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Phenotypic and Genetic Analysis of Familial Congenital Aniridia Caused by a Run-on Mutation in the PAX6 Gene

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Research Article

Keywords: Congenital aniridia, PAX6 gene, Run-on mutation, Haploinsufficiency, Genotype-phenotype correlation

Posted Date: September 2nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10604016/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Congenital aniridia is a rare pan-ocular developmental disorder characterized by iris hypoplasia and foveal hypoplasia, frequently complicated by secondary corneal disease, glaucoma, and cataract. This study evaluates clinical phenotypes and identifies the causative mutation in a Chinese Han pedigree with congenital aniridia, aiming to explore genotype–phenotype correlations and inform genetic counseling and clinical management.

Methods

A three-generation non-consanguineous pedigree of seven members (three affected) was enrolled. Whole-genome sequencing was performed on the proband (II-2, a 31-year-old female) with Sanger sequencing used for validation across all family members. Comprehensive ophthalmic assessments included best-corrected visual acuity, slit-lamp biomicroscopy, and fundus imaging.

Results

Autosomal dominant inheritance was observed. All affected individuals showed variable iris defects, nystagmus, and foveal hypoplasia, with profound early-onset visual impairment. The proband developed progressive keratopathy post-puberty, with vision declining to hand motion; her two children (aged 7 and 3) maintained clear corneas but exihited typical iris and foveal abnormalities. A heterozygous mutation (c.1268 A > T, p.423Leu frameshift) in *PAX6* exon 13 was identified in the proband. Sanger sequencing confirmed complete cosegregation of this mutation with the affected phenotype, while all four unaffected members carried the wild-type allele.

Conclusion

This rare *PAX6* readthrough mutation cosegregates with pan-ocular developmental abnormalities in this pedigree, with notable age-dependent clinical progression. The mutation likely affect PAX6 transcriptional regulation via a C-terminally extended protein, consistent with haploinsufficiency, though the precise pathogenic mechanism requires further study. Our findings expand the *PAX6* mutational spectrum and emphasize the necessity of lifelong systematic ophthalmic surveillance-particularly in pediatric patients- for timely complication management, as well as the critical role of genetic testing in confirming diagnosis and predicting prognosis.

Introduction

Congenital aniridia (OMIM# 106210) is a genetic pan-ocular disorder characterized by partial or complete absence of the iris and foveal hypoplasia[1]. It serves as a clinical paradigm for multiple severe ocular pathologies, including aniridia-related keratopathy (ARK) secondary to limbal stem cell deficiency, glaucoma, later-onset cataract, dry eye disease, meibomian gland dysfunction, and congenital posterior segment defects [2–6]. These manifestations commonly emerge during childhood or adolescence and frequently culminate in profound visual impairment [5, 6]. In addition, an increasing number of on-ocular features have been reported, promoting broader use of the term “aniridia syndrome”.

In the vast majority cases, the underlying etiology involves mutations in the paired-box gene 6 (*PAX6*), which disrupts critical regulatory pathways in ocular morphogenesis [7, 8]. The human *PAX6* gene, located at chromosome 11p13, spans fourteen exons and encodes a 423-amino-acid protein (NM_000280.6). As a transcription factor, PAX6 protein harbors two highly conserved DNA-binding domains-the paired domain (PD) and the homeodomain (HD)-through which it binds target DNA sequences with specificity and orchestrates the expression of numerous developmental genes expressed in the corneal, iris, ciliary body, lens and retinal during eye development [9–11].

To date, over seven hundred *PAX6* genetic variants have been documented (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6). The majority of aniridia-associated variants are heterozygous, arising either sporadic or segregating in an autosomal dominant pattern within families [12]. Most *PAX6* mutations are frameshift, nonsense, or splice-site alterations, which typically result in loss-of-function substitutions or premature truncation of the protein product [1, 13, 14]. Consequently, *PAX6* haploinsufficiency constitutes the principle pathogenic mechanism underlying classic aniridia and its associated ocular anomalies [15].

In this study, we report a Chinese Han pedigree with congenital aniridia showing autosomal dominant inheritance, intrafamilial phenotypic variability, and age-dependent clinical progression. Through whole-genome sequencing followed by Sanger validation, we identified a rare heterozygous readthrough mutation in *PAX6* gene, which abolishes the native stop codon and is predicted to generate a C-terminally elongated PAX6 protein. We further provide detailed ophthalmic phenotyping of all affected individuals across three generations and discuss the potential pathomechanistic implications of this unusual mutation type, as well as its clinical relevance for long-term disease surveillance and genetic counseling.

Methods

Ethics declaration

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki and its subsequent amendments. The study protocol received approval from the Institutional Review Board of the Shanghai Eye Diseases Prevention &Treatment Center (Approval No. EC-20240913-08). Informed consent was obtained from all individual participants or their legal guardians included in the study.

Subjects and clinical data

A total of seven individuals from a three-generation Chinese family with typical aniridia were recruited. The cohort included three affected and four unaffected members. All participants underwent comprehensive ophthalmic examinations, including best-corrected visual acuity (BCVA) assessment, slit-lamp examination, intraocular pressure (IOP) measurement, optical coherence tomography (OCT), and fundus examination.

Experimental methods

DNA samples

Peripheral blood samples were collected from all participating family members. Genomic DNA was extracted using the QIAamp Blood DNA kit (Qiagen, Hilden, Germany; Cat. 51104) in accordance with the manufacturer's instructions.

Genetic analysis and bioinformatics prediction: Whole exome sequencing (WES) was performed on genomic DNA using the Illumina HiSeq 2500 platform). Sequencing reads were aligned to the human reference genome (hg19) using BWA software. Single nucleotide variants (SNVs) and insertions/deletions (INDELs) were called and filtered with GATK software and subsequently annotated using ANNOVA. Variants with a minor allele frequencies greater than 1% in public databases, including gonomAD, dbSNP, 1000 Genomes, and ExAC-were excluded. The pathogenicity of the remaining variants was assessed using Mutation Taster (http://www.mutationtaster.org/) and SIFT (http://sift.bii.a-star.edu.sg/) prediction tools.

Sanger sequencing: The candidate variation identified through WES was validated using Sanger sequencing, based on the NCBI reference sequence of homo sapiens *PAX6* Specific primers were designed to amplify the target region: Forward primer TGCGTGCTTGTTGAAATGCTT and reverse primer AGAACTGAAGCGGCTCTAACA, generating a 589-bp amplicon. Polymerase chain reaction (PCR) amplification was performed using the KOD PCR kit (TOYOBO, Osaka, Japan; Cat. KOD-211) under the following conditions: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 56°C for 30 s, and extension at 68°C for 40 s; with an extension at 68°C for 10 min.

PCR products were evaluated by 1% agarose gel electrophoresis, purified using the FastPure Gel DNA Extraction Mini Kit (Vazyme, Nanjing, China; Cat. DC301), and subsequently sequenced using an ABI 3730xl DNA Analyzer platform (Applied Biosystems, California, USA) according to the manufacturer's instructions. The forward primer for sequencing was: AGGACTCATTTCCCCTGGTGT. All the oligonucleotides were synthesized by Tsingke Technologies (Guangzhou, China).

Results

In this study, we analyzed seven members of a Chinese family, of whom three individuals across two generations were diagnosed with congenital aniridia: a 31-year-old woman (the proband, II-2) and her two daughters, III-1 (aged 7) and III-2 (aged 3). Family pedigree analysis (Fig. 1A) revealed autosomal dominant inheritance. The proband (II:2) presented with low vision and nystagmus since childhood. At age 34, her BCVA deteriorated to hand motion, accompanied by nystagmus, corneal edema, partial iris defect, and macular dysplasia. In contrast, her parents and younger brother (I-1, I-2, and II-1) exhibited normal visual acuity and no ocular abnormalities (Table 1, Fig. 2 and Fig. 3).

Table 1
Clinical features of the family

Cases	Age	BCVA (OD, OS)	IOP (OD/OS)	Aniridia	ARK	foveal hypoplasia
I-1	60	20/20,20/20	16.3/19.0	-	-	-
I-2	55	20/25,20/25	13.7/17.0	-	-	-
II-1	35	20/20,20/20	16.0/16.0	-	-	-
II-2	31	HM, HM	10.0/10.0	+	+	+
II-3	20	20/20,20/20	17.0/17.7	-	-	-
III-1	7	20/200,20/200	16.0/12.0	+	-	+
III-2	3	NA, NA	Tn/Tn	+	-	+*

BCVA, best corrected visual acuity; IOP, intraocular pressure; ARK, aniridia-related keratopathy. NA, not available due to inability to cooperate.

Following a non-consanguineous marriage, the proband gave birth to two children who manifested similar clinical features. Both children presented with poor vision and nystagmus from early childhood. Initial ophthalmic evaluation, prompted by reduced visual acuity and nystagmus, documented iris defects and macular hypoplasia at age 7 and 3 by direct ophthalmoscopy, respectively; notably, both corneas remained transparent (Fig. 1B, 1C).

To identify the causative genetic variant, whole-genome sequencing was performed on the proband (II-2) and the affected ones. A heterozygous likely-pathogenic variant in exon 13 of *PAX6;* NM_000289.4: c.1268 A > T (p.*423Leu frameshift) was identified in exon 13. Sanger sequencing of this gene in all seven family members confirmed that the proband and her two children (III-1 and III-2) were heterozygous carriers of the variant, whereas all unaffected members (I-1, I-2, II-1 and II-3) carried the wild-type allele (Fig. 1D). In this pedigree, the pathogenic genotype completely cosegregated with the disease phenotype.

Discussion

This study systematically characterized clinical manifestations and identified a rare heterozygous *PAX6* variant in a three-generation Chinese Han pedigree with autosomal dominant congenital aniridia. Completely cosegregation between this variant and affected ocular phenotype confirmed its pathogenicity, expanding the mutational spectrum of *PAX6* in East Asian populations and offering further insight into genotype-phenotype correlations of congenital aniridia.

Consistent with established knowledge, all affected family members exhibited the core pan-ocular developmental defects characteristic of *PAX6*-related aniridia, including variable iris hypoplasia, congenital nystagmus and foveal hypoplasia accompanied by early-onset severe visual impairment. Marked intrafamilial phenotypic heterogeneity and age-dependent disease progression were observed within this pedigree. The proband developed progressive ARK after puberty, culminating in vision reduction to hand motion, whereas her two young affected children maintained clear corneal tissue despite typical iris and foveal malformations. This observation strongly supports that foveal and iris anomalies arise during embryonic ocular morphogenesis, while corneal complications represent progressive postnatal degeneration rather than primary developmental lesions [1, 2, 5]. Longitudinal cohort studies have demonstrated that ARK typically emerges in adolescence or early adulthood, driven by limbal stem cell deficiency secondary to persistent *PAX6* haploinsufficiency, which explains why pediatric patients often preserve intact corneal epithelium before puberty[1]. Clinically, this distinction reinforces the importance of stratified surveillance schedules: children require monitoring of refractive error, amblyopia and structural retinal abnormalities, whereas adolescent and adult patients demand intensified screening for corneal opacification, glaucoma and cataract[16].

About one half to two-thirds of aniridia cases are familial, typically inherited in an autosomal dominant manner, which aligns with the segregation pattern observed in this family [1, 6, 17, 18]. The vast majority of *PAX6* mutations associated with aniridia are nonsense, frameshift, or splice-site variants [1, 13, 14], which introduce premature termination codons, leading to nonsense-mediated mRNA decay or production of truncated, functionally deficient proteins. In contrast, run-on (or readthrough) mutation such as the one identified here represent a rare and mechanistically distinct class of variants, it has been reported that this kind of frameshift or run-on variants towards the end of the coding sequence which resulted in an elongated or extended transcript had intermediate levels of aniridia and other aniridia-associated eye abnormalities[19–24]. In the present pedigree, the identified c.1268 A > T variant represents a rare stop-loss/readthrough mutation located in exon 13 of *PAX6*. This run-on mutation identified here was initially reported in a sporadic case and has been observed in both Eastern and Western populations [25, 26], this is the first time that such a mutation has been discovered and confirmed in a Chinese familial pedigree, and reported the genotype-phenotype correlations.

The mutation is predicted to disrupt the native stop codon, thereby allowing translation to proceed into 3'-UTR and generating an elongated protein product with eight additional lysine residues as predicted by Mutation Taster (Fig. 1F). Unlike the most common pathogenic *PAX6* variants, which introduce premature termination codons and active nonsense-mediated mRNA decay, readthrough variants are predicted to generate aberrantly C-terminally extended PAX6 protein products, rather than truncated

peptides or complete allele loss [12]. The resultant structural perturbation of the C-terminal transactivation domain is believed to compromise transcriptional regulation, ultimately manifesting as functional haploinsufficiency. Notably, patients with frameshift or run-on variant of PAX6 gene seem to have a much broader phenotypic spectrum, with aniridia reported at a much lower level and a significant increase in nystagmus, cataracts and foveal hypoplasia [23]. That is consistent with our findings, which also suggests complex modifier effects and that the downstream molecular pathways underlying theses phenotypes remain incompletely characterized.

From a translational perspective, our findings highlight the indispensable role of genetic testing for congenital aniridia. Clinical diagnosis alone cannot reliably predict the timing and severity of secondary complications. Molecular confirmation facilitates accurate genetic counselling for at-risk family members, including prenatal and preimplantation genetic testing options for affected couples [16]. For this pedigree, the two affected children require lifelong annual ophthalmic follow-up to anticipate late-onset corneal disease. Current international guidelines recommend continuous systemic ophthalmic surveillance across the lifespan for all *PAX6* variant carriers, as irreversible vision loss frequently stems from delayed intervention of progressive complications rather than primary congenital malformations [6, 16].

Several limitations should be acknowledged. First, functional studies, including *in vitro* transcriptional activity assays or animal models, are warranted to definitively characterize the molecular consequences of the p.*423Leu frameshift elongated protein. The precise molecular pathway through which such elongation variants lead to haploinsufficiency remains to be fully elucidated and warrants further functional investigation. Second, long-term follow-up data for the two pediatric patients will be essential to delineate the long-term clinical outcomes and to assess whether this specific mutation confers a milder or more aggressive corneal phenotype compared with other *PAX6* variants. Third, given the rarity of readthrough mutations, larger cohort studies are needed to establish whether such variants are associated with unique systemic or ocular manifestations beyond those observed in classic aniridia.

Conclusions

In conclusion, this report describes a rare *PAX6* run-on variant segregating with typical pan-ocular aniridia phenotypes in a Chinese Han family. The prominent age-dependent progression of keratopathy observed here enriches our understanding of variable expressivity in *PAX6*-related disease. Additional functional research and multi-ethnic cohort analyses are necessary to delineate genotype–phenotype relationships for uncommon *PAX6* variants and optimize individualized lifelong clinical management protocols for congenital aniridia patients.

Abbreviations

ARK, aniridia-related keratopathy

PAX6, paired-box gene 6

PD, the paired domain

HD, homeodomain

BCVA, best-corrected visual acuity

IOP, intraocular pressure

OCT, optical coherence tomography

Declarations

Authorship contribution statement

M.H. and S.Z contributed equally: Data curation, Formal analysis, Methodology, Writing – original draft, Writing–review & editing. Y.Y, and J.T: Data curation, Methodology, Formal analysis. Y.W and W.X: Conceptualization, Investigation, Supervision, Writing – review & editing.

Funding

The work was partially supported by Clinical Research Project of Shanghai Eye Disease Prevention Center (No.23LC01009).

Consent for Publication

Written informed consent for publication of identifying ophthalmic images (including anterior segment photographs and retinal color photographs) was obtained from all participants (or their parents/legal guardians, where applicable) included in this study. The authors confirm that all images have been de-identified to the maximum extent possible, and the consent forms are available for review upon request.

Data availability

The datasets of DNA sequencing data generated and analyzed during the current study are available in the Genome Sequence Archive (GSA) for Human via persistent web link: https://ngdc.cncb.ac.cn/gsa-human/ under accession number PRJCA072442.

Conflict of interests

The authors have no competing interests to declare.

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Figures

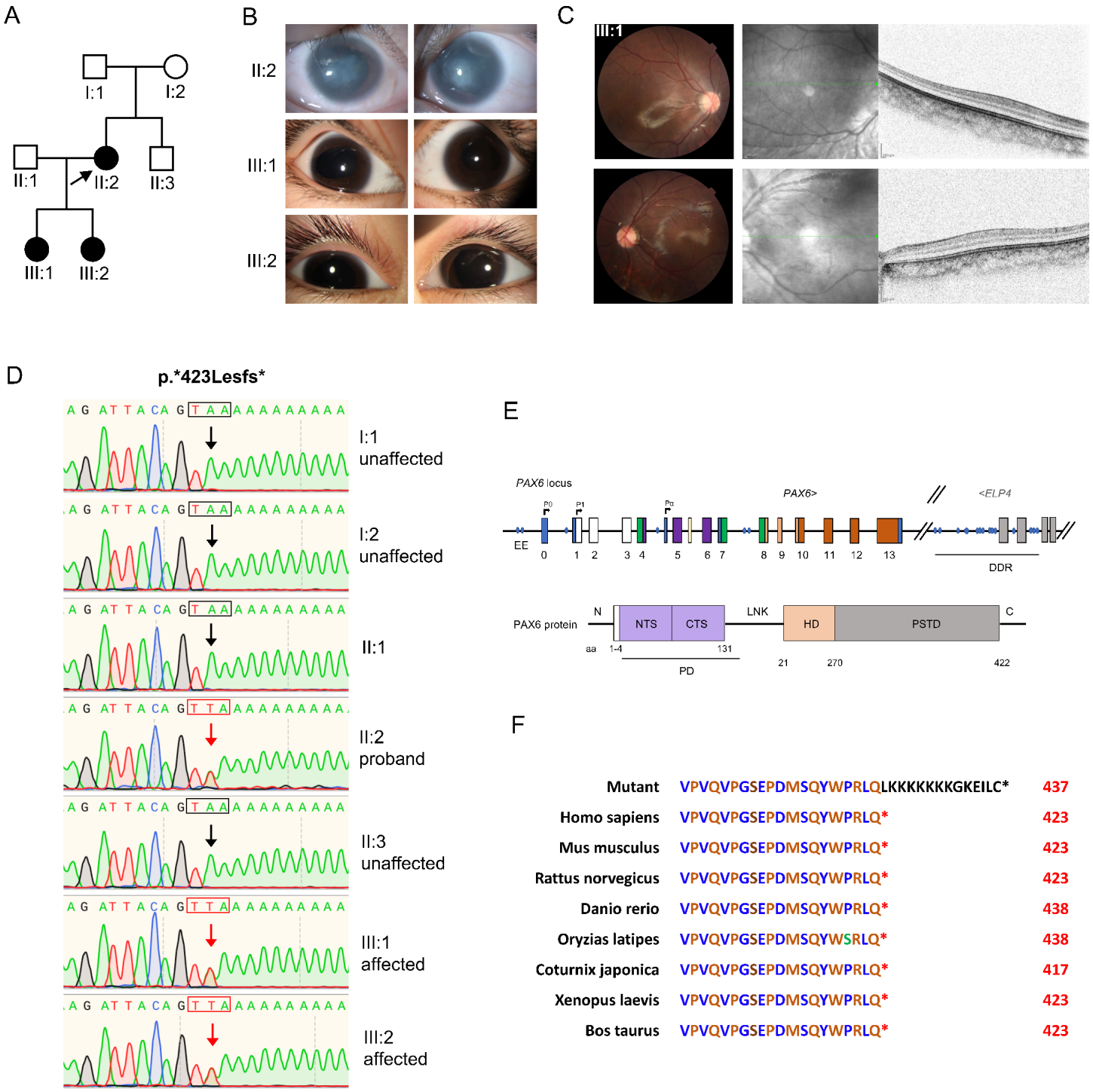


Figure 1

Clinical findings and genetic analysis of the family.

A. Pedigree of the Chinese family with congenital aniridia consistent with autosomal dominant inheritance.

B. Anterior segmental photographs of the three affected patients (proband II-2, III-1 and III-2).

C. Fundus OCT images of patients III-1.

D. Whole-genome sequencing of the proband (indicated by arrow) and Sanger sequencing analysis of all the seven familiy members.

E. Upper panner: Human PAX6 genomic locus, showing promoters P0, P1 and Pα (diagnonally-hatched boxes), along with regulatory elements including the ectodermal enhancer (EE) and downstream regulatory region (DRR). Lower panel: Schematic of the canonical PAX6 protein isoform with functional domains: PD (paired domain), NTS (N-terminal subdomain), CTS (C-terminal subdomain), LNK (linker region), HD (homeodomain), PST (proline-serine-threoine domain); aa: amino acid.

F. Multiple sequence alignment of the last 20 amino acids of PAX6 across various species, and a predicted extended protein sequence resulting from the genomic mutation, as analyzed using Mutation Taster

(https://www.mutationtaster.org/MutationTaster69/info/documentation.html#phylop).

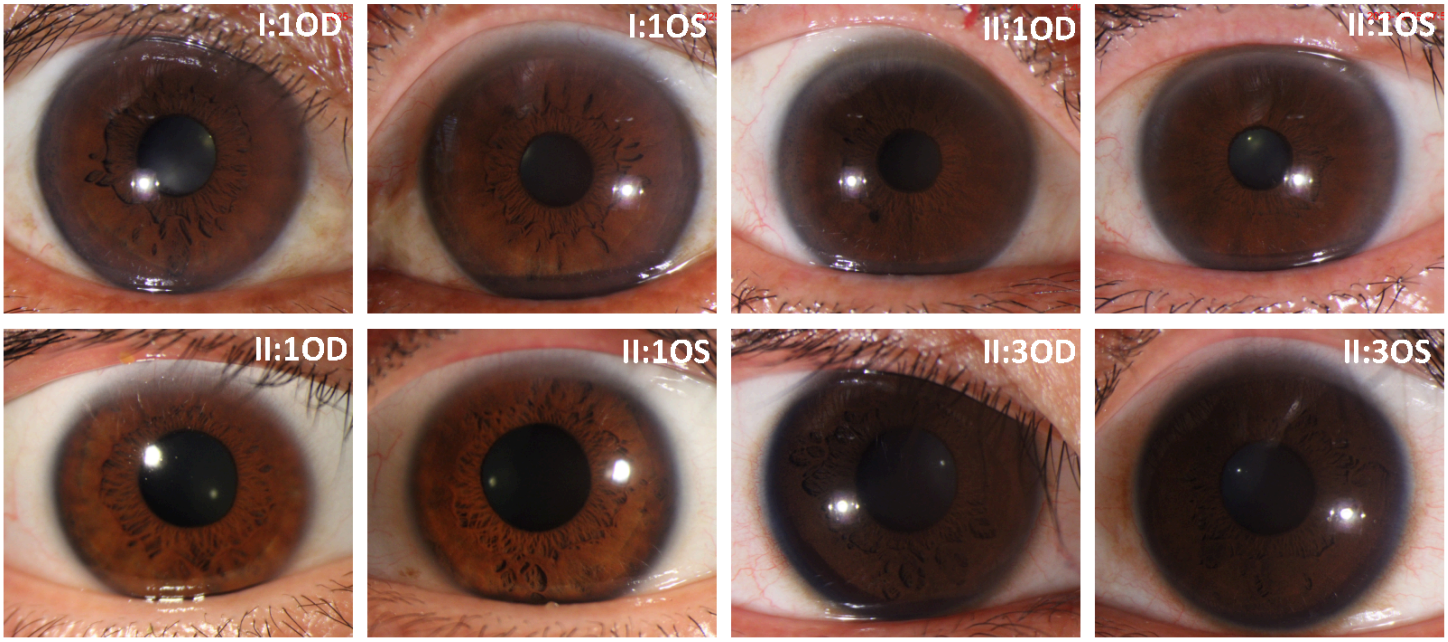


Figure 2

Anterior segmental photography of the unaffected family members.

Slit-lamp examination revealed no anterior segment abnormalities in unaffected family members I:1, I:2, II:1 and II:3.

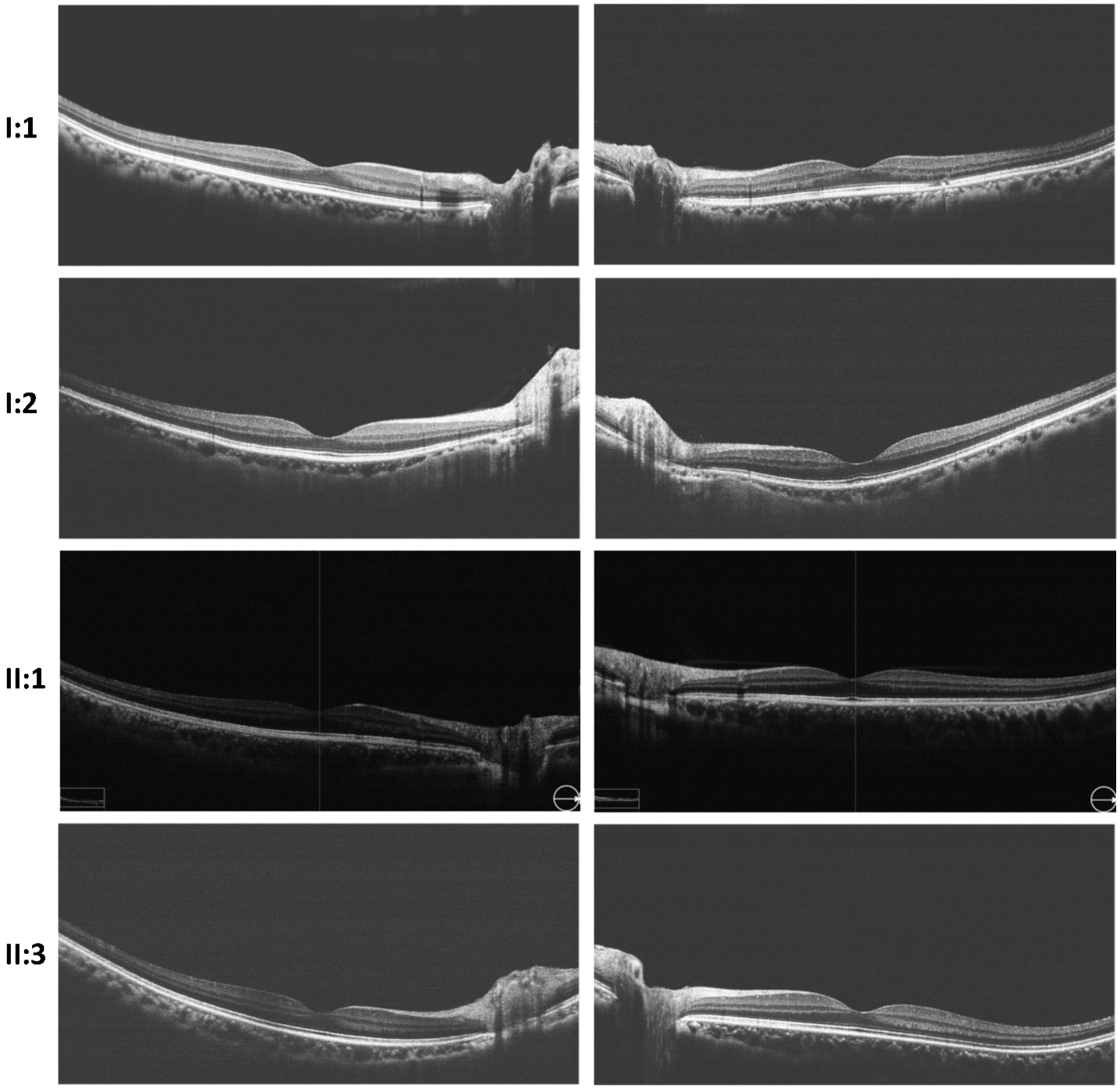


Figure 3

Fundus OCT imaging results from the unaffected family members.

Macular morphology, as assessed by OCT, was unremarkable in the unaffected subjects I:1, I:2, II:1 and II:3.