Congenital Aniridia and the Ocular Surface

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ABSTRACT Aniridia is a congenital pan-ocular disorder caused by haplo-insufficiency of *Pax6*, a crucial gene for proper development of the eye. Aniridia affects a range of eye structures, including the cornea, iris, anterior chamber angle, lens, and fovea. The ocular surface in particular can be severely affected by a progressive pathology termed aniridia-associated keratopathy (AAK), markedly contributing to impaired vision. The purpose of this review is to provide an update of the current knowledge of the genetic, clinical, micro-morphological, and molecular aspects of AAK. We draw upon material presented in the literature and from our own observations in large aniridia cohorts. We summarize signs and symptoms of AAK, describe current options for management, and discuss the latest research

findings that may lead to better diagnosis and new treatment or prevention strategies for this debilitating ocular surface condition.

KEY WORDS aniridia, aniridia- associated keratopathy, congenital aniridia, gene mutations, haplo-insufficiency, iris, *Pax6* gene

I. Introduction

Congenital aniridia is a disorder caused by a haplo-insufficiency of the *Pax6* gene that is often considered the ‘master gene’ for eye development.¹⁻⁴ Although absence of the iris is the hallmark feature of this disorder, the extent of the iris malformation can vary.⁵ The most common ocular features besides the iris defect are nystagmus, foveal hypoplasia, cataract, glaucoma, and aniridia-associated keratopathy (AAK).^{1,5,6}

AAK is one of the most sight-threatening and painful manifestations of congenital aniridia, characterized by a progressive conjunctivalization of the corneal surface with chronic irritation and photophobia. A dysfunctional stem cell niche is thought to give rise to a loss of corneal epithelial phenotype.⁷ In *Pax6*^{+/-} mouse models, there is a developmental delay of the differentiation of the corneal epithelium and a reduction in the number of epithelial cell layers, giving rise to an epithelial fragility.^{7,8} By contrast, the stroma is pathologically thick in mice and in aniridia patients.⁹⁻¹¹ In addition, in patients the tear film is often unstable and the tear film breakup time (TFBUT) is reduced.¹² In a combined Swedish-Norwegian cohort with 124 patients,⁵ the mean best spectacle-corrected visual acuity (BSCVA) was 0.2 (20/100), and in a group of 52 children and teenagers under the age of 20, mean BSCVA was less than 0.3 (20/67). Over the age of 40, most patients with AAK are severely visually handicapped.¹³

The focus of our work has been characterizing and understanding the development and progression of AAK after birth. Although several general reviews of aniridia have been published, very few have focused on the ocular surface and AAK.^{2,8,14} Moreover, they were primarily focused on specific aspects of AAK pathophysiology, examining, for example, cellular-level mechanisms leading to AAK based on data from animal models.⁸ Prior reviews are also limited by lack of current knowledge in the field, as they were published over 10 years ago.^{8,14} A more recent review of aniridia published in 2013 focuses, however, on outcomes of surgical interventions available in the treatment of AAK, thus lacking a broader context in the field.² Herein, we provide a review of current knowledge of AAK, integrating the clinical,

genetic, morphologic, and molecular aspects combined with recent findings reported by our and other research teams as recorded in the PubMed database spanning the years 2003-2015. It is our hope that a deeper understanding of this debilitating condition could lead to its treatment or prevention in the future.

II. Genetic Considerations

PAX6 is expressed in each layer of the developing human eye starting at 49 days post-fertilization, and there are more than 300 *Pax6* gene mutation variants, with penetrance close to 100%.¹⁵ Such mutations can be classified into three main groups^{1,2}:

1. Premature termination codons (**PTC**). These are the most common mutations (>85%) in classical aniridia. Mutations that inactivate one copy of the gene typically result in a severe phenotype. No clear correlation, however, has been shown between location of the mutation and the phenotype.
2. C-terminal extension (**CTE**) mutations. These lead to a run-on sequence predicted to lead to a longer than normal protein. These mutations generally lead to a severe form of aniridia with many complications.
3. Missense mutations. These are mutations that change a single amino acid (substitution) within the protein. These point mutations usually give rise to a milder form of aniridia. They can also cause anterior segment dysgenesis like Peters' anomaly.

Within each mutation group there can be significant variability in the phenotype. Many other genes interact with PAX6, and some phenotypes may be caused by genetic variation in genes downstream of PAX6. Until now, most clinical studies of aniridia have considered patients with *Pax6* gene mutations as a homogeneous group. Clearly, with hundreds of possible mutations, the group exhibits a large heterogeneity, and in the future, clinical assessments and reports would ideally be combined with information on the specific genetic mutation.^{1,2}

III. Knockout Mice Models

Much of the work on the genetics in aniridia derives from animal studies, often performed on a mouse mutant, Small eye (**Sey**) mice.^{7,8,16} Human aniridia and heterozygous *Pax6*^{+/-} haplo-insufficiency in the Sey mice are similar with regard to genotype and phenotype. These mice share many of the ocular features of human aniridia and have been

widely accepted as an animal model. Factors related to the haplo-insufficiency of the *Pax6* gene contribute to a limbal stem cell-associated keratopathy in the mouse models.^{7,8,16,17}

A. Abnormal Corneal Epithelial Differentiation in Mice

Even if 60-70 % of the normal PAX6 protein level is maintained in corneal epithelial cells, serious AAK still results from heterozygous *Pax6*^{+/-} gene transcription.¹⁸ PAX6-regulated downstream genes are essential for cell differentiation. Expression of cytokeratin 12 (**K12**) is regarded as a marker for corneal-type epithelial differentiation, and it has been shown that PAX6 expression is essential to the upward regulation of the K12 gene in human corneal epithelial cells.⁸ Deficiency of K12 makes the corneal epithelium fragile and vacuolated.

A study comparing Sey and wild type mice has revealed peripheral lesions in the *Pax6*^{+/-} corneal epithelium.⁸ The barrier between conjunctival epithelium and the corneal epithelium was disrupted. *Pax6*^{+/-} related opacity formations (both in the epithelium and in the stroma) increased in severity with age, which indicates that the opacities arose from both a degenerative process and a failure of corneal differentiation.⁸ The corneal epithelial cells from adult mice have demonstrated abnormal morphology of the nuclei, mitochondria, and cytoskeleton.^{7,19,20}

PAX6 also regulates the expression of cell adhesion molecules. Davis et al have shown large gaps between corneal epithelial cells and an unusual appearance of the desmosomes in Sey mice.²¹ These results suggest that desmosomes and the intermediate filaments to which they are attached are abnormal and contribute to the loss of adhesion in the *Pax6*^{+/-} mutant cornea.

B. Oxidative Stress, Wound Healing, and Corneal Transparency

In aniridia, corneal epithelial cells are sensitive to oxidative stress, resulting in increased apoptosis and abnormal wound healing. The defective healing is observed both in mice models and clinically, and is related to abnormalities in the cell surface glycoconjugate and deficient and delayed extracellular matrix metabolism.^{1,7,10,20,22,23}

The volume of oxidized proteins increases with age in normal corneas but is more extensive in *Pax6*^{+/-}. Corneal epithelia, both superficial and basal cells in *Pax6*^{+/-} mice, have

demonstrated more severe oxidative modifications.²⁴ The accumulation of oxidized products occurs in parallel with the development of stromal opacities. Oxidative stress keeps *Pax6*^{+/-} cells in a chronic wound state, which can trigger nuclear exclusion of *Pax6*^{+/-} that has been implicated in transdifferentiation of corneal epithelium into non-corneal phenotype.²⁵ This can also lead to abnormal fibroblast/myofibroblast and myoblast expression, suggesting a new pathway linking reduced *Pax6*^{+/-} dosage to aniridia.

One example of the malfunction of keratocytes/fibroblasts/myofibroblasts is their failure to complete the wound healing response, which can lead to the formation of keloid deposits (Figure 1).²⁶ The collagenous extracellular matrix of the corneal stroma can also be disrupted in other ways. In MMP-9 deficiency, there is an accumulation of fibrin and premature infiltration of inflammatory cells into the stroma.²⁷ MMP-9 belongs to an enzyme group known as matrix metalloproteinases, which are regulated by PAX6. In combination with accumulation of pathologic material, the normal collagen-matrix relationship is disturbed, leading to impaired corneal transparency.^{8,10}

In experiments with *Pax6*^{+/-} mice, several insights have been gained into impaired corneal wound healing.^{16,20} One important finding was that increased stromal apoptosis occurred after wounding, and it has been suggested that apoptosis, as well as deficient and delayed extracellular matrix metabolism, aggravated the corneal changes.¹⁶ A summary of the corneal wound healing and stromal response in AAK is given in Table 1.

IV. Clinical Signs and the Stages of Aniridia-Associated Keratopathy

It is generally believed that AAK starts in the corneal periphery with conjunctival tissue overriding the limbal barrier and growing onto the peripheral cornea. A fibrovascular pannus forms and is opaque with the presence of blood vessels and inflammatory cells. The pannus eventually grows over the corneal surface, so that the border between the conjunctiva and the corneal epithelium is found in the peripheral or mid-peripheral cornea. In a later stage, this pannus then extends to cover the whole surface and the stroma is affected, which gives rise to a decreasing visual acuity.

The stages of progression of AAK can be classified according to a numeric scale; however, several scales have been suggested and consensus would be advantageous. In Tables 2 and 3 and Figure 2, examples of previously published clinical grading scales for AAK are given. Despite the differing scales, a threshold between effect on the central cornea

(and thereby visual acuity) and non-vision threatening peripheral pathology should ideally be specified.

V. The Limbal Stem Cells and the Stem Cell Niche

The corneal limbal stem cells are located between the palisades of Vogt, tightly attached to the basement membrane. The PAX6 genetic defect influences the regulation of the stem cells, leading to a breakdown of normal function of the limbal barrier and causing conjunctiva to invade the corneal surface.^{20,31} The limbus area acts as a junctional barrier to separate the cornea and conjunctiva, and there is no evidence that conjunctival epithelial cells can transdifferentiate into corneal epithelial cells that express K12.⁸

Several studies have shown that stem cell plasticity and pluripotency is determined by environmental factors. In mouse models of aniridia, cell proliferation is not reduced, but it is dysfunctional due to an alteration in the stem cell niche.^{7,8} The presence of conjunctival epithelial cells in the cornea may not only be due to an invasion of the conjunctiva. Differentiation of progenitor cells into conjunctival and goblet cells in situ in the cornea may occur in an altered corneal microenvironment. The limbal epithelium has been shown to be intermediate between cornea and conjunctiva.^{8,20} Until recently, however, information about the stem cell niche in aniridia has been limited to the heterozygous *Pax6*^{+/-} mouse models.⁸ The recent advancement of in vivo imaging of the cornea by in vivo confocal microscopy of the cornea (**IVCM**) has enabled a detailed analysis of the limbal stem cell niche in humans. For example, it has been shown that in cases of burn-induced limbal stem cell deficiency (**LSCD**), the presence of the limbal palisades of Vogt (**POV**) after limbal stem cell transplantation indicates success of the graft, while absence of the POV indicates failure.³²

Moreover, IVCM has shown that loss of the POV correlates with loss of central and limbal basal epithelial cells and an increase in limbal dendritic cells.³² The corneal or conjunctival epithelial transition zone and cellular phenotype as revealed by IVCM have also been shown in correlation with the presence or absence of the POV in LSCD.^{33,34} In another series of LSCD patients, IVCM was used to examine the POV and limbal epithelium in early, intermediate, and late clinical stages of LSCD, where one could see that the POV were completely degraded at the early stage.³⁵

The above studies examined LSCD as a heterogeneous group, often with only one or two aniridia patients included. We recently found in a Norwegian cohort of 20 patients with

congenital aniridia that the morphology of the POV strongly correlated with stage of aniridia-related keratopathy.³⁶ This strong structure-function relationship of the POV in aniridia revealed that a progressive morphologic degradation of the POV correlates with the loss of limbal and corneal epithelial phenotype. In grade 0 of keratopathy, POV were present with a normal morphological appearance, but in grade 1 almost half of the eyes had partially degraded POV, while in the remaining eyes the POV were absent. In all higher grades, no POV were present, while dendritic cell density was significantly increased.

IVCM could be a useful tool in the discovery of early degradative changes in the limbal palisades of Vogt that lead to progressive conjunctivalization of the ocular surface. Future studies should focus on longitudinal monitoring of the POV and associated morphology in patients, to clarify the processes of degradation of the limbal stem cell niche and offer potential insights for future treatment.³⁷

VI. Corneal Nerves

It appears that corneal nerves are directly or indirectly involved in the regulation of the corneal epithelial stem cells. It is known that corneal nerves release trophic factors to maintain a healthy corneal epithelium and epithelial turnover including limbal epithelial cell maintenance.^{38,39} Nerve growth factor and its receptor Trk-A have been implicated as potential markers for human corneal epithelial stem cells.^{38,40}

In mouse models, heterozygous *Pax6*^{+/-} adult mice exhibited decreased epithelial innervation and nerve patterns were disrupted.⁴¹ In humans, IVCM has been used to quantify subbasal epithelial nerves in LSCD, where a markedly reduced nerve density has likewise been found in a case series without aniridia patients³⁵ and in a series including two aniridia patients.³⁴

In a Swedish cohort of 16 aniridia subjects where IVCM was used, a wide range of subbasal nerve density was reported, including 3 patients with abnormally high innervation of the central cornea.²⁹ Nerve patterns were disrupted, and esthesiometry indicated that corneal touch sensitivity was reduced below normal levels in 5 of 14 patients. It was postulated that an apparent high subbasal nerve density could reflect better visibility of these nerves in vivo, due to thinning of the corneal epithelium in aniridia.

In a more recent report describing in vivo corneal nerves in a larger Norwegian cohort of 20 aniridia patients, both limbal and central corneal subbasal nerves were examined.³⁶ A

significant correlation was found between limbal epithelial nerve density and grade of keratopathy, while in contrast to the earlier Swedish cohort (where only early-stage keratopathy was examined), the central subbasal nerve density in this larger cohort correlated with grade of keratopathy.³⁶ It is important to note, however, that a reduced presence of corneal subbasal nerves, while linked to the status of the keratopathy, is not specific to aniridia. Indeed, reduced subbasal nerve density has been reported in numerous conditions, such as dry eye disease, diabetes, keratoconus, and after refractive surgery and corneal transplantation (for a review of confocal imaging of corneal nerves in disease, see reference 42).

A decrease in corneal nerves in aniridia was observed as one of multiple pathologic signs in the development of keratopathy with concomitant invasion of inflammatory cells, degradation of limbal POV, opacification of the anterior stroma, and loss of basal epithelial cells.^{29,36} While each of these features in isolation is not necessarily specific to aniridia, their combination appears to mirror the progression of AAK.

VII. Central Corneal Epithelium

Until now the morphology of the central corneal epithelium in AAK has been poorly understood. Although clinical examination by slit lamp microscopy has been used to grade the progression of keratopathy based on peripheral or central corneal involvement, the slit lamp is limited to macroscopic observation. In our Norwegian aniridia cohort, in vivo confocal microscopic imaging showed microscopic-level morphologic changes in the limbal epithelium.³⁶ The central cornea, however, exhibits abnormal morphology in aniridia, even in the earliest stages of keratopathy where the central cornea appears clinically normal and transparent. The results of the morphologic findings of central corneal epithelial status are presented in Figure 3, and have not been previously reported.

In particular, early changes to the epithelial wing and basal cell layers are apparent in stage 0 of AAK and progress through to stage 4. Discrete focal opacities appear in the wing cell layers and increase in size as AAK progresses. Wing cell size also appears to increase, as cell borders become less distinct. In the basal epithelium, inflammatory cells infiltrate and increase in density, with goblet cells and vessels invading in the late stages.

Based on animal models of aniridia⁸ and particularly our clinical findings with in vivo confocal microscopy,^{28,35} we hypothesize that limbal epithelial stem cells are present early in

congenital aniridia, first functioning abnormally for a period, before becoming completely dysfunctional or disappearing. This corresponds to a chain of events culminating in the loss of the limbal epithelial barrier. In the earliest stages (stage 0-1), the limbal stem cell niche in aniridia may be present and functioning but gives rise to epithelial cells with both cornea-like and conjunctiva-like phenotype. In this early stage, stem cell structures such as the limbal POV may be found, and the limbal barrier may be fully or mostly intact. Despite this, conjunctiva-like epithelial cells are produced at the limbus and migrate to the central cornea to form small, isolated 'islands.' Over time, the limbal stem cells begin to produce steadily more conjunctiva-like epithelial cells, in parallel with breakdown of the Palisade structures (stage 1-2). This leads to establishment of larger conjunctiva-like islands in the central and peripheral epithelium, and also appears to coincide with dendritic cell infiltration into the central cornea,^{29,36} although the central and paracentral regions still appear transparent on clinical observation. Eventually (stages 2-4), limbal stem cell function ceases entirely and can no longer give rise to new epithelial cells, resulting in total breakdown of the limbal barrier and invasion of peripheral conjunctiva into the cornea.

Although more clinical and experimental evidence is required to investigate this proposed chain of events and its specificity to AAK versus general LSCD, the possibility of at least partially functioning limbal stem cells at an early stage in congenital aniridia is promising. It may provide an opportunity for future therapies to halt the decline of limbal epithelial stem cell function, or possibly even reverse it.

VIII. Corneal Thickness

The corneal thickness in aniridia patients is usually increased. Brandt et al⁹ and Whitson et al¹¹ have reported an increased central corneal thickness, which highlights the importance of correcting measured values of intraocular pressure for the actual corneal thickness. The corneal thickness can be measured with, for example, ultrasonic pachymetry and anterior segment optical coherence tomography (**OCT**). Central corneal thickness has been reported to vary from $632 \pm 51 \mu\text{m}$ (17 patients,⁹) to $692 \pm 75 \mu\text{m}$ (10 patients,¹¹), compared to a control group where the thickness was $548 \pm 21 \mu\text{m}$.¹¹ Likewise, in Swedish and Norwegian cohorts, reported median central corneal thickness was $642 \mu\text{m}$ (11 patients²⁹) and $623 \mu\text{m}$ (18 patients³⁶), respectively. Although the origins of the increased stromal thickness in aniridia are unknown, elevated numbers of stromal keratocytes or collagen over-production have been postulated as possible causes.¹¹

VIII. The Tear Film

Tear fluid has an important function in the maintenance of the ocular surface, supplying nutrients, proteins, and wound-healing modulating factors that are essential for healthy conjunctival and corneal epithelium.⁴³⁻⁴⁷ Disturbances in secretion or composition of tear film lead to dysfunction of tear film that displays a broad spectrum of symptoms with varying degree of severity associated with many eye pathologies.⁴⁸⁻⁵⁰ The most common is dry eye disease, which can be caused either by deficient tear production or increased tear evaporation. Methods for clinically assessing tear production and function include the Schirmer test, fluorescein-based assessment of TFBUT, and fluorescein and Rose Bengal staining.⁵¹

In aniridia, the TFBUT is often reduced, suggesting tear dysfunction in the majority of patients.^{12,29,52} The extent of tear instability and meibomian gland dysfunction often correlate with the severity of ocular surface disease¹² and are not associated with dry eye syndrome per se.^{52,53} Another important factor is tear film osmolarity, which is one of the principal indicators of tear function and the condition of ocular surface.^{54,55} Increased tear osmolarity is a significant stress factor that activates several stress-signaling pathways resulting in transcription of stress-related genes, such as proinflammatory cytokines TNF- α , IL-1, IL-6, or matrix metalloproteinases.^{46,47}

So far, the osmolarity of aniridic tears has not been assessed and the composition of tear fluid, as well as the expression levels of the particular tear components in aniridia has been largely unknown. Recent research,⁵³ however, revealed different levels of several proteins in aniridic tears when compared with healthy tears. In particular, the levels of α -enolase, peroxiredoxin 6, cystatin S, gelsolin, and apolipoprotein A-1 were decreased in the tears of aniridia patients, and these proteins, along with the increased levels of 80 kDa isoform of vascular endothelial growth factor that corresponds to VEGF-C, may be involved in the pathogenesis of AAK. AAK is associated with the ingrowth of blood vessels into the cornea that compromise visual acuity. The increased tear levels of VEGF in aniridic tears suggest a breakdown of the balance between pro- and anti-angiogenic factors leading to pathological hem- and lymphangiogenesis and the manifestation of AAK. Increased oxidative stress present in AAK may lead to a greater extent of apoptosis in the corneal epithelium and abnormal corneal wound healing.¹⁶ In these processes, peroxiredoxin 6, VEGF, gelsolin, apolipoprotein A-1, and zinc- α 2 glycoprotein may play a substantial role. One study found elevated levels of dinucleotides Ap₄A and Ap₅A in aniridic tears.⁵² The increased levels of

dinucleotides could be related to LSCD as a compensatory mechanism to stimulate the proliferation and differentiation of these cells that are deficient in aniridic eyes.

The anterior lipid layer of tear film contains a large array of nonpolar and polar lipids secreted by the meibomian gland.^{56,57} The main function of this layer is to retard tear evaporation from the ocular surface.⁵⁶ As mentioned, in aniridic eyes the TFBUT is often short,^{29,52} suggesting disturbances in aniridic tear lipidome and the elevated levels of zinc- α 2 glycoprotein,⁵³ a protein known to stimulate lipid degradation, may play a substantial role in modifying the qualities of the tear lipid layer in aniridia.

The molecular mechanisms on the ocular surface involved in the pathogenesis of aniridia and AAK are presented in Figure 4.

IX. Treatment of Aniridia-Associated Keratopathy

Therapeutic management of AAK in aniridia depends on the degree of involvement of the ocular surface. As a general recommendation, treatment should be as minimally invasive as possible to avoid accelerating the progression of AAK.⁵⁸ Moreover, any type of aesthetic surgery to substitute the iris should be avoided.

For patients with well-preserved corneal transparency (stage 0 of AAK), artificial tears without preservatives containing sodium hyaluronate can be used to treat corneal irritation. Hyaluronate-containing artificial tears can effectively improve the ocular surface, as demonstrated in patients with dry eye.⁵⁹

In patients with slight-to-moderate keratopathy, autologous serum eye drops can be used to relieve symptoms from the ocular surface and to improve the surface quality. Serum drop therapy proved superior to conventional therapy with substitute tears, improving the ocular surface and comfort in patients with AAK.⁶⁰ An amniotic membrane transplant (AMT) can considerably improve the environment of the extracellular matrix of the limbal epithelial cells during healing of a corneal wound.⁶¹ AMT has been utilized following pannus removal in AAK, but has provided only temporary improvement in aniridia patients,⁶² with the keratopathy recurring over time.

More invasive treatment to restore the ocular surface in AAK should be considered only in cases of significant reduction of visual acuity and/or recurrent epithelial defects.⁵⁸ Cultured limbal epithelial allograft (**allo-CLET**) surgery has been attempted and the clinical outcome

with 14 eyes of 13 aniridia patients and 4 eyes of 4 patients with Stevens-Johnson syndrome was evaluated using the Clinical Outcome Assessment in Surgical Trials Tool (**COASTL**).³⁷ Postoperative visual acuity was improved in 79% of eyes at 6 months, 71% at 12 months, 64% at 18 months, and 57% at both 24 and 36 months. The COASTL tool showed that following allo-CLET there was a decrease in LSCD severity and an increase in visual acuity up to 12 months post-treatment, but thereafter LSCD severity and visual acuity progressively deteriorated. The allo-CLET surgery provides improvement in ocular surface stability because it temporarily restores the limbal microenvironment, but despite a good immunosuppressant protocol it does not appear to be a long-term solution. Likewise, stem cell culture using autologous cells, which would avoid the adverse effects of graft vs. host reaction, may nevertheless be ineffective in some patients due to a micro-environmental niche in aniridic eyes incapable of supporting and sustaining stem cells in the long term.

Another possibility for treatment of severe corneal opacity is corneal transplantation involving tissue from either a living relative or a cadaveric donor. Surgery using keratolimbal allografts (**KLAL**) should be done early, before stromal scarring is apparent. The risk of corneal rejection, where the transplanted tissue is a chimera of donor and recipient cells, can be seen even many years after transplantation. Therefore, the KLAL should always be combined with immunosuppression therapy, such as steroids, T-cell inhibitors, or anti-metabolites. KLAL can be followed by a penetrating keratoplasty (**PK**) if the central cornea is involved, but keratoplasty alone will result in graft failure⁶² and often increase the extent of AAK.¹⁴ The KLAL treatment, however, in many cases results in rejection and graft failure,⁶³ and in such cases the Boston keratoprosthesis can be an alternative. To avoid this complication, the keratoprosthesis may also be considered as a first-line treatment. The outcome of the Boston type I keratoprosthesis is promising, although possible complications are not uncommon. Therefore, the postoperative care is of great importance.⁶⁴

Surgery using the Boston keratoprosthesis is often combined with cataract extraction and placement of tube shunts for glaucoma. The outcome of surgery is beneficial; however, the intraocular pressure rises after the surgery. A study involving 26 aniridic eyes of 19 patients who underwent keratoprosthesis implantation showed that 14 eyes had improved visual acuity to 20/200 or better from a preoperative 20/300 or worse.⁶⁵ The mean follow-up time was 28.7 $\pm$ 13.5 months. Another study that involved 16 eyes of 15 patients with aniridia showed improved visual acuity in all but one patient from 20/300 to 20/200; however, follow-up time

was short (median of 17 months).⁶⁶ No endophthalmitis occurred during the study, but in one patient corneal melting occurred.

It should be mentioned that all intraocular surgery (including cataract surgery) carries a risk of complications in patients with congenital aniridia, such as postoperative fibrosis. The syndrome is characterized by the development of progressive fibrosis of the anterior chamber and the development of a fibrotic membrane probably arising from the rudimentary iris. A possible mechanism that promotes the formation of fibrotic material may be the proximity or touching of intraocular devices on immature vessels in the rudimentary iris found in aniridia.⁶⁷ The progressive fibrosis can displace the lens with ensuing corneal decompensation. If the ciliary body is included, the prognosis is poor with retinal traction. A treatment option is the Boston type I keratoprosthesis alone or in combination with IOL explantation and removal of the fibrotic membrane.⁶⁸ A recent retrospective study of 110 eyes implanted with the Boston type I keratoprosthesis, however, concluded that aniridia eyes are at especially high risk for postoperative complications and tend to have a shortened retention time of the keratoprosthesis.⁶⁹

In the future, another treatment possibility for the corneal surface afflicted by aniridic keratopathy may be implantation of biosynthetic collagen membranes and biocompatible elastomer nanofibers as an alternative to AMT.⁷⁰ These biomaterials are in the experimental stage and their translation into clinical practice is of great interest.

Regardless of the type of intraocular surgery, there is always a risk for progression of the AAK. Several clinical studies have been carried out to address this issue and assess long-term visual prognosis of corneal and ocular surface surgery in aniridia patients.^{67,71,72} The long-term visual prognosis, however, did not differ as a result of surgery for aniridic keratopathy. A study involving 88 eyes of 45 patients with congenital aniridia revealed that either limbal transplantation (LT) or PK provided very little vision improvement upon long term follow-up (16 to 23 years postoperatively).⁷² A combination of LT with subsequent PK when necessary may be helpful for a period of about 3-5 years as a maximum, after which the disease recurs in the majority of cases.⁷²

Recently, a promising treatment approach using postnatal manipulation of PAX6 dosage in a mouse model has been reported.⁷³ This finding gives hope for the future applicability of gene therapy treatment in early aniridia to reverse major PAX6-related congenital eye defects.

X. Summary and Conclusions

Optimal medical management of aniridia and particularly the ocular surface in AAK requires an understanding of the complexity of its pathophysiology, integrating knowledge from molecular, clinical, micro-morphological, and in the future, genetic⁷⁴ studies. Careful consideration of each treatment modality with the understanding of AAK pathophysiology is a prerequisite for finding an optimal treatment with improved outcome while potentially avoiding the need for surgical intervention with detrimental effects on patient quality of life and vision. Recent findings suggest that early-stage aniridic keratopathy is characterized by development of focal opacities in the basal epithelium, a subbasal nerve deficit, and infiltration of the central epithelium by dendritic cells, as well as tear film instability, increased corneal thickness, and degradation of limbal palisade architecture. These findings along with specific proteins over- and under-expressed in aniridic tears may help to elucidate the pathogenesis of aniridic keratopathy and aid in developing novel strategies that may be used for future treatment. Additionally, gene therapy approaches are at a promising early stage and require further translational studies. The need for development of new therapies is of utmost importance because ‘traditional’ intraocular surgery, including stem cell grafting, keratoplasty, and implantation of keratoprosthetics, has not been successful in the long term and may even worsen the patient’s prognosis.

The present literature review demonstrates that AAK is a challenging condition to understand and manage; however, our knowledge of the pathologic mechanisms contributing to the degradation of the ocular surface is increasing. Current conservative and invasive surgical treatment options for AAK are of only limited success in the long term and carry the risk of associated complications. At the same time, new treatment options at the molecular and genetic level are being proposed and could improve the prospects for aniridia patients in the future. Very little research has been conducted into these new treatment alternatives, but it is imperative that these lines of investigation continue to develop in order to provide much-needed tools in the arsenal against the relentless progression of AAK.

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Table 1. Corneal wound healing and stromal responses in healthy and PAX6 deficient eyes.
(adapted from ref. [8])

	Normal eye (functioning PAX6 gene)	Aniridia (PAX6 mutation)
Gene status	Up-regulation of <i>Pax6</i>	Reduced PAX6 activity
Enzymatic activity	Up-regulation of MMP-9	Deficient MMP-9 activity
Extracellular matrix	Normal matrix remodelling	Aberrant matrix remodelling
Wound healing	Normal healing response	Inflammatory cell pre-infiltration Overexpression of IL-1 Corneal neovascularization Stromal scarring

Table 2. Classification of aniridia-associated keratopathy (AAK)

Aniridia-Associated Keratopathy	Stage
Clear, fully transparent cornea	0
Some cloudiness in the periphery/limbus indicating ingrowth of vessels	1
Minor opacifications with ingrowth of vessels, not disturbing visual acuity	2
Difficulties to investigate retina because of marked central keratopathy with opacification of the corneal stroma and centripetal ingrowth of vessels, reduced visual acuity	3
Opacification of entire cornea	4
End stage, thick opaque pannus, fully vascularized cornea	5

Adapted from reference 28.

Table 3. An alternative classification of aniridia associated keratopathy AAK into three phases (adapted from [30]).

	Erosion/ulcer	Vascular pannus	Signs + symptoms
1. Slight limbal insufficiency	Max 2 recurring erosions or ulcers within 6 months	Not exceeding 1 mm from the limbic arch	Small disorders in absorption of fluorescein, slight epiphora and photophobia
2. Moderate limbal insufficiency	More than 3 recurring erosions or ulcers within 6 months	Involves at least the peripheral half of the cornea +/- subepithelial fibrosis	Permanent instability of the lacrimal film; constant red eye, epiphora and photophobia
3. Severe limbal insufficiency	Permanent signs of corneal erosion	Central cornea involved	Permanent instability of the lacrimal film; constant red eye, epiphora, photophobia and loss of vision

Figure 1. Corneal keloid formations in two aniridia patients. A. Keloids (arrows) appear as coalesced thick, whitish fibrous deposits in the mid-peripheral to peripheral cornea in direct slit illumination. B. A peripheral keloid deposit (arrow) viewed in retroillumination.

Figure 2. Clinical grading of aniridia stage as described in Table 2. In Stage 1, conjunctival tissue containing vessels extends just over the limbal barrier (black arrows). In Stage 2, conjunctivalization of the corneal surface proceeds, encroaching into the peripheral and midperipheral region (white arrows), while the central cornea remains clear. (reproduced with permission from reference 28).

Figure 3. Central corneal epithelial morphology in various stages of AAK. Central corneal involvement becomes clinically apparent only in stages 3 and 4, while microscopically, pathology is evident even in stage 0. A, B. Wing cell layer may appear normal, but can have discrete opacities (arrows). C, D. Basal cells may exhibit early changes in light scatter (arrows). E. Dendritic cell infiltration (arrows) of wing cell layer in stage 2. F. larger focal opacities. (G, H. Accumulation of central dendritic cells in different patients with stage 2 AAK. I. Indistinct cell borders and enlarged wing cells. J. Opacities (white arrow) and dark vacuole-like structures (black arrow) in stage 3 AAK. K. Infiltration of goblet cells (arrows). L. Dense infiltration of inflammatory cells. M. Enlarged wing cells in stage 4. N. Dark vacuoles (arrows) amidst conjunctiva-like epithelial cells. O.

Vessels invade the basal epithelium. P. Infiltration of inflammatory cells and large, dark vacuoles (arrow) in the basal epithelium in stage 4 AAK. All images $400 \times 400\mu\text{m}$.

Figure 4. Proposed molecular mechanisms on the ocular surface involved in the pathogenesis of aniridia and AAK, based on a recent study.⁵³