Review Article

Clinical and molecular aspects of congenital aniridia – A review of current concepts

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Congenital aniridia is a pan ocular disorder characterized by partial or total loss of iris tissue as the defining feature. Classic aniridia, however, has a spectrum of ocular findings, including foveal hypoplasia, optic nerve hypoplasia, nystagmus, late-onset cataract, glaucoma, and keratopathy. The latter three are reasons for further visual compromise in such patients. This entity is often due to mutations in the *PAX6* (Paired box protein Pax-6) gene. Recently, aniridia-like phenotypes have been reported due to non-*PAX6* mutations as in *PITX2, FOXC1, FOXD3, TRIM44*, and *CYP1B1* as well wherein there is an overlap of aniridia, such as iris defects with congenital glaucoma or anterior segment dysgenesis. In this review, we describe the various clinical features of classic aniridia, the comorbidities and their management, the mutation spectrum of the genes involved, genotype-phenotype correlation of *PAX6* and non-*PAX6* mutations, and the genetic testing plan. The various systemic associations and their implications in screening and genetic testing have been discussed. Finally, the future course of aniridia treatment in the form of drugs (such as ataluren) and targeted gene therapy has been discussed.

Key words: Aniridia, genetics, genotype, mutation, *PAX6*, phenotype

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Congenital aniridia (OMIM 106201) is a rare bilateral pan-ocular genetic disorder that affects different parts of the eye presenting at birth. It was first described by Barrata in 1918.[1] As the name suggests, it is characterized by a partial or complete absence of the iris. However, the disease manifestations extend to almost every part of the eye, including the cornea, anterior chamber angle, lens, fovea, and optic nerve.[2,3] Some of its features are present since birth, such as the foveal hypoplasia and gonio-dysgenesis, while other features such as cataract, glaucoma, and keratopathy develop over time. It has a global prevalence of approximately one in 40,000–100,000 live births.[3-5] In the vast majority of the cases, it is caused by a loss of function mutation in the *PAX6* gene either inherited or acquired sporadically. Through this review article, we summarize the clinical features, the concepts of management of comorbidities, and the molecular and genetic basis of congenital aniridia.

Clinical Features

The ocular manifestations of aniridia involve almost every part of the globe [Fig. 1].

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Visual acuity

Visual acuity is highly variable in patients with aniridia, ranging from 20/60 in patients who do not have nystagmus to 20/100–20/200 in most who have it.[3] The visual dysfunction is not determined by the degree of absence of the iris tissue and rather depends on the secondary ocular features such as foveal hypoplasia, nystagmus, limbal stem cell deficiency, corneal opacity, cataract, and glaucoma. Red-green color vision defect is seen in around 60% of aniridia patients.[6]

Iris abnormalities

The degree of absence of iris tissue can be highly variable, ranging from minimal loss wherein it may resemble isolated iris hypoplasia to the total absence of iris tissue. Even in the complete absence of the iris, a thin stump of iris tissue can be visualized on careful gonioscopy [Fig. 2a]. Histopathological evaluation reveals some iris tissue in most cases.[7] Different family members may have different nature of involvement of the iris owing to variable expression of the disease.[8] The milder forms may just lead to abnormal shape of the pupil or corectopia. The iris abnormality per se does not lead to visual loss but may lead to photophobia, loss of depth of focus, and cosmetic concerns. Small iris tissue defects can be opposed using 10-0 polypropylene sutures, while larger defects need some form of iris prosthesis. Currently, there are three types of iris prosthesis [Fig. 3].[9,10] The capsular tension ring-based (CTR)

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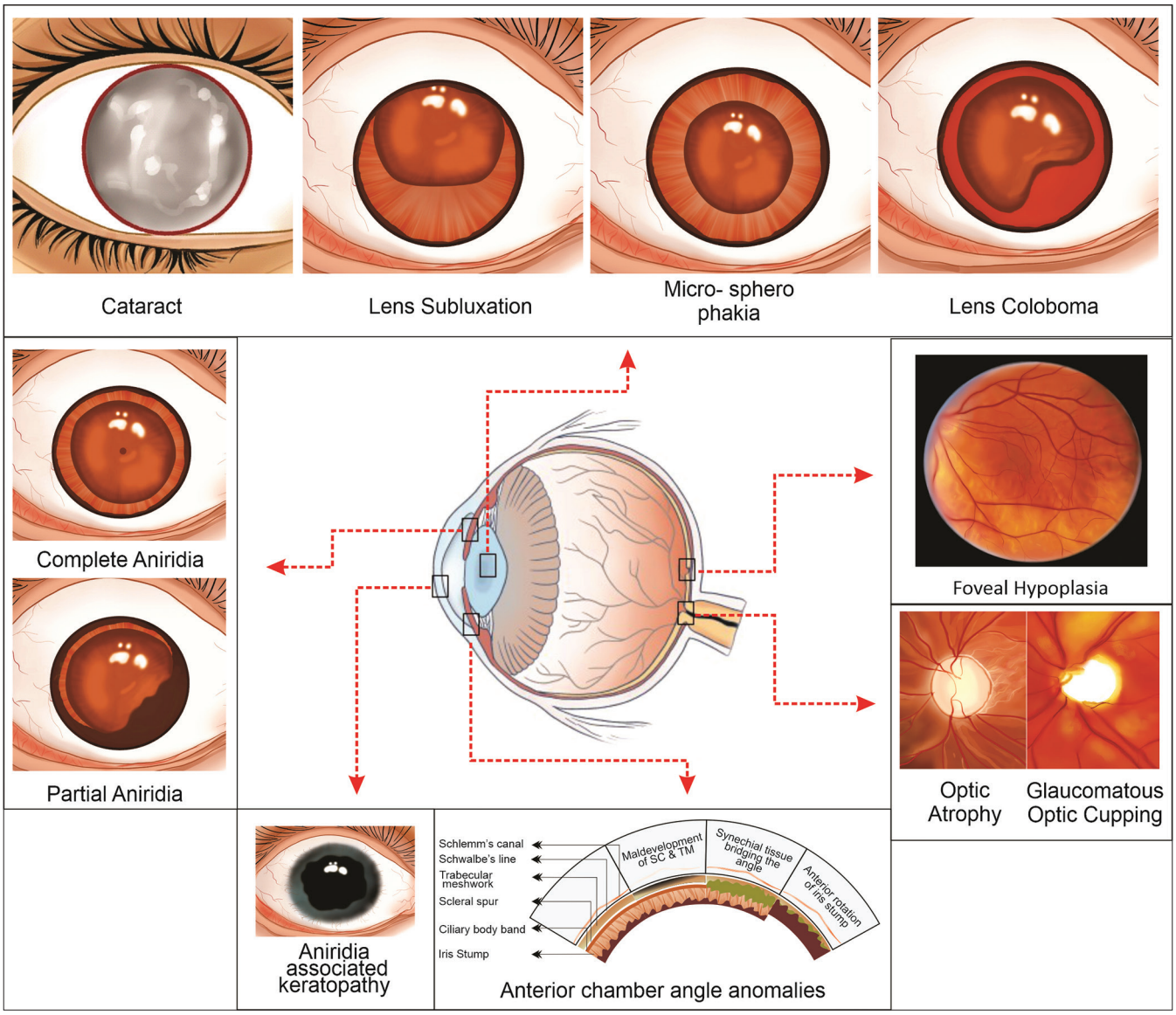

Figure 1: The various ocular manifestations in congenital aniridia

devices (aniridia rings and aniridia segments (Morcher GMBH, Stuttgart, Germany)) can be implanted in the capsular bag during the cataract surgery through the same phacoemulsification wound.[11,12] Two aniridia rings have to be implanted and aligned to create the complete artificial iris [Fig. 4]. A single aniridia segment may be sufficient for a localized area of the defect. A device with two orthogonal segments (Ophtec BV, Groningen, The Netherlands) with a flexible joint is also available and requires a larger incision for implantation.[13] The second type of iris prosthesis is the combined iris–lens implant. This comprises a large 10-mm polymethylmethacrylate (PMMA) diaphragm with a central optical zone and a black periphery. It has curved haptics and a fixation loop for sulcus fixation. The disadvantages of these lenses are that they are rigid, require a large wound for insertion, and cannot be implanted in the bag. Both the combined iris–lens implant and the capsular tension ring-based implants are commonly available in black color and give an artificial appearance. The ring-based implants are also susceptible to fracture and may migrate into the sulcus if the capsular bag is compromised.[12] The third type of iris prosthesis is the hydrophobic silicone elastomer iris diaphragm (Artificiallaris (HumanOptics, Germany)). It was approved by US FDA in 2018 (CustomFlex™artificial iris).[14,15] It can be customized to match the color of the remaining iris or the iris of the other eye, providing excellent cosmesis. It is flexible and can be implanted via a small incision. It can be implanted within the bag or sutured to the sulcus. In aphakic cases, the IOL can be sutured to the back of the prosthesis and the combined device can then be sutured to the sulcus. It may be possible to place the prosthesis in the ciliary sulcus without suture fixation, depending on the amount of iris stump available.[15] The artificial iris diaphragm is recommended to be used in aphakic or pseudophakic eyes. Their use in phakic eyes should be avoided to prevent complications such as cataract and corneal decompensation.[9] All types of prosthetic iris devices may be associated with intraoperative complications such as capsular tear or hyphema and postoperative complications such as new-onset glaucoma, progression of preexisting glaucoma, prolonged inflammation, migration of the implant, and progression of the corneal epithelial disease.[9,10] The use of colored contact lenses to prevent glare has been described in traumatic aniridia;[16] however, their use is limited in

congenital aniridia due to the presence of aniridia-associated keratopathy (AAK). A smart contact lens with artificial iris based on guest–host liquid crystal cells has been studied recently in terms of optical quality in aniridia patients and has been shown to improve vision and reduce retinal illumination.[17] This scleral contact lens entirely spares the limbus and may be a good option for patients who are predisposed to limbal stem cell deficiency.

Retinal abnormalities

Varying degrees of foveal hypoplasia have been reported to be the most common ophthalmological finding following iris hypoplasia (81%–91%).[18-21] Clinically, foveal hypoplasia appears as the absence of the foveal pit and absence of the foveal avascular zone (FAZ) [Fig. 5a and b]. The absence of FAZ may be detected in fundus autofluorescence (FAF) as the absence of the central hypofluorescent spot.[22] Landsend *et al.* (2019) found that the mean intensity of the FAF in macula versus fovea was close to 1 in most aniridia patients.[22] They also reported that 86% of aniridia patients may have a hypopigmented fundus. This can be attributed to the role of *PAX6* in retinal pigment epithelium differentiation.[23] Optical coherence tomography (OCT) can reveal thickening of the central macula and absence of foveal contour [Fig. 5c and d]. The degree of foveal hypoplasia as determined by the structural grading on OCT has been shown to be a predictor of visual acuity in children.[24,25] Fig. 6 shows the Leicester Grading System for Foveal Hypoplasia.[24] Specifically, the presence of outer segment (OS) lengthening correlates with a better best-corrected visual acuity in congenital aniridia.[25] The pattern of foveal differentiation in aniridia leads to an overall increase in central macular thickness, while the outer segment, outer nuclear layer, and Henle's layer are thinner.[25] The subfoveal and parafoveal choroidal thickness has also been found to be reduced in aniridia as compared to control eyes.[26] Recently, OCT angiography in a 7-year-old child revealed the absence of FAZ and dipping of vessels from the superficial vascular complex to the deep vascular complex.[27] Further studies may reveal the importance of retinal microvascular development in the pathogenesis of aniridia. Both multifocal and full-field electroretinography (ERG) have been shown to be affected in the majority of aniridia patients.[28] The amplitudes of the three inner rings are reduced while those of the two outer rings are increased in multifocal ERG. The higher amplitude in outer

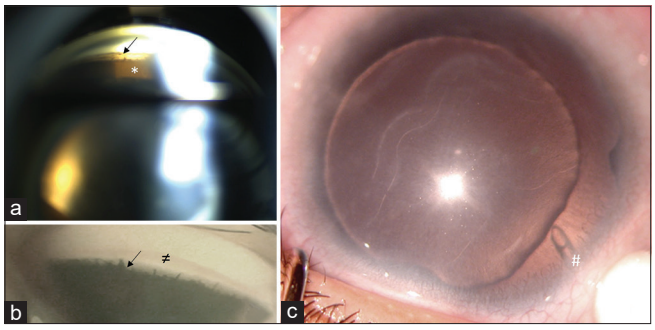

Figure 2: (a) shows the gonioscopic view of anterior chamber angle in a patient with total aniridia. Few goniosynechiae (arrow) and the stump of iris tissue can be seen (*). (b) shows the gonioscopic view of the anterior chamber angle showing extensive goniosynechia. Schlemm's canal (≠) can be seen. (c) shows the anterior segment of a child with aniridia with congenital-onset glaucoma. Buphthalmos and Haab's striae can be seen. The tube of glaucoma drainage device can be noted (#)

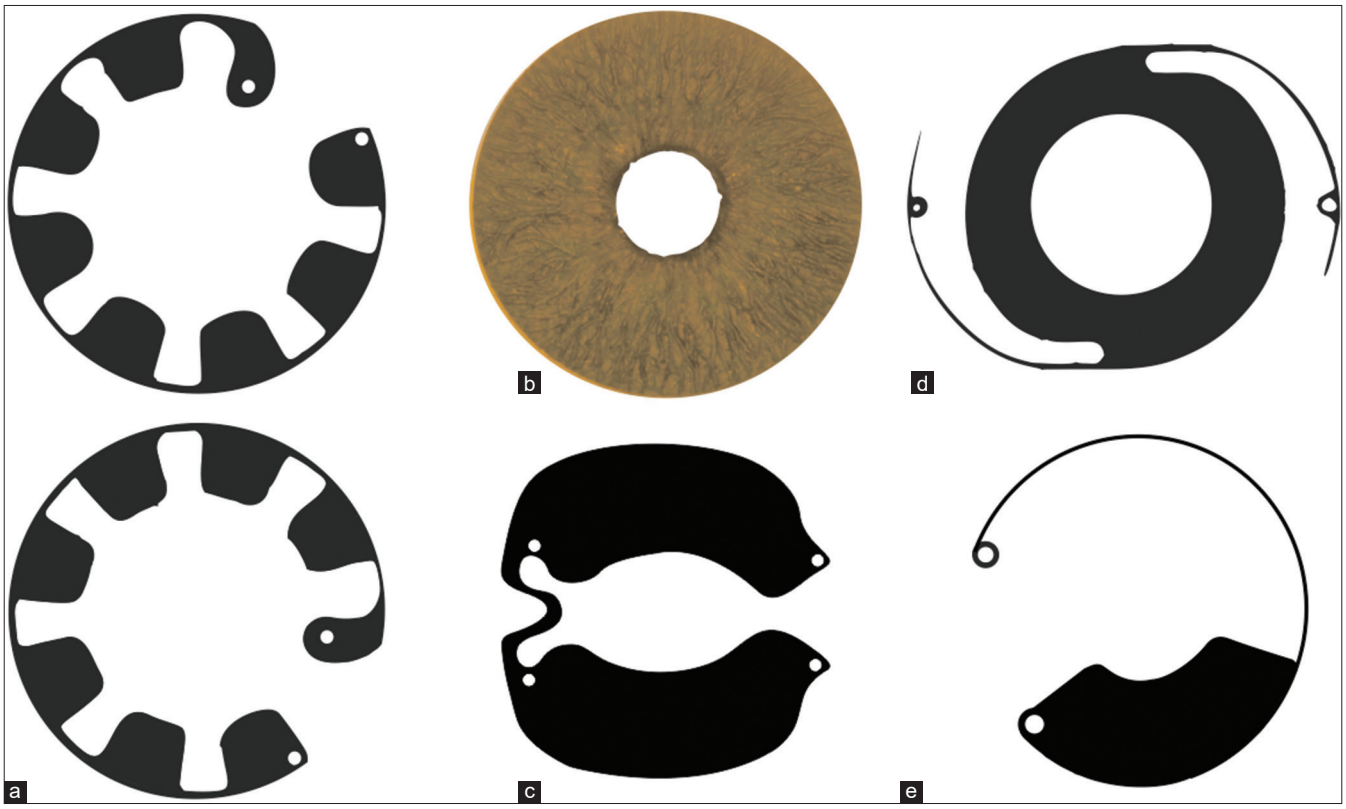

Figure 3: The various types of iris prosthetic devices. (a) shows the dual capsular tension ring implant. (b) shows the foldable silicone elastomer iris diaphragm. (c) shows the orthogonal segment device with a hinge joint. (d) shows the aniridia non-foldable intraocular lens implant. (e) shows the iris segment capsular tension ring implant

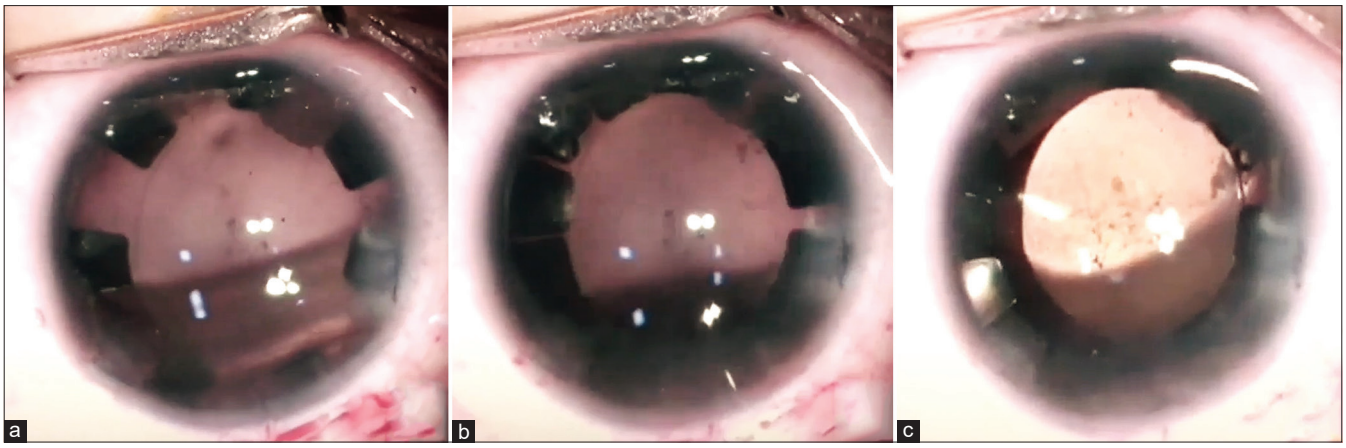

Figure 4: Intraoperative photographs of cataract surgery in aniridia with implantation of dual aniridia rings. (a) is after the implantation of the first ring in the bag, and (b) is after the implantation of both the rings. The interdigitating fans are aligned to create the pseudo iris. (c) shows the status after implantation of intraocular lens

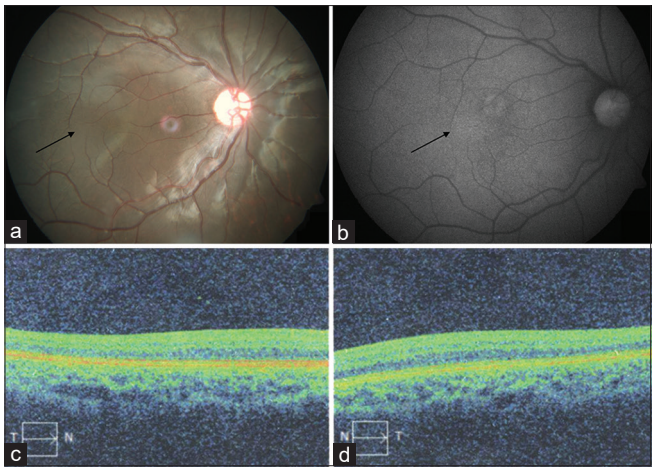

Figure 5: Foveal hypoplasia in aniridia. (a) is the fundus photograph showing loss of foveal pit and absence of foveal avascular zone (arrow). (b) is the red-free image of the same patient showing the absence of the dark spot of the fovea. (c and d) are respectively the optical coherence tomography of the right and left eye of the patient with aniridia showing grade 4 foveal hypoplasia

rings correlates with poorer visual acuity. In the full-field ERG, the amplitude remains unaffected, but the latency of scotopic and photopic b wave, scotopic a wave, and 30-Hz flicker have been found to be prolonged as compared to controls.

Corneal abnormalities

Corneal changes in aniridia are collectively defined under the term AAK. It is characterized by the gradual development of corneal pannus, limbal stem cell deficiency, and progressive opacification of the cornea from the periphery to the center. AAK is age-dependent and can occur as early as 2 years of age, and is probably the second most common clinical feature following foveal hypoplasia with a prevalence reaching up to 90%.[19,21,29-31] The affected individuals experience chronic irritation and photophobia apart from the visual loss due to AAK. Occasionally, central corneal opacities, which unlike AAK are present since birth, may be seen in children with aniridia.[31]

AAK is secondary to a breakdown of the limbal stem cell niche, impaired wound healing, and neural deterioration.[32] The clear central cornea is replaced with the mixed conjunctivo-corneal phenotype in the early grades of AAK and the conjunctival phenotype in the later grades. This manifests clinically as an invading pannus over the cornea. The mean subbasal corneal nerve fiber density is reduced, especially in the PAX6 aniridia, with an increased rate of nerve fiber degeneration. This leads to a loss in corneal sensations and compromised healing. The nerve fiber degeneration results in inflammation. The pro-inflammatory corneal surface is also enhanced by the presence of elevated inflammatory cytokines in the tear film.[32]

The clinical grading of AAK is mainly based on the extent of the corneal opacity and degree of vascularization [Table 1, Fig. 7].[33] The AAK is more severe in *PAX6* mutations compared to non-*PAX6* mutations.[18,34] The central corneal thickness (CCT) is also increased, especially in eyes with significant pannus.[32] However, CCT may be raised to the range of 600–700 microns even in the early grades of AAK despite a clear cornea and normal to increased endothelial cell density.

The management of AAK is determined by its severity. In the early stages, supportive treatment includes preservative-free lubricants. In a study, autologous serum has been shown to stabilize the tear film and reduce corneal epithelial metaplasia.[35] When the keratopathy affects the visual axis, penetrating keratoplasty has been performed but with unsatisfactory outcomes owing to the underlying limbal stem cell deficiency. Recurrence rates from 64% to 85% have been reported.[36,37] Thus, attempts to restore or improve the limbal stem cell niche have been attempted. The various techniques include amniotic membrane grafting,[38] limbal allografting,[39] keratolimbal allografting,[40] cultivated limbal epithelial transplantation,[41] cultivated oral mucosa epithelial transplantation,[42] and a combination of limbal allograft and amniotic membrane transplantation.[43] Most techniques result in stabilizing the ocular surface and halting the progression of the AAK to some extent. Keratoplasty when performed after limbal stem cell augmentation may have a better outcome, especially when combined with systemic immunosuppression.[40] As the disease is progressive, most surgical procedures fail in due course of time. Keratoprosthesis is an option for end-stage AAK with limited success.[44-46] However, various complications such as failure of the prosthesis, formation of a retroprosthetic membrane, glaucoma, and choroidal detachment have been reported.[44-46] Patients operated for AAK need very long-term monitoring due to the progressive nature of the disease.

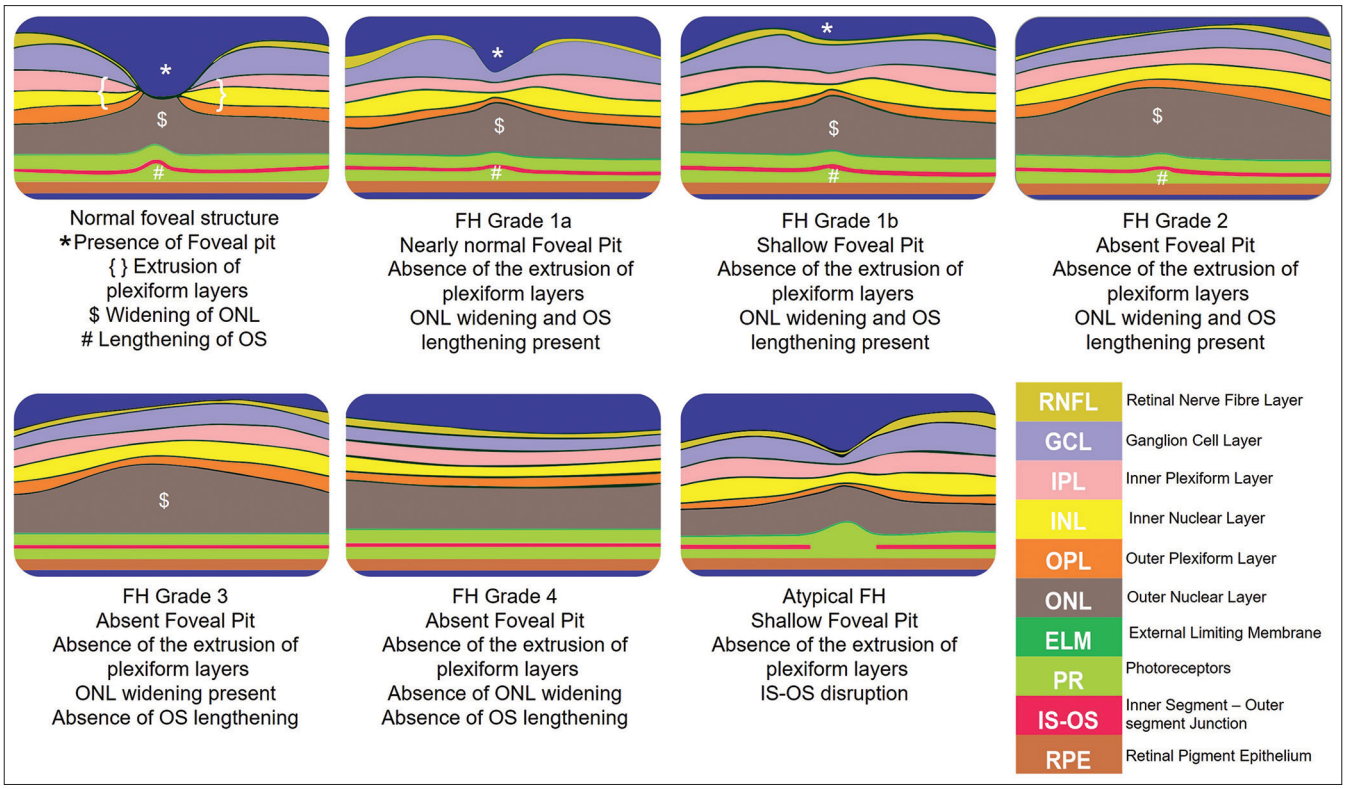

Figure 6: The grades of foveal hypoplasia based on optical coherence tomography of the macula

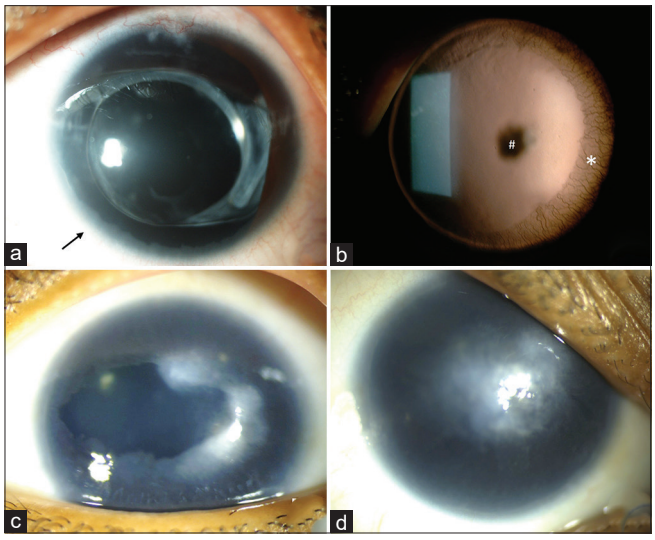

Figure 7: Clinical photographs of various grades of aniridia-associated keratopathy (AAK). (a) shows grade 1 AAK wherein the limbus is compromised with the invasion of conjunctival pannus (arrow). (b) is a retro illumination photograph highlighting the pannus (*). It also shows a posterior polar lenticular opacity (#). (c) shows grade 2 AAK wherein the conjunctival pannus extends centrally but spares the visual axis. (d) shows grade 3 AAK wherein the corneal scarring and conjunctival invasion extends to the central part of the cornea. Note the dull sheen of the corneal surface

Table 1: Clinical grading of aniridia-associated keratopathy

	Description
Grade 0	Intact limbal border without conjunctival tissue or vessels crossing the limbus
Grade 1	Limbal border is compromised, with vessels and conjunctival tissue crossing the border but remaining within approximately one mm from the limbus. Invasion can be localized to one region of the limbus, with other areas of the limbus remaining intact.
Grade 2	Conjunctival tissue with vessels invades the peripheral and mid-peripheral cornea, but at least the central 2-3 mm of the cornea is spared and remains transparent.
Grade 3	Conjunctival tissue invades the central cornea, affecting the central visual axis, and typically the entire corneal surface is covered by a translucent, vascularized pannus.
Grade 4	The ocular surface is opaque and vascularized with a thick pannus, typically of irregular thickness.

Lenticular abnormalities

Lens abnormalities are frequently associated with aniridia, and cataract is the most common association seen. They are reported to be seen in 40%–82% of aniridia patients, and the common morphological types are posterior polar, posterior subcapsular, and total cataract.[19,21,29,30] Other associated lens abnormalities that have been reported include lens subluxation, microspherophakia, lens coloboma, and posterior lenticonus. Cataracts may develop at any age and are usually progressive. Accelerated cataractogenesis is known to occur in the second decade of life, and the median age of presentation is 14 years.[19,47] The sequence of typical lenticular changes in aniridia is illustrated in Fig. 8 a–d. Often, vascularization from the remnant iris tissue is seen on the surface of the cataractous lens. Other morphological types of cataract that are reported frequently include nuclear cataract, mature cataract with

subcapsular edema, and hypermature dehydrated cataract. Anterior polar opacity has been reported least frequently.[47] Common types of lenticular abnormalities in aniridia are depicted in Fig. 8 e–h.

Structurally, the anterior capsule of the cataractous lens is very fragile due to either absence or reduction in basement membrane constituents of the lens capsule, which adds to the challenges during surgery.[48,49] Aniridia associated with cataract results from heterozygous mutation of the PAX6 gene in 90% of cases. Over 60 identified mutations throughout the length of PAX6 genes are associated with aniridia and cataract.[50]

Challenges while doing cataract surgery in aniridia include poor ocular surface, corneal opacification, friable capsule, and reconstruction of the iris and pupil. If corneal transparency is not sufficient for a safe cataract surgery, limbal stem cell transplantation is the best initial approach. Capsulorhexis should be performed with utmost care using modern techniques such as dispersive viscoelastic, capsular staining, and aiming for a smaller capsular opening diameter. Some surgeons prefer a larger capsular opening due to the profibrotic nature of congenital aniridia along with the implantation of iris prostheses to prevent rhexis contraction.[51] The use of CTR has considerably reduced the chances of postoperative lens dislocation, posterior capsular/visual axis opacification, and anterior capsule fibrosis. A posterior capsulorhexis with anterior vitrectomy may be considered in young children in view of the high incidence of visual axis opacification and difficulty in doing YAG laser capsulotomy later due to the associated nystagmus. The other issue in conjunction with cataract surgery is the reconstruction of an optic aperture. This can be achieved using various artificial iris implants as mentioned above.

Complications following cataract surgery in aniridia include postoperative uveitis[19] and development of glaucoma[19,52] in 42%–50% of cases. The complications can lead to endothelial cell loss, progression of corneal epithelial disorders, and persistent damage to limbal stem cells, leading to worsening of keratopathy.[52,53] Contraction of capsular bag may lead to the displacement of iris implants, which in turn can contribute to persistent inflammation and increase in IOP.[53] Incidence of cystoid macular edema is about 18% in these eyes following cataract surgery.[52]

Glaucoma in association with aniridia

Glaucoma in aniridia occurs in 20%–70% of cases.[21,29,32,47,54] Patients generally develop glaucoma in late childhood or early adulthood due to progressive anatomical changes in the drainage angle.[55] The prevalence of glaucoma below 10 years of age is around 15%. Congenital glaucoma with or without buphthalmos is rarely seen [Fig. 2c].[55] Several mechanisms have been described in the development of glaucoma such as maldevelopment of the anterior chamber angle [Fig. 2b], absent Schlemm's canal, and obscuration of the angle with a shapeless, homogenous avascular tissue.[2,55,56] Usually, the angle remains open in infancy. Progressive changes result in the anterior rotation of the rudimentary iris stump and occlusion of the trabecular meshwork (TM). In advanced glaucoma, the entire TM may be covered by the iris.

Glaucoma in patients with aniridia is often resistant to conventional medical and surgical treatment. The worst visual outcomes are associated with familial aniridia, higher IOP at baseline, and in patients undergoing multiple ocular surgeries.[57] Medical therapy remains the first line of treatment. Topical beta-blockers, carbonic anhydrase inhibitors, and prostaglandin analogs can be used. Some eyes may respond to pilocarpine in early glaucoma as it increases the aqueous outflow through unobstructed trabecular meshwork by contracting the ciliary muscle.[2,55] However, most patients eventually require surgical therapy, and all surgical modalities have been attempted. In a study by Sihota *et al.* (2021),[58] medical management was effective in 34.28% of eyes and 65.72% required surgery. Prophylactic goniotomy has been found to be effective in preventing glaucoma in aniridia by Walton *et al.* (1986).[59] A modified goniosurgical technique involving treatment of approximately 200 degrees of the angle to avoid the development of glaucoma with 89% success has also been reported.[60] The technique involves detaching the abnormal tissue between the iris and the TM without incising the TM with no reported surgical complications. However, therapeutic goniotomy seems to be of limited value in advanced cases.[2,56,59] The outcomes of trabeculotomy in aniridic glaucoma are conflicting. A study by Adachi *et al.* (1997)[61] reported a higher success rate with a qualified success of 83% with one or two trabeculotomy procedures over a mean follow-up period of 9.5 years in early glaucoma and children with open angles on gonioscopy in the first year of life. However, others reported trabeculotomy failure.[56] Trabeculectomy has also shown inconsistent results. Some propose trabeculectomy with mitomyin C (MMC) as a reasonable option,[2] while some reports showed poor outcomes.[56,61] Sihota *et al.* (2021)[58] proposed the use of MMC both under the scleral flap and subconjunctivally and the use of releasable sutures to prevent postoperative complications to achieve better results. The lower success in aniridic glaucoma may be related to the limbal pannus and poor ocular surface.[20] The limbus-based conjunctival flap can be preferred in these eyes to prevent injury to the remaining limbal stem cells.[62]

Glaucoma drainage devices (GDDs) have been found to be effective for treating glaucoma associated with aniridia.[2,56] Wiggins and Tomey (1992) reported a success rate of 83% in five out of six eyes that received Molteno implants after multiple intraocular surgeries.[56] A study comparing trabeculectomy with Aurolab aqueous drainage devices in aniridic glaucoma showed that the latter achieved greater IOP reduction at 6 months follow-up, but the success was not sustained at 4 years follow-up.[62] Complications such as flat anterior chamber, hypotony, and tube lens touch can be associated with the implantation of GDDs.[2,20] Cyclodestructive procedures may be performed in refractory cases with some success.[2,63,64] High rates of complications in aniridic eyes have been reported, especially after cyclocryotherapy.[63] However, cyclophotocoagulation with diode laser was found to be effective and safe in children with refractory pediatric glaucoma, including aniridia.[64]

Optic Nerve abnormalities

Optic nerve hypoplasia is seen in 2%–30% of cases in various studies.[18,21,22,65] Some studies have found its association with worse grades of foveal hypoplasia, suggesting that one may influence the development of another.[22] Other optic nerve anomalies apart from glaucomatous optic atrophy are optic nerve aplasia, optic disc pallor, optic disc pit, and morning glory disc.[21,66,67]

Other abnormalities

Nystagmus often accompanies the retinal pathology and is seen in 55%–73% of cases.[21,29,30] Whenever present, it contributes toward visual morbidity. The most common type of refractive error noted is myopia.[30] A study where the authors followed up children with aniridia for 3 years noted a myopic shift in 67%, a

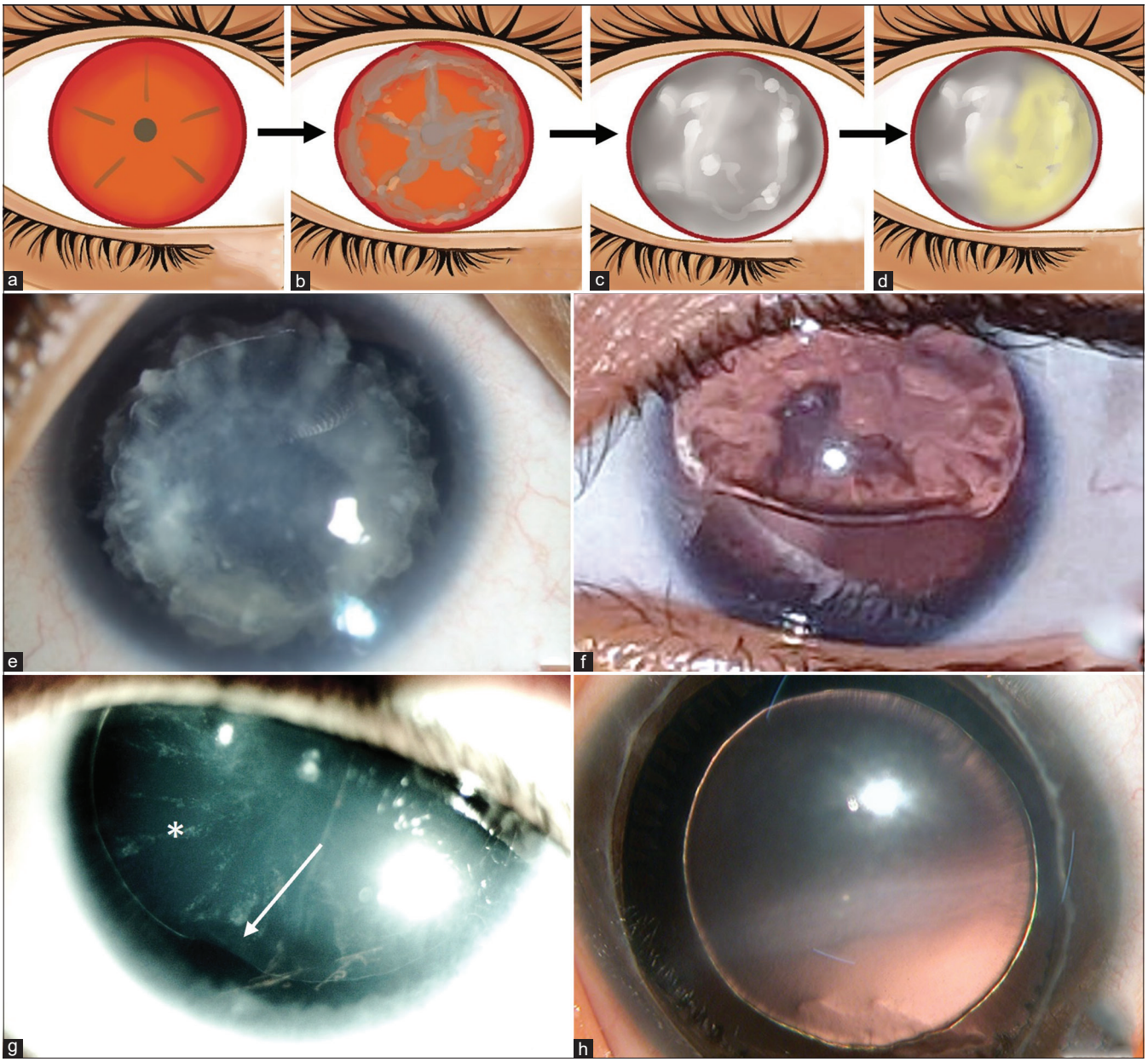

Figure 8: The lenticular abnormalities in aniridia. (a-d) show the typical sequence of cataractogenesis in aniridia starting from posterior polar cataract with radial spokes in the posterior subcapsular area (a), extension of opacities to form a midperipheral ring (b), total cataract (c), and hypermature cataract (d). Figure e shows aniridia and near-total cataract. (f) shows a superiorly subluxated lens with straightening of the inferior border. (g) shows inferior lens coloboma (arrow) and radial lenticular opacities (*). (e) shows a clear microspherophakic lens

hyperopic shift in 33%, and an overall increase in astigmatism in 70%.[29] Other rare clinical manifestations include meibomian gland dysfunction and blepharoptosis.[21,68]

Systemic Associations

Multisystemic manifestations in aniridia are well-known [Fig. 9].[69] Among the sensory developmental defects, anosmia or hyposmia have been observed in aniridic individuals showing *PAX6* haploinsufficiency.[70] There has also been a study indicating hearing and sound perception deficit accompanying *PAX6*-related visual defects.[71] Neurological abnormalities such as autism and intellectual disability have been described in several case reports; however, development delay is rare.[69,72] An association of *PAX6* mutations with the absence of the pineal gland and unilateral polymicrogyria contributing to the pathogenesis of neurodevelopmental disorders such as epilepsy and learning disability has also been studied in animal models.[72] Another unique case highlighting an association between aniridia and bone malformations in the form of absence of the patella was also reported in 1975.[73]

Among the syndromic associations, the most important association of classical aniridia is known to be WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation; OMIM 194072).[74] Around 30% of individuals suffering from sporadic aniridia are likely to develop syndromic features of WAGR.[75] Up to 70% of cases of WAGR syndrome carry a risk of developing a pediatric nephroblastoma called Wilms tumor by 4–7 years of age.[75]

These tumors tend to be bilateral, have a predilection for the male sex, and occur at a younger age when they occur with aniridia. Any child with sporadic aniridia needs to be screened with serial renal ultrasound till 5–7 years of age. Obesity in patients with WAGR and non-WAGR related aniridia has also been reported in some studies.[75]

The other common syndrome associated with aniridia is called Gillespie syndrome (OMIM 206700). It is characterized by a triad of partial aniridia, non-progressive cerebellar ataxia, and intellectual disability.[76] This syndrome presents with partial aniridia due to aplasia of the iris sphincter and a scalloped margin of the iris remnant. Related genetic mutations have recently been identified in the *ITPR1* gene, an inosine triphosphate receptor with calcium channel activity. Truncation mutations in the heterozygous cell system of the *ITPR1* gene showed irregular function which encompasses the IP3-binding domain and varying lengths of the modulatory domain, thus causing the Gillespie syndrome phenotype.[77] Other rare syndromic associations are Aicardi–Goutières syndrome, Duane syndrome, and megalocornea, which have been reported through isolated reports in the literature.[78-80]

Molecular Basis of Aniridia

Aniridia, as an isolated malformation, is an autosomal dominant disorder occurring in the majority due to deleterious mutations in the *PAX6* gene (~90% of the cases).[81] Approximately 60%–70% of cases are familial, and 30%–40% of cases are sporadic.[72] Large inter-family and intra-family variability is observed for the disorder. Mutations in other genes such as *FOXC1*, *PITX2*, *CYP1B1*, *FOXD3*, and *TRIM44* have been found to be linked with aniridia.[18,82,83] While mutations in four genes—*FOXC1*, *PITX2*, *CYP1B1*, and *FOXD3*—may lead to both partial and complete aniridia phenotypes, mutations in the *TRIM44* gene have been shown to be associated with total aniridia.[18] Features of WAGR syndrome occur due to a contiguous gene deletion on the short arm of chromosome 11 (11p13 region) encompassing the *PAX6* and the adjacent *WT1* gene,[75] a transcription factor. Its mutations have been associated with the increased risk of developing Wilms tumor in children and end-stage kidney disease in adults.[74] WAGR syndrome phenotype can be explained by the two "hit" inactivation of *PAX6* and *WT1* in the somatic cells of the kidneys. Complete absence or loss of functional *WT1* protein causes tumorigenesis. However, the likelihood of developing Wilms tumor reduces significantly in a patient >8 years of age.[75]

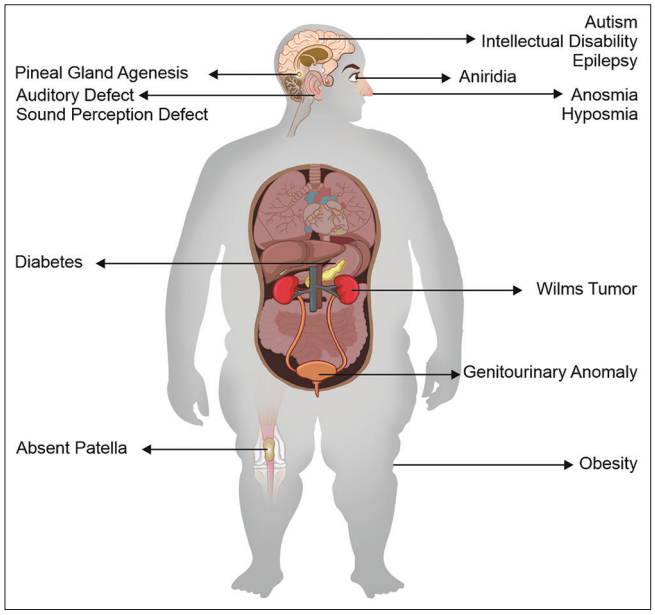

Figure 9: The various systemic abnormalities associated with aniridia. Multisystemic manifestations co-occurring with aniridia, such as Wilms tumor, aniridia, genitourinary anomalies, and mental retardation (WAGR) syndrome features, neurological abnormalities, and obesity are depicted. Rarer phenotypes such as anosmia, auditory defects, diabetes, and absence of patella have also been indicated

Role of PAX6 in aniridia

The *PAX6* gene, containing 14 exons, was the first homeobox gene to be correlated with the pathogenesis of congenital aniridia.[3] It is expressed in the forebrain, gut, early developed eye structures, ventral spinal cord, and endocrine pancreas.[84] It is a crucial transcription factor involved in neurogenesis and oculogenesis.[5] *PAX6* coding and non-coding regions are found to be highly conserved across different species as is identified for mice and *Fugu* fish by sequence analysis.[85] *PAX6* contains three major domains for DNA binding, namely a paired domain, a homeodomain, and a C-terminal transcriptional activation domain [Fig. 10].[86] During development, *PAX6* interacts with other transcription factors and development regulators such as *PAX2* and *SOX2* to produce structural proteins such as crystallins.[87]

Mutations in PAX6 linked with aniridia

Mutations in the *PAX6* gene are recorded in an online database "The Human *PAX6* Mutation Database" (http://pax6.hgu.mrc.ac.uk/). This database was last updated in June 2017 and harbors 491 unique mutations from 1067 patients. Mutations in the *PAX6* gene are highly penetrant. While two-thirds of cases with aniridia are due to loss of function mutations in one copy of *PAX6*, one-third of cases occur due to chromosomal rearrangements.[3] Mutations in the *PAX6* gene may fall in one of the following categories: splice site mutations, frameshift mutations, nonsense mutations, insertions and deletions, and introducing premature termination codons (PTC) resulting in haploinsufficiency.[5,30,75] This phenomenon in aniridia phenotype can be attributed to degraded abnormal mRNA being produced by mutated codons as opposed to prematurely truncated short proteins by a process called nonsense-mediated mRNA decay.[74]

C-terminal extension (CTE) mutations in *PAX6* cause the reading frame to continue protein-coding into the 3′ untranslated region and leads to gain-of-function and severe aniridic phenotype.[3]

Compound heterozygous or homozygous mutations are largely considered inviable to the fetus.[75] Chromosomal aberrations such as deletions, translocations, and inversions encompassing complete or part of the *PAX6* gene can lead to isolated aniridia features.[88] When conserved transcriptional regulatory elements located in the intronic region adjacent to the *PAX6* gene are also included in the chromosome irregularity, classical aniridia can be expected.[3]

While aniridic *PAX6* mutations are mostly due to the formation of truncating protein, missense mutations in *PAX6* are also known to cause non-aniridia phenotypes such as microphthalmia, microcornea, foveal hypoplasia, ocular coloboma, keratitis, congenital cataract, Gillespie syndrome, Peters anomaly, and morning glory disc anomaly.[75-78]

Genotype-phenotype correlations

Although a definitive genotype-phenotype correlation is not established in *PAX6* mutations, several authors have suggested specific correlations in their studied cohorts. Hingorani *et al.* (2009)

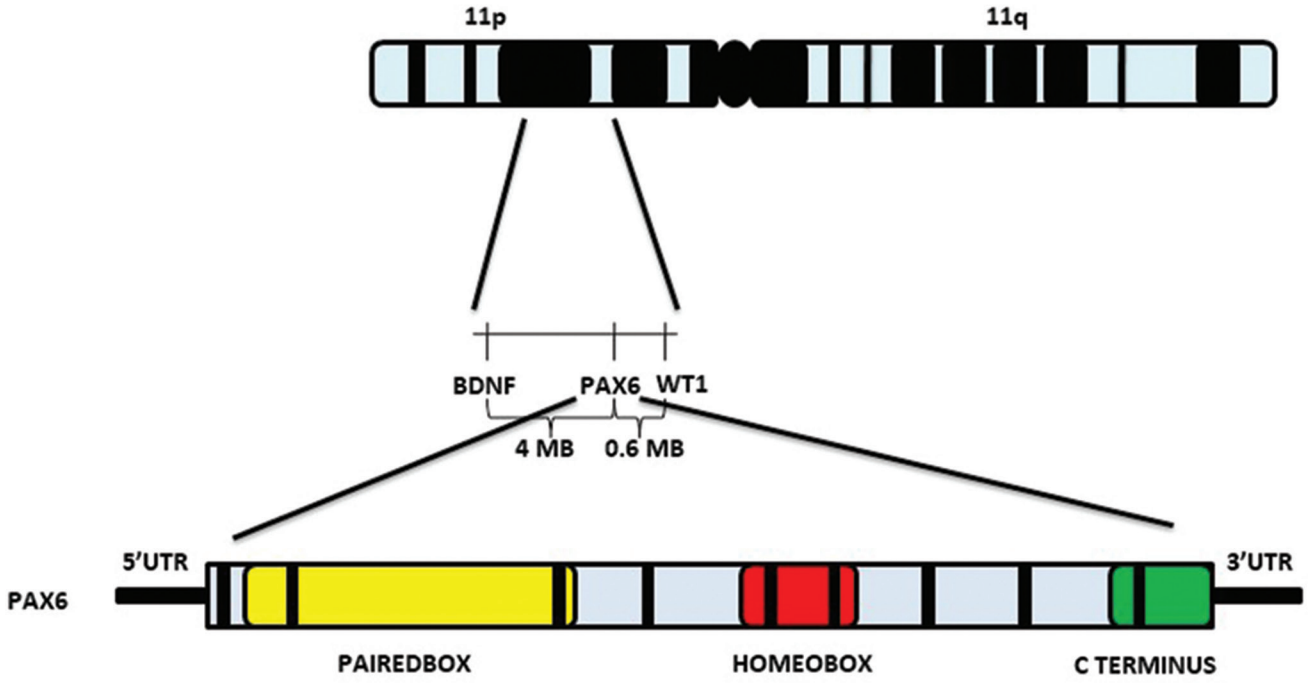

Figure 10: PAX6 gene on chromosome region 11p3. Adjacent genes WT1 and BDNF causing WAGR syndrome and obesity phenotype respectively have also been denoted. The PAX6 gene has 14 exons and contains three major domains for DNA binding, namely a paired domain, a homeodomain, and a C-terminal transcriptional activation domain

[84] analyzed a cohort of 43 *PAX6* mutation-positive patients for phenotypic correlation. The results divided the findings into three main categories: amino acid substitution mutations, loss of protein expression mutations, and CTE mutations. The overall inference indicated that milder iris abnormalities and defects were noted in amino acid substitution mutations, whereas severely affected individuals were either a part of CTE mutation or a loss of protein expression mutations [Table 2].

Another genotype-phenotype correlation analysis for *PAX6* mutations was performed by Yokoi *et al.* (2016)[88] by examining five families with multiple affected members. They reported partial differences in the phenotype of family members harboring the same mutation. This phenotypic heterogeneity was observed for corneal opacity, glaucoma, foveal hypoplasia, and cataracts. This can be attributed to the epigenetic interaction of *PAX6* with other downstream genes. However, no heterogeneity was observed between eyes of the same patient irrespective of maternal or paternal inheritance of mutated allele, signifying similar *PAX6* regulation in both eyes.[88]

In a recent study, Vasilyeva *et al.* (2021)[89] analyzed the phenotypic correlation of variable genotypes in 155 aniridia patients linked with *PAX6* alterations. Their study revealed that patients with 3′-cis-regulatory region deletions had a milder aniridia phenotype without foveal hypoplasia, nystagmus, or keratopathy. This can be attributed to residual promoter function and partial production of *PAX6* protein by the mutant allele as no complex genomic rearrangements were observed in this regulatory deletion.[89]

Case reports and cumulative review articles studying the Indian population have mostly been published from South India and shed light on common mutations and their phenotypes in this population group. A study by Neethirajan *et al.* (2004)[90] examined the genetic mutations in the *PAX6* gene in 28 members with aniridia from six families against controls from the general population. Three novel point mutations in the coding region of *PAX6* were identified. Their correlation to patient phenotype was similar to previous reports from other parts of the world suggesting loss of function mutations causing classic aniridia phenotypes.[90] Specific phenotypic variability with mutations was noted. For example, a proband with c. 715ins5 mutation had sclerocornea with nystagmus. Foveal hypoplasia was observed in aniridic patients with c. 482C>A and c.1201delA mutations. A case with c. 901delA revealed ptosis, microcornea with dislocated cataractous lens, while another with IVS9-12C>T showed peripheral corneal pannus with bullous keratopathy and corneal ectasia.[90]

A comparative analysis of mutations and observations has been reported by Neethirajan *et al.* (2009)[91] in the Indian population [Table 2]. Similar findings were identified across different ethnic groups. However, a high prevalence of frameshift mutations was noted due to frequent consanguineous marriages in India among different religious groups.[88] The authors also observed intra-familial variability for severity of aniridia among members carrying the same mutation and hypothesized the role of upstream and downstream genes from *PAX6* and their pleiotropic effect.[91]

Role of other genes in aniridia

Non-partial as well as complete aniridia has also been reported due to mutations in *FOXC1*, *PITX2*, *CYP1B1*, *FOXD3*, and *TRIM44* genes [Table 3].[92-94]

Molecular diagnosis of aniridia

Molecular diagnosis of aniridia becomes of vital importance for children <8 years of age to rule out the possibility of occurrence of the lethal condition, "Wilms tumor."[95] Even in the absence of genetic testing, all children diagnosed with aniridia should

Table 2: Genotype-phenotype correlation between different mutations in *PAX6* with disease severity in aniridia and its comparison with phenotypes observed in the Indian population

Type of Mutation	Common Phenotype Association[83]	Rare Phenotype Association[83]	Phenotype Association in Indian population[89]
Loss of function Mutation Premature truncation codon mutation; Frameshift deletions	Foveal Hypoplasia Nystagmus Complete absence of Iris Cataract Glaucoma Optic Nerve Dysplasia Visual Acuity: Perception of light to 20/80	Partial iris absence Full iris with abnormal architecture	Aniridia Nystagmus Cataract *Ectopia Lentis* Ptosis Microcornea
Missense Mutations	Partial iris absence Full irides with abnormal architecture Cataracts Visual Acuity: 20/30 to 20/250	Foveal hypoplasia No glaucoma No severe optic nerve malformations	Cataract Macular hypoplasia Ectopic coloboma Glaucoma
C-terminal Extension Mutations	Foveal hypoplasia Keratopathy Cataracts Myopia Mild iris anomalies Visual Acuity: 20/80 to finger counting	Exudative vascular retinopathy Glaucoma	

Table 3: Non-*PAX6* mutations associated with aniridia

Gene	Location	Function	Phenotype along with classic aniridia
FOXC1	6p25	Eye development	Anterior segment malformation Dandy-Walker malformation Redundant umbilical skin tags Hypodontia Malar hypoplasia Glaucoma
PITX2	4q25	Developmental pathways including left-right signaling	Axenfeld-Reiger phenotype Severe iris hypoplasia[90]
CYP1B1	2p21	Oxidative biochemical reactions Anterior chamber development	Primary congenital glaucoma[93]
FOXD3	1p31.3	Development and migration of neural crest cells Formation of melanocytes Maintenance and self-renewal of stem cells[62]	Classic aniridia[62] Vitiligo[91]
TRIM44	11p13	Ubiquitin E3 ligase	Classic aniridia[18]

undergo a renal ultrasound for the same reason as mentioned above. The *WT1* gene located in the proximity of *PAX6* is likely to be altered with small deletions or chromosomal aberrations that lead to sporadic aniridia, hence increasing the risk for WAGR syndrome. However, in the presence of a positive family history of aniridia and in absence of a history of renal neoplasia, it is less likely that the *WT1* gene is mutated.[95]

For young children, chromosomal aberration analysis using multiplex ligation-dependent probe amplification (MLPA) or high-resolution array-based comparative genomic hybridization (aCGH) should be performed to identify known gene duplication/deletion and copy number variations associated with WAGR syndrome.[74,75,95] Chromosomal breakpoints can be detected using fluorescence in-situ hybridization (FISH) to spot rearrangements disrupting the *PAX6* gene.[95] However, in sporadic cases of aniridia, mutation analysis by sequencing the *PAX6* gene via next-generation sequencing or PCR-based Sanger sequencing can identify mutations in nearly 85% of cases.[95]

On occasion, despite strong clinical indication, a mutation in *PAX6* remains unidentified. This can be due to limitations of selected experimental methodology and probe distribution to detect alterations in upstream or downstream regulatory elements and deep introns.[95] Secondary reasons can be attributed to the phenotypic overlap with other ocular disorders caused by mutations in genes such as *FOXC1, FOXE3, FOXD3, CYP1B1, PITX2,* and *PITX3*.[95] A targeted sequencing panel with high coverage and read depths for these mentioned genes can be used for the detection of mutations.

Future directions

Genetic defects in *PAX6* causing haploinsufficiency prove to be a model for gene-targeted therapy. In a study by Roux *et al.* (2018),[96] the authors created a successful cellular model for *PAX6* mutation-positive patients with aniridia-related keratopathy by the introduction of a pathogenic mutation into limbal stem cells by using the CRISPR/Cas9 genome editing technique. Reduced expression of PAX6 in heterozygous mutants was observed, and on introduction of recombinant PAX6 protein, the rescue of disease phenotype could be observed in the cell culture model. This suggested that administration of wild-type PAX6 can

be an encouraging therapeutic approach for aniridia-related keratopathy.[96]

From the topical treatment perspective, it was realized a decade ago that most mutations in *PAX6* causing aniridia are due to PTCs. Thus, a mechanism to override the PTCs by altering the termination process and thereby functionally suppressing the nonsense mutation had therapeutic potential. In principle, the nonsense suppression occurs during mRNA translation, in which a near-cognate aminoacyl tRNA is inserted into the polypeptide, thus replacing the stop codon.[97] This misreading was recognized in animal models as an off-target effect of aminoglycoside antibiotics. However, due to the toxic character of aminoglycosides, a small-molecule chemical analog called Ataluren, PTC124 is now being widely studied.[97] It has been shown to generate a full-length functional protein that reverses the developmental defect following postnatal drug administration in PAX6 mouse models for aniridia. The use of this drug has progressed to a phase-II randomized, double-masked, placebo-controlled study and has shown successful clinical applications for other diseases.[98-100]

Conclusion

Based on the current understanding of aniridia, its pathology, and treatment options, the malformation remains visually debilitating. However, the future points toward developing a better understanding of the epigenetic factors, upstream, downstream regulators, and role of other genes such as *TRIM44* in the pathogenesis of the disorder.[18] Molecular genetics revelations would pave way for better identification and therapy options for classical aniridia and its extraocular associations.

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Conflicts of interest

There are no conflicts of interest.

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