



The genetic architecture of aniridia and Gillespie syndrome

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Abstract

Absence of part or all of the iris, aniridia, is a feature of several genetically distinct conditions. This review focuses on iris development and then the clinical features and molecular genetics of these iris malformations. Classical aniridia, a panocular eye malformation including foveal hypoplasia, is the archetypal phenotype associated with heterozygous *PAX6* loss-of-function mutations. Since this was identified in 1991, many genetic mechanisms of *PAX6* inactivation have been elucidated, the commonest alleles being intragenic mutations causing premature stop codons, followed by those causing C-terminal extensions. Rarely, aniridia cases are associated with *FOXC1*, *PITX2* and/or their regulatory regions. Aniridia can also occur as a component of many severe global eye malformations. Gillespie syndrome—a triad of partial aniridia, non-progressive cerebellar ataxia and intellectual disability—is phenotypically and genotypically distinct from classical aniridia. The causative gene has recently been identified as *ITPRI*. The same characteristic Gillespie syndrome-like iris, with aplasia of the pupillary sphincter and a scalloped margin, is seen in *ACTA2*-related multisystemic smooth muscle dysfunction syndrome. WAGR syndrome (Wilms tumour, aniridia, genitourinary anomalies and mental retardation/intellectual disability), is caused by contiguous deletion of *PAX6* and *WT1* on chromosome 11p. Deletions encompassing *BDNF* have been causally implicated in the obesity and intellectual disability associated with the condition. Lastly, we outline a genetic investigation strategy for aniridia in light of recent developments, suggesting an approach based principally on chromosomal array and gene panel testing. This strategy aims to test all known aniridia loci—including the rarer, life-limiting causes—whilst remaining simple and practical.

Structure and development of the human iris

Sitting between the cornea and lens of the eye, the adult human iris is a 12 mm vivid, multilayered and photoprotective disc. Through control of pupil size in response to light or accommodation, it regulates light passage to the retina and is an optical requirement for sharp vision. It lies anterior to the ciliary body and itself plays a structurally important role in aqueous dynamics and the iridocorneal angle configuration, both of which affect intraocular pressure and glaucoma risk.

A macroscopic view of the iris shows a central pupillary region demarcated from the ciliary region by the iris

collarette (Fig. 1). The sphincter muscle, responsible for miosis (constriction) under parasympathetic nervous control, lies in this inner pupillary region, whereas the dilator muscle, which produces sympathetically mediated mydriasis, lies in the more peripheral ciliary region.

Of the principal components of the iris, the bilayered epithelium and smooth muscle have a different embryological origin to the overlying stroma.

The iris pigmented epithelium is neuroectodermal, developing as an outgrowth from the margins of the optic cup from the third month of gestation—relatively late compared to the rest of the eye (Fig. 1). Specification of iris progenitor cells to a non-neuronal fate is accompanied by the expression of transcription factors including *PAX6* and *OTX1*, and of members of the Wnt signalling pathway (Liu et al. 2007; reviewed by Davis-Silberman and Ashery-Padan 2008). Notch genes are also implicated in this patterning (Bao and Cepko 1997). Recently, two members of the TGF β superfamily (*Bmp4* and *Tgf β 2*) and *Foxc1* have been identified as direct downstream targets of Pax6 in murine iris development (Wang et al. 2016). In addition to evidence that they

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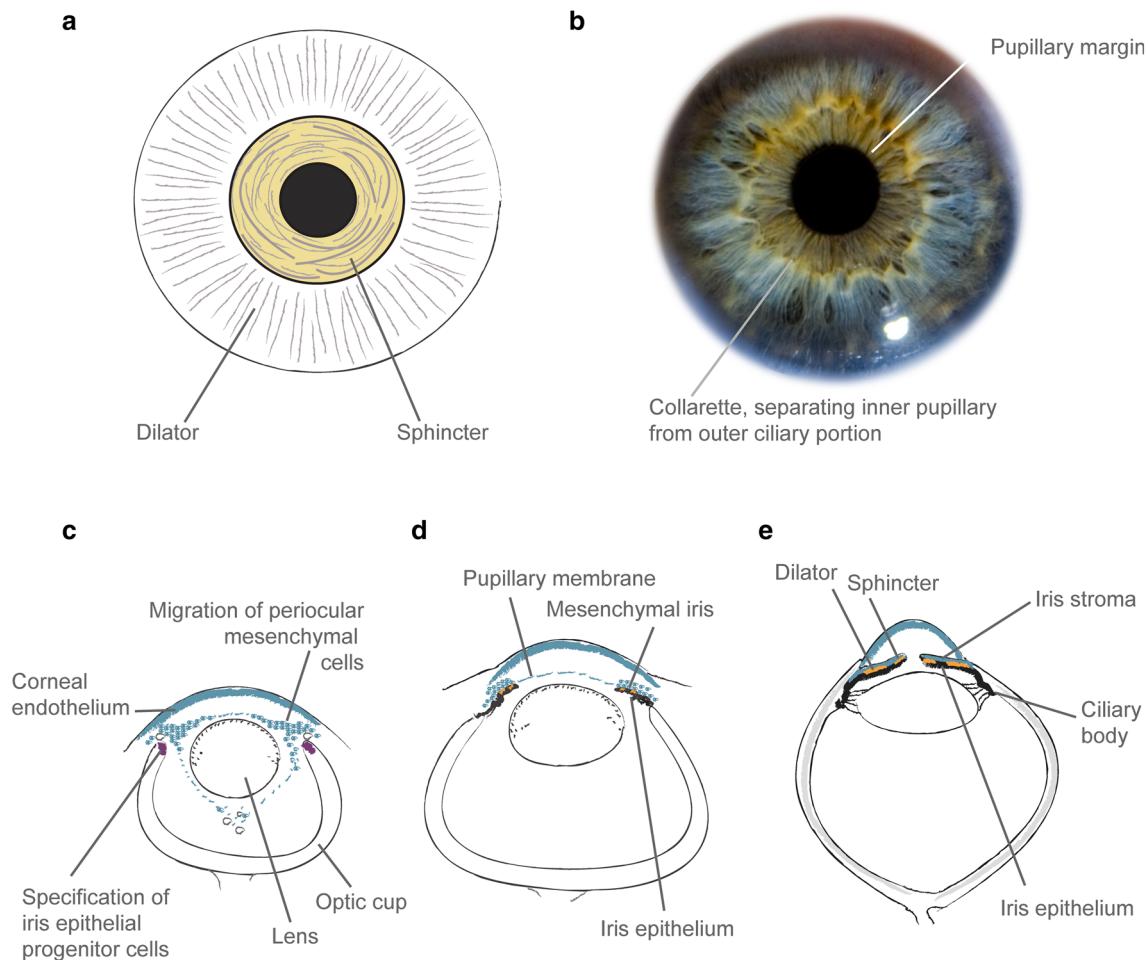


Fig. 1 The structure and development of the human iris. **a** Cartoon of the iris musculature, showing the position of the sphincter (shaded yellow) and dilator muscles. **b** Macroscopic photo of normal adult iris (courtesy of Chris Moody), showing the inner pupillary portion and outer ciliary portion. **c** Cartoon of the developing eye in a 7–8-week-old human embryo, showing the migration of periocular mesenchymal cells (shaded in teal) into the developing anterior chamber, which will go on to form the mesenchymal iris, pupillary membrane and corneal endothelium and stroma. The tips of the optic cup are shaded

in purple showing the specification of iris epithelial progenitor cells. **d** Cartoon of developing human eye at 4–5 months' gestation, showing the iris growing out from the optic cup margins. The iris musculature (shaded orange) is just starting to develop. **e** Cartoon of adult eye, showing a well-formed anterior chamber and iridocorneal angle; the iris stroma, musculature (orange) and epithelium (black); lens attached by zonules to the ciliary muscle. The cartoons in **a**, **b** are derived from multiple sources (primarily Mann 1925)

are expressed in mouse iris development (Chang et al. 2001; Adams et al. 2007; Hägglund et al. 2017), *BMP4* and *BMP7* are highly expressed in developing chick iris smooth muscle (Jensen 2005). The iris sphincter and dilator muscles are rare examples of ectodermally derived muscle (Imaizumi and Kuwabara 1971; Jensen 2005). They are not fully formed histologically in human fetuses until the sixth and eighth months, respectively (Mann 1925).

By contrast, the pupillary membrane and iris stroma are mesenchymal in origin, arising from the third wave of migrating neural crest cells (Williams and Bohnsack 2015). During the second month of gestation, after separation of the lens but before the appearance of iris epithelium, a thin layer of mesodermally derived tissue is visible anterior to

the lens (Mann 1925). The central part of this will become the pupillary membrane, which regresses late in gestation. Mesenchymal cells migrate along the anterior border of the iris epithelium, along with stromal melanocytes also deriving from neural crest (Sturm and Larsson 2009). Retinoic acid and its downstream target *PITX2* are critical to regulating neural crest derivatives in the anterior segment (Williams and Bohnsack 2015). *PAX6* is also expressed during morphogenesis of this part of the iris, though in a more transitory manner and at lower levels than in the ectodermal tissues (Cvekl and Tamm 2004). The *Small eye* rat (*rSey*) shows impaired migration of neural crest cells from the future anterior midbrain to the eye rudiment (Matsuo et al. 1993).

The striking visible colour of the iris is directly related to both the concentration of pigment within iris stromal melanocytes, and the type of melanin (eumelanin: pheomelanin ratio) (Prota et al. 1998). Final adult eye colour is not reached at birth, with iris stromal development continuing postnatally. In Caucasian infants, the iris is bluer for the initial months of life due to lower stromal melanin content (Rennie 2012). Genetically, the *OCA2-HERC2* region of chromosome 15q explains the large majority of variation in human eye colour (Sturm and Larsson 2009).

Classical aniridia

Clinical features

First described in 1818 (Barratta 1818), classical aniridia is a dominantly inherited, usually bilateral, panocular malformation (OMIM 106210). Two comprehensive Scandinavian studies have shown a prevalence of between 1 in 40,000 and 72,000 live births (Grønskov et al. 2001; Eden et al. 2008), in keeping with older US epidemiological data (Shaw et al. 1960). The term aniridia can refer to both the clinical sign and the condition (Table 1). The latter is a spectrum comprising aniridia with foveal plus or minus optic nerve hypoplasia, with later development of cataracts, glaucoma and/

Table 1 Definition of terms used in the description of aniridia and related eye malformations

Term	Working definition
Iris	
Aniridia	1. (Clinical sign) Absence of the iris 2. (Disease) Panocular eye malformation
Complete aniridia	Absence of the iris, with no visible iris tissue (distinction is not made depending on whether any remnant is detectable gonioscopically)
Partial aniridia	Incomplete aniridia with some visible iris rim (e.g., in classical aniridia) or, less commonly, absence of part of the iris (e.g., in Gillespie syndrome; this is a distinct phenotype to classical aniridia consisting of aplasia of the sphincter)
Iris hypoplasia	Unlike aniridia, this is a neutral term which does not imply any particular associated condition. In the context of aniridia, used to describe the minor end of the spectrum of hypoplasia of the iris (cf partial to complete aniridia). The mildest sign of this is iris transillumination
Congenital mydriasis	Fixed dilated pupils. In Gillespie syndrome this is due to aplasia of the sphincter
Iris coloboma	Approximately 6 o'clock defect in the iris resulting from failure of optic fissure closure
Iridolenticular strands	Strands of tissue between the iris and the lens, often seen in Gillespie syndrome or anterior segment dysgeneses
Corectopia	Displacement of the pupil, which may be misshapen
Other anterior segment	
Posterior embryotoxon	An abnormality of the iridocorneal angle resulting in an anteriorly displaced Schwalbe's line. Characteristic of Axenfeld–Rieger syndrome but seen in 15% normal eyes
Keratopathy	A general term for corneal pathology. In the context of aniridia, this is progressive opacification of the cornea over many years, related to limbal stem cell failure
Congenital corneal opacification	Congenital corneal opacification may result from aniridia and from other developmental anomalies of the anterior segment such as failure of lens separation. Terms such as Peters anomaly and sclerocornea are clinical signs and are not helpful aetiologically. The authors recommend the classification outlined by Nischal (2015)
Cataract	Congenital or acquired lens opacity, with the visual prognosis and need for surgery depending on the age of onset, symptoms, morphological type and laterality
Posterior segment	
Foveal hypoplasia	Failure of the specialisation of the centre of the macula for fine detailed central vision, visible on OCT as an absent or shallow foveal dip. A core feature of aniridia (but not Gillespie syndrome) and ocular albinism
Optic nerve hypoplasia	Visible fundoscopically as a small optic disc, classically with a peripapillary halo and altered ring of pigmentation, with reduced vision correlating not with disc size but with papillomacular nerve fibre integrity
Optic nerve coloboma	Excavated optic disc anomaly due to defective optic fissure closure and thus deeper inferiorly, which may involve the whole disc and/or coexist with chorioretinal coloboma and microphthalmia
Other	
Ptosis	Drooping of the upper eyelid

OCT optical coherence tomography

or keratopathy. Ptosis is also sometimes seen as a feature, as in Fig. 2.

The typical iris phenotype is of complete (Fig. 2) or partial aniridia, often with a thin crescent of iris visible. Lesser degrees of iris hypoplasia are also seen. The distinction between partial and total aniridia is of limited use as, armed with a gonioscopy lens to view the iridocorneal angle, even those with apparently complete aniridia tend to have a detectable iris stump (Nelson et al. 1984). However, cases of apparent partial aniridia with aplasia of the sphincter and a scalloped margin are important to note diagnostically (see “Gillespie syndrome” section below).

Whilst congenital glaucoma presenting with buphthalmos is rare in aniridia, glaucoma occurs in approximately half of aniridia patients, in later childhood or in adulthood (Grant and Walton 1974). Mechanisms of glaucoma described in aniridia include open angle glaucoma, rotation of the iris stump causing synechial chronic angle closure, and goniodysgenesis (Netland 2015). Furthermore, some patients undergo childhood cataract surgery which itself carries an independent risk of secondary glaucoma (Mataftsi 2016). The whole spectrum of cataract morphology is seen in aniridia, but they are often polar and associated with capsular fragility that render cataract surgery more difficult (Schneider et al. 2003). Lens subluxation also occurs.

Interestingly, foveal hypoplasia in aniridia occurs without the chiasmal misrouting seen in albinism (Neveu et al. 2005). Foveal hypoplasia usually results in reduced vision, at the level of at least 6/30 (0.7 logMAR), such that most patients are eligible for sight impairment registration. The optical consequences of lack of iris tissue also contribute to this, and strabismic amblyopia (lazy eye) is frequently seen.

Lastly, abnormal corneal epithelial homeostasis and limbal stem cell deficiency in aniridia lead to progressive keratopathy (Douvaras et al. 2013). Signs of aniridic keratopathy are typically apparent from early adulthood, the cornea becoming slowly more cloudy and vascularised. This constitutes the main cause of progressive visual loss in later life (Mayer et al. 2003). Rarely, a congenital corneal opacity may be present due to keratolenticular apposition (Nischal 2015).

Whilst other ocular phenotypes such as microphthalmia are seen in conjunction with *PAX6* mutations (discussed below), this is not considered part of the classical aniridia phenotype. Non-ocular phenotypes, in particular neuro-anatomical and endocrine abnormalities (both potentially asymptomatic) have been identified in studies of aniridia patients. Impaired glucose tolerance has been reported in aniridia (Yasuda et al. 2002), and the prevalence of diabetes may be slightly higher [7% vs 4.5%, though note that the populations of these studies are not directly comparable (Netland et al. 2011; Menke et al. 2015)]. This is supported by functional and animal work on the role of Pax6 in the pancreas (Swisa et al. 2017). Subtle deficits in olfaction and

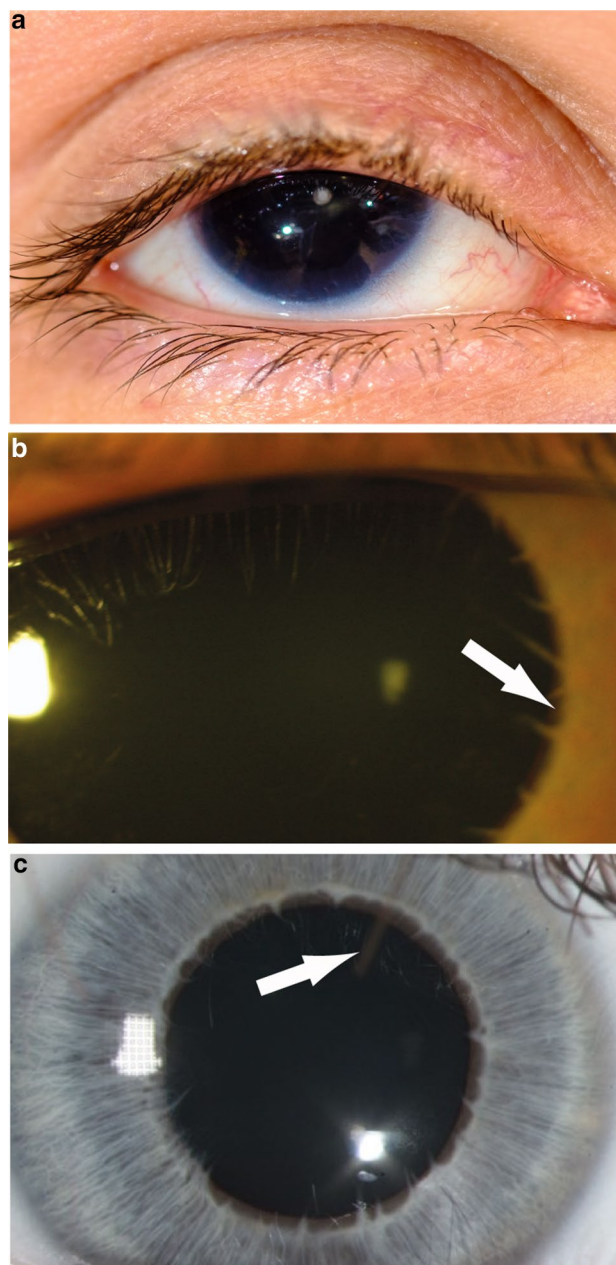


Fig. 2 Iris phenotypes in classical aniridia, Gillespie syndrome and multisystemic smooth muscle dysfunction syndrome. **a** Right eye of an individual with classical aniridia, showing near complete absence of the iris and mild ptosis (image courtesy of David Hall); **b** iris of a Gillespie syndrome patient with an *ITPR1* mutation. Note the scalloped edge of the iris remnant (arrow), with aplasia of the iris central to the collarette (image courtesy of Abhijit Dixit); **c** iris of a patient with an *ACTA2* mutation and multisystemic smooth muscle dysfunction syndrome, showing the aplasia of the iris central to the collarette, with a scalloped pupillary margin and iridolenticular strand (arrow) (image courtesy of Françoise Meire)

hearing have been described (Sisodiya et al. 2001; Bamiou et al. 2007). MRI brain studies have shown structural abnormalities on MRI in the pineal gland, anterior commissure,

corpus callosum and frontoparietal cortical areas (Mitchell et al. 2003; Free et al. 2003; Yogarajah et al. 2016). These are primarily radiological studies with no, or limited (Yogarajah et al. 2016), evidence of potential functional consequences. However, there is evidence of auditory interhemispheric transfer deficits in children with aniridia (Bamiou et al. 2007).

Identification of *PAX6*

Classical aniridia is strongly associated with heterozygous loss-of-function of *PAX6* (*Paired box 6*, OMIM 607108, hg38 chr11:31784792–31811353). Animal models and human patients were key to the identification of this gene in the early 1990s (Hanson et al. 1994). The 25th anniversary was recently commemorated in a review (Cvekl and Caljaerts 2017).

Comparative mapping data had suggested that human aniridia was homologous to the murine *Sey* phenotype (van der Meer-de Jong et al. 1990; Glaser et al. 1990), a strain first reported in 1967 as having microphthalmia in heterozygotes (Roberts 1967)—and later shown to also feature iris hypoplasia, cataracts and corneal opacification (Jordan et al. 1992). *Pax6* was isolated along with other *Pax* genes from mouse expression libraries (Walther et al. 1991). In parallel, the gene was independently identified from positional cloning and shown to be deleted in human aniridia (Ton et al. 1991). Concurrent analysis of *Sey* alleles confirmed the causal link between *Pax6* and semidominant *Sey* (Hill et al. 1991).

Establishing the link between human *PAX6* mutations and the semidominant *Sey*, the authors (Hill et al. 1991) first observed the importance of dosage sensitivity. More recently, this property has been quantified with a haploinsufficiency metric by the Exome Aggregation Consortium (ExAC) (Lek et al. 2016), with an impressive probability of loss-of-function intolerance score, pLI, of 1.00 for *PAX6*. Whilst *PAX6* haploinsufficiency leads to aniridia, whole-gene duplication causes non-aniridic eye malformations, and homozygous loss-of-function is perinatally lethal in humans and mice (Roberts 1967; Glaser et al. 1994; Schedl et al. 1996; Aalfs et al. 1997; Schmidt-Sidor et al. 2009; Schilter et al. 2013).

PAX6 biology and structure

PAX6 is a highly conserved transcription factor critical to the control of both ocular and neural development (Prosser and van Heyningen 1998). In addition to orchestrating the developing eye, it is expressed in the central nervous system and pancreas, as well as in adult tissues such as the cornea, brain and pancreas where it is involved in homeostasis

(Sivak et al. 2003; Hart et al. 2013; Yogarajah et al. 2016; Huettl et al. 2016; Maurya and Mishra 2017).

The canonical human *PAX6* encodes a 422 amino acid, 46.7 kDa protein [UniProt P26367-1 (The UniProt Consortium 2017)], whilst the less abundant, alternatively spliced *PAX6*(5a) isoform (436 amino acid, 48.2 kDa, UniProt P26367-2) contains an extra 14 residues between Gln47 and Val48, encoded by exon 5a (Fig. 3a). *PAX6* contains two DNA-binding domains, the bipartite paired domain (Fig. 3b) and the homeodomain, as well as a proline–serine–threonine-rich C-terminal transactivation domain. The paired and homeodomains can bind to DNA both cooperatively and independently, widening the repertoire of targets (Jun and Desplan 1996). A third isoform has been identified arising from an additional internal promoter (Carrière et al. 1995; Kim and Lauderdale 2006), though the role of such isoforms in humans, potential transcripts of which have been identified in silico, remains an interesting question (Bandah et al. 2008).

The subcellular localisation of *PAX6* is predominantly nuclear. Nuclear localisation signals have been identified in the paired domain and near the homeodomain (Ploski et al. 2004; Tabata et al. 2018). However, non-nuclear localisation has also been observed. In chick retina, *PAX6* was nuclear in the ganglion cell and inner nuclear layers, and cytoplasmic in the photoreceptor and outer nuclear layer (Shin et al. 2003), which may reflect its roles in diverse cellular processes (Simpson and Price 2002). In quail and nematode, paired-less isoforms are found in the cytoplasm (Carrière et al. 1995; Zhang et al. 1998). In the mouse brain, SPARC and *Pax6* have been shown to co-localise and physically interact, and the authors suggest a model in which SPARC facilitates nuclear-cytoplasmic shuttling of *Pax6* (Tripathi and Mishra 2010), a role that has also been shown for Karyopherin 13 (Ploski et al. 2004).

In *PAX6*(5a), the additional 14 amino acids between alpha helices 2 and 3 of the paired domain modulate its binding and transactivation potential, preferentially binding DNA with the C-terminal subdomain, acting as a molecular toggle (Epstein et al. 1994; Azuma et al. 1999). The ratio of *PAX6*(5a) to canonical *PAX6* is higher in adult than embryonic tissue (Kozmik et al. 1997), with the *PAX6*(5a) isoform having a potential role in postnatal eye development (Singh et al. 2002). It is possible that other *PAX6* transcripts whose function is currently uncertain may also have a regulatory role.

PAX6 is thus able to increase its target diversity by alternative splicing as well as through the use of different DNA-binding domains (Simpson and Price 2002). Furthermore, fine-tuning is achieved through cooperative binding with other transcription factors, such as SOX2 (Kondoh et al. 2004; Hu et al. 2017). Targets of *Pax6* include transcription factors [e.g., *Six3* (Goudreau et al. 2002) and

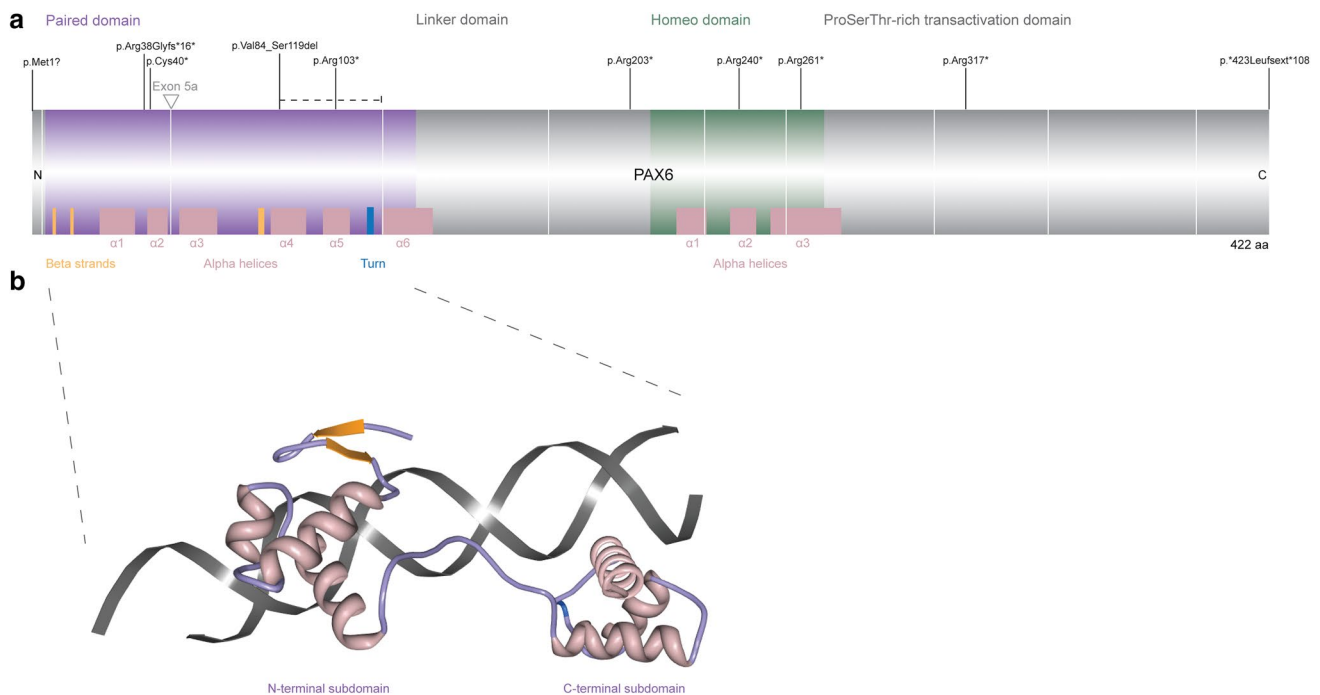


Fig. 3 **a** Protein bar showing the canonical 422-amino acid human PAX6 protein, annotated with the ten commonest aniridia alleles. The domains and main secondary structural elements are shown (UnitProt P26367). The coding exons are 4–13, with exon boundaries indicated by a white vertical line. The location of exon 5a, part of the alterna-

tively spliced isoform PAX6(5a), is indicated. **b** PAX6 paired domain bound to dsDNA. Note the two subdomains, each containing three alpha helices [PDB ID: 6PAX (Xu et al. 1999), from <http://www.rcsb.org>. Image of “6PAX” created using Protein Workshop (Moreland et al. 2005)]

Foxc1 (Wang et al. 2016)], signalling molecules (e.g., Wnt) and crystallins [e.g., alpha A-crystallin (Cvekl et al. 1994)]; for targets in iris development see above. These *PAX6* target genes give rise to diverse ocular phenotypes when mutated in humans: missense mutations in *SIX3* cause microphthalmia, coloboma and holoprosencephaly (MIM #157170); *FOXC1* mutations cause anterior segment dysgenesis (MIM #601631), and alpha A-crystallin (*CRYAA*) mutations cause cataract (MIM #604219). Equally, heterozygous mutations of *SOX2*, *BMP4* and *MAB21L2* are associated with syndromic microphthalmia (MIM #206900, #607932 and #615877, respectively).

Some of the *PAX6* gene regulatory networks have been elucidated. An elegant and phenotypically relevant example is that reciprocal inhibition between Pax6 and Pax2 establishes the retinal-optic nerve boundary in mammals (Schwarz et al. 2000); *PAX2* mutation causes papillorenal syndrome in humans (MIM #120330). More recently, Pax6 was shown by ChIP to directly regulate *Prox1* and *Mab21l1* as part of a lens differentiation network (Sun et al. 2015).

Transcriptional activation of *PAX6* requires the coordinated activity of *cis*-regulatory elements, which are mostly conserved across mammals and zebrafish (Kleinjan et al.

2004; Lakowski et al. 2007). These were initially identified via human translocation cases, detailed below.

PAX6 mutation spectrum

Reflecting the many possible ways of disrupting *PAX6* function, 472 unique sequence variants are reported (LOVD *PAX6* database, version 170616, http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6). All classes of sequence variation are present and no domain of the protein is spared. The ten commonest aniridia variants are marked in Fig. 3. The wider range of phenotypes spans from mild iris hypoplasia to perinatal lethality.

Monoallelic mutations

Likely gene-disruptive variants The commonest intragenic variants are encoded by single nucleotide substitutions resulting in a premature termination codon (PTC), with p.Arg240*, p.Arg317* and p.Arg203* being the three most frequently reported (129 reports in the LOVD database); these mutational hotspots correspond to CpG dinucleotides (Tzoulaki et al. 2005). Frameshifting insertions, deletions and duplications, as well as many splice site mutations,

also fall into this category of likely gene-disruptive variants. PTCs > 50–55 nucleotides upstream of the last coding exon–exon junction are predicted to be subject to nonsense-mediated decay (NMD). Though there could be exceptions to this NMD rule (Isken and Maquat 2007; Kervestin and Jacobson 2012), the known truncating mutations in humans phenocopy null alleles (Tzoulaki et al. 2005). In addition to the classical aniridia phenotype described above, some likely gene-disruptive variants appear to be hypomorphic, though milder phenotypes appear to be more common with missense variants (Hingorani et al. 2009).

Missense variants Missense mutations in *PAX6*, which may lead to production of a full-length protein with altered structure and function (Mishra et al. 2002), have been published associated with both classical aniridia and non-classical phenotypes. These include optic nerve anomalies (Nallathambi et al. 2006), hypomorphic phenotypes such as *ectopia pupillae* (Hanson et al. 1999) and, more severely, microphthalmia (reviewed in Williamson and FitzPatrick 2014; Chassaing et al. 2014). In addition to canonical *PAX6*, two missense variants in the *PAX6*(5a) isoform have been described in five Japanese families (at least three unrelated) with congenital corneal opacification with or without foveal hypoplasia and cataract: p.5aGlu13Arg (1 case) (Nanjo et al. 2004), the rest p.5aVal7Asp, with functional work suggesting subtle changes in binding by *PAX6*(5a) and transactivation (Azuma et al. 1999). The structural and functional protein consequences of many of the missense variants in the database (in addition to many splice site and in-frame deletions) have not been determined experimentally. The functional consequences of missense mutations, unlike most PTCs, are dependent on their location within the protein. For example, missense mutations at the C-terminal amino acid of *PAX6* impair transactivation mediated through the homeodomain (Singh et al. 2001).

C-terminal extensions Frameshift duplications and substitutions predicted to result in C-terminal extensions are notably common (34 database reports; Fig. 3), some with variant phenotypes including exudative retinopathy (Hingorani et al. 2009).

Larger deletions and chromosomal rearrangements Chromosomal rearrangements and whole/partial gene deletions disrupting *PAX6* account for approximately one-third of sporadic aniridia cases, and are also found in some familial cases (Crolla and van Heyningen 2002), such that overall these account for approximately 10% all aniridia cases (Robinson et al. 2008; Bobilev et al. 2015). Contiguous gene deletions causing WAGR syndrome are discussed below.

Variants disrupting the cis-regulation of *PAX6* Via analysis of translocation and inversion breakpoints in aniridia patients, several disease-causing alleles were identified which disrupt the long-range regulation of *PAX6*. These were located 11 kb, 22 kb (Lauderdale et al. 2000), 125 kb and 150 kb (*SIMO* element) (Simola et al. 1983; Fantes et al. 1995) downstream (telomeric) to *PAX6*. Analysis of the *SIMO* element showed that this is an autoregulatory binding site whose disruption impairs *PAX6* maintenance (Bhatia et al. 2013). The phenotypes were identical to heterozygous deletion or PTC-causing mutations of *PAX6*. Mutations in *PAX6* regulatory regions are now more easily detectable by array-based comparative genomic hybridisation. Ansari et al. identified five aniridia patients with deletions involving plausible regulatory regions and suggest a ‘critical region’ for transcriptional activation spanning 244 kb (Ansari et al. 2016).

Biallelic mutations

Biallelic mutations in *PAX6* with two likely gene-disruptive alleles cause severe CNS defects with anophthalmia and perinatal lethality (Glaser et al. 1994; Schmidt-Sidor et al. 2009). A similarly severe ocular phenotype was reported in a viable compound heterozygous child with trisomy 21 (Solomon et al. 2009). Two viable compound heterozygotes families have been published with aniridia or coloboma, and no severe systemic effects, through having a combination of a likely gene-disruptive and presumed mildly hypomorphic missense allele (Grønskov et al. 1999; Chao et al. 2003).

Other forms of *PAX6*-related eye disease

Distinct phenotypes described in association with *PAX6* mutations include Peters anomaly and other anterior segment dysgeneses (Prokudin et al. 2014), coloboma and microphthalmia (Williamson and FitzPatrick 2014), optic nerve anomalies (Azuma et al. 2003; Nallathambi et al. 2006), *ectopia pupillae* and nystagmus (Hanson et al. 1999). Commonly seen are the non-iris features of classical aniridia, either in isolation or combination, namely cataract, foveal hypoplasia, glaucoma and keratopathy (Sale et al. 2002; Hever et al. 2006). Most of these phenotypes can be considered variable expressivity of classical aniridia.

Genetic differential diagnosis of classical aniridia

Whilst classical aniridia is still largely considered a monogenic disorder, rarely aniridia-like phenotypes have occurred with mutations in two other anterior segment disease genes. These include two cases with *PITX2* mutations—one intragenic (Perveen et al. 2000) and one with a telomeric regulatory mutation (Ansari et al. 2016)—and seven cases with

FOXC1 mutations. Three of the latter are missense cases (Ito et al. 2009; Ansari et al. 2016), and four are whole gene deletions (Sadagopan et al. 2015; Ansari et al. 2016). Of these nine aniridia cases, seven had known congenital glaucoma (one unknown), many presenting as infants with buphthalmos, suggesting that this is more common in *FOXC1*- than *PAX6*-associated aniridia.

An aniridia family with a segregating missense mutation in *TRIM44*, ~4 Mb away from *PAX6* on 11p13, was described (Zhang et al. 2015), but we advise caution in ascribing pathogenicity as this is a single pedigree supported by in vitro functional data. There remains a small but significant proportion of aniridia patients with no genetic diagnosis (Ansari et al. 2016).

Following an analysis of 42 *PAX6*-negative aniridia cases with array CGH (comparative genomic hybridisation), which identified some of the cases above (Ansari et al. 2016), the authors were left with 27/42 unexplained cases. Since this publication, the Gillespie syndrome cases in this cohort have been shown to have *ITPR1* mutations (McEntagart et al. 2016; Gerber et al. 2016). We estimate the proportion of unexplained classical aniridia cases to be approximately 5%.

Contiguous deletion syndromes encompassing *PAX6*: WAGR syndrome

Background and clinical features

In 1964, Robert Miller et al. reported the results of a review of the case records of 440 individuals with Wilms tumour, a rare malignancy of embryonic origin affecting the kidney (Miller et al. 1964). Six of these children (1.4%) also had aniridia, which was unlikely to be a coincidence given the incidence of aniridia at that time of 1 in 50,000 live births (Shaw et al. 1960). Each of the children with Wilms tumour and aniridia also had significant intellectual disability or global developmental delay (termed mental retardation at the time). Intellectual disability is not a common association in isolated Wilms tumour or classical aniridia. Three of the six children had microcephaly and one child had contralateral renal hypoplasia. Subsequent to this original report, many other cases were published. This constellation of clinical features has been named either WAGR syndrome (Wilms tumour, aniridia, genitourinary anomalies, mental retardation) or AGR syndrome, depending on the presence or absence of Wilms tumour. For simplicity, we will use WAGR syndrome to cover both groups.

Remarkably few case series or systematic reviews have been published which focus on the clinical aspects of WAGR. The most useful is a series of 54 clinically well-characterised individuals (31 males, 23 females) with

Table 2 Clinical features associated with WAGR+/-O (excluding aniridia, Wilms tumour and intellectual disability): non-canonical WAGR features observed in >2 individuals in a series of 54 WAGR cases (Fischbach et al. 2005)

System	Feature	%
Genitourinary	Cryptorchidism	61.3
Respiratory	Recurrent sinusitis	27.8
Renal	Proteinuria	25.9
Behavioural	Short attention span	22.2
Respiratory	Obstructive sleep apnoea	20.4
Behavioural	Autism spectrum disorders	18.5
Metabolic	Obesity	18.5
Dental	Severe dental malocclusion	16.7
Ocular	Optic nerve hypoplasia	14.8
Musculoskeletal	Scoliosis/kyphosis	14.8
Neurologic	Hypertonia/hypotonia	13
Genitourinary	Hypospadias	12.9
Renal	Focal segmental glomerulosclerosis	11.1
Respiratory	Recurrent pneumonia	11.1
Ocular	Retinal detachment	9.3
Ocular	Strabismus	7.4
Genitourinary	Ambiguous genitalia	7.4
Neurologic	Epilepsy	7.4
Genitourinary	Inguinal hernia	5.6
Musculoskeletal	Hemihypertrophy	5.6
Metabolic	Hyperlipidemia	5.6
Abdominal	Chronic pancreatitis	5.6

WAGR (Fischbach et al. 2005). Clinical features observed in more than two individuals in this series are listed in Table 2. Of these non-canonical WAGR features, obesity has generated the most clinical interest (Marlin et al. 1994; Amor 2002) and has led to the suggestion that WAGR be re-designated as WAGRO (Tiberio et al. 2000).

Cytogenetic analysis

Chromosome 11p13 deletions were first identified in three individuals with WAGR in 1978 (Riccardi et al. 1978). Following detailed mapping of this chromosome region, the causative genes for Wilms tumour and genitourinary anomalies (*WT1*) (Gessler et al. 1990) and classical aniridia (*PAX6*) (Ton et al. 1991) were found to lie within a megabase of each other (Fig. 4). The term “contiguous deletion syndrome” is used to describe distinctive patterns of clinical features caused by haploinsufficiency for more than one gene mapping to the same genomic locus encompassed by a chromosome deletion. WAGR is the paradigm for this type of genetic disorder.

WT1 was identified via homozygous intragenic deletions in isolated Wilms tumours cases (Gessler et al. 1990). This was consistent with *WT1* being a tumour suppressor gene

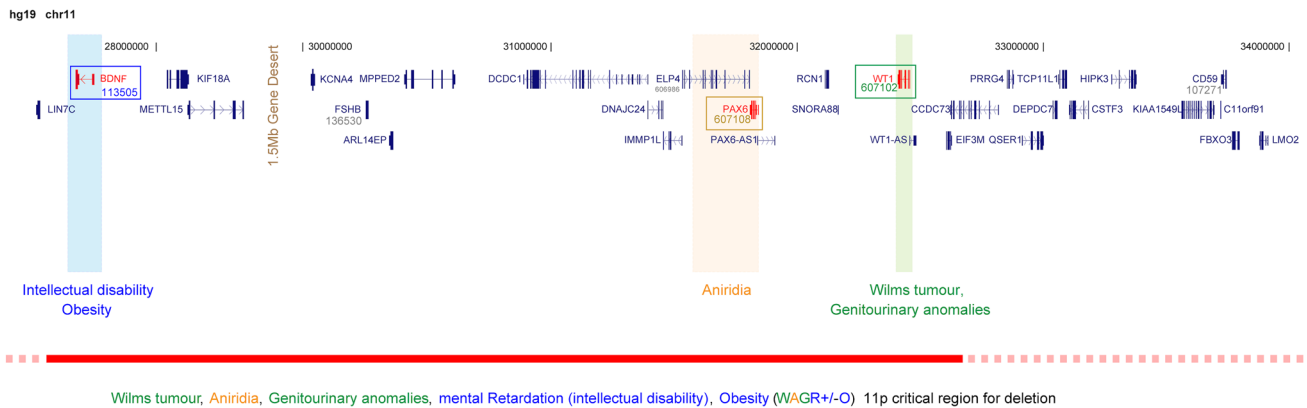


Fig. 4 Molecular pathology of WAGR syndrome. Cartoon of the 11p genomic region which encompasses *PAX6*, *WT1* and *BDNF*. The numbers and lines at the top represent the hg19 human genomic coordinates, below which are the gene models. If the gene is an OMIM morbid gene the cognate OMIM number is given below the gene symbol. The boxed genes are those that have been confidently associ-

operating in a manner consistent with the “two hits hypothesis” (Knudson 1971; Haber et al. 1990), which requires inactivation of both copies of the gene in the relevant tissue to initiate tumorigenesis. *WT1* encodes a zinc finger protein (Wilms tumour protein, UniProt P19544) which functions as a transcription factor and an RNA-binding protein. Heterozygous intragenic, dominant-negative mutations in *WT1* have been identified in several overlapping developmental disorders characterised by Wilms tumour, genital malformations, male-to-female sex reversal, nephropathy, diaphragmatic hernia and gonadoblastoma (Pelletier et al. 1991; Hastie 1992).

The gene—or genes—responsible for the cause of the neurodevelopmental problems and obesity in WAGR is not yet confirmed. Several lines of evidence implicate the gene encoding brain-derived neurotrophic factor (*BDNF*) in both these components of WAGR (Xu et al. 2008; Han et al. 2008, 2013; Shinawi et al. 2011; Rodríguez-López et al. 2013). Heterozygous *Bdnf* knockout mice have hyperphagia, obesity and poorer learning and social behaviours (Lyons et al. 1999; Kernie et al. 2000). In humans, WAGR patients with *BDNF* haploinsufficiency had reduced cognitive functioning, lower adaptive behaviour and higher levels of obesity, compared to those without a *BDNF* deletion (Han et al. 2008, 2013). The *BDNF* locus was also identified as a GWAS hit for obesity (Wen et al. 2012). The genes *SLCIA2* and *PRRG4* have also been implicated in the neurodevelopmental component (Xu et al. 2008). *PAX6* itself has also been proposed, given its known involvement in CNS development and the small proportion of cases with intragenic *PAX6* mutations associated with developmental delay or autism (collated by Davis et al. 2008; Chien et al. 2009).

ated with components of the WAGR syndrome. The bar at the bottom is an empirically-derived critical region for deletions associated with WAGR. However, it is likely there are many possible causes of the intellectual disability associated with WAGR, so it is reasonable to consider that any deletion which encompasses *PAX6* and *WT1* may be WAGR-associated

Relevance to isolated aniridia

Aniridia is usually apparent from soon after birth. Although WAGR-associated deletions represent a rare subset of causative alleles resulting in haploinsufficiency for *PAX6*, it is important to identify such deletions as soon as possible as they have a significant impact on both prognosis and management during infancy and beyond (Fig. 6). Most obviously, individuals who have deletions encompassing both *PAX6* and *WT1* should be screened for Wilms tumour as earlier detection almost certainly improves outcome (Pritchard-Jones et al. 2016). Due to the high incidence of end-stage renal failure in this group (Fischbach et al. 2005), lifelong monitoring of renal function is recommended. It is also reasonable for an individual with a WAGR deletion to be followed up by a paediatrician with expertise in the early recognition and management of intellectual disability and autism.

Gillespie syndrome

Clinical features

Apparent partial aniridia, presenting as congenital mydriasis, is an invariant feature of Gillespie syndrome (GS; OMIM 206700), in association with non-progressive cerebellar hypoplasia and ataxia, intellectual disability and congenital hypotonia. In GS the iris defect is bilateral and has a distinct scalloped appearance resulting from aplasia of the tissue central to the collarette (Fig. 2b). This aplasia appears to be a consequence of abnormal development of the ectoderm-derived sphincter musculature and the

surrounding mesoderm-derived stromal tissues at the distal tip of the foetal iris. In contrast to *PAX6*-associated aniridia, the ocular defects in GS are essentially restricted to the iris and the variable presence of iridolenticular strands. Foveal hypoplasia is rarely observed (McEntagart et al. 2016).

Additional phenotypes are noted infrequently, but multiple cases have shown cardiac (pulmonary valve stenosis, patent foramen ovale), skeletal (affecting the vertebrae or digits), and gastro-oesophageal defects (Dentici et al. 2017; reviewed by Carvalho et al. 2017). The early clinical reports of this syndrome were limited to 12 families (reviewed by Mariën et al. 2008), and they supported X-linked, autosomal recessive and autosomal dominant modes of inheritance.

Identification of *ITPR1* as the causal gene

In 2016, two independent projects utilising whole exome sequencing concurrently identified *ITPR1* (inositol 1,4,5-trisphosphate receptor type 1; OMIM 147265; hg38 chr3:4493348–4847840) as the causative gene in GS (McEntagart et al. 2016; Gerber et al. 2016). These projects studied one familial mother–daughter transmission (Mariën et al. 2008) and 15 singleton cases, with a further five cases reported subsequently (Zambonin et al. 2017; Dentici et al. 2017; Carvalho et al. 2017). GS is now considered a monogenic disorder, as all 22 reported cases with genetic testing had monoallelic or biallelic pathogenic mutations identified in *ITPR1*. Interestingly, 17 of these cases were known to be female, although such a marked sex bias was not evident in the families described in the early clinical reports with no genetic diagnosis. There is no evidence for non-penetrance for *ITPR1* mutations in GS: all individuals with a pathogenic mutation are affected.

ITPR1 biology and structure

The *ITPR1* receptor is a type I intracellular transmembrane calcium release channel that is inositol 1,4,5-trisphosphate (IP₃) responsive [UniProt Q14643, alternative names IP3R1, InsP3R1; reviewed by Patel et al. (1999)]. The subcellular localisation of the receptor is perinuclear: it is primarily in the smooth endoplasmic reticulum (ER), though intranuclear, possibly nucleoplasm reticular, *ITPR1* foci have also been reported (Maeda et al. 1990; McEntagart et al. 2016). The *ITPR1* protein complex is a homotetramer: each subunit is oriented with a large N-terminal region, containing an IP₃-ligand binding site, and a short C-terminal region, both in the cytoplasm; six transmembrane domains contributing to the channel pore in the ER membrane, and inter-transmembrane domain loops in the ER lumen (Jiang et al. 2002). The different subtypes of *ITPR* are ubiquitously expressed, with high levels of *ITPR1* in the brain and particularly in the Purkinje cells of the cerebellum (Maeda et al. 1990).

The expression pattern in the developing iris is not reported. *ITPR1* channel function shows calcium-dependent increased inhibition when the receptor is bound to the *ITPR* interacting protein (*ITPRIP*, previously called *DANGER*; UniProt Q8IWB1), which is highly co-expressed in Purkinje cells and contains an ER/plasma membrane signal peptide and a conserved partial Mab21 domain (van Rossum et al. 2006). In mouse models of *Itpr1* null alleles, heterozygosity elicits mildly impaired motor coordination and minor iris defects distinct from those of GS (Ogura et al. 2001; McEntagart et al. 2016), and homozygosity is embryonic lethal or causes severe ataxia and seizures with early death (Matsumoto et al. 1996; reviewed by Tada et al. 2016).

ITPR1 mutation spectrum

In GS, *ITPR1* mutations have been identified in four families with recessive homozygous or compound heterozygous alleles, and in 16 families with dominant heterozygous alleles (McEntagart et al. 2016; Gerber et al. 2016; Zambonin et al. 2017; Dentici et al. 2017; Carvalho et al. 2017). The families with dominant mutations include the one familial case, with mother–daughter transmission, and a de novo event in a consanguineous family. Parental DNA was available for all but one of the remaining dominant families, and de novo occurrence of the mutation was established in each case. The recessive alleles are predicted to generate premature termination codons that, possibly via exon skipping mechanisms, appear to act as partial rather than full loss-of-function mutations; as the carrier parents of these alleles are unaffected there is no apparent haploinsufficient effect associated with these hypomorphic variants (see below for *ITPR1* haploinsufficiency in non-GS ataxia; Fig. 5).

Given that less than 50% of normal levels of *ITPR1* are necessary to cause GS, it was proposed that the dominant heterozygous alleles associated with this syndrome are not loss-of-function mutations but are dominant-negative mutations. These dominant alleles comprise missense mutations or codon-Lys2596 deletion, and alter C-terminal residues that are located at or near the ER transmembrane domain and the associated ion-transport region of the channel. The restricted and recurrent nature of the dominant Lys2596 deletion and missense mutations, which substitute residues Glu2094, Gly2539, Asn2576, or Phe2586 in the pore of the channel (Fig. 5), indicates that these residues are critical for *ITPR1* function (all numbering from RefSeq Q14643-2, isoform 2; 2743 amino acids). In contrast to other pathogenic variants, these dominant-negative variants are thought to be less destabilising and thereby permit *ITPR1* protein complex formation but with abrogated function (McEntagart et al. 2016; Natan et al. 2016). Furthermore, as a consequence of the homotetrameric structure of *ITPR1*, these mutations are predicted to generate variant subunits that will incorporate

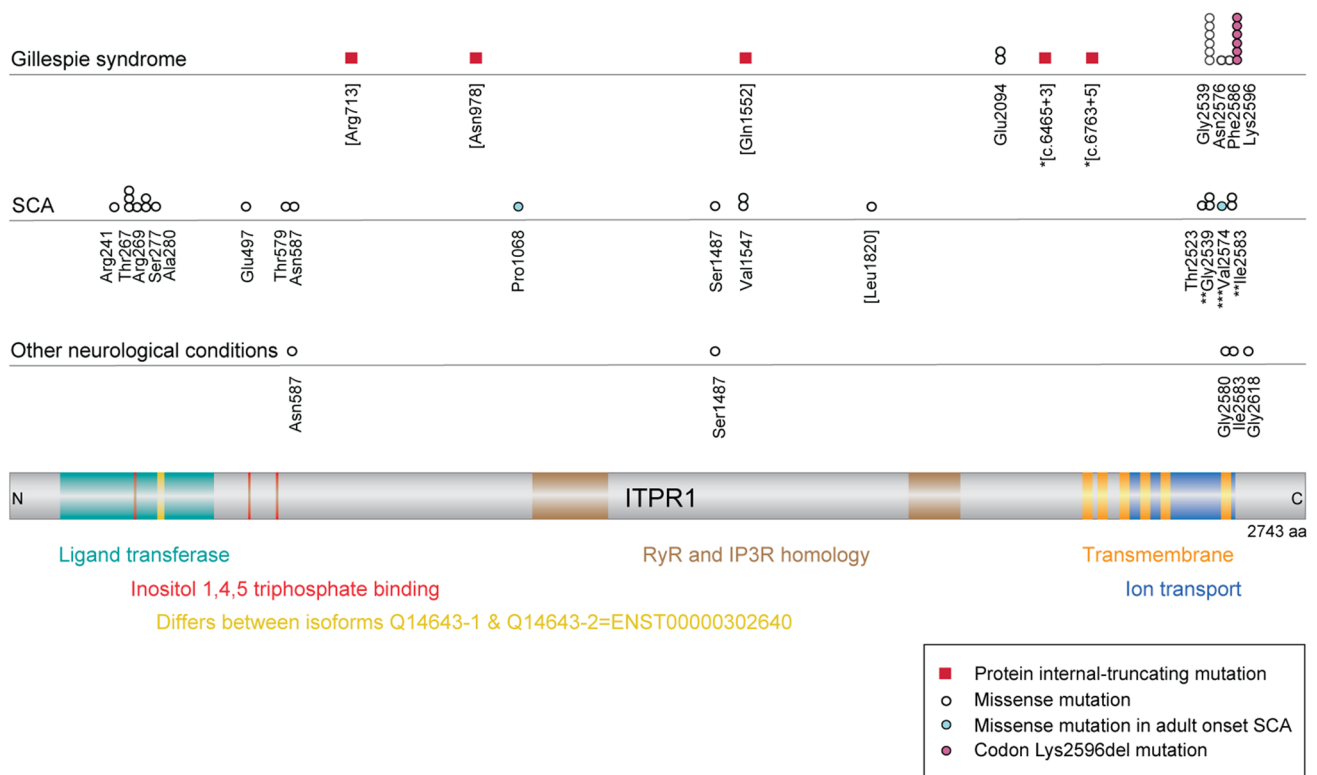


Fig. 5 Spectrum of *ITPR1* pathogenic variants. A linear protein schematic of *ITPR1* is shown in the bottom panel, with domains and features demarcated and labelled in colour. The position of the 15-amino acid insertion in the longer isoform, UniProt Q14643-1, is shown in yellow. Amino acid numbering and domain positions are based on the 2743-amino acid isoform 2: Q14643-2, encoded by the canonical transcript GenBank NM_001168272.1; ENST00000302640. Shown above the protein schematic are all of the published variants associated with Gillespie syndrome and other neurological conditions, and all of the published substitution variants associated with spinocer-

ebellar ataxia (SCA). All variants shown have had their amino acid numbering unified to isoform 2 (to facilitate an accurate collation and comparison of the *ITPR1* dataset) and may therefore differ from the numbering used in the original publication. Variants in brackets indicate recessive alleles, of which *compound heterozygous alleles identified in a single proband; **cases reported as having a SCA29 phenotype and with substitutions at residues associated with Gillespie syndrome (identical variant) or pontocerebellar hypoplasia (different variant); ***variant associated with adult onset SCA in a mother and early onset SCA in her daughter

in to 15/16 protein complexes formed. Decreased calcium release activity was detected in *ITPR1*(Lys2596deletion)-mutant transfected cells; however, the in vivo mechanism(s) underlying the altered function of the mutant protein complex in recessive and dominant GS is not known (Gerber et al. 2016).

Other forms of *ITPR1*-associated disease

Pathogenic mutations in *ITPR1* were first described in two types of spinocerebellar ataxia (SCA), in which iris development is normal. Both of these SCA types are essentially dominant conditions. The late onset, slowly progressive form, SCA15 (OMIM 606658), results from *ITPR1* haploinsufficiency caused mainly by whole gene deletion, but also from stop-gain or rare missense mutations (reviewed by Tada et al. 2016). The congenital/infantile non-progressive form, SCA29 (OMIM 117360), is caused by heterozygous in-frame, mostly missense, mutations that alter

residues predominantly in the cytoplasmic N-terminal portion of *ITPR1* (reviewed by Zambonin et al. 2017). A recessive variant, Leu1820Pro, was recently identified in a SCA29 consanguineous family, where the carrier individuals had asymptomatic cerebellar hypoplasia (Klar et al. 2017). Furthermore, “GS-like” heterozygous variants that substitute residues in the ion-transport channel have been identified in cases with SCA (including reported SCA29 cases with the recurrent GS variant Gly2539Arg) (Valencia et al. 2015; Zambonin et al. 2017; Hsiao et al. 2017), pontocerebellar hypoplasia (Hayashi et al. 2017; van Dijk et al. 2017) or peripheral neuropathy (Gonzaga-Jauregui et al. 2015). De novo substitution variants have also been identified in ataxic cerebral palsy cases with normal neuroimaging (Parolin Schneckenberg et al. 2015) (Fig. 5). These reports demonstrate that recessive and dominant alleles of *ITPR1* are now associated with both GS and SCA, and that additional specific alleles can cause other neurological conditions. The genotype:phenotype pattern is diverse

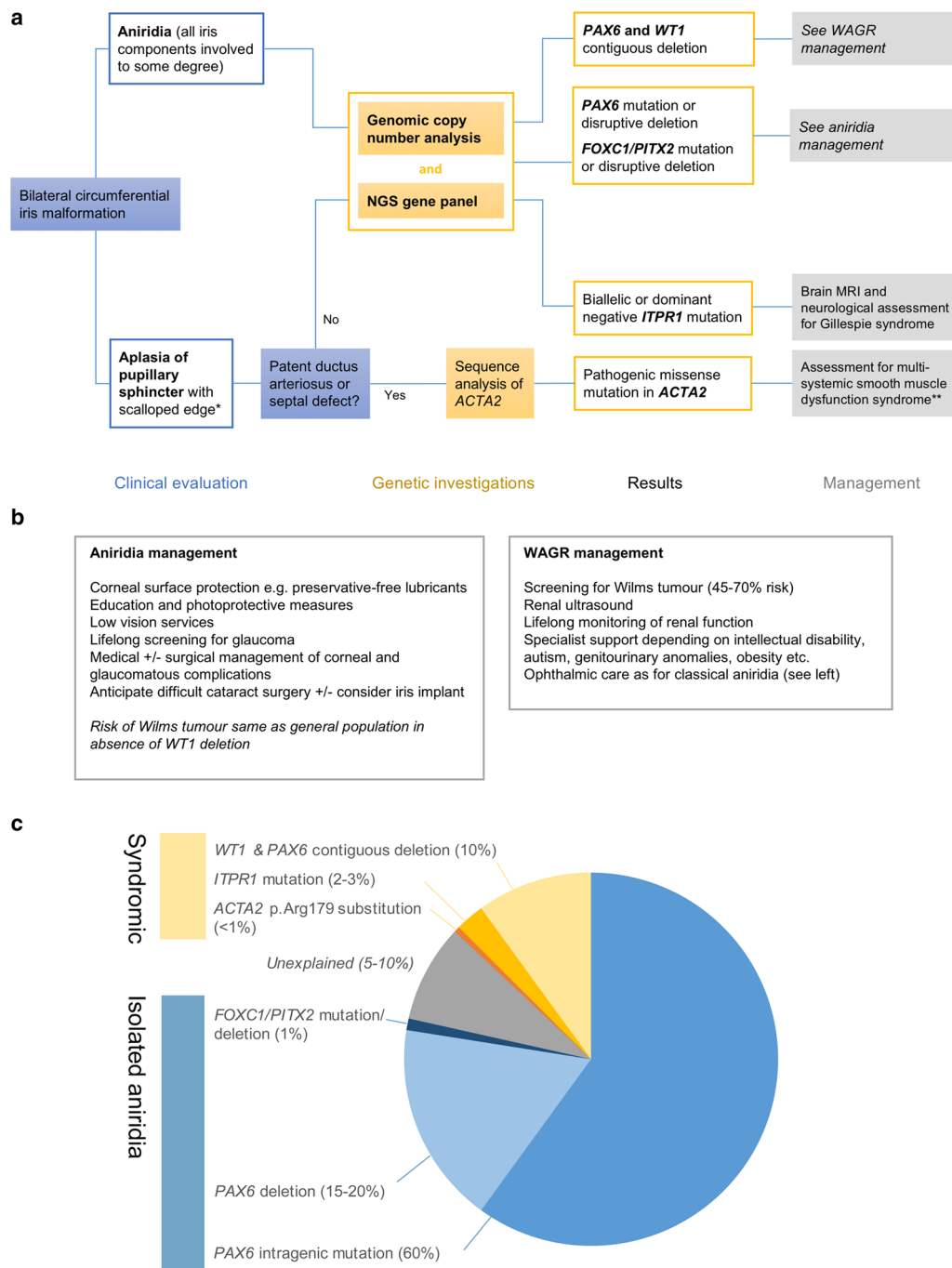


Fig. 6 a Flowchart showing an example approach to the genetic investigation of new congenital aniridia/congenital mydriasis cases. This will usually consist of an aniridia NGS gene panel—in the UK this includes *PAX6*, *FOXC1*, *PITX2* and *ITPR1*—and copy number analysis (e.g., chromosomal array or array-based comparative genomic hybridisation). The latter is important to look for contiguous deletion of *PAX6* and *WT1*. We have suggested the combination of iris and cardiac features which should prompt consideration of *ACTA2* sequencing—this could be tested with other genes such as *ITPR1* depending on clinical judgement. *Gillespie syndrome-like iris (see Fig. 2b, c); **a suggested surveillance regimen for the serious complications of *ACTA2* multisystemic smooth muscle dysfunction

has recently been outlined (Regalado et al. 2018). **b** Brief outline of the management of aniridia and WAGR syndrome. **c** Pie chart showing the genetic causes of isolated and syndromic aniridia. Note that these frequencies are calculated as a percentage of all aniridia cases (using the term broadly and including Gillespie syndrome). For example, whilst *ITPR1* accounts for 2–3% aniridia cases, it is the only known cause of Gillespie syndrome. They are estimated and approximate frequencies derived from published sources (Grønsvov et al. 2001; Crolla and van Heyningen 2002; Robinson et al. 2008; Bobilev et al. 2015; Ansari et al. 2016) or, where no published data is available, estimated from our cohort of > 400 aniridia patients

and indicates that the *ITPR1* protein complex has multiple functional regions that are intricately tissue-specific and that can be aberrantly altered by numerous mechanisms.

Differential diagnosis of Gillespie syndrome

Prior to the identification of *ITPR1* mutations causing GS, two aniridia cases with GS features were reported with *PAX6* mutations: one had a non-coding exon essential-splice mutation and no ataxia or cerebellar abnormalities (Ticho et al. 2006), and the second, with a typical *PAX6* stop-gain mutation, had ataxia but no cerebellar abnormalities (Graziano et al. 2007). In both cases, the iris remnant did not have the scalloped appearance that is characteristic of GS.

A GS-like iris defect is evident in the fixed dilated pupils of individuals with a severe multisystemic smooth muscle dysfunction syndrome (OMIM 613834) caused by Arg179 substitutions in the *ACTA2* gene (actin, alpha 2, smooth muscle, aorta; OMIM 102620; Fig. 2c) (Roulez et al. 2014; reviewed by; Regalado et al. 2018). Cyclic GMP kinase signalling complex studies in tracheal smooth muscle have indicated an interaction between *ITPR1* and alpha-actin (Koller et al. 2003); however, functional analyses of these two proteins in iris tissue are not reported.

Investigations and clinical management

The investigation and management of aniridia cases are multidisciplinary, usually involving genetics, paediatric and ophthalmology services, and even more so in syndromic cases. An example approach to working up new aniridia/congenital mydriasis cases is shown in Fig. 6, with general management recommendations. For the vast majority of aniridia cases, this comprises two tests: an NGS gene panel (that ideally includes *PAX6*, *FOXC1*, *PITX2* and *ITPR1*) and an assessment of copy number such as array CGH. The array is important to detect deletions encompassing *WT1*, which determine the need for continuing Wilms tumour screening (Grønsvov et al. 2001) and monitoring of renal function. In familial aniridia cases, the chance of a *WT1* deletion is very low, with only two WAGR syndrome familial cases reported (Fantes et al. 1992; Robinson et al. 2008). However, the array retains excellent diagnostic value, as the high-resolution assessment of copy number can detect whole gene or regulatory region deletions in *PAX6* (or rarely, *FOXC1/PITX2*) (Ansari et al. 2016; Blanco-Kelly et al. 2017; Franzoni et al. 2017). We therefore have not distinguished between the investigation of sporadic and familial aniridia cases, though clearly in familial cases where the genetic cause is already known, this should be tested directly.

There are two causes of aniridia associated with life-limiting complications. In WAGR syndrome, Wilms

tumour survival rates approach 95% at 4 years, but WAGR carries a late mortality risk from end-stage renal failure (Breslow et al. 2003; Brok et al. 2017). The International WAGR Syndrome Association produce a comprehensive management summary for physicians including screening protocols (<http://wagr.org>). Individuals with *ACTA2* multi-systemic smooth muscle dysfunction are at risk of acute, life-threatening vascular events including aortic dissections and ischaemic strokes in childhood (Roulez et al. 2014); it is important to identify these rare cases early to instigate appropriate surveillance (Regalado et al. 2018).

For patients with a Gillespie syndrome-like iris (Fig. 2b, c), the two known genetic causes are *ITPR1* and *ACTA2* p.Arg179 substitutions. In most cases, the iris defect is detected before the other features of these conditions. However, there is one clinical feature which can aid the decision of whether to test *ACTA2* in addition to *ITPR1*. In the largest series of *ACTA2* p.Arg179 cases (33 individuals), the only feature reliably present at birth in all cases, other than the iris defect, was cardiac: a large patent ductus arteriosus (PDA, 91%) or aortopulmonary septal defect (9%). Based on this and given the serious prognostic implications, it seems reasonable to only test *ACTA2* if both the iris and cardiac features are present.

One of the principal benefits of NGS gene panels for patients with unexplained ocular disease is the number of eye genes that can be tested simultaneously and cost effectively. For example, known *PAX6* target genes, such as *BMP4*, *FOXC1* and *SIX3* discussed above—which are not aniridia genes but cause diverse eye malformations—can be included in such a panel.

If all of the suggested investigations are negative, options for pursuing further investigation include looking for balanced chromosome translocations and whole exome or whole genome sequencing; depending on availability, the latter may be more often pursued in a research rather than diagnostic laboratory setting. In addition to the management outlined in Fig. 6, optical coherence tomography (OCT) is valuable for evaluating foveal hypoplasia, though this is postponed until the child can cooperate. Furthermore, MRI brain may be helpful in classical aniridia if there are relevant neurological symptoms (e.g., olfactory, auditory), as well as in Gillespie syndrome. Lastly, it is also helpful to alert families to the existence of patient organisations, such as Aniridia Network (<http://aniridia.org.uk>), Aniridia Europe (<http://www.aniridia.eu>), Aniridia Foundation International (<http://www.aniridia.net>) and IWSA (<http://wagr.org>).

Knowledge of the specific mutation in aniridia may become important for determining eligibility for future therapies. Ataluren, which targets in-frame nonsense mutations and showed promise in a *Sey* mouse model with a nonsense mutation in the linker region (Gregory-Evans et al. 2014), is in phase 2 clinical trials (STAR trial, ClinicalTrials.gov

ID NCT02647359) (Wang and Gregory-Evans 2015). Ataluren failed to meet the primary endpoint in phase 3 trials for cystic fibrosis (Kerem et al. 2014) and Duchenne muscular dystrophy (McDonald et al. 2017), but showed benefit in a subgroup of the latter. New treatments for the complications of aniridia are also undergoing evaluation. Surgical treatments at the clinical trial stage for aniridia include oral mucosal epithelial transplantation (EU Clinical Trials Register ID 2011-000598-30) and ex vivo expanded corneal limbal stem cell transplantation (UK Clinical Trial Gateway ID, ISRCTN54055321). Both of these aim to treat aniridic keratopathy, the principal cause of progressive visual loss, and as such have the potential to improve quality of life of individuals with aniridia.

Conclusion

In summary, whilst the story of classical aniridia genetics took form nearly three decades ago with the identification of *PAX6*, the cause for Gillespie syndrome has only recently emerged as *ITPR1*. The role of the latter in iris development is not yet clear, and equally many *PAX6*-dependent gene regulatory networks remain poorly understood. Just as *PAX6* has served as a model for dosage-sensitive transcription factors, WAGR syndrome is a paradigm for contiguous deletion syndromes. We have outlined a genetic investigation strategy for aniridia and Gillespie syndrome, including copy number analysis and gene panel testing, and suggest criteria for testing *ACTA2*. Lastly, an estimated 5% of classical aniridia remains unexplained. These cases may be caused by novel mechanisms that disrupt the known genes or by mutations at new loci, which may be elucidated by whole genome sequencing studies.

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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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