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REVIEW

Phenotype–genotype correlations and emerging pathways in ocular anterior segment dysgenesis

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Abstract

Disorders of the anterior segment of the eye encompass a variety of clinical presentations including aniridia, Axenfeld and Rieger anomalies, primary congenital glaucoma, Peters anomaly, as well as syndromal associations. These conditions have a significant impact on vision due to disruption of the visual axis, and also secondary glaucoma which occurs in over 50% of patients. Ocular anterior segment disorders occur due to a complex interplay of developmental, embryological and genetic factors, and often have phenotypic overlaps and genetic heterogeneity. Here we present a review of the clinical features and genes associated with aniridia, Axenfeld and Rieger anomalies, primary congenital glaucoma, Peters anomaly, and syndromic forms of these conditions. We also highlight phenotype–genotype correlations, recent discoveries with next-generation sequencing which broaden known phenotypes, and new anterior segment genes and pathways. We provide a guide towards genetic diagnosis for clinicians investigating patients with anterior segment dysgenesis.

Introduction

The anterior segment of the eye consists of the structures anterior to the vitreous surface (Sowden 2007). These structures include the lens, iris, ciliary body, trabecular meshwork and cornea (Fig. 1a, b). The anterior segment of the eye also contains aqueous humour produced by the processes of the ciliary body. The aqueous humour circulates from the posterior chamber of the anterior segment through the pupil into the anterior chamber. This fluid is then actively transported through the trabecular meshwork into Schlemm's canal and the collecting channels leading to the systemic circulation. These anatomical relationships are important in understanding the altered aqueous humour flow, subsequent raised intraocular pressure and the resultant glaucoma, which may occur in patients with anterior segment disorders.

Disorders of the anterior segment are often grouped together under the term 'anterior segment dysgenesis' (ASD), which includes a number of individual anomalies, some eponymously described [such as Axenfeld–Rieger anomaly (ARA) and Peters anomaly (PA)], as well as combinations of these individual abnormalities. The phenotypic outcomes and nomenclature are complicated due to a number of factors including the complex embryological development of the eye, with contributions from neural crest, mesoderm, surface ectoderm and neuroectodermal derivatives, and interactions between the developing tissues. Disruption of development of these tissues may lead to a variety of phenotypic outcomes, many of which are caused by mutations in a number of different genes. In addition, mutations in one gene may lead to impact on a number of different components of the anterior segment. These factors add to the complexity of the phenotypic and genotypic classification of these conditions.

While many overlaps occur between the embryological, clinical and genetic factors involved in ASD, these will be discussed separately to provide clarity in this complex area.

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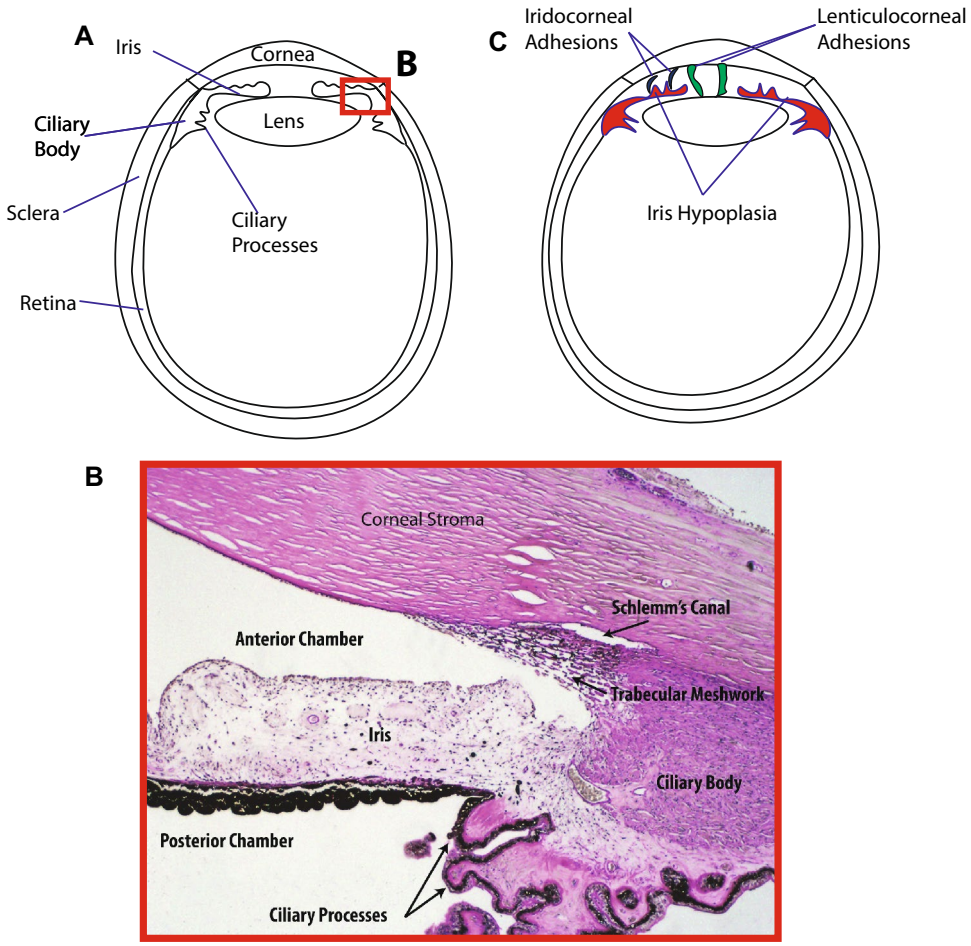

Fig. 1 Structures of the ocular anterior segment and features of anterior segment dysgenesis. On the left side: **a** is a line diagram representing the normal eye with labels corresponding to the cornea, lens, iris, ciliary body, ciliary processes, sclera and retina. Below this, **b** is a boxed region enlarged to show a histological image of angle structures demonstrating the relationships between the ciliary body and processes, iris, trabecular meshwork and cornea. On the right side: **c** is a line diagram representing the abnormal eye with iris hypoplasia (red), iridocorneal adhesions (blue) and lenticulocorneal adhesions (green). (Histological image, courtesy of Associate Professor Michele Madigan, Save Sight Institute, University of Sydney)

The developmental, embryological and clinical classification of ASD will be discussed, followed by review of the ASD phenotypes and their associated key genes. New developments and discoveries in ASD genetics are also discussed, and suggestions are made for a clinical approach to genetic diagnosis in these complex conditions.

Development and embryology of the anterior segment of the eye

Anterior segment ocular structures begin developing during the seventh week of gestation. Periocular mesenchymal cells with contributions from neural crest and mesodermal lineages, contribute to the corneal stroma, endothelium and trabecular meshwork (Davis-Silberman and Ashery-Padan 2008; Gage et al. 2005). During the eighth week, corneal differentiation continues with formation of the corneal endothelium under the influence of Pitx2 expression. The iris arises with contributions from both the anterior margin of the optic cup neuroepithelium and the periocular mesenchyme. The peripheral optic cup is specified to the non-neuronal fate of pigmented epithelial layer of the iris, under the influence of Pax6 expression, and also contributes to the ciliary body epithelium. The ciliary muscle form under the direction of Lmx1b (Nischal and Sowden 2013). Fibrillin-rich zonular fibres produced by the nonpigmented epithelium of the ciliary body, span the narrow cleft between the ciliary body and the lens epithelium and attach to the capsular surface (Shi et al. 2013). Trabecular meshwork differentiation proceeds later, during the fourth month of gestation (McMenamin 1991). Recent mouse work has also implicated the classical angiopoietin/*TEK* signalling axis in Schlemm's canal development, via the angiopoietin ligands *ANGPT1* and *ANGPT2* (Thomson et al. 2017).

Clinical classifications and diagnoses in anterior segment disorders

Clinically, it is useful to consider anterior segment disorder classification based on the anatomical abnormalities that occur, the resultant clinical ophthalmic diagnosis and linkage of this to causative disease genes. Broadly, anterior segment abnormalities can be categorized as abnormalities of the iris, iridocorneal separation, iridocorneal angle drainage

structures, cornea, or combinations of these abnormalities (Fig. 1b, c). Due to dysregulation of aqueous humour flow, approximately 50% of patients with ASD develop glaucoma (Alward 2000), which can have a progressive and severe impact on vision (Reis and Semina 2011). Aniridia is a panocular disorder with additional features including foveal hypoplasia. There are also syndromal forms of these conditions, with a variety of extraocular features.

Aniridia—disorder of the iris with panocular disease

The most well-known anterior segment abnormality affecting the iris is aniridia, or absence of the iris (Fig. 2). Aniridia is a panocular disorder affecting the cornea, anterior chamber, iris, lens, retina, macula and optic nerve(Nelson et al. 1984). There is complete (Fig. 2a) or incomplete iris absence (Fig. 2b) or sometimes other iris abnormalities including ectropion uveae [presence of iris pigment epithelium on the anterior surface of the iris (Fig. 2c)], and iris hypoplasia, which are often considered as part of the spectrum of ARA (Fig. 3d–f) (Hingorani et al. 2009). Additional features due to abnormalities of the anterior segment may include cataract, lens subluxation, corneal opacification and pannus, persistent iris strands, persistent tunica vasculosa lentis and glaucoma. Fundal abnormalities may include foveal hypoplasia and optic nerve coloboma, and strabismus and microphthalmia may also be present. As a consequence of disease affecting both the anterior and posterior segments of the eye, patients with aniridia often have severe visual impairment, with visual acuity worse than 20/100 in 86% of cases (Nelson et al. 1984).

Disorders with iris hypoplasia, peripheral iridocorneal adhesions and associated features—Axenfeld and Rieger anomalies

Axenfeld anomaly is defined by the presence of posterior embryotoxon (Schwalbe's line is visible by external examination) and peripheral iris attachments to Schwalbe's line and the cornea (Fig. 3a–c), which can obstruct or distort angle structures causing glaucoma (Alward 2000; Churchill and Booth 1996; Idrees et al. 2006; Spencer 1996) (Fig. 1a, b). Posterior embryotoxon is a thickening of Schwalbe's line, which is the peripheral termination of Descemet's membrane, which is prominent and anteriorly displaced. It appears as a white line on the posterior cornea when examined with the slit lamp (Fig. 3c) and in isolation it occurs in 15% of the normal population, but the additional feature of peripheral iris attachments indicates the presence of Axenfeld anomaly.

In addition to the features seen in Axenfeld anomaly, Rieger anomaly has additional iris abnormalities including corectopia (ectopic pupils), loss of iris stroma and polycoria (multiple pupils) (Alward 2000) (Fig. 3d–f). As the features of Axenfeld and Rieger anomalies overlap in variable form, they are commonly referred together as the Axenfeld–Rieger anomaly (ARA) (Idrees et al. 2006; Spencer 1996). The types of adhesions that occur are seen typically between the iris and cornea, also termed peripheral anterior synechiae or iridocorneal adhesions (Fig. 1). The association of Axenfeld–Rieger anomaly with systemic abnormalities is termed Axenfeld–Rieger syndrome (Alward 2000) as is discussed later.

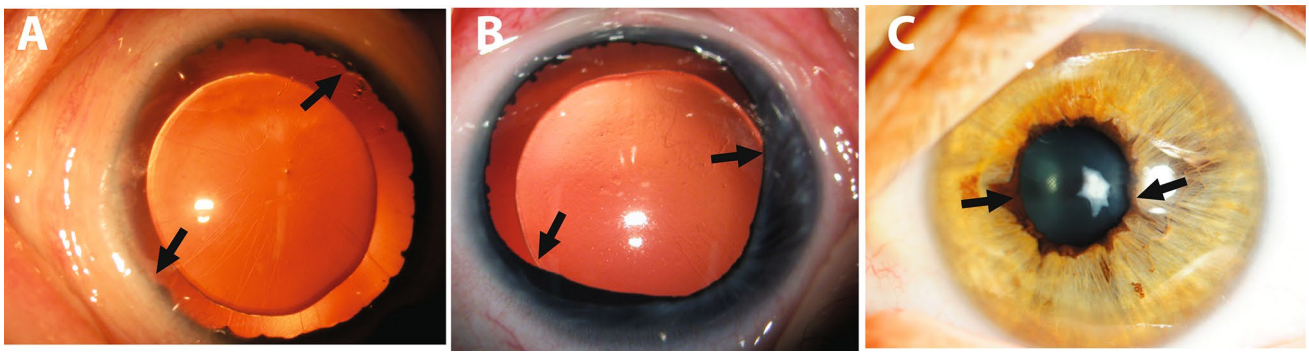

Fig. 2 Ocular anterior segment features of aniridia. **a** Absence of the iris with no iris tissue visible circumferentially to the periphery of the anterior segment only ciliary processes remain (black arrows), **b** partial iris absence with residual iris visible in the periphery, especially between the two black arrows, **c** iris with ectropion uveae (black arrows) which can be seen as an iris feature in patients with mutations in *PAX6*

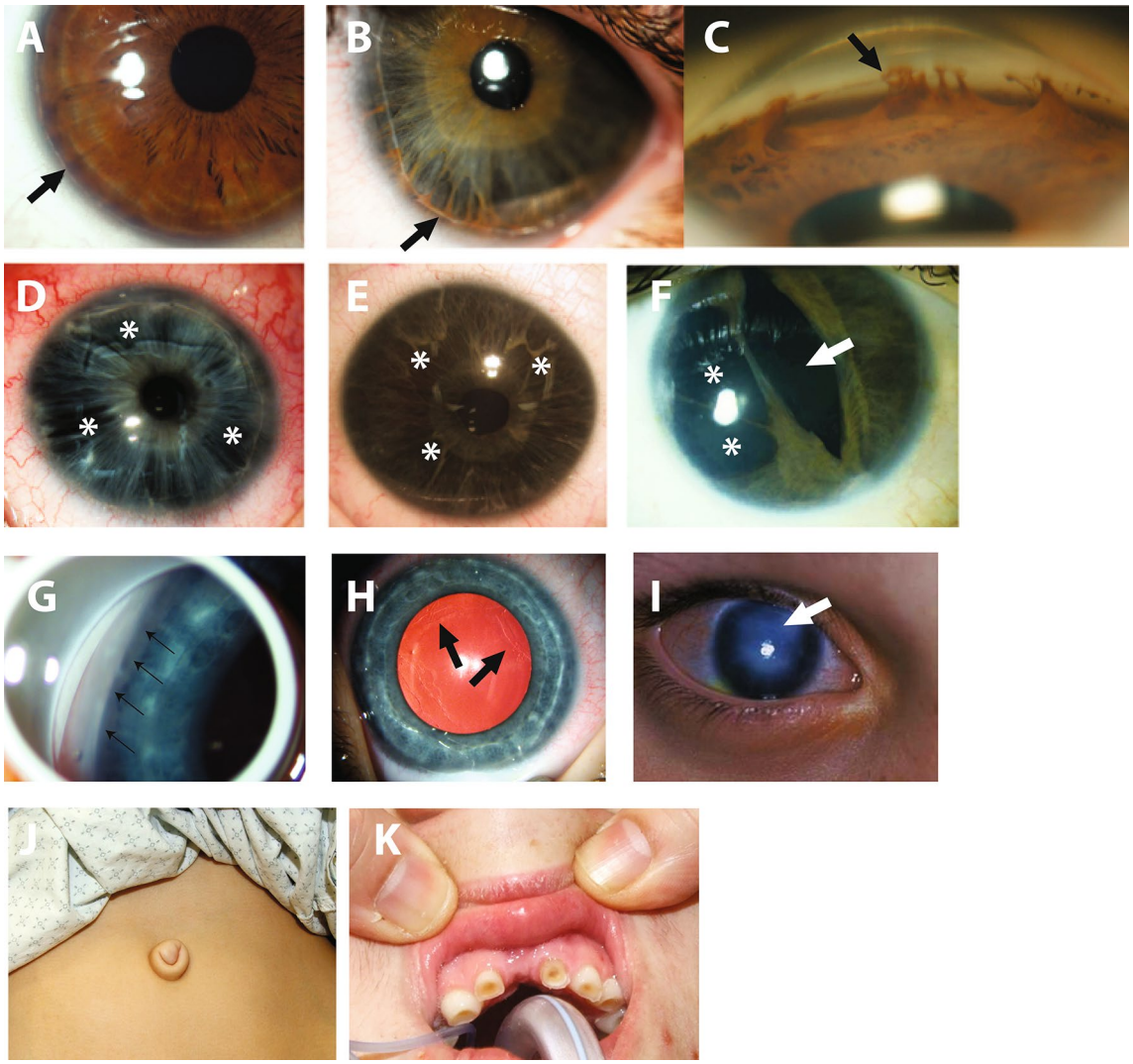

Fig. 3 Ophthalmic features of Axenfeld–Rieger anomaly, primary congenital glaucoma and Peters anomaly and syndromic features in Axenfeld–Rieger syndrome. **a–f** Axenfeld–Rieger spectrum, **a** posterior embryotoxon indicated by black arrow, **b** Axenfeld anomaly showing iris adhesions and processes to posterior embryotoxon (black arrow), **c** gonioscopy view showing the iris processes thin and broad attaching to the posterior embryotoxon (black arrow), **d** polycoria, with multiple regions of iris hypoplasia and loss of iris tissue (white asterix), **e** iris hypoplasia (white asterix) as the only feature of Axenfeld–Rieger, **d** classic Rieger with polycoria (white asterix), and corectopia (white arrow). **g** gonioscopy view of primary congenital glaucoma (PCG) angle with featureless angle tissue (small black arrows) sweeping up from the peripheral iris. **h** PCG: Buphthalmos and Haab Stria (splits in Descemet's membrane showing as tram track lines in peripheral cornea) highlighted here on retro-illumination (black arrows). **i** Peters anomaly, with central corneal opacification (white arrow). **j** Patulous redundant umbilicus and **k** dental features (hypoplastic dentition) of Axenfeld-Rieger syndrome

Disorders of the iridocorneal angle drainage structures—primary congenital glaucoma

Primary congenital glaucoma (PCG) is characterized by infant or early-childhood ocular hypertension, enlarged eye globes (buphthalmos) (Fig. 3g, h), and optic neuropathy, which can result in vision loss and blindness, often despite treatment. The pathogenesis of PCG is still uncertain and the anatomical basis of outflow obstruction remains unclear, although good evidence suggests trabeculodysgenesis relates to embryologic arrest of angle formation with disruption to the trabecular meshwork and outflow pathway (Chang et al. 2013).

Disorders with corneal, lens and multiple anterior segment abnormalities, including Peters Anomaly and sclerocornea

Peters anomaly (PA) occurs when there are iridocorneal and/or lenticulocorneal adhesions, and central corneal opacity, related to defects in the posterior corneal endothelium (Bhandari et al. 2011; Stone et al. 1976) (Figs. 1c, 3i).

This combination of abnormalities causes significant visual impairment due to interruption of the visual axis, often in combination with cataracts, as well as glaucoma due to trabecular meshwork abnormalities (Bhandari et al. 2011; Reis and Semina 2011).

While sclerocornea is not historically included in the definition of anterior segment dysgenesis, it is often clinically described in patients with mutations in ASD genes. Sclerocornea is a broad term used in the literature to describe various presentations with non-inflammatory, non-progressive extension of opaque valcularized scleral tissue onto the periphery or entire cornea (Elliott et al. 1985; Nischal 2007). A more specific term is congenital corneal opacification (CCO), which can be secondary to maldevelopment of the anterior segment of the eye. If CCO is associated with underlying keratolenticular or iridocorneal adhesions, this would be consistent with a diagnosis of PA. In the absence of adhesions, a diagnosis of total CCO (or total sclerocornea) can be made (Nischal and Sowden 2013), and this is usually associated with an abnormal, poorly developed lens (Ma et al. 2018).

Syndromal forms of anterior segment disorders

The most common syndromic forms of anterior segment abnormality are Rieger or Axenfeld–Rieger syndrome (ARS) and Peters Plus syndrome. Others, such as Alagille and SHORT syndromes have anterior segment abnormalities as key diagnostic features. These will be discussed in the following sections with reference to the underlying genetic cause. In addition, there are more than 100 genetic syndromes associated with anterior segment abnormalities (Possum/London Medical Databases trait search). Many of these are chromosomal, due to microdeletion/microduplications of multiple genes, as well as eponymous conditions with no known causative disease gene yet identified, and rare disorders with multiple systemic manifestations such as skeletal dysplasias and intellectual disability syndromes.

Phenotype–genotype classification—anterior segment dysgenesis phenotypes and their major contributing genes

While clinical classification is more straightforward according to the structures involved and different appearances on slit lamp examination, the genetic classification and phenotype–genotype correlations are increasingly complex. This is due to the intricate developmental genetics and embryology of the anterior segment, and the growing number of genes reported with mutations causing ASD and commonly overlapping phenotypes (Tables 1, 2; Fig. 4). In pursuing phenotype–genotype classification, attempts have been made to correlate sites of embryonic gene expression with phenotypic abnormalities seen in patients (Nischal and Sowden 2013). This has led to classification of genes into those with panocular expression such as *PAX6*, those expressed in the migrating periocular neural crest cells such as *FOXC1* and *PITX2*, and those expressed in the developing lens such as *FOXE3* and *PITX3*. While this can be useful as a starting point, there is growing recognition that there are multiple complex events involved in development of the ocular anterior segment (Jamieson and Grigg 2013). This includes gene expression in the developing structure, as well as influence of signals from nearby developing tissues, particularly the lens which plays a key role in induction and influence on anterior segment differentiation (Beebe and Coats 2000; Graw 2003; Jamieson et al. 2002; Ma et al. 2018). This means that for many of these genes, there are overlapping associated phenotypes, and also for each phenotype there are often multiple genetic associations, which makes clinical and genetic diagnosis difficult. To assist the clinician, we will discuss the main phenotypes in ASD and the major genes contributing to the clinically described phenotypes described above, with emphasis on the most common disease genes causing these phenotypes and relationships to embryological factors which may help explain the complex overlapping phenotype–genotype correlations. Table 2 also highlights this link, which may help clinicians to prioritize genes for analysis for any given phenotype.

Aniridia and *PAX6*

Familial and sporadic cases of aniridia are most often due to mutations in *PAX6*, the first gene found to have a role in anterior segment development (Glaser et al. 1992) (Table 1; Fig. 2a–c). *PAX6* is part of the paired box family of DNA-binding proteins, important in altering expression of other genes (van Heyningen and Williamson 2002) with an embryological role in organizing the developing eye as well as central nervous system development (Halder et al. 1995; Walther and Gruss 1991). It is a crucial gene for the differentiation of tissues in the anterior eye segment, and expression of *Pax6* has been found in the neuroectoderm of the optic cup and anterior surface ectoderm during ocular development (Davis and Reed 1996; Walther and Gruss 1991). Homozygous Pax6 null mice have absence of eyes, nasal structures, pancreas and severe brain defects (Aalfs et al. 1997; Glaser et al. 1994; Hill et al. 1991).

PAX6 has two DNA-binding domains, one 128 amino acid paired domain and a 61 amino acid long homeodomain. These two domains act as negative regulators of each other's function (Xu et al. 1995). Over 300 *PAX6* variants have been reported, and over 90% are predicted to be disruptive of transcription or translation (Crolla and van Heyningen 2002; Hingorani et al. 2009; Prosser and van Heyningen

Table 1 Phenotypic features associated with mutations in anterior segment dysgenesis (ASD) genes

Gene	OMIM	Inheritance	Function/gene family	IH	ARA	PA	PCG	CCO	EL	CAT	Additional phenotypes
PAX6	607108	AD	Paired box family—transcriptional regulator in oculogenesis	X		X				X	Aniridia, foveal hypoplasia, keratitis
PITX2	601542	AD	Homeodomain transcription factor	X	X	X					Axenfeld–Rieger syndrome: cardiac abnormalities, craniofacial, hearing loss, redundant umbilicus, hypospadias, anal stenosis
FOXC1	601090	AD	Forkhead transcription factor	X	X	X	X				Axenfeld–Rieger syndrome
CYP1B1	601771	AR	Monomeric mixed function mono-oxygenases		X	X	X				
LTBP2	602091	AR	Latent transforming growth factor-beta binding protein				X		X		Weill–Marchesani syndrome-like (WMS-like) syndrome with microspherophakia
FOXE3	601094	AR/AD	Forkhead transcription factor			X		X		X	Microphthalmia, coloboma, aphakia, sclerocornea
PITX3	602669	AD	Homeodomain transcription factor			X		X		X	
B3GLCT	610308	AR	Beta-1,3-glucosyltransferase			X					Peters plus syndrome: intellectual disability, short stature, cleft lip/palate
COL4A1	120130	AD	Alpha 1 subunit of collagen type IV	X	X	X		X		X	Renal, vascular, brain, cardiac, muscle
PXDN	605158	AR	Peroxidaisin					X		X	
CPAMD8	608841	AR	Complement component 3 family	X					X	X	Iris synechiae, corectopia, ectropion uveae, PCG also reported once

All may be complicated by glaucoma. Note, heterozygous variants in *TEK* and *ANGPT1* have been found in cases with primary congenital glaucoma, but there was marked variation in penetrance and expression associated with these cases. A heterozygous variant in *TRIM44* was found in one family with iris hypoplasia

IH iris hypoplasia, *ARA* Axenfeld–Rieger Anomaly, *PA* Peters anomaly, *PCG* primary congenital glaucoma, *CCO* congenital corneal opacification, *EL* ectopia lentis, *CAT* cataract

Table 2 ASD phenotypes and underlying disease genes

Phenotype	Main gene(s)	Other gene(s) to consider	Comments
Aniridia	*PAX6*	*PITX2*/*FOXC1* (*TRIM44*)	*PAX6* main gene involved in aniridia. Note panocular and milder phenotypes and consider deletion studies for WAGR Syndrome TRIM44 single case report only
Iris hypoplasia/corectopia/polycoria/Axenfeld–Rieger anomaly	*PITX2*/*FOXC1*	*PAX6, COL4A1, CYP1B1, CPAMD8*	*PITX2*/*FOXC1* mutations in 40% cases
Primary congenital glaucoma	*CYP1B1*	*LTBP2, FOXC1* (*TEK, ANGPT1*)	*CYP1B1* main gene involved *LTBP2* may be ethnicity specific Other genes few reports only, with variable penetrance in TEK/ANGPT1
Corneal/lens/including Peters anomaly/multiple ASD abnormalities	*PAX6, PITX2, FOXC1, PITX3, FOXE3*	*CYP1B1, COL4A1,PXDN, GJA8*	Note multiple overlapping phenotypes with each gene Consider *PXDN*/*GJA8* for sclerocornea cases especially if cataracts or abnormal lens present
Syndromal	*B3GLCT* (Peters Plus syndrome) *PITX2*/*FOXC1* (Axenfeld Rieger syndrome)	*JAG1* (Alagille) *PIK3R1* (SHORT)	Testing depending on clinical features

Table of the clinical subclassifications of ASD, and the main genes and secondary genes to consider testing based on phenotype. Some genes are in brackets due to inadequate evidence for pathogenicity based on few reports in the literature. This may change as new evidence is published

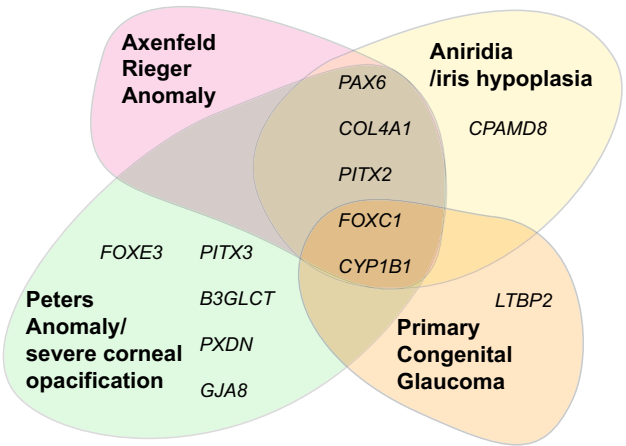

Fig. 4 Anterior segment dysgenesis genes grouped by clinical phenotype. Genes involved in anterior segment dysgenesis (ASD), classified by the clinical subtype of Axenfeld–Rieger, Peters, primary congenital glaucoma, and aniridia/iris hypoplasia. Heterozygous variants in *TEK* and *ANGPT1* have also been suggested to contribute to cases with primary congenital glaucoma, but there was marked variation in penetrance and expression associated with these cases. A heterozygous variant in *TRIM44* was found in one family with iris hypoplasia and further studies are needed to determine the contribution of this gene to anterior segment dysgenesis

1998; Tzoulaki et al. 2005). Most of these mutations lead to *PAX6* haploinsufficiency, which can occur due to three main mechanisms: heterozygous cytogenetic deletions of 11p13 (sometimes involving *PAX6* +/- nearby *WT1*), disruption of the 3′ regulatory region of *PAX6*, or due to intragenic mutations of *PAX6*, such as splicing, nonsense and frameshift mutations (Gronskov et al. 2001; Robinson et al. 2008). This usually leads to the classical presentation of aniridia, with additional panocular consequences including glaucoma, cataract, macular hypoplasia, nystagmus and limbal stem cell deficiency. In contrast, missense mutations, especially those in the DNA-binding paired domain, have been reported in milder and variable phenotypes, causing atypical/milder aniridia and other related disorders including congenital cataracts, foveal hypoplasia, keratitis, optic nerve abnormalities and PA (Azuma et al. 1998; Chauhan et al. 2004; Hingorani et al. 2009; Tzoulaki et al. 2005; Vincent et al. 2003). In addition to the aforementioned domains, there is an alternative splice region in exon 5a containing 14 additional amino acids, which abolishes the N-terminal DNA-binding subdomain of the paired domain (Epstein et al. 1994). Mutations in this region have been reported in PA and foveal hypoplasia (Azuma et al. 1999).

The ocular phenotype of aniridia usually occurs as an isolated feature, but can be part of a broader contiguous gene deletion of 11p13 involving both *PAX6* and the nearby gene *WT1*, leading to Wilm's tumour, aniridia, genitourinary abnormalities and mental retardation (WAGR) syndrome (Fantes et al. 1992). Nonocular associations with mutations in *PAX6* may include multiple central nervous system abnormalities of the anterior commissure, grey matter in the anterior cingulate cortex, cerebellum, temporal/occipital

lobes, corpus callosum and olfactory hypoplasia on MRI (Ellison-Wright et al. 2004; Sisodiya et al. 2001; Valenzuela and Cline 2004) (Fig. 4).

Iris anomalies, peripheral iridocorneal adhesions and genetic factors

While *PAX6* is the main gene associated with aniridia, an increasing number of genes have been associated with iris hypoplasia and additional findings such as corectopia and polycoria such as *PITX2, FOXC1, CYP1B1, COL4A1* and more recently *CPAMD8* (Fig. 4). These will be discussed in more detail in their own sections below, starting with the main ARA genes *PITX2* and *FOXC1*.

Axenfeld and Rieger anomalies and *PITX2, FOXC1*

PITX2 and *FOXC1* are the main genes involved in ARA. They are transcription factors, responsible for DNA binding, and controlling cell migration, differentiation, and downstream gene expression in the eye (Sowden 2007). Heterozygous mutations in these two genes are found in around 40% of patients with classic ARA (Lines et al. 2002; Reis and Semina 2011; Strungaru et al. 2007; Tumer and Bach-Holm 2009) (Tables 1, 2; Figs. 3, 4), and these are the two most studied genes in this disorder. Other phenotypes associated with mutations in these genes include PA, iris hypoplasia, PCG, and aniridia, highlighting their key role in anterior segment development (Table 2; Fig. 4). It is thought that mutations in these genes lead to abnormalities in neural crest migration to the anterior segment (Graw 2003), and that this contributes to the anterior segment anomalies. Both are expressed in the periocular mesenchyme and have a key role in the development of ocular drainage structures, iris, cornea, and trabecular meshwork (Berry et al. 2006; Erickson 2001; Lines et al. 2002). *PITX2* is a paired-like homeodomain transcription factor and *FOXC1* encodes a member of the forkhead transcription factor family. The homeodomain of PITX2 interacts with the C-terminal activation domain of FOXC1, with PITX2 acting as a negative regulator of FOXC1, explaining why they can both cause the same phenotypes (Berry et al. 2006). They have regulatory effects on other key components in eye development such as *PAX6, MAF, PITX3*, the TGFβ pathway and Wnt signalling (Berry et al. 2006; Chen and Gage 2016).

PITX2 is the commonest reported gene with mutations in ASD. These mutations tend to be clustered around the homeodomain, a 60 amino acid bicoid-like DNA-binding homeodomain, and associated with isolated and syndromal forms of ARA, caused by defective binding of downstream DNA targets including *FOXC1*. Over 50 mutations, the commonest being intragenic missense, nonsense and splice site, as well as partial and whole gene deletions, have been reported in this gene. Also, large chromosomal rearrangements, deletions and duplication have been described.

There are also reports of nonocular manifestations of *PITX2* mutations such as congenital heart disease and atrial fibrillation (Gudbjartsson et al. 2007; Lubitz et al. 2014; Sun et al. 2016). Homozygous null pitx2 mice have septal and valvular cardiac defects, pituitary abnormalities, and multiple eye defects (Gage et al. 1999; Lin et al. 1999). Pitx2 also has a role in mouse embryonic development affecting left–right signalling (Cox et al. 2002; Semina et al. 1996; Tamura et al. 1999).

At least 50 mutations are reported in *FOXC1*, and most occur in the forkhead domain—which is a distinct 100 amino acid DNA-binding domain. Mutations are also found in the other domains, such as the N- and C-terminal activation domains, which regulate transcriptional activity, as well as the inhibition domain. *FOXC1* missense, frameshift, and nonsense mutations have been reported, as well as whole gene deletions and even partial duplications, highlighting evidence that gene dosage may play a role in ASD. Apart from ARA, where mutations are likely in isolated cases without syndromal features, mutations have also been reported in PA, glaucoma and PCG (Chakrabarti et al. 2009; Nishimura et al. 2001, 1998; Strungaru et al. 2007; Tumer and Bach-Holm 2009) (Fig. 4).

FOXC1 also has a nonocular role. The mouse foxc1 and foxc2 genes are coexpressed in the heart field, and cardiac neural crest cells (Seo and Kume 2006), and foxc1 homozygous mutations in mice are lethal, with severe skeletal cardiac and ocular defects, but heterozygotes have ASD with irregular pupils, iris hypoplasia, displaced Schwalbe line, and abnormal aqueous drainage (Smith et al. 2000). A small number of ARS patients with missense *FOXC1* mutations have also been reported with congenital cardiac disease, such as valvular dysplasia, outflow tract hypoplasia, and atrial septal defects (Du et al. 2016; Gripp et al. 2013; Khalil et al. 2017).

Apart from *PAX6, FOXC1* and *PITX2*, ARA/iris hypoplasia has also been found to be associated with a number of additional genes including *COL4A1, CYP1B1*, and the recently identified disease gene *CPAMD8* (Cheong et al. 2016).

Rieger or Axenfeld–Rieger syndrome (ARS) and *PITX2, FOXC1*

Rieger or Axenfeld–Rieger syndrome (ARS) is the name given to the combination of ocular ARA, and other systemic extraocular features which include: facial, dental, skeletal and umbilical abnormalities (Fig. 3j, k) (Churchill and Booth 1996; Idrees et al. 2006; Spencer 1996). The variable dysmorphic features include hypertelorism, prominent forehead and telecanthus, as well as redundant umbilical skin,

anal stenosis, hypospadias, growth hormone deficiency, congenital cardiac abnormalities, hydrocephalus and intellectual disability. As for ARA, the main genes where mutations are found to cause ARS are *PITX2* and *FOXC1*. *PITX2* mutations are more commonly associated with systemic features of ARS, whereas mutations in *FOXC1* are more likely to result in isolated ocular involvement (D'Haene et al. 2011; Reis et al. 2012; Strungaru et al. 2007; Tumer and Bach-Holm 2009).

Alagille syndrome and *JAG1, NOTCH2*

The eye involvement in Alagille syndrome is principally posterior embryotoxon in 78–89% of patients(Hingorani et al. 1999), but additional abnormalities with ARA, optic disc drusen, and retinal pigmentary changes have also been described(El-Koofy et al. 2011; Makino et al. 2012). Alagille syndrome is a multisystem disorder with abnormalities of the liver, heart, eyes, face and skeleton. The main clinical issue from this syndrome is cholestasis due to bile duct paucity, as well as congenital cardiac defects, but abnormalities can also arise in the kidneys, neurodevelopment and vasculature. There is marked variable expressivity. Heterozygous mutations in *JAG1* are found in over 90% of patients clinically diagnosed with Alagille, and over 226 mutations are reported in the literature including gene deletions, frameshift, nonsense, splicing and missense mutations. Jagged-1 is an important cell surface ligand for the notch homolog protein 2 receptors, which are part of the Notch signalling pathway involved in cell fate. In eleven families with Alagille who were negative for *JAG1* mutations, heterozygous mutations were found in *NOTCH2* (Harendza et al. 2005; Kamath et al. 2012; McDaniell et al. 2006; Warthen et al. 2006).

SHORT syndrome and *PIK3R1*

The ocular abnormalities in SHORT syndrome include ARA and risk of glaucoma. SHORT is a mnemonic standing for short stature, hyperextensibility, ocular depression (deep-set eyes), Rieger anomaly and teething delay, first described in 1975 (Gorlin et al. 1975). Subsequent reports of this condition have demonstrated partial lipodystrophy, intrauterine growth restriction, short stature, and characteristic facial features with prominent forehead and deep-set eyes. Heterozygous germline mutations in the AKT/mTOR pathway gene *PIK3R1* were initially identified in 16 families with SHORT syndrome (Chudasama et al. 2013; Dyment et al. 2013; Schroeder et al. 2014). Most of the pathogenic variants cluster in the C-terminal SH2 domain of the gene, with a recurrent missense mutation c.1945C > T. This gene encodes the regulatory subunit of PI3K, which is an enzyme activating the AKT/mTOR pathway for cell proliferation and growth. Small deletions, missense and nonsense variants have been reported.

Iridocorneal angle abnormalities, primary congenital glaucoma and genetic factors

The principal and most frequent gene to date associated with PCG is *CYP1B1* (Stoilov et al. 1997). In specific ethnic groups, mutations are reported more rarely in *LTBP2* (Ali et al. 2009; Azmanov et al. 2011; Bejjani et al. 1998; Narooie-Nejad et al. 2009). In addition, mutations in *FOXC1* have been implicated with a limited role in PCG, being found in five families (Medina-Trillo et al. 2016). More recently, variants have been reported in *TEK* (Souma et al. 2016), but there was marked variability in penetrance and expression, so further reports are required to validate the contribution of this gene.

Primary congenital glaucoma and *CYP1B1*

Homozygous or compound heterozygous mutations in *CYP1B1* are primarily responsible for autosomal recessive primary congenital glaucoma. In particular, mutations have been found with high frequency in inbred populations with PCG, but in only 25–27% of cases in populations with mixed ethnicity(Li et al. 2011). A large study in 1220 patients with PCG found mutations in 41.6%,with higher yield in Middle Eastern families, and lower in Caucasian families (Chouiter and Nadifi 2017). This is consistent with a more recent paper (Alsaif et al. 2018) where in a large cohort from Saudi Arabia, mutations were found in 80.8% of 193 patients, with high penetrance. Interestingly, the same study utilized WES in the 34 negative patients, and found no variants in *LTBP2, TEK* or *MYOC*. Instead, they postulated likely pathogenic variants in other genes including *SLC4A11, ADAM9, CPAMD8*, and *KERA*. This highlights the expanding phenotypes associated with known genes, but also a low yield in genetic testing in *CYP1B1*-negative PCG cases, depending on ethnicity. Mutations in *CYP1B1* have also been found in families with PA, first reported in 2006 (Vincent et al. 2006) and also in a Saudi Arabian cohort where homozygous mutations were found in 5/10 families with PA (Edward et al. 2004). Mutations have also been reported in at least two cases of ARA (Chavarria-Soley et al. 2006; Tanwar et al. 2010) (Fig. 4). These mutations are a mix of missense and nonsense/frameshift mutations, and no clear phenotypic correlation with mutation type can be drawn from the small numbers.

As a member of the cytochrome P450 family, *CYP1B1* acts as a monooxygenase, catalyzing reactions for many compounds and their metabolism. The exact mechanism whereby mutations of *CYP1B1* lead to PCG is still unknown. While compound heterozygous or homozygous mutations in

CYP1B1 lead to PCG, it has been suggested that heterozygous mutations may be associated with primary open angle glaucoma (POAG) (Acharya et al. 2006; Lopez-Garrido et al. 2006; Melki et al. 2004; Vincent et al. 2002). In one study, PCG associated mutations in *CYP1B1* caused severe alteration in RA metabolism, whereas those causing steroid metabolism dysfunction without altering retinol metabolism were associated with POAG (Banerjee et al. 2016).

Primary congenital glaucoma, ectopia lentis and *LTBP2*

LTBP2 was the second gene where mutations were reported to cause PCG, found mainly in recessive form in specific ethnic groups such as Pakistani and Gypsy families (Ali et al. 2009). *LTBP2* is a latent transforming growth factor-beta binding protein, composed of 20 EGF (extracellular growth factor)-like domains and 4 transforming growth factor-beta (TGFβ) domains. These TGFβ domains are unique in the LTBP-fibrillin superfamily which includes fibrillin 1 (*FBN1*), the gene for Marfan syndrome which is often diagnosed due to ectopia lentis. *LTBP2* has high expression in human trabecular meshwork and ciliary processes (Narooie-Nejad et al. 2009) and has a role in formation of ciliary microfibrils, which themselves are composed of fibrillins 1 and 2 (Inoue et al. 2014).

Each of the originally reported families had homozygous null (nonsense or frameshift) mutations, and subsequent studies have mostly found mutations in consanguineous Iranian, Gypsy, Indian and Pakistani families, which may highlight an ethnic-specific association with this gene. Interestingly, ectopia lentis was present in some of these families, and recently additional families with autosomal recessive inheritance have been found with mutations in *LTBP2* with additional features apart from glaucoma. Kumar (Kumar et al. 2010) identified a homozygous duplication in *LTBP2* in an Indian family with microspherophakia (small, spherical lenses) without glaucoma, and Desir et al. (2010) identified homozygous null *LTBP2* mutations in patients with megalocornea, microspherophakia and glaucoma. In 2012, Haji-Seyed-Javadi (Haji-Seyed-Javadi et al. 2012) identified a Weill–Marchesani-like syndrome in one Iranian family with skeletal features (short stature and brachydactyly), shallow anterior chambers and ectopia lentis, with a homozygous missense mutation in *LTBP2*.

Peters anomaly, corneal/lens opacities and genetic factors

Peters anomaly is genetically heterogeneous, with mutations in at least 8 genes known to be causative including: *PAX6, PITX2, FOXC1, PITX3, FOXE3, CYP1B1, B3GLCT* (Peters Plus syndrome) and *COL4A1* (Fig. 4) (Aliferis et al. 2010; Bhandari et al. 2011; Dassie-Ajdid et al. 2009; Hanson et al. 1994; Nishimura et al. 2001; Ormestad et al. 2002; Reis et al. 2012; Semina et al. 1998; Vincent et al. 2001; Weh et al. 2014). However, many cases of Peters anomaly lack a genetic diagnosis. While PA encompasses a number of overlapping eye phenotypes, embryologically it is thought to develop due to failure of separation of the lens from the surface ectoderm (Matsubara et al. 2001) and subsequent abnormal adhesions between the lens/iris/cornea.

Many of these eight genes known to cause PA also cause additional phenotypes (both ocular and extraocular). Some are also key transcription factors in lens development and one of the emerging themes is the key role the developing lens plays in the development of the anterior segment, and the multiple abnormalities (sclerocornea, ASD, ARA, PA, glaucoma, microphthalmia) that can occur secondary to interrupted lens development.

Peters anomaly, multiple anterior segment abnormalities and *FOXE3*

FOXE3 is another forkhead transcription factor, involved in a broad number of anterior segment phenotypes, with mutations found in both autosomal dominant and recessive families with cataracts, as well as anterior segment abnormalities, PA, glaucoma and microphthalmia/coloboma (Table 1; Fig. 4). Overall, there are 33 reported mutations in the literature, with frameshift, missense, nonsense and non-stop mutations reported, with most of the missense mutations in the forkhead domain (Anand et al. 2018; Islam et al. 2015).

Heterozygous mutations in this gene have been found in autosomal dominant families with ASD/cataracts (Semina et al. 2001), as well as PA (Ormestad et al. 2002). Other heterozygous mutations have been seen in autosomal dominant families with bilateral microphthalmia, aphakia, sclerocornea and coloboma, as well as PA with congenital cataract, and microphthalmia/coloboma (Iseri et al. 2009). Homozygous or compound heterozygous mutations have been found in patients with bilateral microphthalmia (Reis et al. 2010). A pattern has emerged where heterozygous mutations are usually associated with milder ASD or cataracts, and these are mostly C-terminal extension mutations (Plaisancie et al. 2017). Homozygous or compound heterozygous loss of function mutations cause a more severe ocular phenotype with congenital aphakia, ASD, and extraocular features (Ormestad et al. 2002; Plaisancie et al. 2017). Missense mutations also tend to be recessively inherited and associated with an isolated ocular phenotype. Glaucoma is also a recognized feature in many patients with mutations in *FOXE3* (Anand et al. 2018; Islam et al. 2015). *FOXE3* mutations have also been implicated in thoracic aortic aneurysm and dissection. These missense mutations tend to be clustered in the forkhead domain, in patients without any evidence of ASD (Kuang et al. 2016).

FOXE3 is involved in the formation of the lens placode, vesicle, and anterior epithelium (Blixt et al. 2000), and is expressed at the same time as *PITX3* (Medina-Martinez et al. 2009) which also has a key role in lens formation, and discussed in detail below. Apart from interacting with *PITX3*, another gene *DNAJB1* has been implicated as a downstream transcriptional target (Khan et al. 2016). This gene has been demonstrated to have a key role in lens transparency and development in zebrafish, again highlighting the key role of the lens in anterior segment development.

Peters anomaly, multiple anterior segment abnormalities and *PITX3*

PITX3 mutations in humans cause PA, variable abnormalities of the anterior segment and cataracts (Table 1; Fig. 4). In the literature, only seven mutations are reported in the gene, with one heterozygous missense and six heterozygous frameshift mutations (all clustered in exon 4) in 168 individuals. Around half of these patients have variable manifestations of ASD, including corneal opacification, and majority have congenital cataracts (Anand et al. 2018; Liu et al. 2017; Verdin et al. 2014).

There is a recurrent 17-bp insertion in the C-terminal of *PITX3*. This has been reported in families with isolated congenital cataracts, such as posterior polar cataracts without ASD, and also in families with cataracts and various forms of ASD including corneal opacification, iridocorneal adhesions and glaucoma (Berry et al. 2004; Semina et al. 1998; Summers et al. 2008).

PITX3 belongs to the homeobox transcription factor family and plays a role in epithelial cell maintenance and fibre cell differentiation in the lens, with downstream regulatory effects on the expression of β and γ crystallins (Semina et al. 1997). Again, this highlights the key role of the lens in formation of anterior segment structures, and the impact of aberrant lens formation. This is also demonstrated in mouse studies, where Pitx3 has been implicated as the cause of the recessive ak aphakia mouse with absent lens and eyelids (Semina et al. 2000), as well as the eyl eyeless mouse (Rosemann et al. 2010). The phenotype of the Pitx3 deficient mouse has microphthalmia, as well as other anterior segment anomalies in the pupil, cornea and iris, and these are thought to be secondary to aphakia (Ho et al. 2009; Semina et al. 2000; Wada et al. 2014).

Sclerocornea, microphthalmia, cataracts and *GJA8*

In addition to *FOXE3* and *PITX3*, another key lens gene has recently been discovered to play a role in anterior segment abnormality, especially in sclerocornea. With the advent of next-generation sequencing, the phenotypes associated with known genes is broadening, and our group has reported three families with heterozygous missense mutations in the lens gap junction gene *GJA8* in patients with severe sclerocornea with abnormal lens (Ma et al. 2018). Gap Junction proteins in the lens such as GJA8 and GJA3 have a key role in maintaining lens fibre cell homeostasis (Mathias et al. 2010), and mutations in *GJA8* have previously only been known to be associated with congenital cataracts (Beyer et al. 2013). This broadening of the ocular phenotype associated with *GJA8* mutations has been confirmed by Ceroni et al. (2018). Two patients from our study and one from Ceroni et al, with heterozygous missense mutations affecting p.Gly94, all had sclerocornea with only very rudimentary lens or congenital aphakia, with one patient also demonstrating iris and optic disc coloboma. This emphasises the critical role of the *GJA8* p.Gly94 residue in lens development and impact of lens abnormality on anterior segment and other developmental processes of the eye (Ceroni et al. 2018; Ma et al. 2018). Our patient with a p.Asp51Asn mutation had sclerocornea, bilateral cataracts and microphthalmia, while two patients with the same mutation reported by Ceroni et al. had in addition to cataracts and microphthalmia, a variety of other anterior segment anomalies including persistent pupillary membrane, corneal leukoma and corectopia. Thus, in addition to sclerocornea, other anterior segment abnormalities are possible in association with lens abnormalities and microphthalmia in patients with *GJA8* mutations (Ceroni et al. 2018; Ma et al. 2018).

Peters plus syndrome and *B3GLCT*

Peters plus syndrome occurs due to mutations in *B3GLCT* (Lesnik Oberstein et al. 2006). This is an autosomal recessive disorder of O-glycosylation, and clinical features include Peters anomaly with short stature, cleft lip/palate, brachydactyly, intellectual disability and facial dysmorphism. Rarely, patients can also have congenital cardiac defects (atrial and ventricular septal defects, aortic and pulmonary stenosis), structural brain abnormalities, hearing loss, and genitourinary abnormalities.

B3GLCT is the only gene known to be associated with Peters plus syndrome. Over 20 biallelic mutations have been reported in the literature, including splice site, single nucleotide, deletion, frameshift, and nonsense loss of function mutations (Lesnik Oberstein et al. 2006).

New genes and pathways

The discovery of new genes and pathways involved in ASD highlights an emerging pattern in the types of genes involved in ASD, and some of their clinical consequences. While previously mutations have mostly been reported in the transcription factors *FOXC1, PITX2* and *PAX6*, a number

of additional transcription factors have more recently been shown to be relevant in ASD including *FOXE3* and *PITX3*, as discussed above. These transcription factors have a key role in cell differentiation, embryogenesis and cell migration, as well as lens placode development. Since the lens is such a key structure of the developing eye, mutations in these transcription factors tend to have a global disruptive effect on the anterior segment and carry a high risk of glaucoma. Also, gene-encoding proteins in a number of additional pathways are being discovered with impact on anterior segment development, including *LTBP2* in the TGFβ pathway discussed above, and variants in *PXDN* which reveal the impact of oxidative stress in abnormalities of the anterior segment. Variants in *COL4A1* and the variably expressed variants in *TEK* and *ANGPT1* (Meuwissen et al. 2015; Souma et al. 2016; Thomson et al. 2017) suggest consideration of genes contributing to vascular development as candidate disease genes in anterior segment dysgenesis. These highlight the broadening of associated phenotypes with ASD where there are many extraocular features, especially skeletal, emerging in these disorders, as well as novel genes identified in pathways not previously known to be involved in ASD (Table 1; Fig. 4). While these recently discovered genes do highlight plausible developmental pathways involved in ASD, there are some issues including limited functional data, and variable expressivity and non-penetrance that need to be addressed. Therefore, caution is required before utilizing some of these genes in the human diagnostic setting. As NGS analysis becomes more commonplace, more of these pathways and genes will be discovered. Below, we group emerging pathways in ASD causation identified through new disease gene identifications.

PXDN—oxidative stress pathways in cataract and corneal opacification

Mutations in *PXDN* were found in autosomal recessive families with cataract microcornea from Pakistan and a Cambodian family with glaucoma and severe corneal opacification (Khan et al. 2011). *PXDN* contains a peroxidase domain found in extracellular matrix proteins. The main function of peroxidasin is to metabolise hydrogen peroxide. It has therefore been postulated that *PXDN* acts as an extracellular antioxidant, possibly reducing the buildup of reactive oxygen radicals that could create oxidative stress in ocular tissues and cataracts in the lens.

COL4A1—vascular development in ASD and brain

Mutations in *COL4A1*, a type IV collagen gene, have been found in a wide spectrum of ocular abnormalities including microphthalmia, cataract, ARA, PA, and glaucoma (Table 1; Fig. 4). While mutations in this gene were initially thought to be only responsible for porencephaly, a broadening phenotype including eye malformations has recently emerged, with variable expressivity and intrafamilial variability (Meuwissen et al. 2015). Two COL4A1 peptides and one COL4A2 peptide form a heterotrimer in the endoplasmic reticulum, before secretion into basement membranes where they form important networks with other proteins. The majority of mutations occur in the triple helical domains in conserved glycines, and affect heterotrimer formation. Mao (Mao et al. 2017) performed compelling mouse knockout work showing expression of mutant *COL4A1* in the lens had a dose-dependent impact in ASD, causing iridocorneal adhesions, and abnormal intraocular pressure. This highlights the role of this gene in the ocular anterior segment, and the importance of lens formation in anterior segment development.

TEK/*ANGPT1*—vascular development in PCG and venous malformations

A whole exome sequencing study in 2016 (Souma et al. 2016) found 10 heterozygous loss of function variants in *TEK*, in 189 PCG families. All the variants were found to lead to haploinsufficiency. However, in 6/10 of these families, the heterozygous variants were also found in unaffected family members, usually one parent. Non-penetrance and variable expressivity raise difficulty in interpretation of these variants. *TEK*, an epithelial-specific tyrosine kinase, is expressed in endothelial cells (Partanen et al. 1992) and has a known role in vascular malformations, as gain of function mutations in this gene have been linked to hereditary and sporadic venous malformations (Limaye et al. 2009). *TEK* mouse mutants were found to have abnormal Schlemm's canal and trabecular meshwork, with elevated intraocular pressures, consistent with the pathogenesis of PCG (Limaye et al. 2009; Souma et al. 2016; Thomson et al. 2017). Further work from this group also identified heterozygous variants in *ANGPT1*, a major ligand for *TEK*, in three families with PCG (Thomson et al. 2017). However, in 2/3 of the families asymptomatic carrier parents were found, and the third family had no family segregation analysis. While there are interesting mouse functional studies implicating the angiopoietin/*TEK* pathway in Schlemm's canal formation, further studies are required to elucidate if these are indeed key human disease genes in Mendelian PCG, given the issue of non-penetrance for variants in both *TEK* and *ANGPT1*.

CPAMD8—iris hypoplasia, corectopia, ectropion uveae and cataracts

Compound heterozygous mutations in *CPAMD8* were found using whole exome sequencing in three families with a unique anterior segment phenotype with iris hypoplasia, ectopia lentis, corectopia, ectropion uveae and cataracts

(Cheong et al. 2016). Interestingly, patients had absence of posterior embryotoxon, corneal opacity or any extraocular features, and so these phenotypically did not fit into ARA or PA. *CPAMD8* belongs to the complement component-3/alpha-2-macroglobulin family of genes, involved in innate immunity and damage control (Li et al. 2004). Expression studies suggested a role for this gene in the nonpigmented epithelium of the ciliary body, which produces aqueous humour. Recently, a single family with homozygous frameshift mutations in *CPAMD8* was found in a PCG cohort from Saudi Arabia (Alsaif et al. 2018), with PCG and lens subluxation.

TRIM44—a suggested new iris hypoplasia gene

Variation in *TRIM44*, part of the tripartite motif family, was identified as possibly causing aniridia in a single autosomal dominant four generation Chinese family (Zhang et al. 2015). Two heterozygous missense variants [p.(Ser64Tyr) and p.(Gly155Arg)] in *TRIM44* were found in cis, in affected individuals using linkage analysis and whole genome sequencing. Functional analysis of overexpressed wild-type TRIM44 in HLE-B3 cells showed reduction in expression of PAX6 mRNA and protein, suggesting it could be a negative regulator of PAX6. Mutant TRIM44 with one of the heterozygous missense variants, p. (Gly155Arg) showed an even stronger decrease in PAX6 expression in the HLE-B3 cells suggesting this may lead to the disease phenotype. However, this was not able to be shown in patient cells, since only blood was available and PAX6 mRNA was not detected in the blood cells of the patients or other family members. There have been no further publications of variants in this gene since 2015.

Conclusions

The genetics of anterior segment dysgenesis is complicated by the multiple interacting factors and tissue components required for normal development of the anterior segment of the eye. Consequently, overlapping phenotypes are frequent amongst the genes described in this review (Fig. 4). Interestingly, mutations in *FOXC1* and *PITX2* may lead to almost all the anterior segment dysgenesis phenotypes, highlighting the crucial role of these transcription factors in anterior segment development. In addition, all the genes where mutations may cause ARA (*FOXC1, PITX2, PAX6, COL4A1, CYP1B1*) are also found in PA, highlighting the difficulties in genotype–phenotype correlation. Due to the marked genetic heterogeneity of these conditions, prior to the advent of next-generation sequencing approaches, many patients were only offered very limited genetic testing. As gene discovery and mutation testing becomes more widespread due to the availability of cheaper, more comprehensive testing, the phenotypes associated with the known disease genes are broadening and new disease genes are being identified. One example is demonstrated by our work in congenital cataracts, where mutations in the congenital cataract gene *GJA8* have been shown to cause sclerocornea and microphthalmia, with rudimentary lens (Ma et al. 2018), now also found by others (Ceroni et al. 2018). This highlights the influence of developing lens tissues on other developing anterior ocular structures, and the value of a broad-based sequencing approach such as whole exome sequencing (WES) or whole genome sequencing (WGS) in elucidating novel genotype–phenotype correlations in abnormalities of the anterior segment. In a study of WES in 27 patients with PA (Weh et al. 2014), mutations were found in *PAX6*, as well as possible mutations in a number of other genes: *TFAP2A, NDP, FLNA, SLC4A11, HCCS*. Similarly, a large study of Saudi Arabian PCG families utilizing WES in 34 families found additional mutations in *SLC4A11, ADAM9, CPAMD8*, and *KERA*, and other potential PCG genes *BCO2* and *TULP2* (Alsaif et al. 2018). Many of these genes have not traditionally been reported in PA or PCG, respectively, and while it is difficult from these two papers alone to draw clear conclusions regarding the pathogenicity of these individual genes, it provides further indication of a broadening of phenotypes associated with mutations in genes previously associated with other syndromes or non-ASD phenotypes.

Due to the phenotypic variability and genetic heterogeneity, we suggest a broad approach to patients diagnosed with ASD in the clinical diagnostic setting. First, deletion/duplication studies with microarray should be performed as these structural changes are often associated with ASD and other extraocular features such as facial dysmorphism, congenital abnormalities and intellectual disability. Second, an NGS-based approach, using either a panel of known disease genes or whole exome or genome sequencing examining these genes should be performed. Table 2 summarises phenotype–genotype correlations in ASD, and is a guide for considering which genes to prioritize based on phenotype. Caution is often required in analysing the more recently discovered ASD genes, as there is often limited functional data regarding their pathogenesis, and also possible non-penetrance and variable expressivity further confounding their significance.

Diagnostic yield in ASD is variable. In cases where there is a clear clinical diagnosis of aniridia, examination of *PAX6* will lead to a mutation detection rate of approximately 80–90%. From available studies, we estimate an approximately 30–50% genetic diagnostic detection rate in ASD based on WES in Peters anomaly (Weh et al. 2014), and conventional sequencing of Axenfeld–Rieger anomaly/syndromes (Reis and Semina 2011). Absence of genetic diagnosis using these approaches suggests there may be additional

factors beyond the standard monogenic models contributing to disease pathogenesis, such as multigenic inheritance, or the presence of structural variants and noncoding region mutations not covered by standard diagnostic techniques, as well as the possibility of novel disease genes and phenotypic associations. For those where mutations are not identified, WES and WGS approaches, the use of Matchmaker (Philippakis et al. 2015) or other gene and pathway investigation approaches to find novel variants and disease genes will be important.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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