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Molecular Case Studies

VARIANT DISCREPANCY RESOLUTION

Novel *PAX6* variant in a family with ophthalmologic, pancreatic, and olfactory features

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Abstract Variants in the *PAX6* gene have been associated with ophthalmologic, neurologic, and pancreatic differences. We report on a proband, mother, and affected brother who presented with congenital cataracts and glaucoma at a young age. Nonocular findings are also reported among these family members. After a congenital cataracts next-generation sequencing (NGS) gene panel was found to be nondiagnostic in 2016, a more expanded panel in 2020 revealed a novel variant: c.178T > A; p.Tyr60Asn in exon 6 of the *PAX6* gene in the proband. The variant is also present in the affected mother and affected brother; it is absent in an unaffected brother. The clinical findings of these three relatives, in conjunction with their genetic testing and the associated *PAX6* features reported in the literature, suggest that this novel familial variant may be an underlying etiology for these individuals' ophthalmologic, pancreatic, and olfactory symptoms.

CASE PRESENTATION

The *PAX6* gene encodes a DNA-binding transcriptional factor important for development of the eyes, brain, and pancreas (Xie et al. 2013). Disruption of *PAX6* is known for causing forms of aniridia, keratitis, glaucoma, cataracts, foveal hypoplasia, and microphthalmia (Lee et al. 2008). Neurologic differences, including intellectual disability, cerebellar ataxia, and anosmia, and pancreatic abnormalities, such as diabetes mellitus, have also been reported (Lima Cunha et al. 2019). We present a novel *PAX6* variant found among three family members with ocular, neurologic, and endocrine symptomatology (Table 1).

Most notably, an individual (proband), her mother, and brother were found to have bilateral congenital cataracts in early infancy. These were subsequently removed at 2 mo, 9 mo, and 3 mo of age, respectively. The proband had lens implantations at the time of this surgery. The mother does not have lens implants. The affected brother had lens implantations at 7 years of age. The proband was diagnosed with glaucoma at 6 mo of age. Her mother was diagnosed at 18–19 yr old, and her brother at 6 yr old. Other symptoms experienced by one or more of these related individuals include anosmia, learning difficulties, pancreatitis in adulthood, pancreatic underdevelopment and hypoglycemia in infancy, and type II diabetes mellitus. Figure 1A displays a three-generation pedigree for this family following testing of the proband's first-degree relatives for the *PAX6* variant. The pedigree shows three affected individuals and one unaffected relative, with their associated genetic testing results. Table 1 details the phenotype of the proband and the two relatives who were found to harbor this variant.

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Table 1. Clinical findings among three relatives with *PAX6* variant

Features presumed to be due to *PAX6*	Proband	Mother	Sibling	Alternate explanation
Ophthalmologic				
Bilateral congenital cataracts	Yes	Yes	Yes	
Early-onset glaucoma	Yes	Yes	Yes	
Stromal hypoplasia of the iris	Yes	Yes	No	
Nystagmus	Yes	No	No	
Exotropia	Yes	No	Yes	
Fovea plana	Yes	No	No	
Serous retinal detachment	No	No	Yes	
Coloboma	No	No	No	
Optic nerve hypoplasia	No	No	No	
Aniridia	No	No	No	
Keratitis	No	No	No	
Microcornea	No	Yes	No	
Neurologic				
Reduced olfaction	Yes	Yes	No	
Migraine	Yes	No	No	
Myoclonic tremor	Yes	No	No	
Learning difficulties	Yes	Yes	Yes	
Absent pineal gland	No	No	No	
Hypoplastic anterior commissure	No	No	No	
Hypoplastic corpus callosum	No	No	No	
Endocrine				
Type II diabetes mellitus	No	Yes	No	
Pancreatitis	No	No	Yes	
Neonatal pancreatic underdevelopment	No	No	Yes	
Novel clinical features				
Hypermobile joints	Yes	No	No	Family history of connective tissue disease on maternal and paternal sides
History of orthodontic headgear	Yes	No	No	
Asthma with decreased lung capacity	Yes	No	No	Unrelated
Sinus tachycardia with syncope	Yes	No	No	Unrelated
Hemochromatosis	No	No	Yes	Unrelated

Findings highlighted in green are those reported in Online Mendelian Inheritance in Man (OMIM) in association with pathogenic *PAX6* variants.

TECHNICAL ANALYSIS

All individuals provided consent for genetic testing, and affected individuals provided written consent for inclusion in this manuscript.

Genetic testing using a congenital cataract next-generation sequencing (NGS) panel was nondiagnostic for the proband in 2016. An NGS gene panel performed in 2020 included 86 genes associated with cataracts and revealed a heterozygous variant of uncertain significance (VUS), p.Tyr60Asn in exon 6 of *PAX6* (NM_000280.3 c.178T > A), which has now been reclassified as likely pathogenic, based on PM1, PM2_P, PP3, PP4, and PP1 evidence.

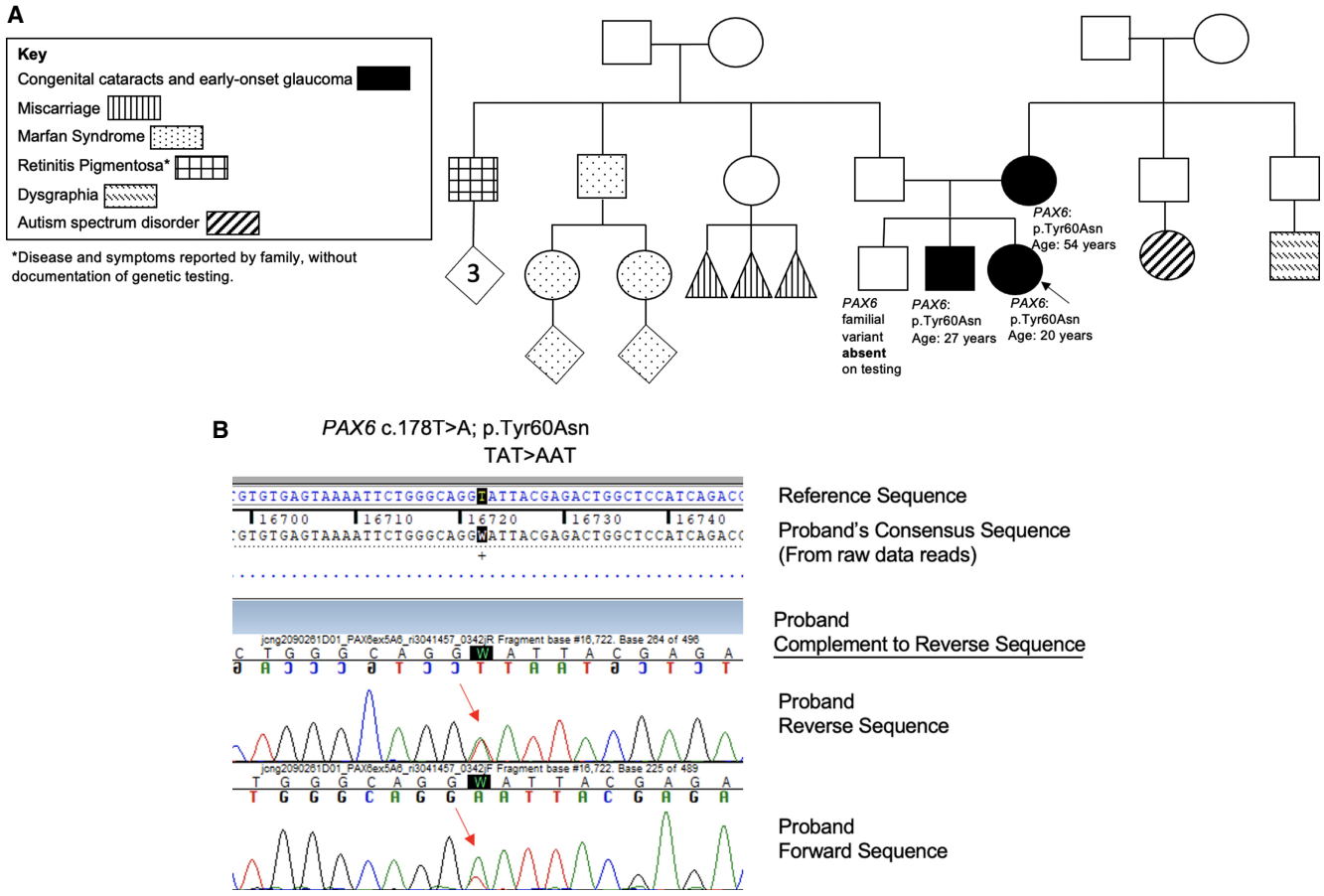

Figure 1. (*A*) A pedigree of the proband (marked by an arrow) and her family members. *PAX6* testing results are denoted next to the proband, her mother, the affected brother, and unaffected brother. Of note, the unaffected brother tested negative for the novel *PAX6* variant. Other diseases and symptoms among the family are denoted by their respective shadings. (*B*) A sequence chromatogram depicting forward and reverse traces obtained by capillary sequencing of *PAX6*, exon 6. The arrows indicate the presence of the heterozygous c.178T > A; p.Tyr60Asn variant. Data obtained by GeneDx, 2021, Cataract Panel.

NGS testing was performed using a proprietary targeted capture system developed by GeneDx for NGS with copy-number variation (CNV) calling (NGS–CNV). The enriched targets were simultaneously sequenced with paired-end reads on an Illumina platform. Bidirectional sequence reads were assembled and aligned to reference sequences based on National Center for Biotechnology Information (NCBI) RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene-specific filtering, data were analyzed to identify sequence and most copy-number variants. Capillary sequencing was performed to confirm the reported variant in the proband (Fig. 1B) and in the two other affected family members. The analysis software program used was Sequencher 4.9, Gene Codes Corporation. Table 2 details the *PAX6* variant segregating with the disease in this family. Table 3 details the damage scores and ranges of the in silico analysis. Each of the three algorithms used suggests that this variant is damaging.

The proband underwent whole genome sequencing in 2021 at a different commercial laboratory to further investigate her extraocular features; this testing revealed only the novel variant in *PAX6*.

Table 2. The proband's genetic findings

Gene	Genomic location	HGVS cDNA	HGVS protein	Zygosity	Parent of origin	Variant interpretation
PAX6	GRCh37/UCSC hg19 Chr 11:31823288A > T	NM_000280.3: c.178T > A	p.Tyr60Asn	Heterozygous	Maternal	Variant of uncertain significance, reclassified as likely pathogenic (PM1, PM2_P, PP3, PP4, PP1)

VARIANT INTERPRETATION

Variant p.Tyr60Asn is a novel missense variant in *PAX6*, located within the α-3 helix, also known as the recognition helix, of the amino-terminal paired domain of the paired box 6 transcriptional regulator (amino acid residues 1–130) (Cross et al. 2020). This region was shown to make multiple contacts with DNA bases per analogous residues in the paired domain crystal structure (Xu et al. 1995). It is a known hotspot region in *PAX6*, where many pathogenic and likely pathogenic missense variants cluster (Cross et al. 2020). In silico analysis supports a deleterious effect on protein structure and function of this variant. The variant has not been observed in large population cohorts (gnomAD).

PAX6 variants are well-known for their effects on eye development, resulting in atrophy of the iris, nystagmus, foveal hypoplasia, cataracts, glaucoma, and corneal keratopathy (Lee et al. 2008; Lima Cunha et al. 2019). The genetic variant in the presented family would therefore explain their specific ocular medical history. Although childhood glaucoma is a potential postoperative complication of cataract surgery, the rate among this population (<2 yr old with bilateral congenital cataracts) is only 6.7%, in a recent meta-analysis (Zhang et al. 2019). Given this complication's relative infrequency, and that congenital malformations related to *PAX6* may promote glaucoma (Davis-Silberman et al. 2005), we suggest that the familial variant may have predisposed this family to developing glaucoma.

Additionally, patients with missense variants, like our patient and her family, often have a less severely affected iris (Lima Cunha et al. 2019). This could explain why the proband and her family do not have the complete aniridia associated with other *PAX6* variants. Figure 2 displays a RetCam photograph of the proband's dilated eye exam under anesthesia.

Variants in *PAX6* have also been associated with obesity and diabetes mellitus because of *PAX6*'s role in pancreatic development (Panneerselvam et al. 2019; Boese et al. 2020); the familial variant, therefore, may have contributed to the brother's and mother's endocrinopathies. The mild neurodevelopmental differences found among affected individuals in this family may be explained by the *PAX6* variant, given previous reported associations with neurodevelopmental abnormalities (Davis et al. 2008; Kikkawa et al. 2019). Furthermore, *PAX6* haploinsufficiency has been reported to cause olfactory dysfunction, which may readily

Table 3. In silico analysis

Prediction algorithm	Result	Score or probability	Range	References
Provean	Damaging	−7.5	<−2.5 is damaging	Variant was run Provean; http://provean.jcvi.org/about.php
MutationTaster	Disease-causing	0.978263334	Value close to 1 indicates high security of the prediction	MutationTaster; https://www.mutationtaster.org/info/documentation.html
PolyPhen-2	Probably damaging	1.0	0.00–1.00, with 1.0 disease-causing	PolyPhen; http://genetics.bwh.harvard.edu/pph2/dokuwiki/about

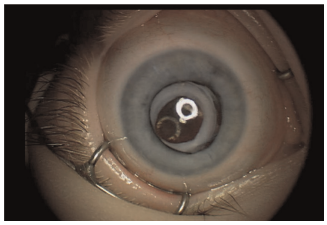

Figure 2. A RetCam photograph taken during a dilated exam under anesthesia of the proband's pupil and iris. Described clinically as iris stromal hypoplasia and slight pupil sphincter underdevelopment. A whitish Soemmering ring can be seen behind her intraocular lens implant, with a central opening.

explain the proband's and her mother's reports of diminished sense of smell (Sisodiya et al. 2001).

Collectively, considering the family's clinical findings and genetic testing results, we classify this variant as likely pathogenic.

SUMMARY

We describe a likely pathogenic variant in *PAX6* present in three relatives with congenital cataracts and glaucoma at an early age. We suggest based on the current literature, clinical findings, and segregation among family members that this variant may be of clinical significance. Notably, the variant was discovered via NGS panel 4 yr after an initially nondiagnostic result. A limitation of ophthalmologic gene panels is the frequency with which they are updated (Méjécase et al. 2020). This family highlights the need to consider retesting patients with nondiagnostic results—particularly those with family history indicating a genetic cause.

ADDITIONAL INFORMATION

Data Deposition and Access

This variant has been deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) under accession number SCV001906465.

Ethics Statement

All individuals provided consent for genetic testing, and affected individuals provided consent for inclusion in this manuscript.

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Author Contributions

J.L.C. conceptualized and designed the study, obtained consent from individuals, and oversaw clinical data analysis and interpretation of genetic test results for the proband and family. K.L.B. performed the clinical data collection and analysis, conducted literature review, and wrote the initial manuscript draft. S.H. organized and facilitated the genetic testing as well as provided genetic counseling for the family. B.W. provided the genomic sequencing methods, data, and associated figures. All authors contributed to the editing of the manuscript and provided approval for the final version.

Competing Interest Statement

B.W. is an employee of GeneDx, Inc. The other authors have no conflicts of interest to disclose.

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