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Revealing hidden genetic diagnoses in the ocular anterior segment disorders

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Purpose: Ocular anterior segment disorders (ASDs) are clinically and genetically heterogeneous, and genetic diagnosis often remains elusive. In this study, we demonstrate the value of a combined analysis protocol using phenotypic, genomic, and pedigree structure data to achieve a genetic conclusion.

Methods: We utilized a combination of chromosome microarray, exome sequencing, and genome sequencing with structural variant and trio analysis to investigate a cohort of 41 predominantly sporadic cases.

Results: We identified likely causative variants in 54% (22/41) of cases, including 51% (19/37) of sporadic cases and 75% (3/4) of cases initially referred as familial ASD. Two-thirds of sporadic cases were found to have heterozygous variants, which in most cases were de novo. Approximately one-third (7/22) of genetic diagnoses were found in rarely reported or recently identified ASD genes including *PXDN*, *GJA8*, *COL4A1*, *ITPR1*, *CPAMD8*, as well as the new phenotypic association of Axenfeld–Rieger anomaly with a homozygous *ADAMTS17* variant. The remainder of the variants were in key ASD genes including *FOXC1*, *PITX2*, *CYP1B1*, *FOXE3*, *and PAX6*.

Conclusions: We demonstrate the benefit of detailed phenotypic, genomic, variant, and segregation analysis to uncover some of the previously "hidden" heritable answers in several rarely reported and newly identified ocular ASD-related disease genes.

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INTRODUCTION

Ocular anterior segment disorders (ASDs) encompass conditions with broad clinical and genetic heterogeneity that affect the structures anterior to the vitreous surface of the eye. There are multiple conditions grouped clinically under the term ASD including aniridia, iris hypoplasia (IH), Axenfeld–Rieger anomaly (ARA) and syndrome (ARS), primary congenital glaucoma (PCG), Peters anomaly (PA), and sclerocornea, and there are many syndromal associations. Phenotypic features may overlap, and there are complex embryonic, genetic, and environmental factors involved in the pathogenesis of this group of disorders. In addition, several genes contribute to multiple phenotypes, adding to the complexity of the phenotype–genotype correlations and genetic diagnostic accuracy.

Despite the successful adoption of next-generation sequencing (NGS) in many genetic conditions, there is a

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lack of systematic investigation of the diagnostic utility of NGS in the full group of ocular ASD patients that may present for genetic diagnosis. Most studies focus on a particular phenotypic subset or gene set, and such studies suggest there may be a detection rate of <10–40% in the broader cohort.[1–3] Our earlier work has highlighted novel genotype–phenotype correlations[4] in the ocular ASDs, and the benefit of analysis of a broader group of genes using genomic approaches to find "missing" genetic diagnoses. In this study, we applied a combination of genomic, phenotypic, and pedigree structure and segregation analyses, aimed at maximizing the genetic diagnostic detection rate in this complex patient group.

MATERIALS AND METHODS

Forty-one probands with a variety of ASD phenotypes were investigated for genetic diagnosis at a major pediatric referral hospital in Sydney, Australia, over a 12-year period. In these patients the predominant presenting phenotype was ocular ASD, and included ARA, ARS, IH, PA, and sclerocornea, with overlapping features such as microphthalmia, cataract or coloboma in some cases, and occasional presence of nonocular features such as intellectual disability, ataxia, or autism (Table 1 and Supplementary Table 1). Cases with the distinct phenotypes of aniridia, known to be predominantly caused by variants in *PAX6*, or primary congenital glaucoma were not included in this study. The majority (35/41) were from a Caucasian background, with a small minority with Asian (3) or Middle Eastern (3) heritage. The majority (37/41) initially presented as sporadic cases, while 4 had a family history suggesting an autosomal dominant mode of inheritance (Supplementary Table 1). Ophthalmological details and samples for genomic DNA extraction were collected from family members when available.

Ethics statement

Informed consent was obtained, including the publishing of photographs where applicable, and the study was approved by the Human Research Ethics Committee of Sydney Children's Hospitals Network, Sydney, Australia.

Structural variation analysis

All probands underwent copy-number variant (CNV) analysis with chromosomal microarray (CMA), on a 400K comparative genomic hybridization (CGH) array platform (Agilent SurePrint G3 Human Microarray, Santa Clara, CA, USA). Samples that underwent genome sequencing (GS) were analyzed for structural variants (SVs) with ClinSV (Minoche et al., in prep; https://github.com/KCCG/ClinSV), utilizing evidence from split-reads, discordant pairs, and depth of coverage to obtain rare, high confidence structural and CNV calls, in accordance with best practice guidelines. Multiplex ligation dependant probe amplification (MLPA) was also performed to validate any deletions (MRC-Holland, Amsterdam, Netherlands).

Next-generation sequencing

NGS was performed using exome sequencing (ES) with Illumina TruSight One Clinical Exome (Illumina, USA) or Agilent SureSelect Exome (Agilent SureSelect V4, Macrogen Inc, Seoul, South Korea). GS was performed on a number of ES-negative samples, new probands, and family samples for segregation analysis, on the Illumina TruSeq Nano HT kit with the Illumina HiSeq X (Illumina Inc, and Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Sydney, Australia).

The library preparation, genomic alignment, variant calling, and annotation were performed as previously described[5–8] with variant filtering undertaken for specific anterior segment, cataract, and microphthalmia/anophthalmia disease genes, as in our previous studies and review of ASD genes[4,5,8] (Supplementary Table 2). Average coverage of the key ASD genes was 93% and 92% above 20× in ES and GS platforms respectively. For negative cases, as well as trio and family samples, rare variants of interest based on in silico analysis, conservation, population databases, and phenotypic data (including pedigree structure) were also examined and manually reviewed for pathogenicity, according to American College of Medical Genetics and Genomics (ACMG) guidelines.[9] All variants reported in this paper have been submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/).

RESULTS

Likely causative variants in 54% of probands, including in 51% of sporadic cases

Overall, pathogenic and likely pathogenic variants in known genes[9] were found in the majority of cases (22/41; 54%), with a detection rate in sporadic cases of 51% (19/37) as well as genetic diagnosis in a very high proportion (75%; 3/4) of familial cases from initial referral (Table 1). Pathogenic or likely pathogenic variants were found in 11 genes (Fig. **1a**). Interestingly, 32% (7/22) of the likely causative variants were in the rarely reported genes *PXDN*, *COL4A1*, *GJA8* (2), *CPAMD8*, and *ITPR1*, and for the first time reported in *ADAMTS17*. The rest of the likely causative variants were identified in the well-known ocular ASD genes (number of cases) *FOXC1* (5) and *PITX2* (4), as well as *CYP1B1* (3), *PAX6* (2), and *FOXE3* (1) (Fig. **1a**).

Of the 19 initially referred sporadic cases where causative variants were found, most were due to de novo autosomal dominant inheritance (Fig. **1b**) while the rest were inherited. Two were found to have autosomal dominant parental inherited variants in *COL4A1* and *FOXC1* respectively, and on re-examination, the parents were found to have subtle features of ocular ASD (Fig. **1b**). In six, autosomal recessive inheritance was identified due to variants in *CYP1B1* (3), *CPAMD8*, *PXDN*, and *ADAMTS17* (Fig. **1b**). Of the three cases initially referred as familial where causative variants were identified, two were confirmed as autosomal dominant, and one was found to have the same homozygous variant in *FOXE3* in a son and his father, in a case of pseudodominance from a highly inbred population group (Fig. **1b**).

Table 1 Patients with likely causative variants.

Patient number	Inheritance before/after testing	Phenotype	Platform	Gene (NM)	Nucleotide change (heterozygous, except where otherwise specified)	Amino acid change	gnomAD MAF	In silico: SIFT, MutTaster, PolyPhen, PhyloP (respectively)	ACMG criteria	Segregation	Novel
1	Spor/new AD	IH & PA	Array	*FOXC1* NM_001453.2	chr6 del :1595464–1716115	n/a	n/a	n/a	n/a	De novo	No
2	Spor/new AD	ARS	Array	*PITX2* NM_153427.1	chr4 del: 111445336–112392782	n/a	n/a	n/a	n/a	De novo	No
5	Spor/new AD	Sclero	ES	*GJA8* NM_005267.4	c.281G>A	p.(Gly94Glu)	Nil	D, D, P, highly conserved	LP (PM2, PM6, PP2, PP3)	De novo	No
9	Spor/AR	ARA	ES	*ADAMTS17* NM_139057.2	hom c.526C>T	p.(Arg176*)	Nil	n/a	P (PVS1, PM2, PM4)	Segregates	Yes
10	Spor/Fam (AD)	ARA	ES	*FOXC1* NM_001453.2	c.516_518dupGCG	p.(Arg173dup)	Nil	n/a	LP (PM1, PM2, PP1, PP3)	Segregates	Yes
11	Fam (AD)/AD	PA	GS CNV	*PAX6* NM_000280.4	del chr11:31822357–31823717	n/a	n/a	n/a	n/a	Segregates	Yes
12	Fam (AD)/AD	ARA	ES	*FOXC1* NM_001453.2	c.518G>A	p.(Arg173His)	Nil	D, D, P, highly conserved	LP (PM1, PM2, PP2, PP3)	Segregates	Yes
14	Spor/likely new AD	ARA	ES	*PITX2* NM_153427.1	c.341dup	p.(Asn115Glnfs*84)	Nil	n/a	P (PVS1, PM2, PM4)	n/k	Yes
15	Spor/new AD	Sclero	ES	*GJA8* NM_005267.4	c.280G>C	p.(Gly94Arg)	Nil	D, D, P, highly conserved,	LP (PM2, PM6, PP2, PP3)	De novo	No
19	Spor/likely new AD	ARS	ES	*PITX2* NM_153427.1	c.250C>T	p.(Arg84Trp)	Nil	D, D, P, highly conserved	P (PS1, PM1, PM2, PP2, PP3)	n/k	No
21	Spor/AR	Sclero	ES	*PXDN* NM_012293.2	Hom c.4085_4086delAG	p.(Gln1362Argfs*22)	1/249,252 (het only)	n/a	P (PVS1, PM2, PM4)	n/k	No
23	Spor/likely new AD	PA	ES	*FOXC1* NM_001453.2	c.1399C>T	p.(Gln467*)	Nil	n/a	P (PVS1, PM2, PM4)	n/k	Yes
25	Spor/new AD	IH	GS trio	*ITPR1* NM_001168272.1	c.7615G>A	p.(Gly2539Arg)	1/249,244 (het only)	D, D, P, highly conserved	LP (PS1, PM2, PP2, PP3)	De novo	No
26	Spor/Fam (AD)	PA	ES	*COL4A1* NM_001845.5	c.634G>A	p.(Gly212Ser)	Nil	D, D, P, highly conserved	LP (PM1, PM2, PP2, PP3)	Segregates	No
28	Spor/AR	ARA	GS trio	*CPAMD8* NM_015692.2	Comp Het c.4549−1G>A	Splice p.(=)	1/249,494 (het only)	n/a	P (PVS1, PS3, PM2, PP5)	Segregates (mat)	No
					c.3149G>T	p.(Gly1050Val)	1/249,372 (het only)	D, D, P, highly conserved	LP (PM2, PM3, PP2, PP3)	Segregates (pat)	Yes
29	Spor/AR	PA	ES	*CYP1B1* NM_000104.3	Hom c.171G>A	p.(Trp57*)	42/233,224 (het only)	n/a	P (PVS1, PM2, PM4)	n/k	No
30	Spor/AR	PA	ES	*CYP1B1* NM_000104.3	Comp Het c.171G>A;	p.(Trp57*)	42/233,224 (het only)	n/a	P (PVS1, PM2, PM4)	n/k	No
					c.1331G>A	p.(Arg444Gln)	Nil	D, D, P, highly conserved	P (PS3, PM2, PM3, PP1, PP5)	n/k	No
32	Fam(AD)/fam (AR)	Multiple ASD	GS	*FOXE3* NM_012186.2	Hom c.720C>A	p.(Cys240*)	7/43,132 (het only)	n/a	P (PVS1, PS3, PM2, PPS5)	Segregates	No
36	Spor/likely new AD	Sclero	GS	*PITX2* NM_153427.1	c.185G>A	p.(Arg62His)	Nil	D, D, P, highly conserved	P (PS1, PM1, PM2, PP2, PP3)	n/k	No
38	Spor/AR	PA	ES	*CYP1B1* NM_000104.3	Comp Het c.171G>A	p.(Trp57*)	21/50,846 (het only)	n/a	P (PVS1, PM2, PM4)	Segregates	No
					c.1200_1209dup	p.(Thr404Serfs*30)	Nil	n/a	P (PVS1, PM2, PM4)	Segregates	No
39	Spor/new AD	PA	ES	*PAX6* NM_000280.4	c.152G>T	p.(Gly51Val)	Nil	D, D, P, highly conserved	LP (PM2, PM6, PP2, PP3)	De novo	No
41	Spor/new AD	IH	ES	*FOXC1* NM_001453.2	c.478_482dup	p.(Met161Ilefs*22)	Nil	n/a	P (PVS1, PM2)	De novo	Yes

Human genome reference GRCh37/HG19 used and NCBI gene reference sequences (NM) provided. gnomAD database v2.1.1 was used (https://gnomad.broadinstitute.org/). ACMG criteria according to ref. [9] References for previously published variants also included in table. In Silico: D, damaging; P, pathogenic.

ACMG American College of Medical Genetics and Genomics, *AD* autosomal dominant, *AR* autosomal recessive, *ARA* Axenfeld–Rieger anomaly, *ARS* Axenfeld–Rieger syndrome, *ASD* anterior segment disorder, *ES* exome sequencing, *Fam* familial, *GS* genome sequencing, *Het* heterozygous, *Hom* homozygous, *IH* iris hypoplasia, *LP* likely pathogenic, *MAF* minor allele frequency, *P* pathogenic, *PA* Peters anomaly, *Sclero* sclerocornea, *Spor* sporadic.

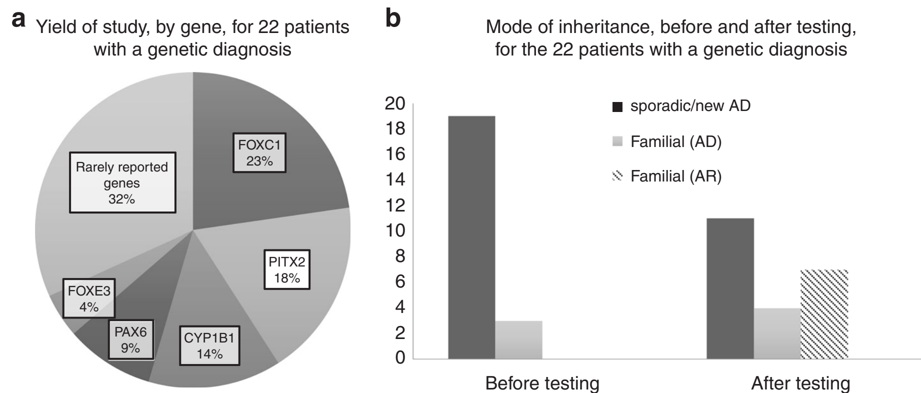

Fig. 1 Yield of study and mode of inheritance. a Yield of study, by gene, for 22 patients with a genetic diagnosis. The proportion of genetic diagnoses found in the relevant genes is shown in this chart. The group of rarely reported genes includes six genes: *COL4A1*, *PXDN*, *CPAMD8*, *ADAMTS17*, *ITPR1*, and *GJA8* (two variants). **b** Mode of inheritance, before and after testing, for the 22 patients with a genetic diagnosis. This figure demonstrates the breakdown of inheritance among the 22 solved cases. On referral, 19/22 were thought to be sporadic, and 3 familial with autosomal dominant (AD) inheritance. After testing, of the sporadic cases, 11 were found to be due to de novo autosomal dominant variants, 6 were due to autosomal recessive (AR) inheritance, and 2 were familial autosomal dominant cases with subtle clinical features in parents. Of the 3 familial cases, 2 were confirmed as familial autosomal dominant and one was found to be due to autosomal recessive inheritance in an inbred population group leading to pseudodominance. Hence overall after testing, there were 11 de novo autosomal dominant cases, 4 familial autosomal dominant cases, and 7 autosomal recessive cases.

Overall, of the 22 cases where causative variants were identified, 3 were due to SVs, and 19 were due to single-nucleotides (SNVs) (Table 1). Two of the SVs were found on CMA, and one SV was found on GS, and this was further validated on MLPA.

Notable variants with a role in collagen and extracellular matrix integrity including *COL4A1*, *PXDN*, *CYP1B1*, and the newly identified ARA phenotype finding due to *ADAMTS17* variation

We identified a number of variants in collagen-related proteins, emphasizing the importance of these in the formation of the ocular anterior segment. In an individual (patient 26) with reportedly sporadic bilateral PA, a heterozygous *COL4A1* p.(Gly212Ser) missense variant was found (Fig. 2a, 3a). This variant was found to be maternally inherited, and the mother was found to have mild iris hypoplasia with iris strands, consistent with a diagnosis of Rieger anomaly (Fig. 2b). This recently reported[10] p.Gly212Ser variant affects a key glycine (G-X-Y) residue of the COL4A1 protein and is expected to disrupt collagen IV heterotrimer formation with COL4A2, as noted for other missense pathogenic *COL4A1* variants associated with ASD (Fig. 3a).[11]

PXDN is a rarely reported gene associated with ocular ASD and sclerocornea, as well as cataract, microcornea, glaucoma, and microphthalmia. It has a key role in collagen IV cross-linking in the basement membrane, another clue in the vital role of this protein in the extracellular matrix (ECM) of the eye. In a proband (patient 21) with severe bilateral sclerocornea (Fig. 2c), glaucoma, and severe developmental delay, a previously reported[12,13] pathogenic homozygous frameshift variant was found in *PXDN* (c.4085_4086delAG, p.[Gln1362Argfs*22]) (Fig. 3b).

A novel likely pathogenic variant in another ECM-related gene, *ADAMTS17*, demonstrates further the role of collagen-related proteins in anterior segment development. In a patient referred with ARA, a novel homozygous nonsense variant in *ADAMTS17* was identified (patient 9, Table 1, Fig. 2d) on manual curation of data for potentially significant pathogenic autosomal recessive variants (Fig. 3c). Homozygous deleterious (frameshift, canonical splice site, nonsense) variants in *ADAMTS17* were found in families with a skeletal and eye phenotype overlapping with Weil–Marchesani syndrome (WMS).[14] WMS is associated with microspherophakia, ectopia lentis, and myopia, but there are no previous reports of an association with ARA. Our patient also had short stature (adult height 150 cm) and ectopia lentis, and the presence of ARA in him broadens the known phenotype of this gene, adding variants in this gene as a new cause of ARA.

In addition, three sporadic cases with severe PA were found with homozygous or compound heterozygous pathogenic variants affecting *CYP1B1* (patients 29, 30, 38, Table 1.[8,15] The cyp1b1 deficient mouse model displays marked loss of collagen and degeneration of the trabecular meshwork.[16] This gene was originally identified as a causative disease gene in autosomal recessive PCG, and findings from this study confirm its significant additional contribution to causation in PA.[8,15] Patient 30 had a very severe Peters phenotype (Fig. 2e), and patient 29 also had severe corneal opacification and iridocorneal adhesions.

Novel variants in rarely reported ocular ASD gene *CPAMD8*, and a syndromal diagnosis in *ITPR1* highlight the importance of deep phenotyping and data reanalysis

Several novel variants were found in rarely reported ocular ASD-associated genes. For these families, availability of pedigree structure information and deep phenotyping greatly aided identification of these causative variants.

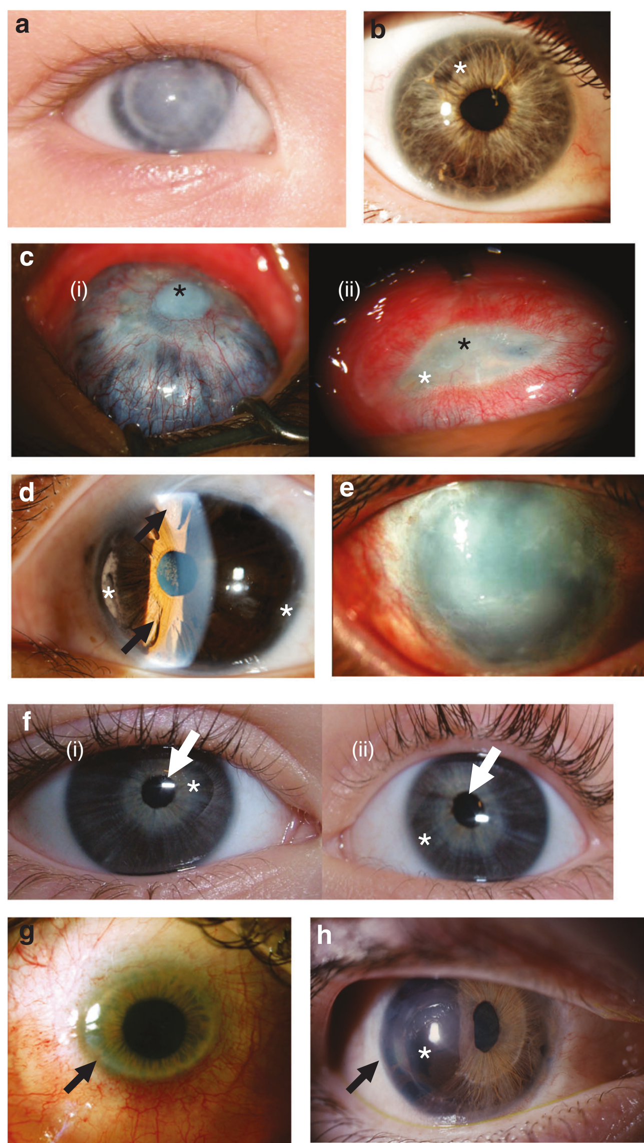

Fig. 2 Representative clinical images of this cohort demonstrate broad range of severity across multiple genotypes. **a** Patient 26 with *COL4A1* heterozygous variant. Photograph of right eye with Peters anomaly and failed corneal graft. **b** Previously undiagnosed affected mother of patient 26, with right eye showing mild features of anterior segment disorder (ASD) including Rieger anomaly, with strands of iris adhesions to the overlying cornea and mild iris hypoplasia (white asterisk). **c** Patient 21 with *PXDN* homozygous variant. Right eye (i) has previously undergone penetrating keratoplasty at age 4 years. Now failed corneal graft with central corneal opacity (black asterisk). Scleromalacia surrounding this with choroidal tissue visible through the residual sclera. Left eye (ii) shows sclerocornea with a residual small oval opaque central corneal tissue (black asterisk) with injected and dilated superficial corneoscleral vessels. No clear view of iris structures through cornea. **d** Patient 9 with homozygous variant in *ADAMTS17*. Left eye slit lamp view of anterior segment demonstrating features of Axenfeld–Rieger anomaly: corectopia, polycoria (arrows), peripheral iridocorneal adhesions, anterior iris stroma hypoplasia (white asterisk). (**e**) Patient 30 with *CYP1B1* variants: left eye shows generalized corneal opacification. In addition, surgical scarring is visible superiorly from previous glaucoma filtration surgery. **f** Patient 28 with *CPAMD8* variants: right (i) and left (ii) eyes of proband showing corectopia of pupils (white arrows) and iris hypoplasia with the iris sphincter muscle visible (white asterisk). **g** Patient 36 with *PITX2* heterozygous variant: image shows the clinical features of primary congenital corneal opacification, commonly termed sclerocornea. This case has central area of clear cornea, which on corneal topography has low (flat) keratometry in the range meeting definition of cornea plana. The peripheral cornea is scleralized (arrow) making identification of the peripheral iris difficult as well. **h** Patient 14 with *PITX2* heterozygous variant: image shows features of Axenfeld–Rieger anomaly with iris hypoplasia (asterisk), corectopia, polycoria, and posterior embryotoxon (black arrow).

At the time of initial ES in patient 28 (Table 1, Fig. 2f) with subluxed lenses, corectopia and iris hypoplasia, no likely pathogenic variants were found. Subsequent trio GS with our updated gene list including *CPAMD8*[17] demonstrated compound heterozygous variants, one a maternally inherited previously reported pathogenic splice site (c.4549–1G>A) variant, and another a novel missense variant (p.[Gly1050Val]) (Fig. 3d), inherited from the father. Interestingly, the father also had milder ocular ASD features with posterior embryotoxon, and some iridocorneal strands. No other known ocular ASD-related variants were found in his genomic analysis. While posterior embryotoxon is known to occur in 10–15% of the general population, the presence of the iridocorneal strands may indicate that heterozygous carriers for *CPAMD8* variants may carry milder ocular changes, as has been noted for other autosomal recessive developmental ocular conditions.[18]

Similarly, reinterrogation of clinical and ES/GS data yielded a pathogenic variant in the recently and rarely reported syndromal gene *ITPR1*. In patient 25, initial analysis of ES data was negative. Subsequent manual review of GS trio data identified a de novo, heterozygous, previously reported[19] pathogenic *ITPR1* variant (c.7615G>A; p.[Gly2539Arg]) in the proband (Table 1, Fig. 3e). This variant was considered in the light of a concurrent clinical re-review at the time, which indicated presence of severe global developmental delay, iris hypoplasia with scalloped margins, and ataxia consistent with the diagnosis of Gillespie syndrome, known to be caused by variants in *ITPR1*.

Novel variants in *FOXC1*, and novel genotype–phenotype correlation in *PITX2* with sclerocornea

Although *FOXC1* is one of the most well-studied genes in ASD, we found four novel variants in this gene. Two novel variants affecting p.Arg173 emphasize the importance of the Wing 2 region of the forkhead domain of *FOXC1*. Case 12 had a dominantly inherited missense (c.518G>A, p.[Arg173His]) variant, and the second proband (case 10) had an in-frame duplication of p.Arg173 (Table 1, Fig. 4a). The duplication variant was also found in the asymptomatic father of case 10, and subsequent detailed examination revealed that he actually had a mild form of ARA with posterior embryotoxon and evidence of iris adhesions. It is noteworthy that p.Arg173 lies in a group of five conserved arginine residues in the highly

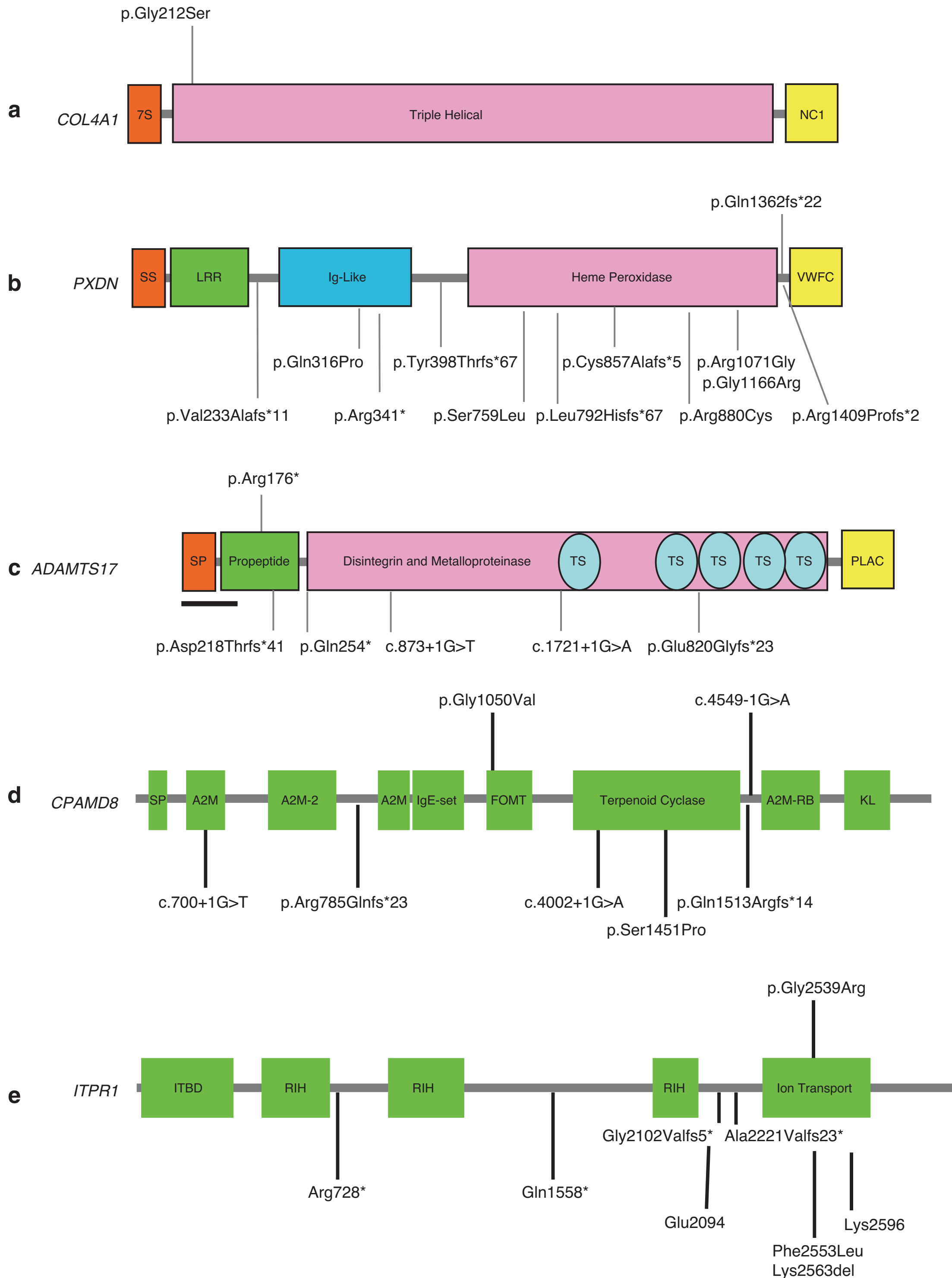

Fig. 3 Variants in extracellular matrix-associated genes *COL4A1*, *PXDN*, *ADAMTS17*, and recently reported anterior segment disorder (ASD) genes *CPAMD8* and *ITPR1*. Variants reported in the rarely reported ASD genes **a** *COL4A1*, **b** *PXDN*, **c** *ADAMTS17*, **d** *CPAMD8*, **e** *ITPR1*. Variants above the gene were found in this study, and previously reported variants are listed underneath the gene diagrams. Note: in *COL4A1* over 50 missense variants, mostly involving glycine residues in the triple helical domain, have been reported in the literature. Several well reported variants are displayed. Also, the *ADAMTS17* variant we report is the first associated with an Axenfeld–Rieger anomaly (ARA) phenotype.

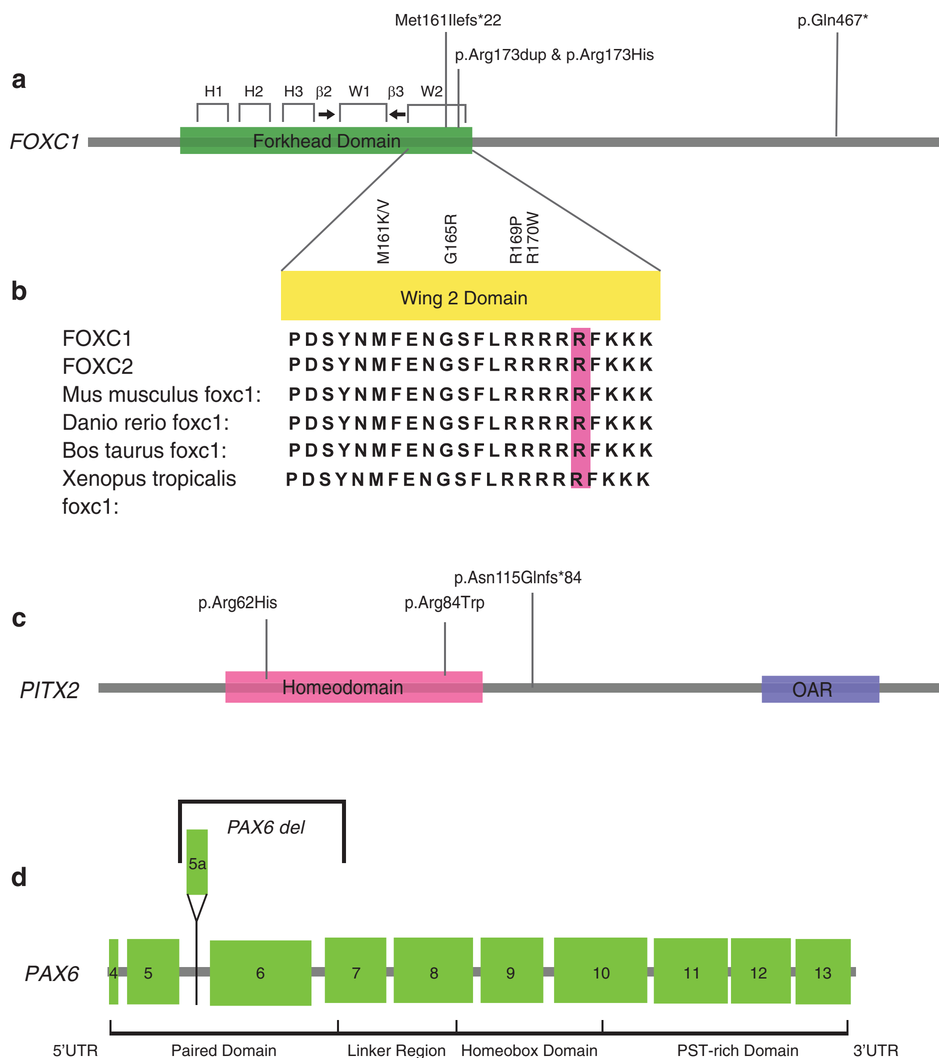

Fig. 4 Novel variants in *FOXC1* Wing 2 domain, *PITX2*, and a *PAX6* deletion identified on genome sequencing (GS). (**a**) *FOXC1* gene, with key domains and regions of the major forkhead domain, with wing 2 (W2) region highlighted. **b** Alignment demonstrates the highly conserved residues of this domain across the *FOXC1* paralog *FOXC2*, and the phylogenetic tree. Previously reported pathogenic variants in the wing 2 domain are labeled, and the Arg173 highlighted in pink. **c** *PITX2* gene and three novel variants found in this study. **d** *PAX6* deletion is also demonstrated, with exon numbering in the diagram.

conserved Wing 2 region of the forkhead domain of FOXC1, which is conserved across species and related FOX proteins (Fig. 4b). Also, even in the more distantly related FOX proteins such as FOXL1/FOXS1 there is a very high degree of homology in this part of the gene across multiple species.[20] Missense variants affecting the nearby p.Arg169 and p.Arg170 have also been identified in patients with ARS,[21,22] and crystal structure analysis and biochemical studies of p.Arg169 indicate a role in the *FOXC1* wing domain DNA binding and transactivation abilities.[22,23] Our work further highlights the likely critical nature of the wing 2 domain in *FOXC1* DNA binding and transactivation.

We also report two novel deleterious pathogenic variants in *FOXC1* in children with ASD and intellectual disability (ID), indicating a possible role for this gene in learning disorders. A girl (patient 23) with PA, intellectual delay, and absent septum pellucidum harbored a novel nonsense pathogenic variant in *FOXC1* (c.1399C>T, p.[Gln467*]) (Table 1, Fig. 4a). A novel frameshift variant (p.[Met161Ilefs*22]) was found in a young boy (patient 41) with iris hypoplasia, corectopia and glaucoma, global developmental delay, and bilateral sensorineural hearing loss (Table 1, Fig. 4a). For both patients, other causes of intellectual delay were not found following baseline testing with chromosome microarray, urine metabolic screen, and fragile X testing, or ES reanalysis for causative variants in known ID genes (Genomics England PanelApp, https://panelapp.genomicsengland.co.uk, Intellectual disability [Version 2.1046]). There have been reports of *FOXC1* deletions in combination with deletions of surrounding genes, causing brain malformations such as Dandy–Walker malformation, partial agenesis of the corpus callosum, and intellectual disability.[24] In a prior study, 1/13 SNV patients, who had a missense p.Met109Val in the forkhead domain of *FOXC1*, was reported to have learning difficulties.[25] This,

combined with our cases, highlights a potential new link between variants in this gene and ID.

Case 36 with a clinical diagnosis of peripheral sclerocornea had a missense pathogenic variant in *PITX2* (c.185G>A, p.[Arg62His]), which was previously found in a family reported to have "ring dermoid" of the cornea. Notably the phenotypic images in this family also showed peripheral sclerocornea,[26] very similar to our patient (Fig. 2g, 4c, Table 1). This highlights peripheral sclerocornea as a new phenotypic link to variation in *PITX2*. In *PITX2*, a novel pathogenic frameshift variant (c.341dup; p.[Asn115Glnfs*84]) was found causing premature truncation of the protein in a patient with ARA (patient 14, Fig. 2h, 4c, Table 1).

CNV detection in *PAX6* facilitated by GS

A novel 1360-bp *PAX6* deletion, was found in a proband (patient 11, Table 1) with PA, and his affected mother who had bilateral partial iris hypoplasia and right ectopia lentis. Initial investigation with ES in the proband was negative. GS was undertaken in the proband, his affected mother, and his two unaffected siblings and no causative SNVs were found. Subsequent SV analysis of GS data found a small 1.4-kb deletion in *PAX6* in the proband and his mother, with breakpoints indicating a deletion of exons 5a, 6, and part of 7 in the paired domain (Fig. 4d) and the deletion was confirmed using MLPA (MRC-Holland P219-B1 PAX6 probemix). Review of CMA data could not find this deletion, as it was beyond the resolution of the Agilent 400K CGH microarray used. Similar deletions are reported in the literature, including one study with an exon 6–7 deletion and sporadic aniridia.[27] Missense variants of the paired domain have also been reported in atypical/milder aniridia and also in PA, as well as congenital cataracts, foveal hypoplasia, keratitis, and optic nerve abnormalities.[28]

DISCUSSION

Deep phenotyping and variant curation for maximization of genetic diagnoses in the ocular ASDs

This study highlights the importance of detailed phenotypic information, pedigree structure, and careful variant curation to find "hidden" variants in the ocular ASD patient cohort that may not be apparent on first analysis. In over 1/3 of the cases (8/22) where causative variants were identified, the variants were novel and required careful clinical review and manual review of sequence data for their identification (Table 1). In addition, approximately one-third of the likely causative variants found in this study were in rarely reported ocular ASD genes with one, in *ADAMTS17*, where this association was identified for the first time (Figs. 1a, 2d, 3c). Identified variants revealed new genotype–phenotype correlations in rarely reported and syndromal ocular ASD genes (Fig. 1a and Fig. 2c, d, f), or were novel variants in key ocular ASD genes revealing new functional domain elements or phenotypic features (Figs. 4a–c, 2g), or in one case required GS analysis for CNV detection (Fig. 4d). This study demonstrates that accurate phenotypic information on the proband and family members, in conjunction with careful analysis of ES/GS variants, and trio and segregation analysis of variants, greatly benefits genetic diagnosis in the ocular ASDs. This approach had a vital role in discovering new genotype–phenotype correlations, broadening the list of candidate disease-causing genes and identifying additional likely causative variants.

In two families, pathogenic variants were found in recently reported ocular ASD-related genes *CPAMD8* (patient 28) and *ITPR1* (patient 25). In *CPAMD8*, the similar clinical features reported in the literature, with absence of posterior embryotoxon, corneal opacity, or any extraocular features, highlight a unique combination of features specific to this gene and a newly emerging genotype–phenotype correlation. Recently an additional family with a homozygous frameshift variant in *CPAMD8* was found in a PCG cohort from Saudi Arabia,[29] with PCG and lens subluxation. With our family, this increases the number of reported families with variants in this gene to five. Similarly, patient 25 initially referred with iris hypoplasia was found to have a syndromal form of ASD with clear genotype–phenotype correlation due to an *ITPR1* variant.

Variants in collagen and extracellular matrix–associated genes highlight novel genotype–phenotype correlations

For three families in this study, variants were found in ECM-related genes *COL4A1* (case 26, Fig. 2a, b, 3a), *ADAMTS17* (case 9, Figs. 2d, 3c) and *PXDN* (case 21, Figs. 2c, 3b). Variants were also found in three cases in *CYP1B1* (cases 29, 30, 38, Fig. 2e), and this gene has emerging evidence for its role in the ECM.[30] In addition, variants in ECM genes of the TGFβ pathway have been found in other anterior segment abnormalities, including *FBN1* (ectopia lentis), *LTBP2* (primary congenital glaucoma), and *ADAMTS10* (ectopia lentis).

Our findings highlight the importance of ECM, collagen, and related genes, in the formation of the anterior segment and scaffolding of tissues to the basement membrane. The ECM has a crucial role in regulating cell adhesion, cellular migration, and tissue morphogenesis. There is increasing evidence pointing to the importance of the periocular ECM in the morphogenesis of the optic cup,[31] with cross-linking of laminin, collagen IV, and other ECM components noted as critical factors.[32] In conditions associated with *COL4A1* variants, eye abnormalities, including ASD, are the second commonest presenting feature.[33] COL4A1 forms a heterotrimer with COL4A2, and is secreted into the ECM to form a scaffolding, with key bonds to other heterotrimers.[11,34] Crucial to the stable formation of these bonds are sulfilamine bonds, and these are catalyzed in a peroxidase reaction by peroxidasin, or *PXDN*.[35] This may explain why the phenotype associated with *PXDN* may be so severe, as demonstrated in case 21 (Fig. 2c) of this study, and as shown in the mouse pxdn nonsense variant model.[36] Also, the association with *PXDN* and intellectual delay has been reported in siblings[37] highlighting the broadening of the reported phenotype with

this gene. In addition, *ADAMTS17* belongs to a family of secreted metalloproteases with an important role in ECM remodeling.[38]

Implications for genetic information for patients and families, and genomic workflow in ASD

Few studies have been performed utilizing NGS in the area of ocular ASD with large cohorts consisting of patients with the variety of ASD clinical diagnoses that present to the clinic. A study using ES in PA[3] found a genetic diagnosis in only a small proportion, and previous studies utilizing conventional sequencing of cohorts with a high proportion of phenotypically defined ARS cases[1] led to a diagnosis rate of around 40% by examining *PITX2/FOXC1*. In ASD patients with a variety of ASD phenotypic subtypes that present for clinical genetic information, whether familial or sporadic, our work highlights the important role of ES/GS and SV analysis, as this leads to a high overall rate of diagnosis (22/41; 54%). Using this approach the highest yields of testing were in ARS (2/3; 67%), ARA (5/9; 56%), and PA (7/11; 64%) (Table **1**).

Of clinical utility and importance for families, this approach led to a revision of the mode of inheritance in 8/19 (42%) of referred sporadic cases where a responsible variant was found, and in 1/4 (25%) of referred autosomal dominant cases (Fig. **1b**). Finding a de novo autosomal dominant pathogenic variant may provide reproductive confidence for families. However, finding an autosomal recessive variant in a supposedly sporadic case means 25% recurrence risk for parents, which happened in six families (cases 9, 21, 28, 29, 30, 38; Fig. **1b**). In one of these families (patient 28) with autosomal recessive *CPAMD8* variants, subsequent detailed clinical review revealed subtle ASD features in the father, and without our genetic diagnostic findings, this could have been interpreted as an autosomal dominant family with variable expression. Also, two cases presenting as sporadic, and therefore low recurrence risk, were found to be familial autosomal dominant cases with 50% recurrence risk. This was after genotype and careful phenotype review revealed an affected parent (families 10 and 26). The parental features were milder, raising the possibility of somatic mosaicism. However, we could find no evidence of this on careful review of the genomic coverage, allele frequency data, and the Sanger sequencing traces.

This is the first study to evaluate the role of ES, GS, and CMA in ASD. Considering the findings of a large meta-analysis in children with suspected genetic diseases,[39] it would have been expected there may be a diagnostic yield of CMA of around 10%, and ES of 36%, with an additional 5% detected by GS. In our study, CMA had a yield of 2/41 (5%). Upon initial analysis of ES data, the vast majority (15/19) of SNV variants were found and with GS, there were an additional 4 SNVs and a single CNV identified. However, the 4 SNVs found on GS would all have been found from ES, if it had been performed first-line rather than GS (cases 32 and 36), and clinical correlation and an updated gene list had been prioritized (cases 25 and 28). Hence, from a platform perspective of our cohort, using ES first, followed by the other approaches, would lead to a 46.3% (19/41) diagnostic yield with ES, with CMA finding pathogenic deletions in an additional two cases (extra 4.9%) and GS an additional one case (extra 2.4%), to give an overall genetic diagnosis rate of 22/41 (54%). If GS had been used as the first-line and only platform, then it would be expected to pick up all cases giving the same overall detection rate. At this stage, it is difficult to assess the yield and cost effectiveness of ES versus GS in a cohort examined over time with many confounding factors, as has been raised elsewhere in the literature.[40] Apart from discovering small SVs, the enhanced yield of GS may lie in the discovery of additional potential regulatory variants, which would require further studies for variant interpretation. We would advise that clinical genetics and genetic eye services should consider performing either ES or GS as a first-line test in ASD, with SV analysis and trio/segregation analysis for additional yield. In addition, patients should first have detailed ophthalmic and clinical review for syndromal features, as well as parental review for undiagnosed autosomal dominant disease.

Conclusion

We have identified a high diagnostic rate in a cohort of families with ASD, using ES and GS with CNV analysis. These findings add to our understanding of the genetics of ASD, and also highlight the complex heterogeneous nature of this condition, with many syndromal associations and novel genotype–phenotype correlations. This study also has significant implications in recurrence risk counseling for families, and highlights the importance of relying on an accurate genomic diagnosis, rather than pedigree information alone, for genetic counseling. Some of our cohort remain unsolved, and this may be due to additional undiscovered genes, or further variants within known genes such as 5'UTR promoter, *cis*-regulatory, deep intronic, and other difficult to interpret regions of the genome. These conditions warrant further study to uncover their full genetic basis.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-0854-x) contains supplementary material, which is available to authorized users.

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DISCLOSURE

The authors declare no conflicts of interest.

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REFERENCES

1. Reis LM, Semina EV. Genetics of anterior segment dysgenesis disorders. Curr Opin Ophthalmol. 2011;22:314–324.
2. Reis LM, Tyler RC, Volkmann Kloss BA, et al. PITX2 and FOXC1 spectrum of mutations in ocular syndromes. Eur J Hum Genet. 2012;20:1224–1233.
3. Weh E, Reis LM, Happ HC, et al. Whole exome sequence analysis of Peters anomaly. Hum Genet. 2014;133:1497–1511.
4. Ma AS, Grigg JR, Prokudin I, Flaherty M, Bennetts B, Jamieson RV. New mutations in GJA8 expand the phenotype to include total sclerocornea. Clin Genet. 2018;93:155–159.
5. Ma AS, Grigg JR, Ho G, et al. Sporadic and familial congenital cataracts: mutational spectrum and new diagnoses using next-generation sequencing. Hum Mutat. 2016;37:371–384.
6. Ma AS, Grigg JR, Jamieson RV. Phenotype-genotype correlations and emerging pathways in ocular anterior segment dysgenesis. Hum Genet. 2018;138:899–915.
7. Nash BM, Symes R, Goel H, et al. NMNAT1 variants cause cone and cone-rod dystrophy. Eur J Hum Genet. 2018;26:428–433.
8. Prokudin I, Simons C, Grigg JR, et al. Exome sequencing in developmental eye disease leads to identification of causal variants in GJA8, CRYGC, PAX6 and CYP1B1. Eur J Hum Genet. 2014;22:907–915.
9. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424.
10. Matias-Perez D, Garcia-Montano LA, Cruz-Aguilar M, et al. Identification of novel pathogenic variants and novel gene-phenotype correlations in Mexican subjects with microphthalmia and/or anophthalmia by next-generation sequencing. J Hum Genet. 2018;63:1169–1180.
11. Meuwissen ME, Halley DJ, Smit LS, et al. The expanding phenotype of COL4A1 and COL4A2 mutations: clinical data on 13 newly identified families and a review of the literature. Genet Med. 2015;17:843–853.
12. Patel N, Khan AO, Alsahli S, et al. Genetic investigation of 93 families with microphthalmia or posterior microphthalmos. Clin Genet. 2018;93:1210–1222.
13. Zazo-Seco C, Plaisancie J, Bitoun P, et al. Novel PXDN biallelic variants in patients with microphthalmia and anterior segment dysgenesis. J Hum Genet. 2020;65:487–491.
14. Morales J, Al-Sharif L, Khalil DS, et al. Homozygous mutations in ADAMTS10 and ADAMTS17 cause lenticular myopia, ectopia lentis, glaucoma, spherophakia, and short stature. Am J Hum Genet. 2009;85:558–568.
15. Vincent A, Billingsley G, Priston M, et al. Phenotypic heterogeneity of CYP1B1: mutations in a patient with Peters' anomaly. J Med Genet. 2001;38:324–326.
16. Teixeira LB, Zhao Y, Dubielzig RR, Sorenson CM, Sheibani N. Ultrastructural abnormalities of the trabecular meshwork extracellular matrix in Cyp1b1-deficient mice. Vet Pathol. 2015;52:397–403.
17. Cheong SS, Hentschel L, Davidson AE, et al. Mutations in CPAMD8 cause a unique form of autosomal-recessive anterior segment dysgenesis. Am J Hum Genet. 2016;99:1338–1352.
18. Ng WY, Pasutto F, Bardakjian TM, et al. A puzzle over several decades: eye anomalies with FRAS1 and STRA6 mutations in the same family. Clin Genet. 2013;83:162–168.
19. McEntagart M, Williamson KA, Rainger JK, et al. A restricted repertoire of de novo mutations in ITPR1 cause Gillespie syndrome with evidence for dominant-negative effect. Am J Hum Genet. 2016;98:981–992.
20. Jackson BC, Carpenter C, Nebert DW, Vasiliou V. Update of human and mouse forkhead box (FOX) gene families. Hum Genomics. 2010;4:345–352.
21. Gripp KW, Hopkins E, Jenny K, Thacker D, Salvin J. Cardiac anomalies in Axenfeld–Rieger syndrome due to a novel FOXC1 mutation. Am J Med Genet A. 2013;161A:114–119.
22. Murphy TC, Saleem RA, Footz T, Ritch R, McGillivray B, Walter MA. The wing 2 region of the FOXC1 forkhead domain is necessary for normal DNA-binding and transactivation functions. Invest Ophthalmol Vis Sci. 2004;45:2531–2538.
23. Clark KL, Halay ED, Lai E, Burley SK. Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. Nature. 1993;364:412–420.
24. Aldinger KA, Lehmann OJ, Hudgins L, et al. FOXC1 is required for normal cerebellar development and is a major contributor to chromosome 6p25.3 Dandy-Walker malformation. Nat Genet. 2009;41:1037–1042.
25. D'Haene B, Meire F, Claerhout I, et al. Expanding the spectrum of FOXC1 and PITX2 mutations and copy number changes in patients with anterior segment malformations. Invest Ophthalmol Vis Sci. 2011;52:324–333.
26. Xia K, Wu L, Liu X, et al. Mutation in PITX2 is associated with ring dermoid of the cornea. J Med Genet. 2004;41:e129.
27. Bobilev AM, McDougal ME, Taylor WL, Geisert EE, Netland PA, Lauderdale JD. Assessment of PAX6 alleles in 66 families with aniridia. Clin Genet. 2016;89:669–677.
28. Tzoulaki I, White IM, Hanson IM. PAX6 mutations: genotype–phenotype correlations. BMC Genet. 2005;6:27.
29. Alsaif HS, Khan AO, Patel N, et al. Congenital glaucoma and CYP1B1: an old story revisited. Hum Genet. 2018;138:1043–1049.
30. Safari I, Suri F, Haji-Seyed-Javadi R, Yazdani S, Elahi E. The p.Gly61Glu mutation in CYP1B1 affects the extracellular matrix in glaucoma patients. Ophthalmic Res. 2016;56:98–103.
31. Kwan KM. Coming into focus: the role of extracellular matrix in vertebrate optic cup morphogenesis. Dev Dyn. 2014;243:1242–1248.
32. Bryan CD, Casey MA, Pfeiffer RL, Jones BW, Kwan KM. Optic cup morphogenesis requires neural crest-mediated basement membrane assembly. Development. 2020;147:dev181420.
33. Zagaglia S, Selch C, Nisevic JR, et al. Neurologic phenotypes associated with COL4A1/2 mutations: expanding the spectrum of disease. Neurology. 2018;91:e2078–e2088.
34. Mao M, Kiss M, Ou Y, Gould DB. Genetic dissection of anterior segment dysgenesis caused by a Col4a1 mutation in mouse. Dis Model Mech. 2017;10:475–485.
35. Sirokmany G, Kovacs HA, Lazar E, et al. Peroxidasin-mediated crosslinking of collagen IV is independent of NADPH oxidases. Redox Biol. 2018;16:314–321.
36. Yan X, Sabrautzki S, Horsch M, et al. Peroxidasin is essential for eye development in the mouse. Hum Mol Genet. 2014;23:5597–5614.
37. Choi A, Lao R, Ling-Fung Tang P, et al. Novel mutations in PXDN cause microphthalmia and anterior segment dysgenesis. Eur J Hum Genet. 2015;23:337–341.
38. Hubmacher D, Schneider M, Berardinelli SJ, et al. Unusual life cycle and impact on microfibril assembly of ADAMTS17, a secreted metalloprotease mutated in genetic eye disease. Sci Rep. 2017;7:41871.
39. Clark MM, Stark Z, Farnaes L, et al. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. NPJ Genom Med. 2018;3:16.
40. Schwarze K, Buchanan J, Taylor JC, Wordsworth S. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genet Med. 2018;20:1122–1130.

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