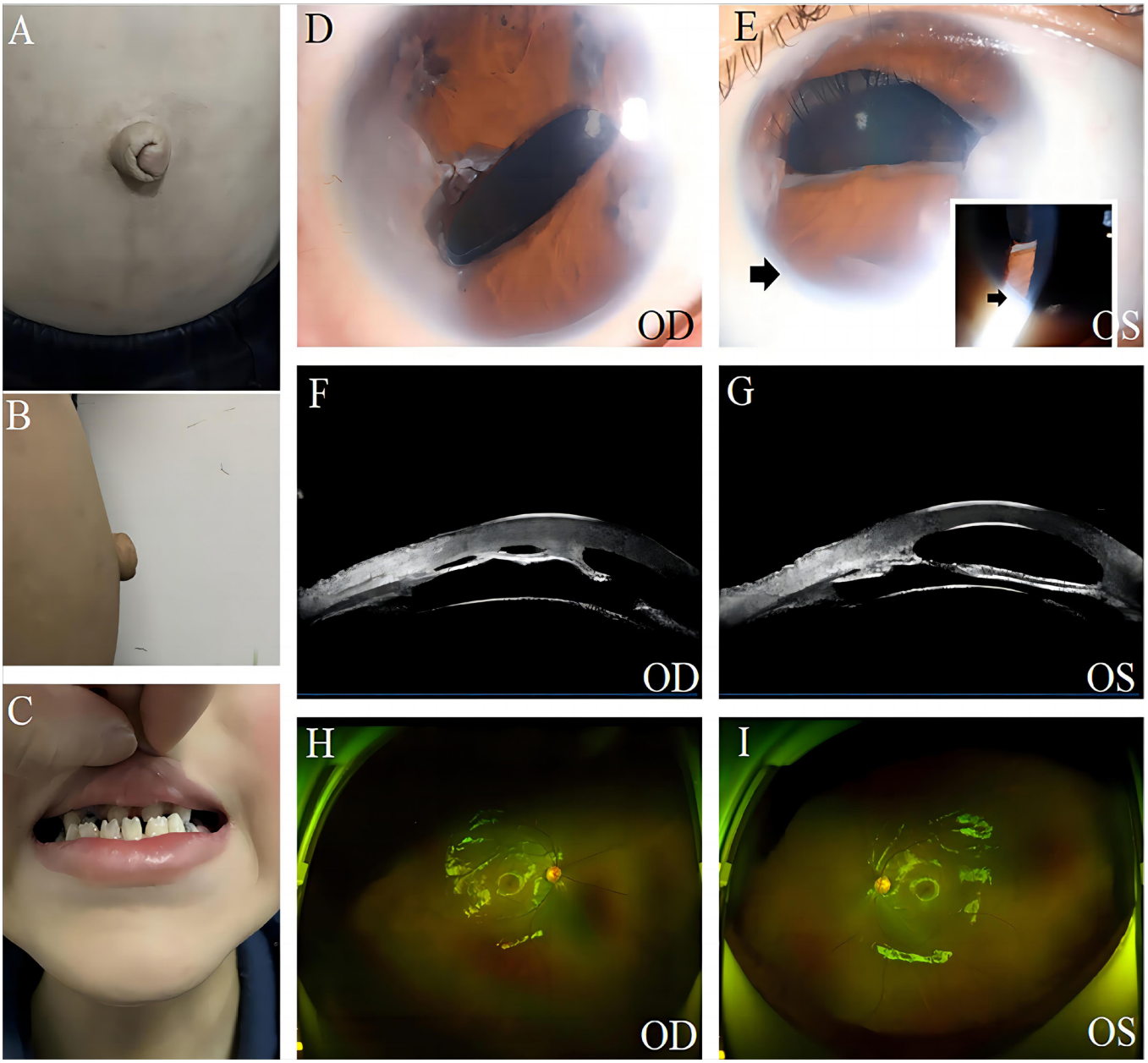

Figure 2. Clinical features of Patient 2. **A**, **B**: Systematic examination revealed a protuberant umbilicus. **C**: Dental examination revealed widely spaced teeth as well as some abnormally shaped teeth. **D**, **E**: Slit-lamp images showed posterior embryotoxon (black arrowhead), corectopia, and iris hypoplasia in both eyes. **F**, **G**: UBM revealed high insertion of the iris root and iris anterior synechia. **H**, **I**: Scanning laser ophthalmoscopey (SLO) indicated that the cup-to-disk ratios were 0.3 (OD) and 0.4 (OS).

and their father have two breakpoints on Chromosome 4. On Chromosome 4, the breakpoint 110617776 sequence is followed by 162481408, and the breakpoint 116769011 sequence is followed by 162481423. The sequences 162481409 to 162481422 have a small 14-bp deletion (Figure 3B). Thus, we identified a complex rearrangement involving a 6.15 Mb deletion on Chromosome 4, including the *PITX2* coding region (Hg38; chr4:110617776–116769011) and a 45.71 Mb inversion (Hg38; chr4:116769011–162481408), and a 14-bp deletion (Hg38; chr4:162481409–162481422). However, Sanger sequencing showed that the breakpoints on Chromosome 4 are also present in the patients' father. The translocation breakpoint removing a portion of the *PITX2* 3′UTR in the father did not have any clinical effects. We speculate that there is insertion translocation in the father, which is a complex chromosomal rearrangement that requires at least three breakpoints on the related chromosomes, and thus, he had no clinical symptoms. Insertion translocations are rare, balanced chromosomal rearrangements with an increased risk of imbalances for offspring. This suggests that the father is a balanced rearrangement carrier, while the children have unbalanced rearrangements. Additional analysis to identify the location of the insertion could not be performed due to limited sample availability. Combined with previous studies, we found that deletion affecting cytogenic bands 4q25q26 can cause different clinical manifestations (Table 3).

DISCUSSION

ARS is a group of clinically and genetically heterogeneous developmental disorders, which cause eye defects and non-ocular systemic defects. Ocular symptoms include anterior segment dysplasia of the eye with iris dysplasia, multiple pupils, posterior embryotoxon, and glaucoma [1,17]. ARS can also lead to non-ocular systemic defects, including distinctive craniofacial dysmorphism, hearing loss, dental abnormalities, umbilical skin defects, and heart defects [18,19]. ARS Type 1 usually features an ocular and systemic phenotype and is caused by variants in *PITX2*. In our study, WGS revealed that the entire coding region of the *PITX2* genes was deleted in the proband. Based on their clinical presentation and genetic testing results, the patients in this study were classified as Type 1. Both had eye defects, dental abnormalities, craniofacial malformations, and umbilical abnormalities. On Chromosome 4, microdeletions near the *PITX2* may also be associated with abnormal brain development [20 –23]. Intellectual disabilities were observed in the younger brother, with manifestations of neurodevelopmental abnormalities also present in the proband. The most dangerous complication of ARS is glaucoma, which develops in more than 50% of patients and can lead to total and irreversible blindness within a few years [24,25]. The proband was diagnosed with glaucoma.

On Chromosomes 4q25 and 6p25, *PITX2* and *FOXC1* variants account for 71% of the instances of ARS [9]. In addition to the typical intragenic variants (missense, nonsense, splicing, and intragenic deletion/insertion), large deletions affecting *PITX2* and *FOXC1* have been reported. *PITX2* and *FOXC1* encode developmentally relevant transcription factors that regulate the expression of downstream target genes by binding to specific DNA sequences. Transcription factors play an important role in embryonic development, and their expression is subject to strict spatiotemporal regulation.

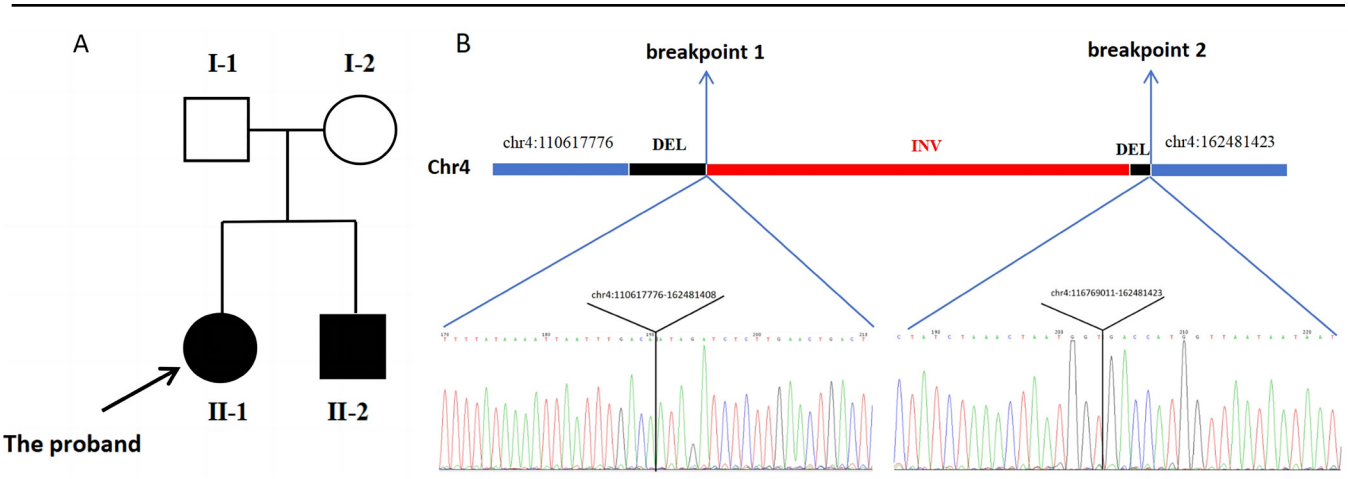

Figure 3. Pedigree chart and rearrangement schematic. **A**: Females are indicated by circles and males by squares. Shading indicates an affected individual, and arrows indicate the proband. **B**: A schematic diagram of the complex rearrangement in the proband, which includes the 6.15 MB deletion (including *PITX2*), an inversion of approximately 45.71 MB, and a 14-bp deletion. Black bands indicate deletion regions and red bands indicate inversion regions.

Table 3. Clinical manifestations of 4q25q26 deletions with ARS.

Ref-PMID	Age(years)	Deletion	size	Eye	Facial	Dental	Umbilicus	Heart	Hearing	Other
20881290	<1	4q25q26	7.46 Mb	ARS	FA	DA	RU	–	–	GD, MD, Mental retardation
22569110	21	4q25q26	6.4 Mb	ARS, GL	FA	DA	RU	None	None	Low thyroid
22569110	<1	4q25-q28	19.2 Mb	ARS, GL, CA	FA	Natal tooth	RU	VSD	HL	GD
20358612	1	4q25-q31	28 Mb	ARS, GL, Strabismus, High myopia	FA	DA	RU	None	None	GD, LA, LD
24715413	11	4q25–4q27	9.5 Mb	ARS, GL	FA	DA	RU	Heart murmur	None	GD, ASD, LD
24715413	7	4q25q26	8.5 Mb	ARS	FA	DA	UH	None	-	GD, MD, LA Dyspraxia
24715413	7	4q25q26	4 Mb	ARS, Strabismus	FA	DA	UH	-	None	GD, LA, Iron deficiency anemia, Seizures,
9132488	-	4q25q26	-	ARS, GL	FA	DA	-	-	-	Dyscrania
12647202	1	4q25–4q27	-	ARS, GL	FA	DA	UH	Patent ductus arteriosus and an atrial septal defect	-	GD, LA, Hypotonia, Seizures, Piebaldism
1863994	<1	4q25–4q27	-	ARS	FA	-	-	Atrial septal defect		LA, GI, Hypotonia, Seizures, Apnea, Dilated lateral ventricles
7717415	<1	4q25–4q27	-	External ocular anomaly	FA	DA	-	-	-	GD, Hypotonia, Otitis, LA, Fetal alcohol syndrome
9429145	7	4q25–4q27	-	ARS, Strabismus	FA	DA	RU	VSD, WPWS	None	GD, Aggression, Tantrums, Biting
The present study. 2023	11	4q25q26	6.15 Mb	ARS; GL	FA	DA	RU	None	None	ASD, Shortening of the upper labial frenulum, Proteinuria
The present study. 2023	9	4q25q26	6.15 Mb	ARS	FA	DA	RU	None	None	ASD, Shortening of the upper labial frenulum

Abbreviations: ARS, Axenfeld-Rieger syndrome (any combination of posterior embryotoxon, irido-corneal adhesions, iris hypoplasia, and pupillary anomalies); GL, glaucoma; FA, facial anomaly; DA, dental anomaly; RU, redundant periumbilical skin; UH, umbilical hernia; GD, growth disorder (short stature, failure to thrive, and delayed development); ASD, autism spectrum disorder; MD, Meckel's diverticulum; GI, gastrointestinal; CA, cataract; LA, Limb abnormality; LD, Learning difficulties; VSD, Ventricular septal defect; WPWS, Wollf-parkinson-white-syndrome; HL, hearing loss; –, unknown.

Therefore, variations in *FOXC1* or *PITX2* expression may lead to ARS [19,26–28]. *PITX2* and *FOXC1* act synergistically during individuals' eye development, particularly that of the anterior segment. These two genes interact in the regulation of common downstream target genes in specific cell lines [29]. Interestingly, the variants in our case included not only two deletions but also an inversion. Although similar deletions and many breakpoints in the region harboring *PITX2* have been reported, this specific rearrangement had not been reported. A description of ARS-associated deletions, which also include 4q25q26, as well as descriptions of their clinical manifestations, are provided in Table 3.

Herein, we identified two deletions and one inversion variant on Chromosome 4 in a family with ARS. Some of the clinical presentations in our patients were unusual and atypical for ARS. Among the 86 missing genes, we sought to identify genes associated with the patients' symptoms. *NEUROG2*, *UGT8*, *NDST4*, and *ZGRF1* were associated with cognitive impairment and neurodevelopmental phenotypes. Mental retardation was found in ARS patients harboring a 4q25q26 deletion [30], which includes *NEUROG2* and *UGT8*. In another study, developmental delay was also reported in ARS patients with a 4q25-q28.2 deletion, including *NEUROG2*, *UGT8*, and *NDST4* [19]. Similarly, Titheradge et al. reported ARS patients with 4q25q26 or 4q25-q27 deletions who had developmental delays, learning difficulties, difficulty with pronunciation, and autism spectrum disorder. In these cases, deleted genes included *NEUROG2*, *UGT8*, and *ZGRF1* [21]. *NEUROG2* is a transcription factor that belongs to the basic helix–loop–helix (bHLH) family and is involved in cell fate and neuronal differentiation within various areas of the central nervous system [31]. *UGT8* is an enzyme of the uridine diphosphate glycosyltransferase family associated with the production of 3-O-sulfogalactosylceramide, a major component of the myelin sheath in the central and peripheral nervous systems [32]. *NDST4* also plays an important role in the adult brain and embryonic development [33]. In a family chain analysis, *ZGRF1* was found to be associated with childhood apraxia of speech, a severe form of speech sound disorder [34].

Despite extensive research, the potential genetic causes remain unknown in approximately 30% of ARS cases, with efforts in this direction still ongoing [9]. The current treatment for patients with ARS primarily focuses on glaucoma management. It is critical to regularly evaluate IOP and optic nerve development in patients owing the high risk of glaucoma and the fact that it is frequently diagnosed in childhood. Current glaucoma treatments include medications and surgery. However, reports have demonstrated that medical treatments do not successfully reduce IOP or hinder glaucoma progression in ARS patients with *PITX2* or *FOXC1* variants [35]. Surgical treatment has thus emerged as an alternative option, including laser iridotomy, laser trabeculoplasty, laser cycloablation, trabeculectomy, aqueous shunt devices, and minimally invasive glaucoma surgeries. The proband underwent micropulsed ciliary photocoagulation twice. Fortunately, the proband's IOPs were temporarily controlled through surgery. In conclusion, our findings broaden the range of known *PITX2* variants and may be useful for genotype–phenotype analyses in patients with ARS.

Appendix 1. Supplementary Table 1.

To access the data, click or select the words "Appendix 1." Genes contained in the 4q25 and 4q26 deletion fragments(NCBI RefSeq Annotation GCF_000001405.40-RS_2023_03).

Appendix 2. Supplementary Table 2.

To access the data, click or select the words "Appendix 2." Variants identified in the proband from WGS datasets.

ACKNOWLEDGMENTS

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