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Editors

Aniridia

Recent Developments in Scientific and Clinical Research

Springer

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Preface: A Special Eye to Aniridia

Rare diseases such as aniridia are a challenge. Not only for the people affected, who have to face their diagnosis every day, but also for research scientists, physicians, health and social policy makers who want to improve the understanding of the inherently complex mechanisms of these disorders and to ensure clinical appropriateness and equity of care to the patients.

In the last two decades the issue about rare diseases has been raised in many countries and in different ways, and it has been clearly established that facing this challenge requires a strong international networking and cooperation among many parties: scientists and research centres, clinicians capable of a multidisciplinary approach, health and social care institutions, patients' organizations.

As far as aniridia is concerned, patient associations began gathering people and contacting scientists and physicians in the 1990s, thus eventually building a network on both sides of the Atlantic Ocean. Nowadays, Aniridia Foundation International in the USA and Aniridia Europe (that represents patients in more than 20 countries in Europe) are well-established organizations strongly committed to promote research on the disease, to provide reliable and scientifically grounded medical information and to improve the quality of care for all patients. A growing number of scientists and physicians are joining this network, in order to exchange expertise, collect and confront data on a significantly increased critical mass of patients, present new findings and discuss controversial issues or case reports.

The rationale behind this book is to convey and make available an updated, scientifically correct and clinically appropriate knowledge on aniridia that has been built through the common efforts of scientists, clinicians and patients from different countries.

The authors contributing to this book belong to an international panel of experts in vision science and clinical ophthalmology and have all had a long-term involvement in research and/or treatment of aniridia. The range of topics discussed include the clinical management of the ocular conditions associated to aniridia (cataract, glaucoma, ocular surface disorders, nystagmus, among others), but also the genetic basis of the pathology and the options available to patients. Some chapters are focused on the paediatric patients to offer guidance to the parents as this is a

very important stage for an affected individual. This will further ensure a careful, conservative and comprehensive approach to the disease and its manifestations. It will also accompany the process of vision's acquisition in the best possible way and to minimize the consequences of the visual impairment on the general development of the child.

The aim of this book is therefore to provide a state-of-the-art information on the ocular problems affecting patients with aniridia. We believe that this book will not only be useful for the ophthalmologists but also for the geneticists, general practitioners, paediatricians and low vision experts. This will also facilitate the patients to understand the pathology and guide them in taking difficult, yet conscious and collaborative decisions.

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Chapter 1
Introduction – What Is Aniridia: Epidemiology, Clinical Features and Genetic Implications

Giuseppe Damante and Angela Valentina D'Elia

Abstract Aniridia is characterized by congenital hypoplasia of the iris and alterations of other structures of the eye, including cornea, crystalline lens, optic nerve, and retina. Patients suffer from early onset of nystagmus, photophobia, amblyopia, and severely decreased visual acuity. In 70 % of cases, aniridia is inherited in an autosomal dominant fashion, while it is sporadic in about 30 % of cases. In the great majority of patients, this disease is caused by heterozygous mutations in the *PAX6* gene, which encodes for a transcription factor, very well conserved along phylogeny and critical for eye morphogenesis. Aniridia-causing mutations can be of various types, from single base substitution to large chromosomal deletions. All of them determine a loss of function of the gene. When chromosomal deletions are large and involve the *WT1* gene, subjects suffer from the WAGR (Wilm's tumor, Aniridia, Genitourinary abnormalities, mental Retardation) syndrome. Both prenatal or postnatal genetic test is available. It is indicated when isolated or WAGR is present, as well as other eye disorders potentially associated with *PAX6* mutations. Genetic testing is useful for differentiating aniridia caused by mutations only in the *PAX6* gene from those forms associated with the deletion of contiguous genes.

Keywords Aniridia • *PAX6* • *WT1* • Genetic test

Introduction

Aniridia (from Greek, meaning "without" [an-] and "iris" [-iridia]) is an extremely rare eye condition. Its prevalence in Norway and Sweden is estimated to be 1:76,000 population and 1:70,000 population, respectively [1]. The estimated point prevalence

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is 1 in 40,000 live births in Denmark [2] and 0.42 in 100,000 live births in Spain [3]. It affects males and females equally.

Aniridia (Online Mendelian Inheritance in Man [OMIM] 106210) is characterized by congenital hypoplasia of the iris which can vary considerably from milder forms to complete bilateral aplasia.

Besides the lack of iris tissue, aniridia also shows alterations of other structures of the eye: cornea, crystalline lens, optic nerve, and retina. The fovea, the central part of the retina which enables detailed vision, and the optic nerve are often not fully developed (hypoplasia/dysplasia). This is associated with the early onset of nystagmus, photophobia, amblyopia, and severely decreased visual acuity.

In adolescents and adults aniridia can manifest itself with keratopathies, including central epithelial defects, corneal opacities, peripheral vascular pannus, and limbal stem cell deficiency. A further decrease in vision occurs with the development of cataracts, lens displacement and glaucoma [4].

In 70 % of cases, aniridia is inherited in an autosomal dominant fashion, while it is sporadic in about 30 % of cases [5]. It is caused by mutations in the *PAX6* gene (located on chromosome 11p), which plays an important role in cell differentiation and embryonic development, as it is involved in the morphogenesis of the eye, the olfactory bulb, the neural tube, the brain, and non-central nervous system organs such as the pancreas and the intestines [2]. In the majority of persons with aniridia, there is a loss of function of one copy of the gene *PAX6* intragenic mutations are observed in two-thirds of cases, whereas chromosomal rearrangements are found in about one third (deletions, translocations, and inversions). The mutations can affect the structural gene or the regions of other genes that regulate development (e.g., *SOX2*), adhesion cells, and structural proteins of the cornea and lens.

Clinically, aniridia may manifest itself as an isolated eye abnormality without apparent systemic involvement or as part of a more complex constellation of conditions. Large alterations in chromosome 11p, comprising *PAX6* and the adjacent *WT1* gene, lead to a contiguous gene syndrome, the WAGR syndrome (Wilms tumor, Aniridia, Genitourinary abnormalities, and mental Retardation) [6].

The Gillespie syndrome (OMIM 206700), another extremely rare congenital condition, is characterized by aplasia of the pupil border, cerebellar ataxia, and delayed psychomotor development. Gillespie syndrome is genetically distinct from aniridia, although *PAX6* mutations have been described in two persons with a phenotype similar to the Gillespie syndrome.

Aniridia is registered in Orphanet, the reference portal for information on rare diseases and orphan drugs, under the number ORPHA77.

The PAX6 Gene

Aniridia is caused by mutations of the *PAX6* gene that encodes a highly conserved transcription regulator involved in the ocular development of animals from the fruit fly (Drosophila melanogaster) to humans [7–9]. The *PAX6* gene was cloned in 1991

[10] and in 1992 a cDNA homologue was isolated from mouse embryo [11]. The human and mouse proteins show nearly complete sequence homology and both proteins are members of the PAX protein family, comprising nine members that share a paired domain. Each of the genes encoding PAX proteins has a tissue-specific expression; each PAX protein is involved in the development and function of one or more organs. The paired domain is about 120 amino acids long and is responsible for specific interactions with DNA sequences. The PAX6 protein interacts with the DNA sequences through the homeodomain which extends for about another 60 amino acids at the C terminal of the paired domain [12].

The *PAX6* gene is highly conserved phylogenetically. Nearly all animals have at least one gene very similar to human *PAX6*. For example, the fruit fly has a gene that encodes the paired domain and the homeodomain which has extended sequence homology with the human *PAX6* gene; it is called eyeless (ey) because some of its mutant allelic variants are associated with ocular structure anomalies [13].

In humans, the *PAX6* gene is located on the short arm of chromosome 11 (11p13), about 22.4 kb long and comprising 14 exons [9]. The mature transcript of PAX6 is about 2.7 kb long [10]. PAX6 transcription is regulated by two promoters, P0 and P1, which are differently regulated by elements in cis and activated in tissue-specific fashion [14, 15].

The protein encoded by the PAX6 gene, in addition to the domains for interaction with DNA (paired and homeodomain), has a domain at the C terminal (PST), rich in proline, serine, and threonine.

Preceding the PST region is a linker region, 78 amino acids long, which contains a high percentage of glycine (16.7 %) and glutamine (12.8 %) residues [9].

The paired domain is subdivided into an N-terminal subdomain (residues 1–60) containing a beta short motif and three alpha-helices arranged in a helix-turn-helix motif, and a C-terminal subdomain (residues 77–133) containing three alpha-helices. There do not appear to be direct protein-protein interactions between the two subdomains.

The homeodomain is a protein domain with about 60 amino acids and is characterized by three alpha-helical-like structures (helix I, II and III) folded into a compact globular structure [16, 17]. The tissue-specific expression of the PAX6 gene is identical in the mouse and humans. It is expressed in various tissues during embryonic development and in the adult organism.

PAX6 plays a centrally important role in the complete development of the eye lens and the transcriptional activation of its structural genes, such as the zeta-crystallins [18, 19]. It also plays a determinant role in the differentiation of pluripotent progenitors of the retinal cells and in maintaining their tissue-specific expression [20, 21]. The presence of the isoform containing exon 5a ensures for correct eye growth [21].

The *PAX6* gene is expressed during the earliest stage of embryonic development of the pancreas and continues to be expressed in adult endocrine cells. Mutant mice homozygous for PAX6 lack cells able to produce pancreatic glucagon, suggesting that the gene is essential for the differentiation of pancreatic alpha cells [22]. In addition, PAX6, by binding to common elements in the promoters of genes for insulin, glucagon and somatostatin, activates the gene promoters that encode these hormones

[23]. Reports have described cases of patients presenting aniridia and diabetes associated with *PAX6* mutations, suggesting that the two conditions share a common regulating mechanism [24].

In the nervous system, PAX6 controls the migration and differentiation of several specific progenitors of neural brain cells. The presence of PAX6 in association with Emx2 factor regulates the formation of cortical areas and confers area identity to diverse cells [25, 26]. Analysis of its genetic expression in mutant mice has shown that PAX6 regulates the expression of Neurog2 in the spinal cord and differentially controls distinct enhancers along the dorsoventral axis [27]. Radial glial cells, precursors of astrocytes, are ubiquitous in the central nervous system during its development.

Experimental studies have shown that cells isolated from the cortex of mice mutant for *PAX6*, have less neurogenic potential, suggesting the importance of PAX6 in the differentiation of the central nervous system [28].

PAX6 is also involved in the development of Rathke's pouch and the anterior pituitary gland; its expression is essential for the differentiation of various types of cells (somatotropic, lactotropic, thyrotropic) along the dorsoventral axis of the adenohypophysis [29].

A study on the molecular basis for hypophyseal dysfunctions in the mouse and humans identified 12 transcription factors considered critical for hypophyseal development and function, including the *PAX6* gene [30].

Genetic Basis of Aniridia

Aniridia is transmitted in autosomal dominant fashion. Each gene in every cell is present in two copies (alleles) one each from both parents. A disorder is referred to as dominant when it is expressed in a heterozygous person (i.e., a person with only one mutant allele). The affected person transmits the mutation on average to 50 % of his or her children, irrespective of the sex of the child. Most persons with aniridia (about 70 %) have a parent with the condition (familial aniridia), whereas the remaining 30 % do not (sporadic aniridia) [5]. Sporadic aniridia arises from a new mutation during gametogenesis. The rate of pathogenetic mutation of the *PAX6* gene is about 10^{-5} to 10^{-6}, meaning that each healthy individual has a probability between 1:100,000 and 1:1,000,000 of having a child with aniridia caused by a new mutation.

Aniridia may manifest itself clinically as an isolated ocular anomaly caused by point mutations in *PAX6* or by deletions of the structural gene or the regions regulating its expression. In 15 % of cases, aniridia is the clinical expression of the WAGR syndrome (Wilms tumor, a rare kidney cancer; Aniridia; Genitourinary abnormalities; and mental Retardation) which is caused by a cytogenetically visible deletion in the 11p13 band or by a submicroscopic deletion involving the *PAX6* gene and the adjacent *WT1* gene [6].

An interactive database for the analysis of *PAX6* mutations is available at http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6. The database currently contains

information on 359 mutations, 92 % of which are associated with congenital eye defects and 8 % apparently neutral polymorphisms [31, 32]. The pathogenic mutations comprise: nonsense mutations (36 % of the total); frameshift deletions or insertions (24 %); missense mutations (17 %); splicing mutations (12 %); in frame deletions or insertions (6 %); and run on mutations (5 %). Nonsense mutations introduce a premature stop codon; in splicing mutations and frameshift deletions or insertions, the protein following the mutation is strongly altered and therefore nonfunctional.

These three categories of mutations constitute over 72 % of all pathogenic mutations identified to date [6, 31–34]. Of the pathogenic mutations present in the database, about 90 % are associated with aniridia and about 10 % with other phenotypes such as a foveal hypoplasia, microophthalmia, and optic nerve defects [31, 32]. Among the mutations responsible for aniridia, few missense 2 % mutations encode proteins with a likely loss of function [32, 35–38]. Of the 29 mutations known to be associated with eye defects (without aniridia), 69 % are missense mutations [32].

This means that aniridia is more often associated with mutations that lead to complete inactivation of the protein (nonsense mutations, frameshift, splicing, deletion of the entire gene or a significant part of it), whereas other ocular phenotypes are associated with missense mutations. This is probably because missense mutations lead to changes in a single amino acid. This class of mutations does not completely inactivate protein function but rather modifies it, resulting in a phenotype different from aniridia.

Missense mutations are generally located in the paired domain (exons 5, 5a, 6, and 7) and are associated with phenotypes that affect the tissues involved in aniridia, such as the fovea, the optic nerve, and the iris [39, 40].

The mutations that introduce a premature stop codon have presumably a negative dominant effect in that the PAX6 protein trunk containing only the domains for DNA binding could acquire a major capacity for binding the target sequences without activating the genes downstream and thus interfere with normal protein function [41, 42].

It could be expected that the mutations that truncate the normal protein sequence of PAX6 are associated with a less severe form of the condition (or do not lead to its development) if the mutation alters only the C-terminal of the proteins while sparing the functional domains. Actually, however, genotype-phenotype correlations of mutations in the database suggest that the position of the truncating mutation does not have a precise role, hence the phenotype consequences in vivo. The truncating mutations associated with aniridia are not correlated with their location [32]. It is possible that nonsense-mediated decay is the pathogenically responsible molecular mechanism. Nonsense- mediated decay is the mechanism through which mRNAs containing a premature stop codon are decayed before they can produce large amounts of protein trunks [43]. The available data suggest the hypothesis that aniridia is due to haploinsufficiency because of the loss of allele function.

This does not appear to be due to premature termination of the protein but rather to the nonsense-mediated delay mechanism [31, 32].

The majority of patients (80–90 %) with aniridia are heterozygous for *PAX6* mutations [44] (see also the database mentioned above). In humans, homozygous mutations (i.e., when both alleles carry the mutation) are lethal and cause a phenotype similar to that seen in the mouse, characterized by anophthalmia and central nervous system defects [45]. Also other organisms with homozygous *PAX6* mutations present anomalous phenotypes, for example, small eye mice, eyeless Drosophila, and Caenorhabditis elegans [13, 46–48]. Homozygous small eye mice die shortly after birth, have no eyes or nasal cavities and present brain defects [7].

Genetic Analysis

Point mutations of the *PAX6* gene are identified by DNA sequencing. The deletions (small and large) are identified with molecular (multiple ligation-dependent probe amplification [MLPA]) or cytogenetic techniques (fluorescent in situ hybridization [FISH]). In these cases the possible deletion of the *WT1* gene, associated with the risk of Wilms tumor in the WAGR syndrome, is evaluated.

The sensitivity of genetic testing (i.e., a test's ability to identify a mutation) is less than 100 %. In the WAGR syndrome, cytogenetic screening has a sensitivity of about 70 %. In isolated aniridia, the complete panel of molecular tests has a sensitivity of about 65 %.

When a pathogenic mutation is detected in a person with aniridia, screening can be extended to other family members. To pregnant women may be offered prenatal genetic testing (chorionic villous sampling CVS or amniocentesis).

Theoretically, preconceptional genetic testing is another possibility, analyzing the first polar globule of an affected mother.

In cases of de novo mutation, the neonate should be tested for the possible involvement of the *WT1* gene, due to the higher risk of developing Wilms tumor.

Genetic analysis of *PAX6* is indicated when isolated or syndromic aniridia (WAGR) is present, as well as other disorders potentially associated with PAX6 mutations (Peters anomaly, papillary ectopia, foveal hypoplasia, coloboma, optic nerve hypoplasia).

Medically, genetic testing is useful for differentiating aniridia caused by mutations only in the *PAX6* gene from those forms associated with the deletion of contiguous genes.

References

1. Edén U, Iggman D et al (2008) Epidemiology of aniridia in Sweden and Norway. Acta Ophthalmol 86:727–729
2. Grindley JC, Davidson DR, Hill RE (1995) The role of Pax-6 in eye and nasal development. Development 121:1433–1442

3. Bermejo E, Martínez-Frías ML (2002) Defectos congénitos oculares:algunos aspectos clínicos y epidemiológicos. Bol ECEMC: Rev Dismor Epidemiol 1:43–48
4. Tornqvist K (2008) Aniridia: sight-threatening and hard to cure. Acta Ophthalmol 86:704–705
5. Valenzuela A, Cline RA (2004) Ocular and nonocular findings in patients with aniridia. Can J Ophthalmol 39:632–638
6. Crolla JA, van Heyningen V (2002) Frequent chromosome aberrations revealed by molecular cytogenetic studies in patients with aniridia. Am J Hum Genet 71:1138–1149
7. Hill RE, Favor J et al (1991) Mouse Small eye results from mutation in a paired-like omeobox containing gene. Nature 354:522–525
8. Jordan T, Hanson I et al (1992) The human PAX6 gene is mutated in two patients with aniridia. Nat Genet 90:41–54
9. Glaser T, Walton DS, Maas RL (1992) Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. Nat Genet 2:232–238
10. Ton CC, Hirvonen H et al (1991) Positional cloning and characterization of a paired box -and homeobox – containing gene from the aniridia region. Cell 67(6):1059–1074
11. Ton CC, Miwa H, Saunders GF (1992) Small eye (Sey): cloning and characterization of the murine homolog of the human aniridia gene. Genomics 13(2):251–256
12. Stuart ET, Kioussi C, Gruss P (1993) Mammalian Pax genes. Annu Rev Genet 27:219–236
13. Quiring R, Walldorf U et al (1994) Homology of the eyeless gene of Drosophila to the Small eye gene in mice and aniridia in humans. Science 265:785–789
14. Plaza S, Dozier C et al (1995) Quail Pax-6 (Pax- QNR) mRNAs are expressed from two promoters used differentially during retina development and neuronal differentiation. Mol Cell Biol 15:3344–3353
15. Plaza S, Saule S, Dozier C (1999) High conservation of cis-regulatory elements between quail and human for the Pax-6 gene. Dev Genes Evol 209:165–173
16. Tsao DH, Gruschus JM et al (1995) The three dimensional solution structure of the NK-2 homeodomain from the Drosophila. J Mol Biol 251:297–307
17. Esposito G, Fogolari F et al (1996) Analysis of the solution structure of the homeodomain of rat thyroid transcription factor 1 by 1H-NMR spectroscopy and restrained molecular mechanics. Eur J Biochem 241:101–113
18. Ashery-Padan R, Marquardt T et al (2000) Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. Genes Dev 14:2701–2711
19. Richardson J, Cvekl A, Wistow G (1995) Pax-6 is essential for lens-specific expression of z-crystallin. Proc Natl Acad Sci U S A 92:4676–4680
20. Marquardt T, Ashery-Padan R et al (2001) Pax6 is required for the multipotent state of retinal progenitor cells. Cell 105:43–55
21. Mann RS (2004) Two Pax are better than one. Nat Genet 36:10–11
22. St-Onge L, Sosa-Pineda B et al (1997) Pax6 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. Nature 387:406–409
23. Sander M, Neubuser A et al (1997) Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. Genes Dev 11:1662–1673
24. Yasuda T, Kajimoto Y et al (2002) PAX6 mutation as a genetic factor common to aniridia and glucose intolerance. Diabetes 51:224–230
25. Glaser T, Jepeal L et al (1994) PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. Nat Genet 7:463–471
26. Bishop KM, Goudreau G, O' Leary DD (2000) Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. Science 288:344–349
27. Scardigli R, Schuurmans C et al (2001) Crossregulation between neurogenin2 and pathways specifying neuronal identity in the spinal cord. Neuron 31:203–217
28. Heins N, Malatesta P et al (2002) Glial cells generate neurons: the role of the transcription factor Pax6. Nat Neurosci 5:308–315

29. Kioussi C, O'Connell S et al (1999) Pax6 is essential for establishing ventral-dorsal cell boundaries in pituitary gland development. Proc Natl Acad Sci 96:14378–14382
30. Cushman LJ, Camper SA (2001) Molecular basis of pituitary dysfunction in mouse and human. Mamm Genome 12:485–494
31. Prosser J, van Heyningen V (1998) PAX6 mutation reviewed. Hum Mutat 11:93–108
32. Tzoulaki I, White IM, Hanson IM (2005) PAX6 mutations: genotype-phenotype correlations. BMC Genet 6:27–39
33. Fisher E, Scambler P (1994) Human haploinsufficiency – one for sorrow, two for joy. Nat Genet 7:5–7
34. Fantes J, Redeker B et al (1995) Aniridia-associated cytogenetic rearrangements suggest that a position effect may cause the mutant phenotype. Hum Mol Genet 4:415–422
35. Tang HK, Chao LY, Saunders GF (1997) Functional analysis of paired box missense mutations in the PAX6 gene. Hum Mol Genet 6:381–386
36. Azuma N, Hotta Y et al (1998) Missense mutations in the PAX6 gene in aniridia. Invest Ophthalmol Vis Sci 39:2524–2528
37. Vincent MC, Pujo AL et al (2003) Screening for PAX6 gene mutations is consistent with haploinsufficiency as the main mechanism leading to various ocular defects. Eur J Hum Genet 11:163–169
38. Chauhan BK, Yang Y et al (2004) Functional interactions between alternatively spliced forms of Pax6 in crystallin gene regulation and in haploinsufficiency. Nucleic Acid Res 32(5):1696–1709
39. Azuma N, Yamaguchi Y et al (2003) Mutations of the PAX6 gene detected in patients with a variety of optic-nerve malformations. Am J Hum Genet 72:1565–1570
40. Hanson I, Churchill A et al (1999) Missense mutations in the most ancient residues of the PAX6 paired domain underlie a spectrum of human congenital eye malformations. Hum Mol Genet 8:165–172
41. Singh S, Tang HK et al (1998) Truncation mutations in the transactivating region of PAX6 result in dominant-negative mutants. J Biol Chem 273(34):21531–21541
42. Duncan MK, Cvekl A et al (2000) Truncated forms of Pax6 disrupt lens morphology in transgenic mice. Invest Ophthalmol Vis Sci 41:464–473
43. Byers PH (2002) Killing the messenger: new insights into nonsense-mediated mRNA decay. J Clin Invest 109:3–6
44. van Heyningen V, Williamson KA (2002) PAX6 in sensory development. Hum Mol Genet 11(10):1161–1167
45. Hodgson SV, Saunders KE (1980) A probable case of the homozygous condition of the aniridia gene. J Med Genet 17:478–480
46. Matsuo T, Osumi-Yamashita N et al (1993) A mutation in the Pax-6 gene in rat small eye is associated with impaired migration of midbrain crest cells. Nat Genet 3:299–304
47. Chisholm AD, Horvitz HR (1995) Patterning of the Caenorhabditis elegans head region by the Pax-6 family member vab-3. Nature 377:52–55
48. Zhang Y, Emmons SW (1995) Specification of senseorgan identity by Caenorhabditis elegans Pax-6 homologue. Nature 377:55–59

Chapter 2
Optical Coherence Tomography Imaging in Patients with *PAX6* Mutations

Mervyn G. Thomas and Irene Gottlob

Abstract *PAX6* mutations result in pan-ocular phenotypes which include iris defects, ranging from subtle iris defects to subtotal aniridia. In addition to iris defects, foveal hypoplasia and nystagmus are common phenotypes associated with *PAX6* mutations. In this chapter, using optical coherence tomography (OCT), we show examples of the range of arrested retinal development associated with *PAX6* mutations. Most of the patients with *PAX6* mutations have grade 1 to grade 3 foveal hypoplasia. One of the challenges in obtaining reliable posterior segment scans is related to anterior segment opacities. We also show the potential of anterior segment OCT in detecting iris abnormalities in patients with *PAX6* mutations.

Keywords PAX6 mutation • Aniridia • Foveal hypoplasia • Nystagmus • Optical coherence tomography

The phenotypic spectrum associated with *PAX6* mutations is extensive. Previous studies have shown that all patients with *PAX6* mutations have some form of iris anomalies [1] which can range from complete aniridia to iris stromal hypoplasia [2]. There have been previous reports of *PAX6* mutations with no clinical evidence of iris defect [3–5]. Recently we reported a family, harbouring a missense mutation of the *PAX6* gene, with autosomal dominant nystagmus, foveal hypoplasia, presenile cataracts but there were no iris defects [5]. Traboulsi et al. reported four cases of aniridia however no associated *PAX6* mutations were identified on sequence analysis. The authors speculate that this could be due to mutations of other genes, suggesting that 10–20 % of patients with aniridia have mutations of other genes thus representing the genetic heterogeneity associated with aniridia [6]. However, recently it has been shown that mutation within an ultraconserved cis-element located 150 kb downstream from *PAX6* can also result in aniridia [7]. Mutation within this enhancer element causes loss of enhancer activity, resulting in defective

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maintenance of PAX6 expression. Therefore although the predominant phenotype associated with *PAX6* mutation is aniridia there are reports of aniridia without *PAX6* mutations and similarly there are reports of *PAX6* mutations without iris defects.

In addition to the classical phenotype of aniridia, patients with *PAX6* mutations also quite commonly have nystagmus (95 % of aniridia patients) and foveal hypoplasia (86 % of aniridia patients). In comparison to nystagmus and foveal hypoplasia, optic nerve hypoplasia was less common in patients with *PAX6* mutations (23 % of aniridia patients) [1]. Traditionally these phenotypes were characterised based on ophthalmological examination. However, now it is possible to document the retinal phenotypes using optical coherence tomography (OCT) and also obtain quantitative retinal thickness measurements.

OCT is a technique for obtaining cross sectional images non-invasively in biological systems with wide applications in the fields of ophthalmology, cardiology and dermatology. OCT uses low-coherence interferometry to produce a two-dimensional image of optical scattering from tissues [8]. In the field of ophthalmology, it is routinely used to diagnose, determine prognosis and monitor therapeutic response of acquired and congenital retinal disorders. The previous generation time domain OCT's were limited in both axial resolution (approximately 10 μm) and scanning speeds (approximately 400 A-scans/s). With the advent of spectral domain OCT (SD-OCT) it is now possible to obtain high (typically <6 μm) and ultra-high resolution images of the retina and optic nerve head with very fast scanning speeds (typically faster than 20,000 A-scans/s) [9]. This is a major improvement in both resolution and scanning speeds, making it possible to visualise the retina at much greater detail and also obtaining scans in patients with nystagmus. Thus SD-OCT is likely to have a major role in phenotyping patients with *PAX6* mutations, since as discussed above, most patients with *PAX6* mutations have nystagmus.

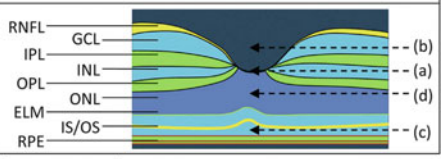
There are only a limited number of studies looking at the retinal structure in patients with *PAX6* mutations. This is mainly due to the limitations in scanning speeds and axial resolution as discussed above. Using an SD-OCT we have recently shown that it is possible to obtain reproducible retinal thickness measurements in patients with nystagmus [10]. One of the earliest studies looking at retinal structure in patients with *PAX6* mutation was using a time domain OCT in 2008 [11]. Bredrup and colleagues studied a large Norwegian family with nystagmus, corneal opacities, corectopia, iris hypoplasia and foveal hypoplasia. They were able to obtain OCTs in five out of nine affected patients. The five examined patients had absence of a foveal pit (fovea plana) and continuous retinal layers through the centre of the retina [11]. Traboulsi et al. identified four cases of aniridia with no *PAX6* mutations, OCT was performed in one out of the four patients and it revealed mild foveal hypoplasia [6]. Gregory-Evans et al. identified four family members with a nonsense *PAX6* mutation (p. Q178X), they were able to obtain SD-OCTs in two out of the four members which showed abnormal dome shaped macular profile and the entire macula was abnormally thick. They were not able to obtain retinal OCTs in the other two patients due to corneal opacities. Interestingly they were able to obtain anterior segment OCTs in all four patients which showed thickened central cornea, truncated iris root, rounded and ill-defined iris remnants [12].

In 2011 we developed a structural grading system for patients with foveal hypoplasia [13]. The purpose of this grading system was to be able to predict visual acuity based on foveal morphology across patients from different disorders. This included patients with *PAX6* mutations, albinism, isolated cases and achromatopsia. Normal foveal development occurs in stages in which the pit formation for the incipient fovea starts at fetal week 25 and the foveal pit continues to develop postnatally [14]. Disruption of this developmental process leads to foveal hypoplasia. During development of the fovea, there is (1) centrifugal displacement of cells of the inner retina toward the periphery, (2) centripetal migration of cone photoreceptors toward the location of the incipient fovea, and (3) cone specialization of the foveolar cones [14, 15]. Because of the centrifugal displacement of the inner retinal cells, the foveal depression continues to deepen until 15 months after birth, and this is seen as complete extrusion of the inner nuclear and plexiform layers posterior to the foveola. The centripetal migration of the cone photoreceptors is represented by the outer nuclear layer (ONL) widening. The cone outer segment undergoes both a decrease in diameter and an increase in length (i.e., cone specialization); this allows an increase in foveolar cone packing density [15]. The cone specialization is represented on OCT by the OS lengthening. The grading system used takes into account each of these developmental steps [13] (Fig. 2.1).

We studied the retinal morphology in ten patients with *PAX6* mutations. One of the major challenges in obtaining good quality retinal OCTs were due to anterior segment opacities or cataracts. One of the ten patients had very poor quality foveal scans hence had to be excluded. We found that patients with *PAX6* mutations had different degrees of arrested retinal development. Most patients had a grade 1 foveal hypoplasia; however there were some patients with either grade 2 or 3 foveal hypoplasia. Their visual acuity was closely related to the degree of retinal development as ascertained by the grades [13]. Examples of foveal hypoplasia associated with *PAX6* mutations are shown in Figs. 2.2 and 2.3. We have recently started using anterior segment OCT in patients with *PAX6* mutations and albinism. Sheth et al. showed that in patients with albinism there is significant thinning of the iris in comparison to controls [16]. Using similar scanning parameters described in Sheth et al. we have obtained high resolution images of the anterior segment including the cornea and iris in patients with *PAX6* mutations. An example of sectorial iris hypoplasia and an iris stump is shown in Fig. 2.4.

Previous studies have shown good genotype-phenotype correlation based on mutation type and which domains are affected. Mutations resulting in a premature termination codon and C-terminal extension have been shown to be associated with severe pan-ocular phenotypes. Phenotypes associated with missense mutations have been reported to be variable depending on mutation location (i.e. which domain is affected). Certain missense mutations within the paired domain have been shown to result in reduced DNA binding and altered transcriptional activation function [17, 18]. Normal foveal morphology with no nystagmus and relatively good visual acuity has been described in patients with p. G36R mutation suggesting that the function of this mutant protein is only moderately impaired [1]. Similarly we described a large family with autosomal

a

Normal foveal structural features detectable using optical coherence tomography	Illustration
(a) Extrusion of plexiform layers (b) Foveal pit (c) OS lengthening (d) ONL widening	

b

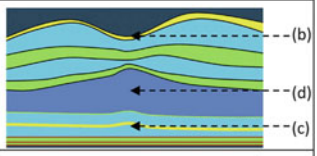
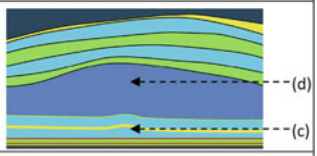
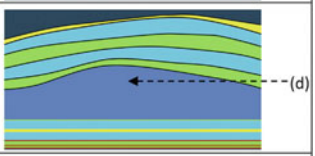
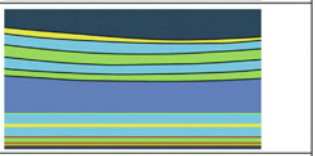
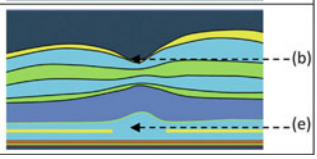
Grade of foveal hypoplasia	Structural features detected on optical coherence tomography	Present or absent	Illustration
1	**(a) Extrusion of plexiform layers** (b) Foveal pit – Shallow (c) OS lengthening (d) ONL widening	**(a) Absent** (b) Present (c) Present (d) Present	
2	**(a) Extrusion of plexiform layers** **(b) Foveal pit** (c) OS lengthening (d) ONL widening	**(a) Absent** **(b) Absent** (c) Present (d) Present	
3	**(a) Extrusion of plexiform layers** **(b) Foveal pit** **(c) OS lengthening** (d) ONL widening	**(a) Absent** **(b) Absent** **(c) Absent** (d) Present	
4	**(a) Extrusion of plexiform layers** **(b) Foveal pit** **(c) OS lengthening** **(d) ONL widening**	**(a) Absent** **(b) Absent** **(c) Absent** **(d) Absent**	
Atypical	**(a) Extrusion of plexiform layers** (b) Foveal pit – Shallow (e) IS/OS disruption	**(a) Absent** (b) Present (e) Present	

Fig. 2.1 (**a**) Illustration showing the unique features of a normal fovea detectable on optical coherence tomography. (**b**) Illustration of typical and atypical grades of foveal hypoplasia. All grades of foveal hypoplasia had incursion of inner retinal layers. Atypical foveal hypoplasia also had incursion of the inner retinal layers. Grade 1 foveal hypoplasia is associated with a shallow foveal pit, outer nuclear layer (*ONL*) widening, and outer segment (*OS*) lengthening relative to the parafoveal ONL and OS length, respectively. In Grade 2 foveal hypoplasia, all features of grade 1 are present except the presence of a foveal pit. Grade 3 foveal hypoplasia consists of all features of grade 2 foveal hypoplasia except the widening of the cone outer segment. Grade 4 foveal hypoplasia represents all the features seen in grade 3 except there is no widening of the ONL at the fovea. Finally, an atypical form of foveal hypoplasia also is described in which there is a shallower pit with disruption of the inner segment/outer segment (*IS*/*OS*) junction, possibly a sign of photoreceptor degeneration. The atypical form of foveal hypoplasia is seen with achromatopsia, whereas grades 1 through 4 are seen with albinism, PAX-6 mutations, and isolated cases. *ELM* external limiting membrane, *GCL* ganglion cell layer, *INL* inner nuclear layer, *IPL* inner plexiform layer, *OPL* outer plexiform layer, *RNFL* retinal nerve fibre layer, *RPE* retinal pigment epithelium (Reproduced with Permission from Thomas et al. [13])

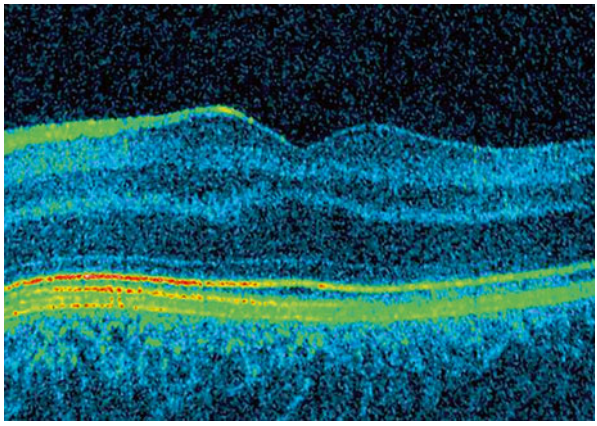


Fig. 2.2 Example of grade 1 foveal hypoplasia in a patient with *PAX6* mutation. Features of grade 1 foveal hypoplasia include: rudimentary foveal pit, incursion of inner retinal layers posterior to the foveola, widening of outer nuclear layer and lengthening of outer segment

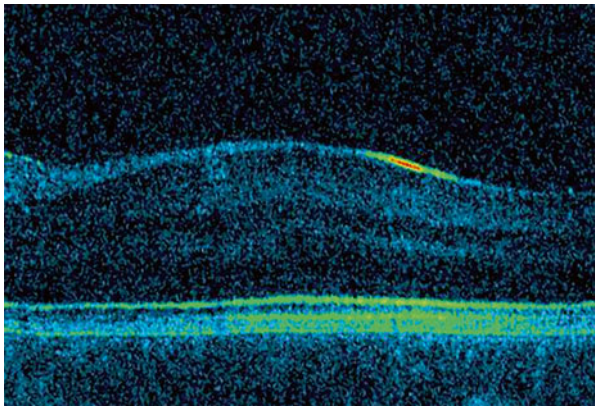


Fig. 2.3 Example of grade 3 foveal hypoplasia in a patient with *PAX6* mutation. Features of grade 3 foveal hypoplasia include: absent foveal pit, incursion of inner retinal layers, widening of the outer nuclear layer but no lengthening of outer segment

dominant nystagmus, foveal hypoplasia and presenile cataracts associated with a missense mutation resulting in the amino acid variation p. P76R within the paired box domain [5]. Other variants close to codon 76, such as: p. G72S, p. G73D and p. S74G were reported to be associated with foveal hypoplasia [1, 4, 19]. PAX6 consists of two DNA-binding domains, the paired box domain and the homeodomain. The variants described above are all located within the paired box domain and, specifically, within the linker subdomain, and are thus likely to result in reduced DNA binding and altered transcriptional activation function as previously demonstrated with the help of functional assays using paired box domain missense mutant proteins [20]. Although modifier genes could also contribute to the phenotype, there have been consistent reports that missense mutations within the PAX6 domain linker region are associated with milder phenotypes. If modifiers are involved, it is plausible that they reside in the region tightly linked to or even within the paired box domain [4, 5]. However to date there are no OCT studies systematically looking at retinal/anterior segment phenotypes in relation to the genotype.

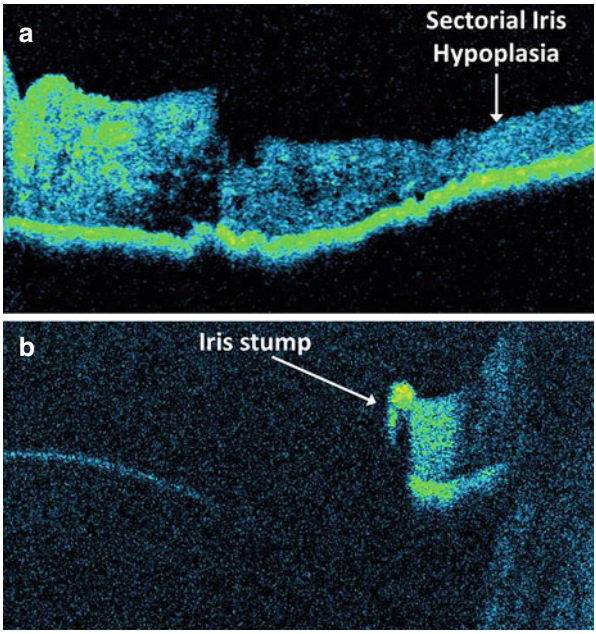


Fig. 2.4 Example of anterior segment OCT images in patients with *PAX6* mutation showing iris sectorial hypoplasia (**a**) and an iris stump (**b**)

Conclusions

In conclusion there is great need for collaborative research in recruiting and combining OCT datasets of patients with *PAX6* mutations. As discussed above there are only a handful of studies, which are mostly case reports, looking at the retinal OCTs in patients with *PAX6* mutations. To date there is only one case report which has shown the potential of anterior segment OCT in patients with *PAX6* mutations [12]. With SD-OCT we can now obtain reliable scans in these patients with nystagmus [10]. Moreover we can also obtain anterior segment OCTs which will have a role in identifying subtle iris defects which are not clinically detectable. This will provide a deeper understanding of the genotype-phenotype correlations and morphological abnormalities associated with *PAX6* mutations.

References

1. Hingorani M, Williamson KA, Moore AT, Van Heyningen V (2009) Detailed ophthalmologic evaluation of 43 individuals with PAX6 mutations. Invest Ophthalmol Vis Sci 50(6): 2581–2590
2. Gronskov K, Rosenberg T, Sand A, Brondum-Nielsen K (1999) Mutational analysis of PAX6: 16 novel mutations including 5 missense mutations with a mild aniridia phenotype. Eur J Hum Genet: EJHG 7(3):274–286
3. Nallathambi J, Neethirajan G, Shashikant S, Vijayalakshmi P, Sundaresan P (2006) PAX6 missense mutations associated in patients with optic nerve malformation. Mol Vis 12:236–242

4. Dansault A, David G, Schwartz C et al (2007) Three new PAX6 mutations including one causing an unusual ophthalmic phenotype associated with neurodevelopmental abnormalities. Mol Vis 13:511–523
5. Thomas S, Thomas MG, Andrews C et al (2014) Autosomal-dominant nystagmus, foveal hypoplasia and presenile cataract associated with a novel PAX6 mutation. Eur J Hum Genet: EJHG 22(3):344–349
6. Traboulsi EI, Ellison J, Sears J et al (2008) Aniridia with preserved visual function: a report of four cases with no mutations in PAX6. Am J Ophthalmol 145(4):760–764
7. Bhatia S, Bengani H, Fish M et al (2013) Disruption of autoregulatory feedback by a mutation in a remote, ultraconserved PAX6 enhancer causes aniridia. Am J Hum Genet 93(6):1126–1134
8. Huang D, Swanson EA, Lin CP et al (1991) Optical coherence tomography. Science 254(5035):1178–1181
9. Kiernan DF, Mieler WF, Hariprasad SM (2010) Spectral-domain optical coherence tomography: a comparison of modern high-resolution retinal imaging systems. Am J Ophthalmol 149(1):18–31
10. Thomas MG, Kumar A, Thompson JR, Proudlock FA, Straatman K, Gottlob I (2013) Is high-resolution spectral domain optical coherence tomography reliable in nystagmus? Br J Ophthalmol 97(4):534–536
11. Bredrup C, Knappskog PM, Rodahl E, Boman H (2008) Clinical manifestation of a novel PAX6 mutation Arg128Pro. Arch Ophthalmol 126(3):428–430
12. Gregory-Evans K, Cheong-Leen R, George SM, Xie J, Moosajee M, Colapinto P, Gregory-Evans CY (2011) Non-invasive anterior segment and posterior segment optical coherence tomography and phenotypic characterization of aniridia. Can J Ophthalmol 46(4):337–344
13. Thomas MG, Kumar A, Mohammad S et al (2011) Structural grading of foveal hypoplasia using spectral-domain optical coherence tomography a predictor of visual acuity? Ophthalmology 118(8):1653–1660
14. Hendrickson AE, Youdelis C (1984) The morphological development of the human fovea. Ophthalmology 91(6):603–612
15. Youdelis C, Hendrickson A (1986) A qualitative and quantitative analysis of the human fovea during development. Vision Res 26(6):847–855
16. Sheth V, Gottlob I, Mohammad S et al (2013) Diagnostic potential of iris cross-sectional imaging in albinism using optical coherence tomography. Ophthalmology 120(10): 2082–2090
17. Azuma N, Yamaguchi Y, Handa H, Tadokoro K, Asaka A, Kawase E, Yamada M (2003) Mutations of the PAX6 gene detected in patients with a variety of optic-nerve malformations. Am J Hum Genet 72(6):1565–1570
18. Tand HK, Chao LY, Saunders GF (1997) Functional analysis of paired box missense mutations in the PAX6 gene. Hum Mol Genet 6(3):381–386
19. Chao LY, Mishra R, Strong LC, Saunders GF (2003) Missense mutations in the DNA-binding region and termination codon in PAX6. Hum Mutat 21(2):138–145
20. Hanson I, Churchill A, Love J et al (1999) Missense mutations in the most ancient residues of the PAX6 paired domain underlie a spectrum of human congenital eye malformations. Hum Mol Genet 8(2):165–172

Chapter 3
Aniridic Glaucoma: Diagnosis and Treatment

Giorgio Marchini, Marco Toscani, and Gabriele Vizzari

Abstract Aniridia is a bilateral iris aplasia/hypoplasia, associated with other ocular anomalies arising during the childhood: nystagmus, photophobia, amblyopia, keratopathies, cataract and lens luxation, glaucoma, fovea and optic nerve hypoplasia. In 6–75 % of cases aniridia is accompanied by a dysgenetic secondary glaucoma caused by an iridogoniodysgenesis for abnormal migration of neural crest neuroectodermal cells, and a higher vulnerability of the optic nerve head for possible microstructural alterations in lamina cribrosa. Congenital glaucoma associated with aniridia is uncommon. The poor young patient collaboration for several clinical and instrumental analyses entails in many cases the need of examinations under general anesthesia. Medical therapy represents the first step, whereas low-responsive patients may undergo laser treatments (transscleral diode laser cyclophotocoagulation or cyclocryotherapy) and/or surgery (trabeculectomy with or without antimetabolites). Refractory cases, frequently with an early onset, require glaucoma drainage devices (Molteno implant, Ahmed valve, or Baerveldt tube shunt). A prophylactic goniotomy can be performed with a long-term effectiveness in reducing risks of aniridic glaucoma onset or progression.

Keywords Aniridic glaucoma • Iridogoniodysgenesis • Glaucoma treatment

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Introduction

Aniridia is defined as bilateral iris aplasia or hypoplasia, associated with other ocular anomalies of various kinds which are arising mostly late during the childhood: nystagmus, photophobia, and amblyopia (visual acuity reduced to 1–2/10) can accompany other diseases, more frequent in the advanced age, like keratopathies (central corneal epithelial alterations, corneal opacities, peripheral pannus, limbal stem cell deficiency), cataract and lens luxation, glaucoma, fovea and optic nerve hypoplasia [1–3].

Epidemiology and Genetics

It is a matter of an extremely rare pathology (incidence rate of 1:64,000–1:100,000), with autosomal dominant inheritance, showing a sporadic (30 %) or hereditary (70 %) form [4–7].

In primary cases, there is an isolated aniridia in absence of other systemic manifestations: the genetic base of this malformation is Paired box gene 6 (PAX6) gene mutation in chromosoma 11 or deletion of its expression regulating region.

In syndromic cases, aniridia is a part of a more complex clinical context defined by the acronym Wilms tumour, Aniridia, Genitourinary anomalies, mental Retardation (WAGR), including Wilms tumour (nephroblastoma), aniridia, genitourinary anomalies (hypospadias, cryptorchidism, genital ambiguity, urethral stenosis, ureteral anomalies, gonadoblastoma) and mental retardation: in this case de novo 11p13 deletions involving PAX6 and the adjacent Wilms tumour gene 1 (WT1) oncosuppressor gene can be identified [6–11].

Gillespie syndrome (autosomal recessive), defined by the triad congenital aniridia (with posterior synechiae), cerebellar ataxia, and mental retardation, merits mention [12].

Finally, post-traumatic or iatrogenic cases can be considered as incomplete forms of aniridia [13].

Pathogenesis

In 6–75 % of cases aniridia is accompanied by a dysgenetic secondary glaucoma, with childhood or teen-aged onset, caused by iridogoniodysgenesis for abnormal migration of neural crest neuroectodermal cells, impeding the aqueous humour outflow through anterior chamber angle with different mechanisms, often simultaneously: permanence of rotated and anteriorized iris rough draft, trabecular meshwork

and Schlemm canal dysgenesis, peripheral anterior synechiae [5, 14, 15]. Another hypothesis is the existence of microstructural alterations in optic nerve head lamina cribrosa connective tissue, resulting in a higher vulnerability to intraocular pressure (IOP) insults [16].

Congenital glaucoma associated with aniridia, presenting buphthalmos and corneal edema since birth, is uncommon [17–21].

Finally, it is necessary to remember the risk of causing intraocular hypertension or impairing an actual glaucoma as a consequence of black diaphragm intraocular lens (IOL) implantation, usually employed in congenital or posttraumatic aniridia: this probably occurs because of magnitude and stiffness of the IOL loops which produce compression on trabecular meshwork [22–27].

Diagnosis

Diagnostic analyses to execute are as follows:

- Applanation tonometry (sec. Perkins or sec. Goldmann): monitoring of the IOP is of primary importance to start and adjust the treatment.
- Central corneal thickness (CCT): usually increased in case of aniridia (>630 μm), useful to interpret the IOP measurements [16, 28–30].
- Manual ultrasound biometry: discovery of augmented axial length due to anteroposterior bulbar enlargement allows estimating intraocular hypertension progression.
- Gonioscopy: to disclose rotation and anteriorization of the residual peripheral iris basal tissue, invading angular structures with goniosynechiae.
- Ultrasound biomicroscopy (UBM): dynamic high-definition echographic study of the anterior segment morphology, possible also with corneal opacities [31].
- Anterior segment optical coherence tomography (AS-OCT): tomographic exam of the anterior chamber angle [32].
- Fundoscopy: to research signs of optic disc (excavation, dysgenesis) and fovea (hypoplasia) alterations.
- Standard automated perimetry (SAP): visual field defects indicate an advanced glaucomatous opticopathy, even if sometimes they can be expression of associated macular or disk anomalies.
- Glaucoma Diagnosis (GDx), Heidelberg Retina Tomography (HRT), retinal nerve fiber layer OCT: recent diagnostic methods permit to measure thickness of nerve fiber bundles, precociously locating pathologic thin regions.

The poor young patient collaboration for several clinical and instrumental analyses entails in many cases the need of examinations under general anesthesia. This kind of narcosis, soft and quite brief, exposes to risks justified anyway by the advantages of a correct and prompt diagnosis.

Differential Diagnosis

- Rieger anomaly: anterior segment mesenchymal dysgenesia with iris atrophy, corectopia, pseudopolycoria, childhood glaucoma, posterior embryotoxon (Schwalbe line thickening).
- Iris coloboma: sectorial absence of iris tissue.

Treatment

In recent years, the scientific literature about this topic is expanding but suffers anyway from limitations (considering the Evidence-Based Medicine criteria) because of a sample size too much small to allow a statistically significant comparison of effectiveness among different treatments, moreover without a control group.

However, the collected experiences suggest that medical therapy with antiglaucoma and miotic drugs represents the first weapon to use, whereas low-responsive patients may undergo laser treatments (outcomes obtained with transscleral diode laser cyclophotocoagulation or cyclocryotherapy were better than with argon laser trabeculoplasty) and/or surgery (trabeculectomy with or without antimetabolites was more effective and log-lasting than trabeculotomy or goniotomy) [33–42].

Refractory cases, frequently with an early onset, require glaucoma drainage devices (Molteno implant, Ahmed valve, or Baerveldt tube shunt) to achieve an high percentage of success (66–100 %) [32, 43–49].

Furthermore, it is important to remember that trauma provoked by surgical acts on corneal limbal structures can alter the weak equilibrium of this fragile tissue. Keratoplasty for corneal opacities or phacoemulsification with intraocular lens implantation for early cataract (frequently associated with aniridia) could have intraocular hypertension or progression of pre-existing glaucoma as postoperative complications, probably as a consequence of a chronic profibrotic flogistic stimulus on angle structures [4, 50–53].

A prophylactic goniotomy can be performed with a long-term effectiveness in reducing risks of aniridic glaucoma onset or progression [5, 54–58].

Finally, there isn't a surgical procedure indicated as elective surgery and predictably effective in a sufficient percentage of patients. The following table shows the outcomes published in literature about aniridic glaucoma surgical therapy (Table 3.1).

Follow-Up

A yearly follow-up visit is recommended for IOP measurement, optical nerve head examination, and visual field (if possible considering nystagmus, corneal opacities, cataract).

Table 3.1 Treatment of congenital glaucoma associated with aniridia: surgical procedures and outcomes

Authors	Review	Year	Patients	Aniridic glaucoma (eyes)	Therapy		
					Eyes	Procedures	Success[a]
Panda A, et al.	Indian J Ophthalmol	1982	16	11	11	Drugs	6
					5	Cyclocryo	1
					1	Trabeculectomy	1
Walton DS.	Trans Am Ophthalmol Soc	1986	16	28	28	Prophylactic goniotomy	25 (89,3 %)
Wiggins RE Jr, Tomey KF.	Arch Ophthalmol	1992	10	17	20	Cyclocryo	5
					2	Cyclodiode	0
					2	Trabeculotomy	0
					15	Trabeculectomy	1
					6	Tube (Molteno)	5
Mandal AK, et al.	Ophthalmology	1997	13	2	2	Trabeculectomy	2
Adachi M, et al.	Ophthalmology	1997	16	29	12	1st Trabeculotomy	6
					6	2nd Trabeculotomy	4
					17	1st surgery [trabeculectomy/ goniotomy/tube (Molteno)]	3
					14	2nd surgery [trabeculectomy/ goniotomy/tube (Molteno)]	8
Filous A, et al.	Cesk Slov Oftalmol	1998	11	22	22	Drugs	13 (59,1 %)
					9	Cyclocryo/trabeculectomy	6
Wagle NS. et al.	Ophthalmology	1998	49	8	8	Cyclocryo	0
Mandal AK, et al.	Ophthalmic Surg Lasers	1999	29	2	2	Trabeculectomy	2
Chen TC, Walton DS.	Arch Ophthalmol	1999	33	55	55	Prophylactic goniotomy	49 (89,1 %)
					6	Goniotomy and drugs	6

(continued)

Table 3.1 (continued)

Authors	Review	Year	Patients	Aniridic glaucoma (eyes)	Therapy		
					Eyes	Procedures	Success[a]
Esquenazi S, Amador S.	Ophthalmic Surg Lasers	2002	1	2	2	Trabeculectomy	2
Arroyave CP, et al.	Am J Ophthalmol	2003	5	8	8	Tube	8
Yalvac IS, et al.	J Cataract Refract Surg	2004	1	1	1	Tube (Ahmed)	1
Menezo JL, et al.	Eur J Ophthalmol	2005	8	4	3	Drugs	1
					3	Cyclodiode	2
					1	Tube (Ahmed)	1
Lanzagorta-Aresti A, et al.	Eur J Ophthalmol	2007	3	4	4	Tube (Ahmed)	4
Yu WH, et al.	Zhonghua Yan Ke Za Zhi	2008	8	5	1	Drugs	1
					1	Trabeculectomy	0
					4	Cyclodiode	2
Low S, et al.	J AAPOS	2008	25	1	1	Trabeculotomy/trabeculectomy	1
Aslam SA, et al.	Ophthalmology	2008	35	40	22	Drugs	16 (72,7 %)
					1	Trabeculectomy	1
					3	Cyclodiode	2
					2	Tube (Baerveldt)	2
Edén U, et al.	Acta Ophthalmol	2008	52	28	28	Drugs	20 (71,4 %)
					8	Trabeculectomy/tube (Molteno)	?
Moreker M, et al.	Indian J Ophthalmol	2009	1	2	2	Drugs	0
					2	Trabeculectomy	2
Diago T, et al.	Arch Soc Esp Oftalmol	2009	1	2	2	Trabeculectomy	2
Kulkarni SV, et al.	J Glaucoma	2010	8	4	4	Goniotomy	1

Zeppa L, et al.	Eur J Ophthalmol	2010	15	1	1	Trabeculectomy	0
					1	Tube (Ahmed)	1
Lee H, et al.	J Pediatr Ophthalmol Strabismus	2010	11	9	9	Drugs	1
					1	Cyclodiode	1
					2	Goniotomy	0
					4	Trabeculectomy	1
					1	Tube (Ahmed)	1
					5	Needling and tube (Ahmed)	5
Terasaki H, et al.	Jpn J Ophthalmol	2010	1	2	2	Vitrectomy and endocyclodiode	2
Park SH, et al.	Korean J Ophthalmol	2010	31	31	31	Drugs	22 (71,0 %)
					6	Trabeculectomy	6
					3	Tube (Ahmed)	3
Almousa R, Lake DB	Int Ophthalmol	2014	15	8	8	Tube (Ahmed)	7

NB: number of procedures can exceed total number of evaluated eyes also considering multioperated refractory cases. Treatments are reported following the validated sequence: medical therapy, laser treatment, penetrating surgery and drainage implant

[a]Success is defined as IOP <21 mmHg at 12 months

Conclusions

We consider that diagnostic workup and periodic follow-up should consist of tonometry, ultrasound biometry, and fundoscopy with optic disk evaluation. The visit has to be completed with GDx, HRT, or retinal nerve fiber layer OCT and with a visual field analysis, if age, nystagmus, and patient compliance permit these exams.

In case of non responsive patients towards medical therapy with antiglaucoma and miotic drugs, the sequence of surgical treatment should be as follow: goniotomy, trabeculotomy combined or not with trabeculectomy, drainage implant.

References

1. Lee HJ, Colby KA (2013) A review of the clinical and genetic aspects of aniridia. Semin Ophthalmol 28(5–6):306–312
2. Ninios K, Jonescu-Cuypers CP, Seitz B (2011) Glaucoma with primary iris malformations. Axenfeld-Rieger syndromes, ICE syndromes (essential iris atrophy, Chandler's syndrome, Cogan-Reese syndrome), aniridia. Ophthalmologe 108(6):585–593; quiz p. 594
3. Tornqvist K (2008) Aniridia: sight-threatening and hard to cure. Acta Ophthalmol 86(7):704–705
4. Park SH, Park YG, Lee MY, Kim MS (2010) Clinical features of Korean patients with congenital aniridia. Korean J Ophthalmol 24(5):291–296
5. Lee H, Khan R, O'Keefe M (2008) Aniridia: current pathology and management. Acta Ophthalmol 86(7):708–715
6. Hingorani M, Moore A (2003) Aniridia. In: Pagon RA, Bird TD, Dolan CR, Stephens K (eds) GeneReviews. University of Washington, Seattle [updated 2008 Aug 12]
7. Nelson LB, Spaeth G, Nowinski TS, Margo CE, Jackson L (1984) Aniridia: a review. Surv Ophthalmol 28:621–642
8. Zhang X, Zhang Q, Tong Y, Dai H, Zhao X, Bai F, Xu L, Li Y (2011) Large novel deletions detected in Chinese families with aniridia: correlation between genotype and phenotype. Mol Vis 17:548–557
9. Fischbach BV, Trout KL, Lewis J, Luis CA, Sika M (2005) WAGR syndrome: a clinical review of 54 cases. Pediatrics 116(4):984–988
10. Wolf M, Zabel B, Lorenz B, Blankenagel A, Ghorbani MB, Schwenn O, Wildhardt G (1998) Molecular genetic study of the PAX6 gene in aniridia patients. Ophthalmologe 95(12):828–830
11. Ivanov I, Shuper A, Shohat M, Snir M, Weitz R (1995) Aniridia: recent achievements in paediatric practice. Eur J Pediatr 154(10):795–800
12. Edén U, Beijar C, Riise R, Tornqvist K (2008) Aniridia among children and teenagers in Sweden and Norway. Acta Ophthalmol 86(7):730–734, Epub 2008 May 19. Erratum in: Acta Ophthalmol. 2009; 87(2):242
13. Muzaffar W, O'Duffy D (2006) Traumatic aniridia in a pseudophakic eye. J Cataract Refract Surg 32(2):361–362
14. Margo CE (1983) Congenital aniridia: a histopathologic study of the anterior segment in children. J Pediatr Ophthalmol Strabismus 20(5):192–198
15. Grant WM, Walton DS (1974) Progressive changes in the angle in congenital aniridia, with development of glaucoma. Trans Am Ophthalmol Soc 72:207–228
16. Dimasi DP, Burdon KP, Craig JE (2010) The genetics of central corneal thickness. Br J Ophthalmol 94(8):971–976

17. Singh B, Mohamed A, Chaurasia S, Ramappa M, Mandal AK, Jalali S, Sangwan VS (2014) Clinical manifestations of congenital aniridia. J Pediatr Ophthalmol Strabismus 51(1):59–62
18. Diago T, Harto M, Serra I, Pardo D, Montero J, Díaz-Llopis M (2009) Aniridia, congenital glaucoma and white corneas in a newborn baby. Arch Soc Esp Oftalmol 84(11):573–576
19. Khan AO, Aldahmesh MA, Al-Amri A (2008) Heterozygous FOXC1 mutation (M161K) associated with congenital glaucoma and aniridia in an infant and a milder phenotype in her mother. Ophthalmic Genet 29(2):67–71, Erratum in: Ophthalmic Genet. 2008; 29(4):190
20. Lise-Schneider B, Calvas P, Roche O, Lambert JC, Dufier JL, Costet-Fighiera C (2007) Glaucoma with aniridia and isolated congenital glaucoma in siblings: contribution and limits of genetics. J Fr Ophtalmol 30(1):44–48
21. Lee WB, Brandt JD, Mannis MJ, Huang CQ, Rabin GJ (2003) Aniridia and Brachmann-de Lange syndrome: a review of ocular surface and anterior segment findings. Cornea 22(2): 178–180
22. Aslam SA, Wong SC, Ficker LA, MacLaren RE (2008) Implantation of the black diaphragm intraocular lens in congenital and traumatic aniridia. Ophthalmology 115(10):1705–1712
23. Lanzagorta-Aresti A, Menezo-Rozalen JL, Martínez-Costa R, Cisneros-Lanuza A (2007) Ahmed valve for elevated intraocular pressure associated with black diaphragm lenses for aniridia. Eur J Ophthalmol 17(3):437–440
24. Menezo JL, Martínez-Costa R, Cisneros A, Desco MC (2005) Implantation of iris devices in congenital and traumatic aniridias: surgery solutions and complications. Eur J Ophthalmol 15(4):451–457
25. Wong VW, Lam PT, Lai TY, Lam DS (2005) Black diaphragm aniridia intraocular lens for aniridia and albinism. Graefes Arch Clin Exp Ophthalmol 243(5):501–504
26. Beltrame G, Salvetat ML, Chizzolini M, Driussi GB, Busatto P, Di Giorgio G, Barosco F, Scuderi B (2003) Implantation of a black diaphragm intraocular lens in ten cases of post-traumatic aniridia. Eur J Ophthalmol 13(1):62–68
27. Reinhard T, Sundmacher R, Althaus C (1994) Iris black diaphragm intraocular lenses in traumatic Aniridia. Klin Monatsbl Augenheilkd 205(4):196–200
28. Freedman SF (2008) Central corneal thickness in children-does it help or hinder our evaluation of eyes at risk for glaucoma? J AAPOS 12(1):1–2, comment pp 7–10
29. Whitson JT, Liang C, Godfrey DG, Petroll WM, Cavanagh HD, Patel D, Fellman RL, Starita RJ (2005) Central corneal thickness in patients with congenital aniridia. Eye Contact Lens 31(5):221–224
30. Brandt JD, Casuso LA, Budenz DL (2004) Markedly increased central corneal thickness: an unrecognized finding in congenital aniridia. Am J Ophthalmol 137:348–350
31. Engels BF, Dietlein TS, Jacobi PC, Krieglstein GK (1999) Ultrasound biomicroscopy diagnosis of congenital glaucoma. Klin Monatsbl Augenheilkd 215(6):338–341
32. Lee H, Meyers K, Lanigan B, O'Keefe M (2010) Complications and visual prognosis in children with aniridia. J Pediatr Ophthalmol Strabismus 47(4):205–210
33. Low S, Hamada S, Nischal KK (2008) Antimetabolite and releasable suture augmented filtration surgery in refractory pediatric glaucomas. J AAPOS 12(2):166–172
34. Brémond-Gignac D (2007) Glaucoma in aniridia. J Fr Ophtalmol 30(2):196–199
35. Kirwan JF, Shah P, Khaw PT (2002) Diode laser cyclophotocoagulation: role in the management of refractory pediatric glaucomas. Ophthalmology 109(2):316–323
36. Okada K, Mishima HK, Masumoto M, Tsumamoto Y, Tsukamoto H, Takamatsu M (2000) Results of filtering surgery in young patients with aniridia. Hiroshima J Med Sci 49(3):135–138
37. Wallace DK, Plager DA, Snyder SK, Raiesdana A, Helveston EM, Ellis FD (1998) Surgical results of secondary glaucomas in childhood. Ophthalmology 105(1):101–111
38. Wagle NS, Freedman SF, Buckley EG, Davis JS, Biglan AW (1998) Long-term outcome of cyclocryotherapy for refractory pediatric glaucoma. Ophthalmology 105(10):1921–1926; discussion pp 1926–1927
39. Filous A, Odehnal M, Brůnová B (1998) Results of treatment of glaucoma associated with aniridia. Cesk Slov Oftalmol 54(1):18–21

40. Adachi M, Dickens CJ, Hetherington J Jr, Hoskins HD, Iwach AG, Wong PC, Nguyen N, Ma AS (1997) Clinical experience of trabeculotomy for the surgical treatment of aniridic glaucoma. Ophthalmology 104(12):2121–2125
41. Wiggins RE Jr, Tomey KF (1992) The results of glaucoma surgery in aniridia. Arch Ophthalmol 110(4):503–505
42. Panda A, Sood NN, Agarwal HC (1982) Management of secondary glaucoma in aniridia. Indian J Ophthalmol 30(4):311–313
43. Zeppa L, Romano MR, Capasso L, Tortori A, Majorana MA, Costagliola C (2010) Sutureless human sclera donor patch graft for Ahmed glaucoma valve. Eur J Ophthalmol 20(3):546–551
44. Park HY, Lee NY, Park CK (2009) Risk factors of shallow anterior chamber other than hypotony after Ahmed glaucoma valve implant. J Glaucoma 18(1):44–48
45. Brauner SC, Walton DS, Chen TC (2008) Aniridia. Int Ophthalmol Clin 48(2):79–85
46. Trigler L, Proia AD, Freedman SF (2006) Fibrovascular ingrowth as a cause of Ahmed glaucoma valve failure in children. Am J Ophthalmol 141(2):388–389
47. Chen TC, Bhatia LS, Walton DS (2005) Ahmed valve surgery for refractory pediatric glaucoma: a report of 52 eyes. J Pediatr Ophthalmol Strabismus 42(5):274–283
48. Arroyave CP, Scott IU, Gedde SJ, Parrish RK 2nd, Feuer WJ (2003) Use of glaucoma drainage devices in the management of glaucoma associated with aniridia. Am J Ophthalmol 135(2):155–159
49. Beauchamp GR, Parks MM (1979) Filtering surgery in children: barriers to success. Ophthalmology 86(1):170–180
50. Chew HF, Ayres BD, Hammersmith KM, Rapuano CJ, Laibson PR, Myers JS, Jin YP, Cohen EJ (2009) Boston keratoprosthesis outcomes and complications. Cornea 28(9):989–996
51. Vanathi M, Panda A, Vengayil S, Chaudhuri Z, Dada T (2009) Pediatric keratoplasty. Surv Ophthalmol 54(2):245–271
52. López-García JS, García-Lozano I, Rivas L, Martínez-Garchitorena J (2006) Congenital aniridia keratopathy treatment. Arch Soc Esp Oftalmol 81(8):435–444
53. Schwartz GS, Holland EJ (2001) Iatrogenic limbal stem cell deficiency: when glaucoma management contributes to corneal disease. J Glaucoma 10(6):443–445
54. Swanner JC, Walton DS, Chen TC (2004) Prevention of aniridic glaucoma with goniosurgery. Int Ophthalmol Clin 44(1):67–71
55. Chen TC, Walton DS (1999) Goniosurgery for prevention of aniridic glaucoma. Arch Ophthalmol 117(9):1144–1148
56. Chen TC, Walton DS (1998) Goniosurgery for prevention of aniridic glaucoma. Trans Am Ophthalmol Soc 96:155–165; discussion pp 165–169
57. Walton DS (1986) Aniridic glaucoma: the results of gonio-surgery to prevent and treat this problem. Trans Am Ophthalmol Soc 84:59–70
58. Walton DS (1986) Aniridia with glaucoma. In: Epstein DL (ed) Chandler and Grant's glaucoma. Lea & Febiger, Philadelphia, pp 506–511

Chapter 4
Management of Glaucoma in Congenital Aniridia

Peter A. Netland

Abstract Glaucoma in aniridia usually develops during childhood, due to either open- or closed-angle mechanisms. In our study of Aniridia Foundation International (AFI) members, approximately half of the subjects developed glaucoma, with glaucoma diagnosis at average age 13.6 years and median age 8.5 years. The majority of patients were treated surgically for glaucoma. Average central corneal thickness is increased in aniridia, which may be a consideration for assessment of intraocular pressure. Although surgical procedures vary, clinicians often use glaucoma drainage implants to treat aniridic glaucoma. Regular monitoring during childhood, with prompt recognition of elevated intraocular pressure and effective management, may prevent vision loss due to glaucoma in aniridia.

Keywords Aniridia • Glaucoma • Goniotomy • Trabeculotomy • Trabeculectomy • Glaucoma drainage implants • Cyclophotocoagulation

Introduction

Glaucoma is a potentially vision-threatening problem that is commonly encountered in aniridia patients. Although this condition may develop at any time in life, glaucoma usually develops during childhood or even young adulthood. Aniridia patients require regular examinations during childhood to allow diagnosis and early treatment of glaucoma, which may be asymptomatic. With accurate measurement of increased intraocular pressure, glaucoma is suspected in aniridia patients. Glaucoma can be diagnosed when changes of the optic nerve occur, due to this elevated intraocular pressure and/or visual field loss occurs. In children with aniridia, prompt recognition of glaucoma and effective management can prevent irreversible vision loss.

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Mechanisms of Glaucoma

The glaucoma in aniridia may be due to open- or closed-angle mechanisms [1, 2]. In aniridia patients with glaucoma, the anterior chamber angle usually is open, with increased resistance of aqueous flow through the conventional outflow pathway from the trabecular meshwork into Schlemm's canal. Onset of glaucoma in infancy in aniridia is uncommon, and may involve iridotrabeculodysgenesis, absence of Schlemm's canal, or other mechanisms [3, 4]. The angle may be closed in aniridic patients when the stump of residual iris covers the trabecular meshwork in the anterior chamber angle. Although there is only a small amount of iris remaining in most aniridia patients, this may cause closure of the irido-corneal angle by the iris remnant, which may be progressive and increasing over time [5]. In an ultrasound biomicroscopic (UBM) study, the trabecular-iris angle of aniridic eyes with glaucoma was not significantly different from that of eyes without glaucoma, suggesting that open-angle configuration is more common than closed-angle in aniridic glaucoma [6]. In this UBM study, hypoplasia of not only the iris but also the ciliary body were found in aniridia patients, perhaps due to similar influence of abnormal embryologic development of mesoderm or neuroectoderm in aniridia.

The Prevalence of Glaucoma in Aniridia

The majority of patients with aniridia develop glaucoma in their childhood, adolescent or early adult years. In aniridia patients, the reported incidence of glaucoma ranges from 6 to 75 %, but the majority of studies show an incidence of glaucoma of approximately 50 % [7]. In a survey of 54 patients with WAGR syndrome, investigators identified 44 % with glaucoma [8]. In a survey of 33 Canadian aniridia patients, glaucoma was present in 30 % and was the main cause of vision loss [9]. Most reports have described onset during the preadolescent or early adolescent years [10–13]. Therefore, there is a high likelihood of development of glaucoma, but it may take years to develop. For this reason, aniridic patients are monitored for glaucoma from birth through adulthood.

Examination of the Eye in Aniridia

Even if glaucoma is not detected initially, it is important for children with aniridia to have regular examinations of the eye, because the development of glaucoma can occur at any time in childhood. These examinations are directed towards identifying ophthalmic problems that are commonly associated with aniridia.

A complete examination is often possible in the office, without the use of anesthetics. In some instances, a mild sedative, such as chloral hydrate syrup, may be administered. If a complete examination cannot be performed in the office, or

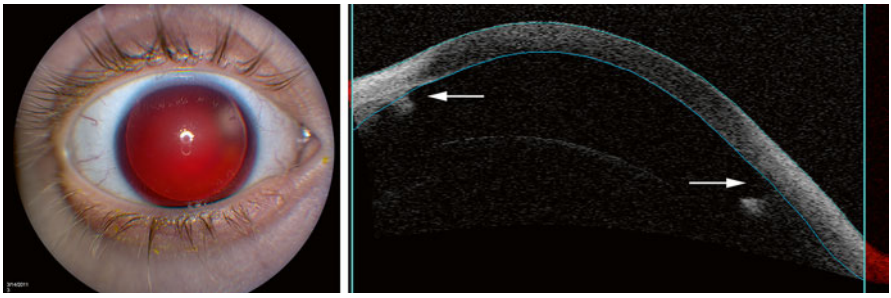

Fig. 4.1 (*left*) Anterior segment image of an 11-year old girl with aniridia and glaucoma. (*right*) Anterior segment optical coherence tomography (OCT) shows the residual iris tissue and an open anterior-chamber angle (*arrows*)

when there is uncertainty about the clinical findings, an examination under anesthesia is warranted. Examination under anesthesia does not always require intubation of the patient, as the anesthesiologist may be able to use a laryngeal mask or a facemask for ventilation. Older children (age 2–4 years) may require an occasional examination under anesthesia to provide good quality examination of the intraocular pressure and other findings.

During the initial office visit, the examiner will elicit any symptoms, and will question the parents (or the older child him/herself) regarding any visual problems. Nearly all children with aniridia have photophobia resulting from absence of iris tissue. However, other classic symptoms of congenital glaucoma, such as blepharospasm and tearing, usually are not present in patients with aniridia, who often acquire glaucoma later in childhood. The patient's vision is measured to determine if a refractive error would be corrected with glasses. In younger children, it may be difficult to assess the vision accurately, and specialized testing for visual acuity may be performed.

In most instances, a complete ocular examination, including slit lamp examination, tonometry, gonioscopy, and optic nerve evaluation, can be performed in the office in children over the age of 5 years old and, with some training, in children younger than 5 years old. In aniridia patients, it is important to assess the cornea for aniridic keratopathy and the lens for cataract. Timing the examination of an infant to occur when the child is placated by a bottle feeding can allow a complete examination of these younger children. Gonioscopic examination, ultrasound biomicroscopy (UBM), or anterior segment optical coherence tomography (OCT) can distinguish open-angle from closed-angle mechanism (Fig. 4.1).

Measurement of Intraocular Pressure

In determining whether the aniridic patient has developed glaucoma, the intraocular pressure should be assessed on a regular basis. This measurement of the intraocular pressure can be performed with an applanation (usually Goldmann) or by electronic

(Tonopen) tonometer. The Perkins handheld applanation tonometer allows the measurement of the intraocular pressure at any angle, including when the patient is lying down. These measurements require a drop of topical anesthetic to dull sensation on the cornea, and then application of the tonometer to the surface of the eye. The rebound tonometer (Icare Finland Oy, Vantaa, Finland) does not require anesthetic, and can frequently obtain measurements of the intraocular pressure in awake children, which has greatly reduced the need for examination under anesthesia. The intraocular pressure in infants can be obtained while feeding or distracted with a pacifier, and older children are usually cooperative if clearly instructed. Children ages 2 years to 3–4 years can present the greatest challenge in obtaining accurate IOP readings.

The normal intraocular pressure for a child should be in the mid- to low-teens, and certainly not above 20–21. Sometimes, because of crying or difficulties in obtaining the measurement, the intraocular pressure may be overestimated. In this situation, it is important to try and get a measurement of the intraocular pressure under sedation, to make sure that the true reading is not elevated. In children, there is no ideal method of measuring the intraocular pressure. Our preference for clinic is the rebound tonometer, although the gold standard Goldmann or Perkins applanation tonometry should be performed whenever possible. During an examination under anesthesia, we most commonly use the Perkins applanation tonometer. Tonopen, pneumotonometry, dynamic contour tonometry (DCT), and other techniques may also provide helpful measurements of the intraocular pressure.

Applanation tonometry measurements may be influenced by the thickness of the cornea. Studies have demonstrated an increased central corneal thickness in aniridic patients [14–17]. In 1 study of 16 eyes with aniridia, the average corneal thickness was 692 ± 75 μm, compared with 548 ± 21 μm in controls ($P < 0.001$) [15]. Specular microscopy has demonstrated normal endothelial cell counts and structure in aniridia [15, 16]. Thicker central corneal thickness measurements were found in aphakia and aniridia compared with anterior segment dysgenesis and uveitis [17]. Increased central corneal thickness could influence the measurements of intraocular pressure, potentially leading to overestimation of intraocular pressure in some aniridic patients.

Assessment of the Optic Nerve

Another important component of the examination of the aniridic patient is the examination of the optic nerve, looking for any evidence of glaucoma damage. The fundus is examined using direct or indirect ophthalmoscopy. The appearance of the optic nerve is carefully assessed for evidence of glaucomatous damage. Careful drawings are very helpful, and also photographs can be taken of the optic nerve to provide a baseline for future comparisons. Whenever possible, retinal nerve fiber layer thickness should be assessed by optical coherence tomography (OCT). Optic nerve cupping occurs much more quickly and at lower pressures in children, as

compared to adults. Similarly, in children, a decrease in cupping can occur within hours or days after control of intraocular pressure. This is especially marked in infants below 1 year of age. In adults, reversal of cupping after normalization of intraocular pressure rarely, if ever, occurs. Ophthalmoscopy can also be performed to examine the retina, in particular to determine if the patient has a hypoplastic or absent fovea. In aniridia patients, the foveal region may be evaluated by OCT of the macula.

Visual Field Testing

Peripheral visual field defects can be measured in older children and young adults with perimetry, but it is difficult to perform visual field testing on infants and young children. Visual field examinations can be performed at 5–6 years of age, but the patient's short attention span and poor fixation often prevent a detailed study. The older and more cooperative the child, the more detailed the examination. By the age of 8–10 years old, most children can cooperate for a full visual field examination [18].

Clinical Course of Glaucoma in Aniridia

In a study of 83 members of Aniridia Foundation International (AFI) members, the prevalence of glaucoma was 46 % [7]. As shown in Fig. 4.2, the cumulative percentage of aniridia patients diagnosed with glaucoma abruptly rose during childhood, then continued to slowly increase through adulthood. The average age at diagnosis of glaucoma was 13.6 ± 15.0 years, with a median age of 8.5 years (range 0–58 years) [7]. Of 38 subjects with aniridia and glaucoma, 76 % were treated medically, and 58 % had been treated surgically. In subjects with glaucoma, the

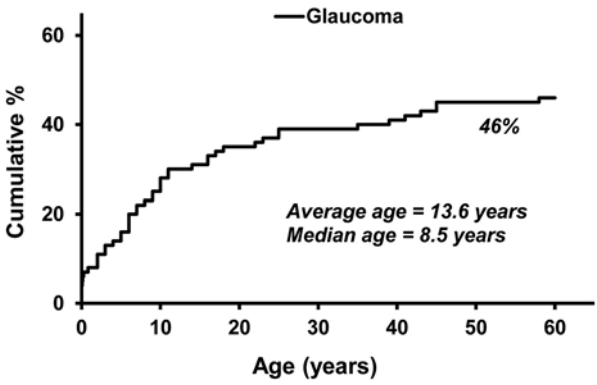

Fig. 4.2 Cumulative percentage of aniridia patients diagnosed with glaucoma. Diagnosis of glaucoma was found in 38 of 83 patients with aniridia (46 %). Most patients were found to have glaucoma in childhood, with continued increase of glaucoma diagnosis into adulthood (Data from reference [7])

mean number of glaucoma medications was 1.8 ± 1.3, and the number of surgical procedures was 1.7 ± 2.0 [7]. These findings indicate that glaucoma associated with aniridia most commonly occurs during childhood or adolescence and often requires surgical treatment.

Medical Treatment of Glaucoma in Aniridia

Medical therapy plays an important adjunctive role in the treatment of glaucoma in aniridia. It is often possible to control the intraocular pressure over a long period of time with medical therapy. However, in more than half of aniridia patients with glaucoma, surgical therapy is required for definitive control of the elevated intraocular pressure.

Primary medical therapy is commonly initiated with a trial of topical prostaglandin therapy. Beta blocker drops, can be effective, but should not be used in children who have asthma or other breathing problems due to possible pulmonary side effects. Carbonic anhydrase inhibitors can be given as drops or as a systemic medication. Although safe for pediatric use, oral carbonic anhydrase inhibitors can cause troublesome side effects in some children, such as malaise, fatigue, and loss of appetite. In order to minimize the possibility of systemic side effects, topical carbonic anhydrase inhibitors are preferred by many clinicians. Pilocarpine and other similar cholinergic drugs may not be as helpful. In young children, adrenergic agonists such as brimonidine should be used with caution, as they can cause strong sedative effects. If patients respond well to an appropriately-chosen medication, they may be able to achieve good long-term control of the intraocular pressure, and forego, or, at least forestall, glaucoma surgery.

Surgical Treatment of Glaucoma and Aniridia

Although medical therapy is often tried as the initial therapy, most patients require surgery to provide long-term control of the intraocular pressure. After surgery, however, some patients may still require additional treatment with medical therapy. There are many options for surgical therapy of glaucoma, and the exact choice of procedure will depend upon the specific clinical problems of the individual aniridic patient with glaucoma.

Prophylactic Goniotomy

Progressive angle closure may occur in aniridia. After monitoring the situation, the ophthalmologist may choose to perform goniotomy with synechialysis to open to the angle and prevent further closure [19]. Thus, further elevation of the intraocular

pressure may be avoided in some patients. This approach is uncommon in clinical practice, due to low prevalence of progressive angle-closure in aniridia patients.

Therapeutic Goniotomy or Trabeculotomy

A report of treatment of one aniridia patient with goniotomy by Otto Barkan in 1953 described control of intraocular pressure during 9 months follow-up [20]. Goniotomy is usually not helpful in aniridia patients [21], although the procedure may be considered in young children less than 3 years old with aniridia, or in older aniridic children with a closed anterior chamber angle. An alternative to goniotomy is trabeculotomy, which may be useful when corneal opacity prevents a view of the anterior chamber angle required to perform goniotomy. Some surgeons prefer trabeculotomy over goniotomy because they are more familiar and comfortable with a technique that utilizes the operating microscope. Unlike goniotomy, trabeculotomy does not require passing a knife over the lens, which is not covered by the iris in aniridia. Modest success using trabeculotomy for treatment of aniridic glaucoma has been reported [22], especially when the procedure is performed early in life. The choice of goniotomy and trabeculotomy depends on the specific clinical situation of the patient and the preferences of the surgeon.

Trabeculectomy

Trabeculectomy may be performed to reduce intraocular pressure in older children, or in those patients who have failed previous goniotomy or trabeculotomy. Poor success rates have been reported using trabeculectomy without antifibrosis drugs in aniridic glaucoma [22, 23]. Anti-fibrosis drugs, such as mitomycin-C, may improve the short-term success rate sufficiently to consider this procedure in patients with aniridia. However, clinicians remain concerned about the likelihood of long-term success in aniridia, which is a pro-fibrotic entity with potential for severe ocular surface problems. In some instances, trabeculectomy may be combined with trabeculotomy [24]. This is a more common procedure in areas, such as India and the Middle East, where initial trabeculotomy alone is not as successful. Also, in older children, trabeculotomy may be combined with trabeculectomy, if the surgeon feels that a combined technique will give a better chance of success.

Glaucoma Drainage Implants

Many clinicians prefer glaucoma drainage implants for primary glaucoma surgery in aniridia patients, with long-term ocular surface problems and pro-fibrotic tendencies that may threaten long-term success of conventional filtration surgery.

Also, when other types of glaucoma filtration surgery have failed, clinicians may choose to use a glaucoma drainage implant. The specific type of implant varies depending on the preferences of the surgeon for the individual patient [25]. In one study of eight eyes in five aniridia patients, the success rate was 88 % at 1 year after drainage implant surgery [26]. In another study of eight aniridic eyes treated with Ahmed Glaucoma Valve implantation, intraocular pressure control was successful in 87 % of eyes at 12 months [27]. In nine eyes with aniridia and corneal opacity, successful intraocular pressure control was achieved with endoscopic vitrectomy with pars plana glaucoma tube shunt implantation [28]. In aniridia patients, we frequently place the tube in the ciliary sulcus, with a polyglactin suture under a clear-cornea patch graft, which can be treated with laser suture lysis.

If there is a planned procedure around the limbus or the cornea, such as a limbal stem cell transplant or keratoprosthesis, glaucoma drainage implants are often required, because they can be performed despite extensive limbal scar tissue. Keratoprosthesis is increasingly used for treatment of advanced aniridic keratopathy [29]. Aniridia patients, with and without pre-existing glaucoma, usually experience increased intraocular pressure after keratoprosthesis. The Ahmed Glaucoma Valve controlled the intraocular pressure in approximately 81 % of patients after keratoprosthesis implantation [30]. When glaucoma drainage implants are not effective in controlling the intraocular pressure in patients with keratoprosthesis and glaucoma, adjunctive medical therapy or cyclophotocoagulation is often effective [31].

Cyclodestructive Procedures

Cyclodestructive procedures are often used when other types of filtration surgery have failed, or their potential for success is low. The eye may have poor to no vision, or may have the worst vision of the two eyes. Cyclodestructive procedures are generally not used as a primary surgical procedure for aniridia, because of the limited long-term success and the risk of vision-threatening complications. If other surgical treatments have been performed and the intraocular pressure remains elevated, an adjunctive treatment using a cyclodestructive procedure may be helpful [31]. Transcorneal argon laser ciliary body photocoagulation and adjunctive medical therapy was effective in controlling the intraocular pressure in one patient with 5 years follow-up [32].

Aniridia Fibrosis Syndrome

Vision-threatening intraocular fibrosis was noted after ocular surgery in 6 of 80 aniridia patients (8 %) [33]. Aniridia is a pro-fibrotic syndrome, but the adverse effects of fibrosis on the success of glaucoma surgery is poorly understood at this

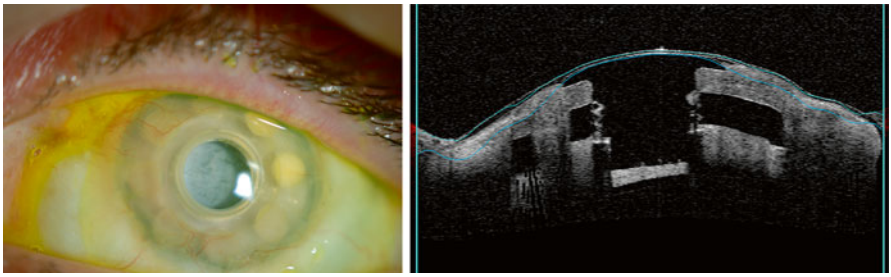

Fig. 4.3 (*left*) Slit lamp biomicroscopy image of a 63-year old woman with aniridia, keratoprosthesis, and aniridia fibrosis syndrome, with retroprosthetic membrane. (*right*) No angle structures or patent glaucoma drainage implant tube are visible in the anterior segment OCT image. Note the dense retroprosthetic membrane, which is in contact with the ciliary body

time. We have observed obstruction of glaucoma drainage implant tubes in patients who have developed aniridia fibrosis syndrome (Fig. 4.3).

Other Surgical Procedures for Glaucoma Associated with Aniridia

Procedures such as Trabectome, iStent, and suprachoroidal drainage devices, may have a role in treatment of aniridia patients, depending on the specific clinical situation and the clinician's judgment. More information is needed about the results of minimally-invasive glaucoma surgery in aniridia patients.

Long Term Care

In patients who have not been diagnosed with glaucoma, follow-up visits every 4–6 months during childhood and even into young adulthood are recommended. Frequent follow-up is helpful to identify glaucoma at its earliest onset. Early identification can allow timely treatment and prevent visual loss. In patients who have developed glaucoma and have had treatment for glaucoma, the frequency of follow-up depends on the severity of the problem.

The success of long-term care and treatment is very dependent on the coordination of different ophthalmic specialists. It is important to identify refractive errors and treat any amblyopia. Other eye problems, such as cataract and pannus, should be identified and treated as needed. A multi-disciplinary approach, incorporating clinical care of the patient, is usually most effective. This multi-disciplinary approach includes not only the ophthalmologist, but also teachers, mobility instructors, low vision specialists, and the parents.

Possible Future Therapies

The diagnosis and treatment of glaucoma associated with aniridia will, no doubt, continue to improve over time. At this time, diagnosis of glaucoma, restoration of optic nerve function, and gene-based therapy are active areas of investigation, and may lead to tangible improvements in the therapy of aniridia patients.

Conclusion

Aniridia is often associated with glaucoma, which usually develops in mid-late childhood or early adulthood. Medical treatment may be helpful, but patients often require surgical treatment for aniridic glaucoma, usually with glaucoma drainage implants. Treatments are effective for glaucoma associated with aniridia. Close monitoring, early identification, and effective treatment of glaucoma may prevent damage to the optic nerve and vision loss in aniridic glaucoma.

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References

1. Mandal AK, Netland PA (2006) The pediatric glaucomas. Elsevier, Edinburgh
2. Netland PA (2010) Glaucoma problems associated with aniridia. In: Nerby JA, Otis JJ (eds) Aniridia and WAGR syndrome: a guide for patients and families. Oxford University Press, New York, pp 69–81
3. Margo CE (1983) Congenital aniridia: a histopathologic study of the anterior segment in children. J Pediatr Ophthalmol Strabismus 20:192198
4. Baulmanna DC, Ohlmanna A, Flugel-Kocha C, Goswamib S, Cveklb A, Tamm ER (2002) Pax6 heterozygous eyes show defects in chamber angle differentiation that are associated with a wide spectrum of other anterior eye segment abnormalities. Mech Dev 118:3–17
5. Grant WM, Walton DS (1974) Progressive changes in the angle in congenital aniridia, with development of glaucoma. Am J Ophthalmol 78:842–847
6. Okamoto F, Nakano S, Okamoto C, Hommura S, Oshika T (2004) Ultrasound biomicroscopic findings in aniridia. Am J Ophthalmol 137:858–862
7. Netland PA, Scott ML, Boyle JW, Lauderdale JD (2001) Ocular and systemic findings in a survey of aniridia subjects. JAAPOS 15:562–566
8. Fischbach BV, Trout KL, Lewis J, Luis CA, Sika M (2005) WAGR syndrome: a clinical review of 54 cases. Pediatrics 116:984–988
9. Valenzuela A, line RA (2004) Ocular and nonocular findings in patient with aniridia. Can J Ophthalmol 39:632–638
10. Edén U, Beijar C, Riise R, Tornqvist K (2008) Aniridia among children and teenagers in Sweden and Norway. Acta Ophthalmol 86:730–734

11. Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L (1984) Aniridia. A review. Surv Ophthalmol 28:621–642
12. Gramer E, Reiter C, Gramer G (2012) Glaucoma and frequency of ocular and general diseases in 50 patients with aniridia: a clinical study. Eur J Ophthalmol 22:104–110
13. Singh B, Mohamed A, Chaurasia S, Ramappa M, Mandal AK, Jalali S, Sangwan VS (2014) Clinical manifestations of congenital aniridia. J Pediatr Ophthalmol Strabismus 51: 59–62
14. Brandt JD, Casuso LA, Budenz DL (2004) Markedly increased central corneal thickness: an unrecognized finding in congenital aniridia. Am J Ophthalmol 137:348–350
15. Whitson JT, Liang C, Godfrey DG, Petroll WM, Cavanagh HD, Patel D, Fellman RL, Starita RJ (2005) Central corneal thickness in patients with congenital aniridia. Eye Contact Lens 31:221–224
16. Park SH, Park YG, Lee MY, Kim MS (2010) Clinical features of Korean patients with congenital aniridia. Korean J Ophthalmol 24:291–296
17. Lopez JP, Freedman SF, Muir K, Duncan L, Stephens D, Atenafu E, Levin AV (2011) Central corneal thickness in children and adolescents with pediatric glaucoma and eye disorders at risk of developing glaucoma. J Pediatr Ophthalmol Strabismus 48:108–116
18. Kerr NC, Litchford DW, Dong Q, Netland PA (2005) Reliability and artifact in automated visual field testing of children. Ann Ophthalmol 37:185–189
19. Chen TC, Walton DS (1999) Goniosurgery for prevention of aniridic glaucoma. Arch Ophthalmol 117:1144–1148
20. Barkan O (1953) Goniotomy for glaucoma associated with aniridia. AMA Arch Ophthalmol 49:1–5
21. Brauner SC, Walton DS, Chen TC (2008) Aniridia. Int Ophthalmol Clin 48:79–85
22. Adachi M, Dickens CJ, Hetherington J Jr, Hoskins HD, Iwach AG, Wong PC, Nguyen N, Ma AS (1997) Clinical experience of trabeculotomy for the surgical treatment of aniridic glaucoma. Ophthalmology 104:2121–2125
23. Wiggins RE Jr, Tomey KF (1992) The results of glaucoma surgery in aniridia. Arch Ophthalmol 110:503–505
24. Lawrence SD, Netland PA (2012) Trabeculectomy versus combined trabeculotomy-trabeculectomy in pediatric patients. J Pediatr Ophthalmol Strabismus 49:359–365
25. Ishida K, Mandal AK, Netland PA (2005) Glaucoma drainage implants in pediatric patients. Ophthalmol Clin N Am 18:431–442
26. Arroyave CP, Scott IU, Gedde SJ, Parrish RK 2nd, Feuer WJ (2003) Use of glaucoma drainage devices in the management of glaucoma associated with aniridia. Am J Ophthalmol 135:155–159
27. Almousa R, Lake DB (2014) Intraocular pressure control with Ahmed glaucoma drainage device in patients with cicatricial ocular surface disease-associated or aniridia-related glaucoma. Int Ophthalmol 34:753–760
28. Shaikh AH, Khatana AK, Zink JM, Miller DM, Petersen MR, Correa ZM, Riemann CD (2014) Combined endoscopic vitrectomy with pars plana tube shunt procedure. Br J Ophthalmol 98:1547–1550
29. Hassanaly SI, Talajic JC, Harissi-Dagher M (2014) Outcomes following Boston type 1 keratoprosthesis implantation in aniridia patients at the University of Monteral. Am J Ophthalmol 158:270–276
30. Netland PA, Terada H, Dohlman CH (1998) Glaucoma associated with keratoprosthesis. Ophthalmology 105:751–757
31. Rivier D, Paula JS, Kim E, Dohlman CH, Grosskreutz CL (2009) Glacuoma and keratoprosthesis surgery: role of adjunctive cyclophotocoagulation. J Glaucoma 18:321–324
32. Sbordone S, Romano V, Savastano A, Piccirillo V, Savastano MC, Bifani M (2013) Aniridia-associated with lens coloboma and secondary glaucoma treated with transcorneal argon laser ciliary body photocoagulation: a case report. Case Rep Ophthalmol 4:44–47
33. Tsai JH, Freeman JM, Chan CC, Schwartz GS, Derby EA, Petersen MR, Holland EJ (2005) A progressive anterior fibrosis syndrome in patients with postsurgical congenital aniridia. Am J Ophthalmol 140:1075–1079

Chapter 5
Clinical and Surgical Management of Cataract in Congenital Aniridia

Dominique Brémond-Gignac

Abstract Aniridia consists in a complex malformation of the eye with congenital absence of iris. This genetic rare disease can cause severe visual impairment occurring from various mechanisms. Ocular clinical signs in aniridia may associate glaucoma, most common complication with limbal insufficiency leading to keratopathy, cataract, ptosis, foveal aplasia or a microphthalmia. The cataract in aniridia must be identified with its specificities in order to adjust the treatment according with other ocular signs and complications of the disease. In aniridia, cataract is usually reduced in infancy to mild opacities or partial lens opacification. The main treatment aims to correct ametropia, potentially induced strabismus, nystagmus and amblyopia in case of anisometropia or cataract asymmetry. When visual acuity becomes low, time of cataract surgery must be discussed. The assessment of the low vision due to an occlusive cataract must be confirmed. Other factors as foveal aplasia, corneal opacities, glaucoma may influence visual acuity and will not be corrected by cataract surgery. Different techniques of cataract surgeries are available and adapted for cataract in aniridia however the surgeon must be aware of high rate of complications as glaucoma, fibrosis and ocular surface impairment. Phacoemulsification and classical intraocular lens, artificial iris or combinated intraocular lens with diaphragm can be performed very carefully. A regular follow-up of the patient must be performed in order to detect complications.

Keywords Cataract • Aniridia • Artificial iris • Visual impairment • Ocular surface • Glaucoma • Fibrosis

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Introduction

Aniridia consists in a congenital absence of iris with a panocular malformation and the incidence varies from 1/56,000 [1] to 1/76,000 [2] and 1/96,000 [3]. This complex embryologic malformation involves iris, trabéculum, macula and cornea with limbal stem cells deficiency. Aniridia causes usually a major visual impairment due to different factors. Ocular associated clinical signs include cataract, glaucoma (most common complication), limbal insufficiency with keratopathy, ptosis, nystagmus, foveal aplasia optic nerve hypoplasia or microphthalmia. Many patients will have congenital opacities or mild cataract and later will develop an obstructive cataract. In a Familial aniridia with preserved ocular function Elsas [4] found only 18 % of cataract in affected patients. The cataract formation in aniridic patients was reported by the age of 20 years-old from 50 % [5] to 85 % [6]. So the cataract is a usual ocular sign in aniridic patients. In a relatively rare condition, aniridic patients can present ectopia lentis (18–35 %).

Clinical Examination

In aniridic patients, most common symptoms are photophobia, nystagmus and low vision. In most families with aniridia visual acuity is less than 20/60 in all patients and less than 20/200 in over 60 %. Nystagmus is a quite constant sign in 85–92 % of cases [6]. In order to evaluate the part of cataract in the low vision of the aniridic patient a complete ocular examination must be performed. At ocular examination at slit lamp the form of the cataract varies congenital, classical anterior polar, pyramidal, lamellar rings, nuclear opacities, opacities in wheels and cortical spokes (Fig. 5.1). Cataract increases in prevalence with age. A recent patient self-reported study brings 71 % of aniridia patients with a mean age of 25 years-old had a cataract [7]. Aniridic twins have been described with bilateral congenital cataract presenting

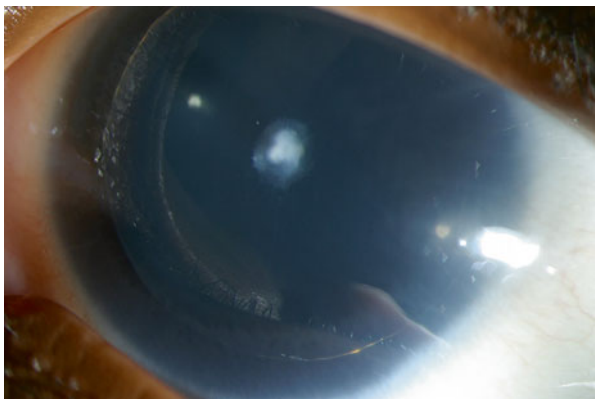

Fig. 5.1 Anterior polar cataract in aniridia patient

a WAGR syndrome associating Wilms tumor, Aniridia, Genitourinary malformation and mental Retardation [8]. A bilateral cataract in infancy was also described in WAGRO syndrome associating WAGR and Obesity [9]. Some partial Wachendorf membrane can be sometimes observed even in complete aniridia. A precise visual acuity evaluation with systematic cycloplegia in children allows the best optical correction associated with tinted glasses as needed. Myopia is common and probably induced by the low vision. If a strabismus is present the treatment aims to correct amblyopia however considering the nystagmus and the limited effect of occlusion. Different ocular explorations can be performed to evaluate the severity of the case of the aniridic patient. Abnormal tear film stability and meibomian gland dysfunction are newly identified factors inpatients with aniridia. Both correlate to the severity of ocular surface disease. Impression cytology is informative in diagnosing various degrees of limbal stem cell deficiency in aniridia eyes [10]. Corneal OCT and corneal topography as Pentacam provides information on quality of ocular surface, corneal status and lens opacities. Pachymetry is useful as central measurement is thicker than in general population and overestimate ocular pressure if a corrective coefficient is not associated [11]. Keratometry and biometry allow a power calculation of the artificial lens that could be requested. Macular OCT, RNFL OCT and ERG provide an evaluation of the macular function and help in the comprehension of the different parts of low vision of aniridia patients. In young children regular ocular examinations must be performed under general anesthesia to control ocular pressure, perform biometry and evaluate lens opacities and retinal status.

Medical Treatment

After ocular examination optical correction is prescribed. The cycloplegia allows a full correction that must be provided to aniridia children. As in all patients presented aniridia, dark glasses or tinted contact lenses may be helpful for photophobia and light sensitivity. As esotropia is the more common form described full correction of hyperopia is useful to correct strabismus. Treatment of amblyopia is also essential in children and can use occlusion if the nystagmus is absent or mild. The close follow-up is important to perform to detect anomalies of young patients. The use of contact lens must be carefully evaluated with the status of ocular surface to avoid corneal complications as ulcers or opacifications. Due to dry eye and limbal stem cell deficiency lubricant preservative free may be used to preserve ocular surface.

Surgical Treatment

Cataracts are extracted in aniridic patients if they produce a significant decrease in visual acuity in addition to the visual loss inherent to aniridia proper. In many patients with cataract even with extensive lens opacities the visual acuity is

relatively preserved and compatible with the foveal hypoplasia. Cataract surgery is best deferred in these patients because of low potential for visual improvement and increased risks of complications as glaucoma or corneal dystrophy. Surgical technique of lens extraction is classical by phacoemulsification but in order to avoid more limbal insufficiency incision by sclera tunnel is recommended instead of corneal incision. Classical phacoemulsification allows the removal of the crystalline lens and the intraocular lens is implanted preferentially in the bag (Fig. 5.2). Parameters of phacoemulsification must be adjusted to reduce pressure during surgery. The use of specific viscoelastic during surgery is controversial. Some zonular anomalies can be associated to aniridia and may lead to a difficult surgery management. Schneider [12] studied five eyes from four aniridia patients. A thinning of the anterior capsule was found comparing with normal eyes. Greater awareness of anterior capsule fragility in some aniridia patients may reduce the risk of capsule complications and lead to safer surgical outcomes.

Glaucoma is the main cause of acquired visual loss in aniridia and develops in 50–75 % of cases in late childhood or early adulthood. So the patient will commonly associates glaucoma before or after the surgery. Complications of cataract surgery in aniridic patients must be known and if available prevented. Limbal stem cell deficiency results in corneal opacities and impaired ocular surface. After cataract surgery lacrimal supplementation is provided to avoid new corneal opacities that will affect the vision. Glaucoma may preexist to the cataract surgery planned. Post operative high pressure of the eye has to be detected and followed. Glaucoma surgical procedure could be necessary to control ocular pressure and preserve vision in the outcome of cataract procedure. Tsai reported a progressive fibrosis syndrome after cataract surgery (or tube for glaucoma surgery) that could lead to a anterior chamber fibrosis causing entrapment and displacement of the intraocular lens. An

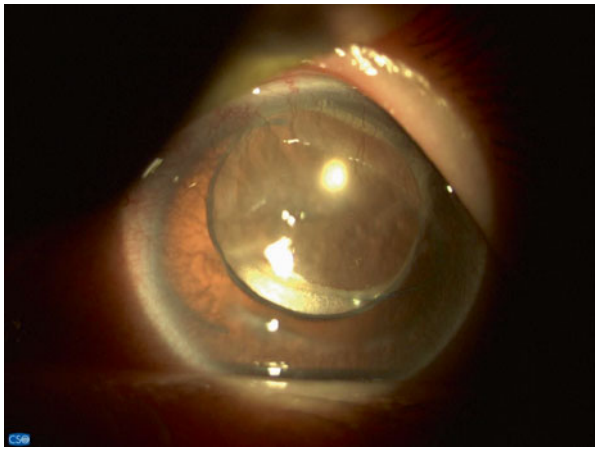

Fig. 5.2 Classical intraocular lens implanted in a congenital aniridia patient with severe ocular surface impairment

endothelial decompensation is also observed. An early surgical treatment to remove the fibrotic membrane is recommended by authors [13]. Some complications of posterior segment may also occur as retinal detachment or choroidal haemorrhage.

The time of surgery can associate a correction of absence of iris. Iris prosthetic devices for complete or partial restoration of an iris diaphragm have been developed [14]. Specific intraocular lenses manufactured with colored iris on the optic or specific capsular tension rings may provide an artificial diaphragm. Different manufacturers with various devices are available in Europe for more than 15 years as in United States these intraocular lenses are not yet FDA approved. These implants provide a better cosmetic appearance and reduce light sensitivity of the patient. The evaluation of these implants is difficult due to other ocular damages of aniridia (Fig. 5.3). Li reports a case of bilateral implantation of an intraocular lens and capsular tension rings for congenital aniridia with glare improvement however duration of the follow-up is not specified. Reinhard [15] reported black diaphragm aniridia intraocular lens implanted with long term follow-up. Of the 19 eyes implanted with a mean follow-up of 46 months visual acuity improved in 14 eyes however 4 developed glaucoma deterioration or 4 glaucoma onset, 2 a cystoid macular edema, 3 a chronic endothelial loss and 4 a deterioration of ocular surface. Two eyes had to be explanted with glaucoma. The technique uses two rings that are rotated to form a confluent iris. Aslam [16] reported 40 eyes with black diaphragm intraocular lens implanted in aniridia. Fifteen were of congenital origin. Increasing of glaucoma occurs in 25 % of patients after cataract surgery. In contrast with traumatic aniridia, no significant improvement of visual acuity was seen in the 15 eyes with congenital aniridia, the authors conclude that implantation of the black diaphragm intraocular lens in congenital aniridia therefore should be approached with caution, because the recreation of an iris diaphragm does not confer the expected optical benefits in these eyes. The results of these iris prostheses in aniridic patients cannot be compared to those implanted in traumatic aniridia because of complications specific to aniridia patients.

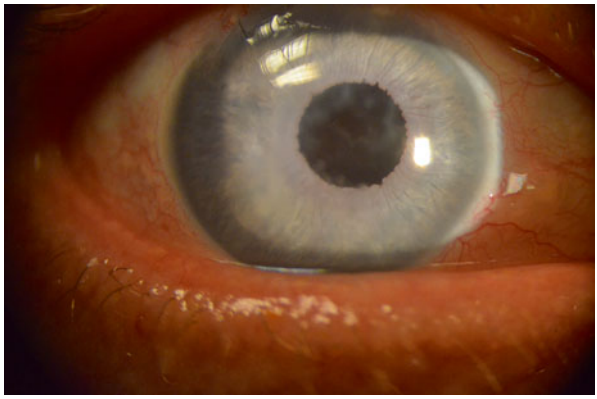

Fig. 5.3 Artificial iris implanted in aniridia patient (Courtesy of Pr. Chiambaretta)

Conclusion

Aniridia is often responsible for a severe impairment. Cataract in aniridia patients commonly is well tolerated and not the main cause of low vision. Aniridia requires a medical and surgical treatment adapted from infancy to adult life. If the visual acuity is compatible with foveal aplasia and ocular surface impairment, cataract surgery must be differed until the lens opacification demonstrates to be responsible of the visual impairment. Intraocular lens with or without iris replacement can be chosen. The surgical technique and the device must be chosen according to the patient's expectations and balance of advantage/risk of the intraocular or the device implanted due to the possible complication as increase ocular pressure, ocular surface impairment, retinal complications or anterior fibrosis syndrome.

References

1. Shaw M, Falls H, Neel J (1960) Congenital aniridia. Am J Human Genet 12:389–415
2. Eden U, Iggman D et al (2008) Epidemiology of aniridia in Sweden and Norway. Acta Ophthalmol 86:727–729
3. Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L (1984) Aniridia. Rev Surv Ophthalmol 28:621–642
4. Elsas FJ, Maumenee IH, Kenyon KR, Yoder F (1977) Familial aniridia with preserved ocular function. Am J Ophthalmol 83(5):718–724
5. Layman PR, Anderson DR, Flynn JT (1974) Frequent occurrence of hypoplastic optic disks in patients with aniridia. Am J Ophthalmol 77(4):513–516
6. Traboulsi E (1998) Genetic disease of the eye. Oxford University Press, New York
7. Netland PA, Scott ML, Boyle JW 4th, Lauderdale JD (2011) Ocular and systemic findings in a survey of aniridia subjects. J AAPOS 15(6):562–566
8. Bremond-Gignac D, Gerard-Blanluet M, Copin H, Bitoun P, Baumann C, Crolla JA, Benzacken B, Verloes A (2005) Three patients with hallucalpolydactyly and WAGR syndrome, including discordant expression of Wilmstumor in MZ twins. Am J MedGenet A 134(4):422–425
9. Bremond-Gignac D, Crolla JA, Copin H, Guichet A, Bonneau D, Taine L, Lacombe D, Baumann C, Benzacken B, Verloes A (2005) Combination of WAGR and Potocki-Shaffer contiguous deletion syndromes in a patient with an 11p11.2-p14 deletion. Eur J Hum Genet 13:409–413
10. Jastaneiah S, AL-Rajhi AA (2005) Association of aniridia and dry eyes. Ophthalmology 112(9):1535–1540
11. Brandt JD, Casuso LA, Budenz DL (2004) Markedly increased central corneal thickness: an unrecognized finding in congenital aniridia. Am J Ophthalmol 137:348–350
12. Schneider S, Osher RH, Burk SE, Lutz TB, Montione R (2003) Thinning of the anterior capsule associated with congenital aniridia. J Cataract Refract Surg 29(3):523–525
13. Tsai JH, Freeman JM, Chan CC, Schwartz GS, Derby EA, Petersen MR, Holland EJ (2005) A progressive anterior fibrosis syndrome in patients with postsurgical congenital aniridia. Am J Ophthalmol 140(6):1075–1079
14. Neuhann IM, Neuhann TF (2010) Cataract surgery and aniridia. Curr Opin Ophthalmol 21(1):60–64
15. Reinhard T, Engelhardt S, Sundmacher R (2000) Black diaphragm aniridia intraocular lens for congenital aniridia: long-term follow-up. J Cataract Refract Surg 26(3):375–381
16. Aslam SA, Wong SC, Ficker LA, Maclaren RE (2008) Implantation of the black diaphragm intraocular lens in congenital and traumatic aniridia. Ophthalmology 115(10):1705–1712

Chapter 6
The Ocular Surface in Aniridia

Paolo Rama, Maurizia Viganò, and Karl Anders Knutsson

Abstract In aniridia, ocular surface alterations arise after several years, in distinction to congenital anomalies such as absence of the iris and cataract. In patients with aniridia, the cornea is transparent at birth and gradually loses transparency from 18 to 20 years of age due to the formation of a superficial vascular pannus determined by limbal stem cell deficiency. It is currently not clear whether this process is due to congenital anomalies of the limbal stem cells or to alterations of their regulation. In its early stages, limbal stem cell deficit usually causes problems related to the corneal epithelium such as: recurrent erosions and persistent epithelial defects leading to reduced visual acuity, pain and photophobia. In the following stages, with the absence of corneal epithelium, the ocular surface is covered by conjunctiva-derived epithelium. The conjunctival epithelium determines chronic inflammation that induces symptoms such as burning sensation and photophobia. In the later stages, the conjunctival epithelium may completely cover the cornea causing severe visual impairment. In the initial stages, treatment is focused on ocular surface lubrication. When corneal opacity is present, different treatments such as keratoplasty, keratoprosthesis, allogenic limbal stem cell transplantation and transplantation of oral mucosa epithelium autologous stem cells have been experimented.

Keywords Aniridia • Limbal stem cell deficiency • Ocular surface • Limbal stem cell transplantation • Corneal transplantation

Ocular Surface

The ocular surface is a complex functional unit comprising the conjunctiva, the limbus, the cornea, the tear film, nervous system and loco-regional immune system [1]. All of these components of the system guarantee an equilibrium which is fundamental for maintaining corneal transparency.

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The conjunctiva is a mucous membrane that covers the internal surface of the lids and extends to the superficial part of the eye towards the limbus. Its principal functions are the production of the mucous layer of the tear film and protection of the ocular surface through the immune system and antibacterial/antiviral actions [2].

The sclerocorneal limbus is the transition zone between the cornea and bulbar conjunctiva. Clinical and experimental research suggests that the basal cells of the limbal epithelium are the stem cells of the corneal epithelium [3–5]. The main characteristic of a stem cell is the capacity to divide asymmetrically: a daughter cell remains stem cell while the second undertakes a path of irreversible differentiation. The latter cells are denominated transit amplifying cells and are characterized by a great proliferative capacity capable of providing a high number of epithelial cells necessary for the constant renewal of the corneal epithelium and restoring cell loss in case of trauma [6–9].

The cornea is a transparent, avascular lamina in continuity with the surrounding sclera. It is composed of five layers, which from the outside to the inside are: epithelium, Bowman's membrane, stroma, Descemet's membrane and endothelium. The main role of the cornea is to function as a positive lens in order to focus rays of light onto the retina together with the help of the crystalline lens. This function is guaranteed by maintenance of transparency [10].

The cornea is the most innervated tissue in the organism. The nerve endings are composed of sensitive myelinated fibers from the first branch of the trigeminal nerve. These give rise to a high density network of nociceptors which explains the sensitivity of the cornea to external stimuli. The corneal sensorial innervation composes the afferent part of the two arch reflexes of lacrimation and blinking. The trigeminal fibers also have a trophic role towards the corneal epithelium, stimulating mitosis and favoring repair processes [11].

Alterations of the Ocular Surface in Aniridia

In aniridia, ocular surface alterations arise after several years, in contrast to congenital anomalies such as absence of the iris and cataract [9, 11–17].

Cornea and Limbus

In patients affected by aniridia, the cornea is transparent at birth and gradually loses transparency from 18 to 20 years of age due to the formation of a superficial vascular pannus. Some studies have hypothesized that the invasion of this neovascular tissue is caused by limbal stem cell deficit [17, 18]. It is currently not clear whether this process is due to congenital anomalies of the limbal stem cells, their reduction in number or to alterations of their regulation. Understanding these mechanisms is fundamental to plan new therapeutic strategies.

In its early stages, limbal stem cell deficit usually determines problems related to the corneal epithelium such as: recurrent erosions, persistent epithelial defects, opacity and fibrosis leading to reduced visual acuity, pain and photophobia [19, 20].

In the following stages, with the absence of corneal epithelium, the ocular surface is covered by epithelium deriving from the conjunctiva: this process is known as "corneal conjunctivalization." [13]. The conjunctival epithelium determines chronic inflammation capable of inducing symptoms such as foreign body sensation, burning sensation and photophobia. In the later stages, the conjunctival epithelium may completely cover the cornea causing severe visual impairment.

Persistent inflammation leads to the formation of white-gray nodular lesions resembling Salzmann nodular degeneration which are initially located in the corneal periphery in a ring-like pattern and later in the central cornea [21]. After years of ocular surface inflammation, a corneal pannus is formed determining central stromal scarring and neovascularization [22, 23]. Increased corneal thickness is a typical structural element in patients affected by aniridia observed even before the onset of edema. Some authors have reported average corneal thicknesses of 631 μm in a series of 32 eyes of 17 patients, about 100 μm thicker than normal corneas [21, 24].

Diagnosis

Limbal stem cell deficit may present several clinical characteristics. In the initial phase, recurrent or persistent epithelial defects determine pain and photophobia. In the late stages, corneal ulcers may be observed until a vascularized corneal pannus is formed; stabilizing the situation but strongly compromising visual acuity. Fluorescein staining can be used to distinguish corneal epithelium, which when intact is impermeable to the dye, from conjunctival epithelium which is more permeable. The epithelium can be analyzed with impression cytology, which allows distinguishing of corneal and conjunctival epithelium in a more precise manner [16]. This test is rapid, easy to perform and consents identification of the epithelium with specific colorations [25–28]. Impression cytology is however relatively invasive and often determines an area of epithelial defect which may be painful for patients and may take even weeks to heal in eyes affected by aniridia. It is therefore a diagnostic tool that must be utilized only in selected cases in order to respond to a specific doubt or question. Confocal microscopy has been recently used for diagnosis of limbal stem cell deficit. It is less invasive than impression cytology and may in the future consent to quantify damage to limbal stem cells, follow the progression of disease and evaluate the effectiveness of medical or surgical treatment [21, 29–31].

The tear film can be altered in aniridia. While the aqueous component of the tear film seems unaffected, some studies implicate a possible alteration of the mucous and lipid layers. Two studies suggest that the cause of dry eye in aniridia is due to the deficit of the mucous component with reduction of conjunctival goblet cells, while other studies observe an increase of these cells [12, 32]. Contradictory results

can also be found in relation to the lipid layer component as one study [32] indicates mild blepharitis which is not implicated in tear film alterations while another study [12] highlighted a stenosis and atrophy of the meibomian gland orifices in 77.8 % of patients. Further research has hypothesized that dystrophic epithelium, secondary to the limbal deficit, may alter the correct adhesion and distribution of the tear film on the corneal surface determining an analogous situation to dry eye disease [12, 32].

Recommendations

- Slit lamp examination is of paramount importance for diagnosis of the corneal manifestations of aniridia.
- If the cornea is opaque, anterior segment optical coherence tomography may be useful for determining the precise depth of opacity and permits examination of the remaining structures of the anterior chamber.
- To diagnose limbal stem cell deficiency, clinical slit lamp examination is fundamental.
- Impression cytology is a more specific test utilized to better characterize the severity of limbal stem cell deficiency. It is however more invasive than slit lamp examination and may cause epithelial defects which in these patients do not resolve promptly as in normal subjects.
- Confocal microscopy is a less invasive test compared to impression cytology and may have an increasing role in the future as it permits quantification of limbal stem cell deficit, progression of disease and response to medical and surgical treatments.

Treatment of Ocular Surface Alterations in Aniridia

Ideally, the treatment for aniridia lies in the correction of the genetic defect associated to the pathology; however the true perspectives of this approach are currently unknown and current treatments target the manifestations of the disease.

In case of dry eye, topical lubricant drops or gels may be used; preservative free drops are encouraged. During night time, the use of ointments can prolong the effect of lubrication. Autologous serum drops have also been utilized as they have a composition similar to natural tear film [14, 33]. However, these drops require a difficult preparatory phase, may potentially become contaminated and are generally used in cases non responsive to artificial lubricants. Lastly, scleral contact lenses may provide benefits as they maintain a small quantity of fluid between the ocular surface and contact lens.

In case of recurrent epithelial defects, maximal topical lubricant therapy is encouraged and soft contact lenses may help alleviate symptoms, protect the corneal surface and aid re-epithelization. Eye lid closure is usually not considered and is up to the patient as there is no proven benefit in accelerating re-epithelization. Amniotic membrane grafting can be used in cases non responsive to therapy and is able to reduce pain and promote epithelial closure. The tissue is immunologically inert and is characterized by anti-inflammatory, bacteriostatic and anti-angiogenic properties which stimulate the growth of healthy epithelium [34, 35]. In cases of corneal opacity, corneal transplantation with lamellar or penetrating techniques is usually not successful because the graft epithelium must be gradually replaced by the recipient epithelium. If the recipient corneal limbus presents alterations, it will not be able to provide healthy epithelium, but conjunctival epithelium will migrate forming a vascularized pannus over the corneal graft. For these reasons, corneal transplantation is generally contraindicated, except for very few selected cases [36].

Limbal stem cells harvested from living related donors or cadaver eyes have been experimented in patients affected by aniridia. Even though initial results were encouraging, long term results show that repeated episodes of rejection determine failure causing the reformation of corneal pannus [18, 37, 38]. This observation is confirmed by other studies demonstrating that after allogenic limbal stem cell transplantation it is not possible to isolate donor epithelial cells in the recipient cornea several years after treatment [39, 40].

Recent studies are focusing on transplantation of oral mucosa epithelium autologous stem cells [15]. It is too early to hypothesize whether this technique can be successful in guaranteeing a transparent cornea.

Current research is focusing on the concept of creating a synthetic cornea. Currently, osteo-odonto-keratoprosthesis is the only alternative to corneal transplantation in cases of total bilateral limbal deficiency without adequate tear production. This reconstructive technique was developed in the 1960s and utilizes a tooth lamina to create a biological support as an alternative to the cornea, with less risk of extrusion compared to synthetic prostheses [41–43]. Other kerathoprostheses, such as the Boston KPro, show good short term results but may give rise to complications after many years and must be evaluated carefully beforehand [44–49].

Recommendations

- In cases of dry eye, topical lubricant drops or gels may be used; especially preservative free preparations.
- During night time, ointments can offer longer term protection.
- In more severe cases, autologous serum may be used, keeping in mind the potential risks related to contamination and infection.

- Scleral contact lenses can offer advantages by protecting the ocular surface by maintaining a small quantity of fluid between the lens and ocular surface.
- In case of epithelial defects, maximum lubricant therapy together with therapeutic soft contact lens application should be encouraged.
- In cases of a persistent epithelial defect, a soft therapeutic contact lens can be kept in place until the epithelial defect resolves. The lens should be replaced every 2–3 weeks. In resistant cases, amniotic membrane graft can be successful in reducing symptoms and epithelial healing.
- When the cornea is opaque and a corneal vascularized pannus is present:
  - Corneal transplantation is usually contraindicated (both lamellar and penetrating types).
  - Limbal stem cell grafts from living-related or cadaver donors have limited duration.
  - Complete results from studies investigating the possibility of transplanting epithelium from other districts (such as oral mucosa) are currently unavailable.
  - Considering the development of artificial corneas, osteo-odonto-keratoprosthesis gives the best long term results but is a very complex procedure adequate for few selected patients.
  - Other keratoprostheses, such as Boston KPro, have shown good short-term results but are often associated with complications in the long-term and thus must be carefully evaluated.
- In cases of loss of corneal transparency without pannus formation, corneal transplantation may be considered. In these cases lamellar keratoplasty is the preferred technique.

References

1. Tsubota K, Tseng SCG, Nordlund ML (2002) Anatomy and physiology of the ocular surface. In: Holland EJ (ed) Ocular surface disease: medical and surgical management. Springer-Verlag, New York
2. Gipson IL (1994) Anatomy of the conjunctiva, cornea and limbus. In: Smolin G, Thoft RA (eds) The cornea, 3rd edn. Little, Brown and Company publisher, Boston
3. Schermer A, Galvin S, Sun TT (1986) Differentiation related expression of a major corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. J Cell Biol 103:49–62
4. Cotsarelis G, Cheng SZ et al (1989) Existence of slowcycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. Cell 57:201–209
5. Pellegrini G, Golisano O et al (1999) Location and clonal analysis of stem cells and their differentiated progeny in the human ocular surface. J Cell Biol 145:769–782
6. Barrandon Y (1993) The epidermal stem cell: an overview. Dev Biol 4:209–215
7. Lavker RM, Miller S et al (1993) Hair follicle stem cells: their location, role in hair cycle, and involvement in skin tumor formation. J Invest Dermatol 101:16–26

8. Morrison SJ, Shah NM, Anderson DJ (1997) Regulatory mechanisms in stem cell biology. Cell 88:287–298
9. Miller SJ, Lavker RM, Sun TT (1997) Keratinocyte stem cells of cornea, skin, and hair follicle. In: Potten C (ed) Stem cells. Academic, New York
10. Rieger G (1992) The importance of the precorneal tear film for the quality of optical imaging. Br J Ophthalmol 76:157–158
11. Muller LJ, Marfurt CF et al (2003) Corneal nerves: structure, contents and function. Exp Eye Res 76:521–542
12. Jastaneiah S, Al Rajhi AA (2005) Association of aniridia and dry eyes. Ophthalmology 112:1535–1540
13. Kruse FE (2002) Classification of ocular surface disease. In: Holland EJ, Mannis MJ (eds) Ocular surface disease: medical and surgical management. Springer-Verlag, New York
14. Lopez-Garcia JS, Rivas L et al (2008) Autologous serum eyedrops in the treatment of aniridic keratopathy. Ophthalmology 115:262–267
15. Nakamura T, Inatomi T et al (2007) Phenotypic investigation of human eyes with transplanted autologous cultivated oral mucosal epithelial sheets for severe ocular surface diseases. Ophthalmology 114:1080–1088
16. Nelson LB, Spaeth GL et al (1984) Aniridia: a review. Surv Ophthalmol 28:621–642
17. Nishida K, Kinoshita S et al (1995) Ocular surface abnormalities in Aniridia. Am J Ophthalmol 120:368–375
18. Tseng SC (1989) Concept and application of limbal stem cells. Eye 3:141–157
19. Dua HS, Gomes JA et al (2004) The amniotic membrane in ophthalmology. Surv Ophthalmol 49:51–77
20. Gatinel D, Hoang-Xuan (2000) Le déficit en cellules souches limbiques. J Fr Ophtalmol 23:718–728
21. Edén U, Fagerholm P et al (2012) Pathologic epithelial and anterior corneal nerve morphology in early-stage congenital aniridic keratopathy. Ophthalmology 119:1803–1810
22. Collinson JM, Chanas SA et al (2004) Corneal development, limbal stem cell function, and corneal epithelial cell migration in the Pax6 (+/−) mouse. Invest Ophthalmol Vis Sci 45:1101–1108
23. Holland EJ, Djalilian AR, Scwartz GS (2003) Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. Ophthalmology 110: 125–130
24. Brandt JD, Casuso LA, Budenz DL (2004) Markedly increased central corneal thickness and unrecognized finding in congenital Aniridia. Am J Ophthalmol 137:348–350
25. Elder MJ, Hiscott P, Dart JK (1997) Intermediate filament expression by normal and diseased human corneal epithelium. Hum Pathol 28:1348–1354
26. Donisi PM, Rama P et al (2003) Analysis of limbal stem cell deficiency by corneal impression cytology. Cornea 22:533–538
27. Espana EM, Di Pascuale MA et al (2004) Characterization of corneal pannus removed from patients with total limbal stem cell deficiency. Invest Ophthalmol Vis Sci 45:2961–2966
28. Singh R, Joseph A et al (2005) Impression cytology of the ocular surface. Br J Ophthalmol 89:1655–1659
29. Patel DV, Sherwin T, McGhee CN (2006) Laser scanning in vivo confocal microscopy of the normal human corneoscleral limbus. Invest Ophthalmol Vis Sci 47:2823–2827
30. Shortt AJ, Secker GA et al (2008) Ex vivo expansion and transplantation of limbal epithelial stem cells. Ophthalmology 115:1989–1997
31. Miri A, Alomar T et al (2012) In vivo confocal microscopic findings in patients with limbal stem cell deficiency. Br J Ophthalmol 96:523–529
32. Rivas R, Murube J (2003) Impression cytology study of dry eyes in patients with congenital aniridia. Arch Soc Esp Oftalmol 78:615–622
33. Quinto GG, Campos M, Behrens A (2008) Autologous serum for ocular surface diseases. Arq Bras Oftalmol 71:47–54

34. Dua HS, Azuara-Blanco A (2000) Limbal stem cells of the corneal epithelium. Surv Ophthalmol 44:415–425
35. Grueterich M, Tseng SC (2003) Ex vivo expansion of limbal epithelial stem cell: amniotic membrane serving as a stem cell niche. Surv Ophthalmol 48:631
36. Tiller AM, Odenthal MT et al (2003) The influence of keratoplasty on visual prognosis in aniridia: a historical review of one large family. Cornea 22:105–110
37. Holland EJ, Schwartz GS (1996) The evolution of epithelial transplantation for severe ocular surface disease and proposed classification system. Cornea 15:549–556
38. Daya SM, Watson A et al (2005) Outcomes and DNA analysis of ex vivo expanded stem cell allograft for ocular surface reconstruction. Ophthalmology 112:470–477
39. Henderson TR, Coster DJ, William KA (2001) The long term outcome of limbal allografts: the search for surviving cells. Br J Ophthalmol 85:604–609
40. Sharpe JR, Daya SM et al (2007) Survival of cultured allogeneic limbal epithelial cells following corneal repair. Tissue Eng 13:123–132
41. Strampelli B (1963) Osteo-odontokeratoprosthesis. Ann Ottalmol Clin Ocul 89:1039–1044
42. Falcinelli G, Falsini B et al (2005) Modified osteoodonto- keratoprosthesis for treatment of corneal blindness: long-term anatomical and functional outcomes in 181 cases. Arch Ophthalmol 123:1319–1329
43. Chammartin M, Goldblum D et al (2009) Case report of osteo-odonto keratoprosthesis (Strampelli) and of Dacron keratoprosthesis (Pintucci). Klin Monatsbl Augenheilkd 226:180–183
44. Bakhtiari P, Chan C et al (2012) Surgical and visual outcomes of the type I Boston Keratoprosthesis for the management of aniridic fibrosis syndrome in congenital aniridia. Am J Ophthalmol 153:967–971
45. Chan CC, Holland EJ (2012) Infectious keratitis after Boston type 1 keratoprosthesis implantation. Cornea 31:1128–1134
46. Kang JJ, de la Cruz J, Cortina MS (2012) Visual outcomes of Boston keratoprosthesis implantation as the primary penetrating corneal procedure. Cornea 31:1436–1440
47. Rudnisky CJ, Belin MW et al (2012) Risk factors for the development of retroprosthetic membranes with Boston keratoprosthesis type 1: multicenter study results. Ophthalmology 119:951–955
48. Colby KA, Koo EB (2011) Expanding indications for the Boston keratoprosthesis. Curr Opin Ophthalmol 22:267–273
49. Robert MC, Harissi-Dagher M (2011) Boston type 1 keratoprosthesis: the CHUM experience. Can J Ophthalmol 46:164–168

Chapter 7
Aniridic Keratopathy: Conservative Approaches

José Santiago López García and Isabel García Lozano

Abstract Aniridia is a panocular disorder that involves many structures of the eye. Aniridic keratopathy is caused by a primary dysfunction of the limbal stem cells, probably by a limbal microenvironment alteration caused by the PAX6 gene mutation. Keratopathy, together with cataract and glaucoma, are the main causes of progressive visual loss in patients with aniridia, and it represents the main source of non visual symptoms in these patients. It is very important to classify the keratopathy in order to plan the therapeutic management. Similar to others patients with limbal deficiency, the treatment should be focused on repopulating the sclerocorneal limbus of limbal stem cells and/or on restoring the microenvironment surrounding them in order to ensure the expansion and survival of the epithelial cells. The therapeutic management will depend on the degree of ocular surface involvement: In patients with sub-clinical or slight limbal deficiency, the treatment with preservative-free lubricants could be sufficient. In patients with moderate keratopathy, the treatment with autologuos serum or amniotic membrane transplantation may be a useful (although temporary) measure to enhance the survival and expansion of limbal stem cells. Finally, patients with severe keratopathy need a source of limbal stem cell.

Keywords Aniridic keratopathy • Autologuos serum eyedrops • Amniotic membrane transplantation • Limbal dysfunction

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Introduction

Aniridia is an uncommon congenital bilateral disease affecting 1 in 65,000–90,000 live births and is caused by mutation of the PAX6 gene [1]. It is a panocular disorder that involves many structures of the eye such as cornea, anterior chamber, lens, retina and optic nerve [2]. Congenital aniridia can be familiar or sporadic. Familiar aniridia use to be autosomal dominant with incomplete penetrance and expressivity. Sporadic aniridia is frequently associated with WARG syndrome (Wilms' tumor, aniridia, genitourinary abnormalities and mental retardation) [3]. Clinical findings in aniridia include photophobia and epiphora, decreased vision, foveal, optic nerve and iris hypoplasia, strabismus, nystagmus, amblyopia, glaucoma and lens abnormalities such as cataracts or lens subluxation [4].

Aniridic keratopathy (AK) occurs in 20–90 % of patients [3, 5]. Corneal changes include recurrent erosions and ulcerations of corneal epithelium, tear film instability, dry eye, chronic pain, corneal vascularization, progressive corneal opacification, and blindness [6]. AK is caused by a primary dysfunction of the limbal stem cells, probably by a limbal microenvironment alteration caused by the PAX6 gene mutation [7].

Pathogenic Bases in Aniridia Keratopathy

Although AK has been traditionally attributed to limbal stem cell deficiency [8], current evidence based on clinical observations and animal models of aniridia, suggest that the proliferative potential of limbal stem cells may not initially be affected, and this corneal alteration may be related to an abnormality in the limbal stem cell microenvironment [9]. Mutations in the PAX6 gene have been identified in a high proportion of patients with aniridia. Normal expression of the PAX6 gene is necessary for the normal development of the eye. This gene plays an important role in the epithelial cell proliferation, migration and differentiation [10]. The PAX6 gene is essential for the cytokeratin-12 expression; a cytoskeleton protein restricted to corneal epithelium and directly regulated by PAX6. Reduced levels of cytokeratin 12 and 5 were found in PAX6 mutation. Keratins constitute the intermediate filaments of the epithelial cytoeskeleton and their alteration is associated with epithelial cell fragility and disorders. These cytokeratins perform a vital role in cell-to-cell binding. PAX6 is essential for the expression of cell adhesion molecules like as desmoglein and α and β catenin. These molecules are responsible for the maintenance of cytoskeletal architecture, desmosome assembly, microtubule organization, ability of cells to migrate in wound healing and reinforcement of membrane attachments [11–14]. These alterations cause a fragile corneal epithelium that clinically is manifested by epithelial erosion and persistent epithelial defects. On the other hand, the PAX6 gene also contributes to the metabolism of extracellular matrix. The matrix degradation is mediated by a group of enzymes known as matrix metalloproteinases (MMP) [15]. The PAX 6 regulates the MMP9 or Gelatinase-B

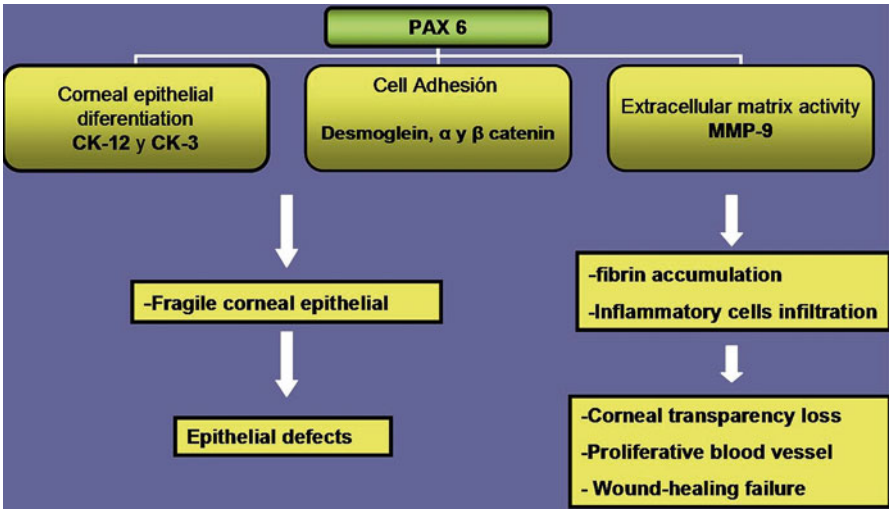

Fig. 7.1 Pathogenic bases in aniridic keratopathy

expression in the cornea [16]. This enzyme is crucial in wound-healing extracellular matrix remodelation. The Gel-B absence causes a fibrin accumulation and inflammatory cell infiltration that clinically is manifested by loss of corneal transparency and proliferative blood vessel stimulus. This situation generates a chronic wounded state (Fig. 7.1). Moreover, the pattern of corneal innervations is modulated by PAX6 and the corneal nerves play an essential role in the maintenance of ocular surface through provision of neurotrophic support [17].

Corneal Involvement in Aniridia Keratopathy

AK is clinically manifested as a primary limbal stem cell deficiency. Keratopathy, together with cataract and glaucoma, are the main causes of progressive visual loss in patients with aniridia, and it represents the main source of non visual symptoms in these patients.

The natural course of AK presents several stages of progression. Signs of keratopathy appear in the first decade of life with thickening of the peripheral corneal epithelium and without clinical manifestation. In the second decade, the patients manifest red eye and chronic irritation, and show a thin and superficial vascularization in the peripheral cornea that gradually advances into the central cornea. It is common the pain, photophobia and recurrent corneal epithelial erosions. In later stages, the keratopathy progress until the whole cornea is involved with a large increase in central corneal thickness. The central cornea is affected and the subepithelial infiltrates, stromal opacifications and vascularization cause a significant visual loss (Fig. 7.2).

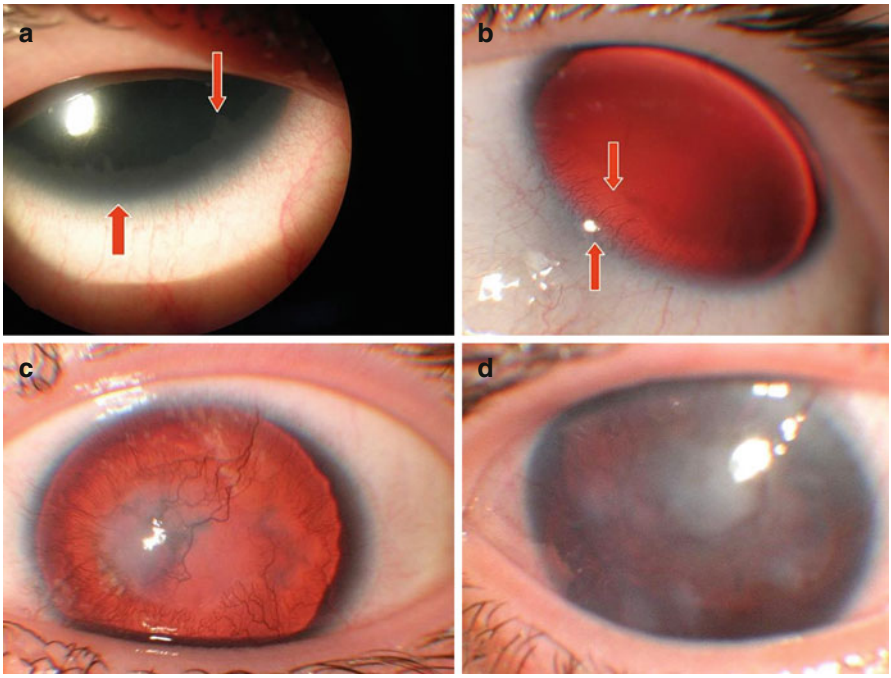

Fig. 7.2 Natural course of aniridic keratopathy. Thickening of the peripheral corneal epithelium in the first decade of life (**a**). Thin and superficial vascularization in the peripheral cornea nn the second decade (**b**) that gradually advances into the central cornea (**c**). In later stages (**d**), the keratopathy progress until the whole cornea is affected and the subepithelial infiltrates, stromal opacifications and vascularization cause a significant visual loss

Aniridic Keratopathy Classification

It is very important to classify the keratopathy in order to plan the therapeutic management of these patients. There are some classifications as Mackman [18], but we use a classification based in signs, symptoms and the severity of squamous metaplasia determined by impression cytology as previously we published [19, 20]. According to the severity of limbal deficiency, the patients are splitted into four levels or stages of development. A patient is considered to show slight Limbal Stem Cell Deficiency (LSCD) (grade 1) when he/she relates two or less episodes of corneal erosion within the last 6 months. These patients show a pannus less than 1 mm from the limbal area and abnormalities in the absorption of fluorescein, with minimal photophobia or epiphora. A patient is considered to show moderate LSCD (grade 2) when he/she refers three or more episodes of corneal erosion or persistent epithelial defects during the last 6 months. In these patients, the vascular pannus, with or without sub-epithelial fibrous tissue, involves under half of the corneal periphery and the instability of the lacrimal layer, photophobia, epiphora and red eye are common. A patient is considered to exhibit severe LSCD (grade 3) when the

central cornea is affected by neovascular pannus and stromal opacity. Epiphora, photophobia and red eyes are constant, as well as a loss of vision and corneal erosion. In the grade 0 or subclinical LSCD, we include eyes with etiological processes related to limbal deficiency who do not express associated clinical signs, although, they show a grade 1–2 of squamous metaplasia.

Conservative Management in Aniridic Keratopathy

In the past, the approach to AK was based on supporting treatment with topical lubricants, therapeutic contact lenses or tharsorraphy. When the patients developed severe corneal opacity, penetrating keratoplasty was adopted with very negative results due to recurrence of pre-surgical corneal alteration [21]. Similar to others patients with limbal deficiency, the treatment of these patients should be focused on repopulating the sclerocorneal limbus of limbal stem cells and/or on restoring the microenvironment surrounding them in order to ensure the expansion and survival of the epithelial cells. Although the progress in the understanding of the underlying mechanism of AK in the last years has allowed a more adequate therapeutic approach, we have not an effective treatment and many of the conventional therapeutic strategies only yield a temporary improvement.

The therapeutic management of these patients will depend on the degree of ocular surface involvement:

Slight or Subclinic Keratopathy

In patients exhibiting sub-clinical or slight limbal deficiency (grade 0 and 1), the treatment with preservative-free lubricants could be sufficient. Tear film disorder and dry eye syndrome have been reported in relation with congenital aniridia [4, 22]. In these patients the aqueous layer produced by lacrimal glands does not change as demonstrated by the normal Shirmer's test in all patients but the most severe eyes [4]. The cause of dry eye in aniridia is related to poor tear film quality produced by the lipid layer dysfunction and the corneal epithelial disorders. The lipid layer alteration is caused by meibomian gland dysfunctions, with stenosed atrophic meibomian orifices, that change the lipid layer and facilitate tear evaporation. The dry eye severity is related with the keratopathy grade.

Although there are many tear drops in the market, we prefer to use tears of sodium hyaluronate. Preliminary studies have demonstrated that artificial tears of sodium hyaluronate exhibits rheological characteristics and an adherence to epithelium capacity higher than others viscosizing agents. Hyaluronic acid is a long but flexible molecule which behaves as a non-Newtonian fluid, i.e., its viscosity depends on the degree of movement. This pseudo-plasticity makes sodium hyaluronate solutions more comfortable for the eye and at the same time increasing

the adhesion to the corneal epithelium and the persistence time on the ocular surface [23]. The sodium hyaluronate solution adheres very well to the mucin fraction of the pre-corneal tear due to its mucoadhesive properties which, together with its water-retaining capacity, make it perform as a stable complex on the ocular surface. This covering capacity has a protective effect which combined with a direct effect, regulated by CD44 receptors, on cell migration and proliferation, enhancing the cicatrization processes [24]. These receptors are expressed at the corneal and conjunctival level and participate in many cell processes and functions [25].

The aim with these patients is to improve their symptoms such as protection against sunlight by means of dark glasses, or preferences for humid environments. The corneal erosions should be treated just like any other patients by occlusion and topical antibiotics. In patients with slight keratopathy, we have tried treatment cycles with autologous serum and have found a subjective and objective improvement in the reduction of corneal erosion.

Moderate Keratopathy

In patients with moderate keratopathy (grade 2), the treatment with artificial tears is not enough. In these patients, autologuos serum (AS) or amniotic membrane transplantation (AMT) may be a useful (although temporary) measure to enhance the survival and expansion of limbal stem cells.

The treatment with AS has proven to be an efficient method for stimulating the stability of corneal and epithelial cells by supplying a number of Growth Factors (GF) which are scarce due to ocular dryness associated to the majority of processes coursing with epithelization disorders. In patients with dry eye, AS provides some epitheliotrophic factors such as EGF, βFGF, vitamin A, fibronectin, α2 macroglobulin, and neural growth factors that ease the proliferation, migration and adhesion of epithelial corneal cells. Furthermore, facilitates the mucin expression and this may contribute to the beneficial effects in patients with dry eye. They are by nature non-allergenic and their biochemical and biomechanical properties are similar to normal tears [26, 27].

Because AK is caused by a primary dysfunction of the limbal stem cells microenvironment [7], the epitheliotrophic factors presented in AS can help to treat the corneal changes that occur in these patients by a stem cell niche improvement. AS significantly improves Schirmer and BUT levels in patients with aniridia. The epithelial surface development, a better mucin expression and an improvement in meibomian dysfunction after AS treatment improve tear stability and, therefore, BUT levels [27].

The treatment with AS greatly helps corneal epithelialization. Recurrent erosions are frequent complications in patients with AK caused by a defective adhesion of the basal epithelial layers to the underlying basement membrane. Clinically, it is manifested by repeated episodes of irritation, pain, epiphora and ocular hyperemia. A decrease in the erosion recurrence rate has been reported in patients with slight and moderate AK treated with AS [27].

Conjunctival goblet cell hyperplasia in aniridia was first described by Seefelder in 1909 [28], and later by Jastaneiah and Al-rajhi [4]. However, other authors found a decrease in the conjunctival goblet cells by impression cytology [22].

The presence of goblet cells on the corneal surface is considered to provide clinical evidence of limbal stem cell deficiency [8]. We can find goblet cells in corneal impression cytology in patients with moderate and severe limbal deficiency. AS improves significantly the epithelial squamous metaplasia in all patients. These findings and the better tear stability resulted in all the patients showing a subjective clinical improvement after AS therapy in comparison with the prior treatment with artificial tears.

In conclusion, AS has biochemical and biomechanical properties similar to normal tears, it is non allergenic and has antimicrobial and optic properties. It contains epitheliotrophic factors that are thought to be responsible for the therapeutic effect over ocular surface disorders. AS improves the AK in all patients but especially in patients with slight or moderate severity. In these patients, AS was superior to conventional therapy with substitute tears for improving ocular surface and subjective comfort. This treatment is recommended alone or combined with other tear substitutes in patients with slight or moderate severity. In patients with severe keratopathy, the serum can be used in addition to a limbal transplantation.

However, the use of these eyedrops involves drawbacks which require a new approach in order to optimize the therapy and diminish the need of drops administrations and the frequency of blood extractions required by patients in ongoing chronic treatment [6]. A greater knowledge of the active Growth Factors (GF) present in autologous serum as well as of their behavior under different circumstances would help to develop more efficient preparations.

Conventional dilution of AS with saline solution requires that the patient must administer drops every 2–3 h due to the short duration of the physiological effect on the ocular surface. This entails a significant problem for patients at their work or productive activity as the eye drops must be kept in a refrigerator to maintain the activity of the epitheliotrophic factors and to reduce the contamination risk caused by the lack of preservatives. The idea of utilizing other viscosizing agents as vehicles for the serum to increase their duration and effect on the ocular surface and diminish the number of applications is highly attractive, although not all tear substitutes with these characteristics are useful in clinical practice.

Previously, we have reported as the use of sodium hyaluronate for the dilution of serum and the use of containers with an adapted filter, optimize the therapy with AS [29, 30]. The hyaluronate molecule is mainly hydrophilic and displays a strong affinity with water, although it also exhibits non-polar areas that facilitate bonding with lipids. The polar and non-polar areas repel each other causing the molecule to expand and occupy a large three-dimensional space in the form of a flexible ball of yarn. This space or "domain" has great importance in its physiological behavior. Small molecules such as water, electrolytes and nutrients are able to diffuse freely within this domain, but larger molecules such as proteins or GF exhibit slower diffusion. This property of sodium hyaluronate renders it very useful as a vehicle for epitheliotrophic factors present in AS, extending the contact of GF over the ocular surface, increasing their effect and reducing the frequency of drop instillations

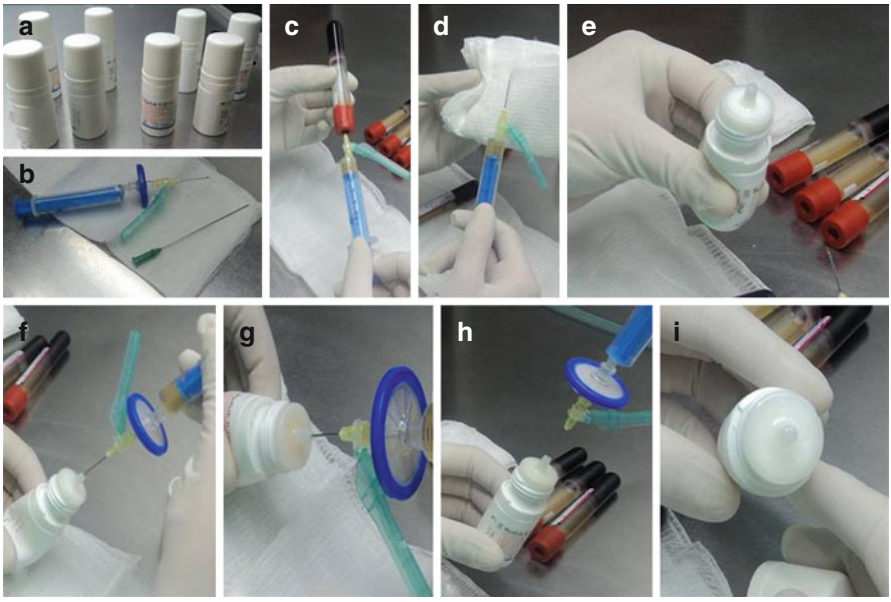

Fig. 7.3 Preparation of eyedrops in containers with filter. Hyabak containers (**a**). 20G intravenous needle and rounded tip, syringes and Millipore filter (**b**). Extraction of 1.25 ml of serum (**c** and **d**). To avoid any reflux after introducing the serum in the container, slight pressure is exerted on the container to remove air and generate negative pressure that facilitates the entry of the serum (**e**). (**f**) The intravenus needle or the rounded tip cannula is introduced about 5–6 mm into the dispensing tip, taking care not to perforate the membrane and introducing the serum very slowly, observing how the filter and the dispensing tip become yellowish due to the presence of the serum (**g**). When the serum has been fully introduced, the needle is slowly withdrawn (**h**). The negative pressure inside the container facilitates the entry of air which in turn causes the introduction of the serum remaining in the dispensing tip and filter inside the container, without any serum remaining within the tip which exhibits its usual whitish appearance (**i**)

compared to conventional AS diluted with saline solution [30]. The AS diluted with sodium hyaluronate is better tolerated by the patients, and its effect on tear stability, fluorescein and rose Bengal staining, BUT and squamous metaplasia of the corneal and conjunctival are significantly higher than those obtained with the preparations diluted with saline solution [30]. This eyedrops can be instilled every 4–6 h according to the severity of the baseline pathology. This posology is compatible with an active and productive lifestyles and it allows most patients to apply their eye drops at home without having to go to great lengths to maintain the samples at a cool temperature. On the other hand, the use of containers with an adapted filter significantly reduces the contamination rates. This way extending the use of such container by patients for up to 4 weeks without virtually any contamination risks [29] (Fig. 7.3). Both procedures improve the lifespan of these eyedrops while reducing the amount of serum required for the treatment. This feature can be used to decrease the blood needed to prepare the eyedrops or to reduce the frequency of blood collection as well as to prolong the use of these containers longer.

In our routine clinical practice, 40 cc of collected blood allow us to obtain between 18 and 20 cc of serum that allows us to prepare six to seven containers (10 cc) with 20 % AS diluted with sodium hyaluronate. If we use a drop every 4–6 h and if the container can be used for a month, we may treat a patient for 6–7 months with just one single blood collection.

References

1. Jordan T, Hanson I, Zaletayev D et al (1992) The human PAX6 gene is mutated in two patients with aniridia. Nat Genet 1:328–332
2. Nelson LB, Spaeth GL, Nowinski TS et al (1984) Aniridia. A review. Surv Ophthalmol 28:621–642
3. Nishida K, Kinoshita S, Ohashi Y et al (1995) Ocular surface abnormalities in aniridia. Am J Ophthalmol 120:368–375
4. Jastanciah S, Al-Rajhi AA (2005) Association of aniridia and dry eyes. Ophthalmology 112:1535–1540
5. Holland EJ, Djalilian AR, Schwartz GS (2003) Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. Ophthalmology 110:125–130
6. Gomes JA, Eagle RC Jr, Gomes AK et al (1996) Recurrent keratopathy after penetrating keratoplasty for aniridia. Cornea 15:457–462
7. Ramaesh T, Collinson JM, Ramaesh K et al (2003) Corneal abnormalities in Pax6+/− small eye mice mimic human aniridia-related keratopathy. Invest Ophthalmol Vis Sci 44: 1871–1878
8. Dua HS, Azuara-Blanco A (2000) Limbal stem cells of the corneal epithelium. Surv Ophthalmol 44:415–425
9. Ramaesh K, Ramaesh T, Dutton GN, Dhillon B (2005) Evolving concepts on the pathogenic mechanisms of aniridia related keratopathy. Int J Biochem Cell Biol 37:547–557
10. Collinson JM, Quin JC, Buchanan MA et al (2001) Primary defects in the lens underlies complex anterior segment abnormalities of the Pas6 heterozygous eye. Proc Natl Acad Sci U S A 98:9688–9693
11. Simpson TI, Price DJ (2002) Pax6; a pleiotropic player in development. Bioessays 24:1041–1051
12. Chiambaretta F, Blanchon L, Rabier B et al (2002) Regulation of corneal keratin-12 gene expression by the human Kruppel-like transcription factor 6. Invest Ophthalmol Vis Sci 43:3422–3429
13. Tseng SC, Li DQ (1996) Comparison of protein kinase C subtypes expression between normal and aniridic human ocular surface: implications for limbal stem cell dysfunctions in aniridia. Cornea 15:168–178
14. Davis J, Duncan MK, Robison WG Jr, Piatigorsky J (2003) Requirement for Pax6 in corneal morphogenesis: a role in adhesion. J Cell Sci 116:2157–2167
15. Sivak JM, Fini ME (2002) MMPs in the eye: emerging roles for matrix metalloproteinases in ocular physiology. Prog Retin Eye Res 21:1–14
16. Sivak JM, Mohan R, Rinehart WB et al (2000) Pax-6 expression and activity are induced in the reepithelializing cornea and control activity of the transcriptional promoter for matrix metalloproteinase gelatinase B. Dev Biol 222:41–54
17. Leiper LJ, Ou J, Walczysko P et al (2009) Control of patterns of corneal innervation by PAX6. Invest Ophthalmol Vis Sci 50:1122–1128
18. Mackman G, Brightbill FS, Optiz AK (1979) Corneal changes in aniridia. Am J Ophthalmol 87:497–502

19. López-García JS, García-Lozano I, Rivas L et al (2006) Congenital aniridia keratopathy treatment. Arch Soc Esp Oftalmol 81:435–444
20. López-García JS, Rivas L, García-Lozano I (2006) Corneal epithelium squamous metaplasia determination as diagnostic factor in limbal deficiency. Arch Soc Esp Oftalmol 81:281–288
21. Tiller AM, Odenthal MT, Verbraak FD et al (2003) The influence of keratoplasty on visual prognosis in aniridia: a historical review of one large family. Cornea 22:105–110
22. Rivas L, Murube J, Rivas A, Murube E (2003) Impression cytology study of dry eyes patients with congenital aniridia. Arch Soc Esp Oftalmol 78:615–622
23. Vico E, Quereda A, Benitez-del-Castillo JM et al (2005) A comparative study of 0,15% sodium hyaluronate versus polyvynyl alcohol in treatment of dry eyes. Arch Soc Esp Oftalmol 80:387–394
24. Gomes JAP, Amankwah R, Powell Richards A, Dua HS (2004) Sodium hyaluronate (hyaluronic acid) promotes migration of human corneal epithelial cells in vitro. Br J Ophthalmol 88:821–825
25. Hornof M, de la Fuente M, Hallikainen M et al (2008) Low molecular weight hyaluronan shielding of DNA/PEI polyplexes facilitates CD44 receptor mediated uptake in human corneal epithelial cells. J Gene Med 10:70–80
26. López-García JS, García-Lozano I, Rivas L et al (2007) Use of autologous serum in ophthalmic practice. Arch Soc Esp Oftalmol 82:9–20
27. López-García JS, Rivas L, García-Lozano I, Murube J (2008) Autologous serum eye drops in the treatment of aniridic keratopathy. Opthalmology 115:262–267
28. Seefelder R (1909) Aniridia. Graefes Arch Ophthalmol 70:65
29. López-García JS, García-Lozano I (2012) Use of containers with sterilizing filter in autologous serum eyedrops. Ophthalmology 119:2225–2230
30. López-García JS, García-Lozano I, Rivas L et al (2014) Autologous serum eye drops diluted with sodium hyaluronate: clinical and experimental comparative study. Acta Ophthalmol 92:e22–e29

Chapter 8
Lamellar and Penetrating Keratoplasty in Congenital Aniridia

Sandra Planella, María Fideliz de la Paz, and Juan Alvarez de Toledo

Abstract Aniridia is a rare panocular disorder affecting the cornea, anterior chamber, iris, lens, retina, macula and optic nerve. It occurs as a result of abnormal neuro-ectodermal development secondary to a mutation in the PAX6 gene, linked to 11p13 chromosome. In this group of patients, one of the causes of progressive loss of vision and morbidity is keratopathy derived from the dysfunction of limbal stem cell deficiency. The absence of this important limbal structure suggests the origin of the epithelial abnormalities involving a progressive corneal opacification, sub-epithelial fibrosis and neovascularization. The management of ocular surface diseases in aniridia is complex but has changed in recent years. The progresses in the understanding of the mechanisms involved in cellular renewal of the cornea have allowed an adequate therapeutic approach of these patients. The current treatments for aniridic keratopathy are to replace the limbal stem cells through kerato-limbal allograft with or without subsequent keratoplasty for visual rehabilitation. Based on our experience, Descemet's membrane and endothelium

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complex in aniridic patients with keratopathy, has shown to be normal. For that reason, we propose that patients with advanced aniridic keratopathy could be candidates for deep anterior lamellar keratoplasty associated to limbal allograft instead of penetrating keratoplasty.

Keywords Aniridia • Keratopathy • Limbal stem cell deficiency • Penetrating keratoplasty • Lamellar keratoplasty

Introduction

Aniridia is an uncommon bilateral congenital, panocular disorder affecting not only the iris but also the cornea, anterior chamber angle, lens, retina and optic nerve as well as life-threatening associations. It occurs as a result of abnormal neuroectodermal development secondary to a mutation in the PAX6 gene, linked to 11p13 chromosome as described by [1–3]. Loss of function of one copy of the PAX6 gene can be identified in about 90 % of aniridia cases. About two-thirds of all aniridia cases are familiar, showing autosomal dominant inheritance with high penetrance. In the majority of cases with familial inheritance, an autosomal dominant inheritance pattern with almost complete penetrance has been described [4]. However, variations in expression have also been seen to occur. The remaining sporadic cases carry de novo mutations that will be dominantly inherited in further generations.

Aniridia is usually first diagnosed at a clinical level by a pediatrician, often following parental concerns about the baby's vision. Differential diagnoses should include anterior segment developmental abnormalities (e.g. Axenfeld-Rieger syndrome and Peters anomaly), iris coloboma, traumatic iris injury, WAGR, albinism, Gillespie syndrome and other causes of infantile nystagmus and reduced vision [5, 6]. Following clinical diagnosis of aniridia in an infant, it is important to assess family history.

Associated congenital abnormalities of the anterior segment include cilliary body hypoplasia, cataract, ectopia lentis, and anomalous development of the anterior chamber angle, microcornea, peripheral corneal pannus and keratopathy due to limbus dysfunction, dry eye and hypoplasia of Bowman's membrane. The alterations in the posterior segment include foveal hypoplasia, diffuse retinal dysfunction as shown in electro-retinography tests, impaired visual acuity with nystagmus, strabismus, glaucoma and optic nerve hypoplasia [7–9]. These defects, in combination, usually cause a formidable barrier to normal visual function. The age at presentation is generally at infancy when the parents notice abnormalities in the pupil. Glaucoma develops at either the pre-teens or the teenage level. Cataracts may occur before puberty, and its risk increases with age [10]. Corneal tissue is often involved and its progressive deterioration significantly affects vision throughout the years. Medical or surgical treatment of the corneal alterations in congenital aniridia patients represents a major challenge nowadays.

Aniridia-Related Keratopathy

Congenital aniridia patients develop aniridia related keratopathy, which is characterized by a progressive corneal opacification and pannus that occur due to anomalies in the ocular surface such as limbal stem cell deficiency and dry eye [11]. A significant correlation has been established between keratopathy and age [12, 13]. In the majority of individuals aniridia related keratopathy (ARK) manifests in the first decade of life, as thickened irregular whitish epithelium in the peripheral cornea [13]. The main cause for ARK is still not clear. It has been reported in almost 78 % of cases that there are micro-environmental changes, as well as the genetic defect of PAX6 [14]. The corneal epithelial cells have numerous adhesion mechanisms, both intra- and inter-cellular as well as with the extra-cellular matrix. Said mechanisms include "tight-junctions", "gap-junctions", desmosomes and adhering unions. In addition, there are a large variety of adhesion molecules such as catenins, integrins, desmogleine and desmocholine that make the corneal epithelium highly resistant to external attacks. It has been reported that in CA there is a reduction in desmogleine as well as beta- and alpha-catenin, the synthesis of which seems to be regulated by gene PAX6, which gives rise to spaces between epithelial cells [15]. These biochemical and pathological changes make the corneal surface very fragile and weakens the function of the epithelial barrier.

It is frequent to find in our daily practice patients with alterations of the ocular surface caused by a limbal stem cell deficiency syndrome, which in most cases is caused by external agents like chemical burns. In congenital aniridia, it appears to be due to a dysfunction in the limbal stem cells' microenvironment [15]. Patients with aniridia can remain without symptoms, if there is only a partial limbal insufficiency, until an external factor acts upon the limbus, overthrowing the fragile balance, which maintained the integrity of the corneal epithelium. For example, ARK worsens often after surgery that involves excessive manipulation of the limbus, or after the chronic application of topical medications to treat the aniridia-associated glaucoma. These surgical or toxic aggressions appear to be enough to disrupt the fragile balance that maintains the corneal epithelium's self-renewing process in aniridia.

Briefly, ARK is caused by a combination of factors: an abnormally differentiated epithelium, abnormal intercellular adhesion, impaired healing response, limbal stem-cell deficiency and the infiltration of conjunctival cells and new vessels on the cornea [14, 15]. Special attention has to be given to the clinical stage of ARK. It is important to classify the keratopathy in order to plan the therapeutic strategies in these patients. There is a globally accepted and standardized classification of ARK. Mackman (1979) originally described the most used staging (Fig. 8.1) and it includes four stages [4, 11].

- Grade 0: Peripheral and central cornea not affected.
- Grade 1: Partial affectation of limbal epithelium.
- Grade 2: Near total affectation of the limbus without central opacification.
- Grade 3: 360° affectation of the limbus with central corneal opacification.

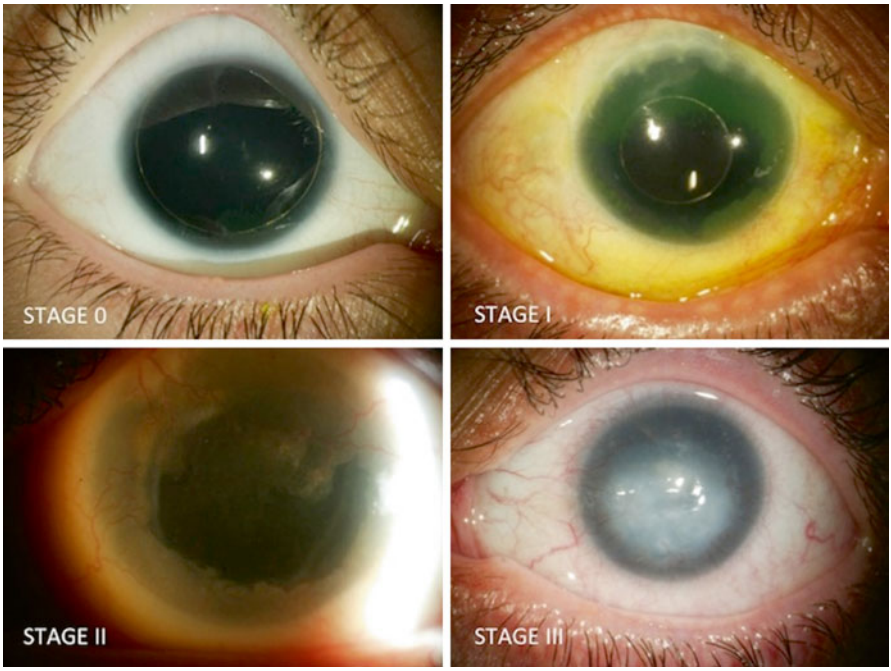

Fig. 8.1 Staging of aniridia associated keratopathy. In *stage 0*, no alteration is found in the peripheral cornea. In *stage I* thickened white fluorescein positive epithelium grows centripetally from the limbal area. In *stage II* new vessel in growth is present with invasion of the corneal apex and recurrent epithelial erosions appear often. In *stage III* we observe the presence of Salzmann's type nodular degeneration with 360° neovascularisation which opacifies the cornea and infiltrates sub-Bowmann's stroma

Although it does not appear in all patients, ARK is a frequent cause of ocular morbidity. It is interesting to highlight that in general, aniridic patient's corneal stromal pachymetry is thicker than in the normal population. Many published studies described a central corneal thickness average in aniridic patients between 630 and 690 μ compared with the average of 550 μ in the normal population. This fact should be taken into account when measuring intraocular pressure. Some patients could be treated unnecessarily with the consequent pharmacological toxicity to the cornea and ocular surface [8, 9, 12]. Therefore, an adequate therapeutic approach could be extremely useful to improve the quality of life of these patients.

Limbal Stem Cell Deficiency in Aniridia

The clinical and histopathological morphology of the limbus in aniridic patients has been shown to be abnormal. The alteration of this important limbal structure suggests the primary origin of the epithelial abnormalities [11, 15]. When the

corneal limbus is severely damaged, conjunctival epithelium replaces the corneal epithelium, which causes significant visual deterioration. The limbal epithelial stem cells (LEST) have self-renewal capabilities and therefore, allow the corneo-scleral limbus to serve as a barrier. Multiple findings that led to the understanding that LEST are located in the palisades of Vogt (PV). PV are radially oriented fibro-vascular structures located 1–2 mm from limbo-corneal junction. They are more prominent in the upper and lower quadrants. Their morphology is believed to create an optimal microenvironment filled with stem cell nutrients and growth factors but also regulates the process of cell division [16]. Limbal stem cell deficiency (LSCD) inhibits ocular surface restoration and may result in ocular irritation, epiphora, blepharospasm, photophobia, pain, severe visual impairment, recurrent epithelial erosions and even bacterial infections with the risk of eye perforation [16, 17]. In congenital aniridia there is also meibomian gland dysfunction and abnormal tear film, with reduced tear break-up time and reduced tear meniscus [18, 19]. This chronic aggression on the ocular surface epithelium will produce a reaction of the latter in the form of metaplasic transformation. The epitheliopathy in aniridic patients is typically accompanied by superficial neovascularization that advances centripetally for years to ultimately involve the entire corneal surface. The progression can affect the entire thickness of cornea. Sub-epithelial fibrosis and stromal scarring predisposes to recurrent erosions, corneal ulceration and chronic pain. It may progress in a variable manner to completely cover the cornea, further compromise vision requiring corneal transplantation [20]. The opacification of the cornea in aniridia following repeated episodes of erosion and ulceration may be caused by a deficiency in matrix metallo-proteinase 9 (MMP-9), which is also regulated by PAX6 gene. Matrix metallo-proteinases are responsible for the degradation of collagen during normal cell remodeling and wound healing. In PAX6 mutation in animal models, MMP-9 deficiency results in the accumulation of fibrin and the infiltration of inflammatory cells. This disrupts the orderly arrangement of the collagen fibrils of the cornea, and results in subsequent loss of transparency [21]. The morphological changes of cornea and limbus vary in ARK; in vivo confocal microscopy is a promising tool to determine the degree of LSCD in patients with ARK. Thickness of the central cornea is usually very increased [9, 15, 22], frequently involving neovascularization, sub-epithelial fibrosis, changes in Bowman's membrane and keratinization. Accordingly, squamous metaplasia that occurs before the keratinization process can be identified by impression cytology, facilitating an earlier diagnosis and improved therapeutic approach. The presence of goblet cells in corneal impression cytology demonstrates the invasion of epithelial conjunctival phenotype cells within the central cornea area. Impression cytology also facilitates studying the epithelial phenotype by marking with monoclonal antibodies of selective cyto-keratines of each cellular lineage [12].

What are the options ophthalmologists have to surgically treat the ARK? Amniotic membrane has effectively been used as a temporary patch to promote healing of the ocular surface by reducing inflammation and scar formation. Limbal autograft transplantation was described for the first time in 1989 and in the past decades transplantation of the limbal tissues either autograft or allograft (Fig. 8.2)

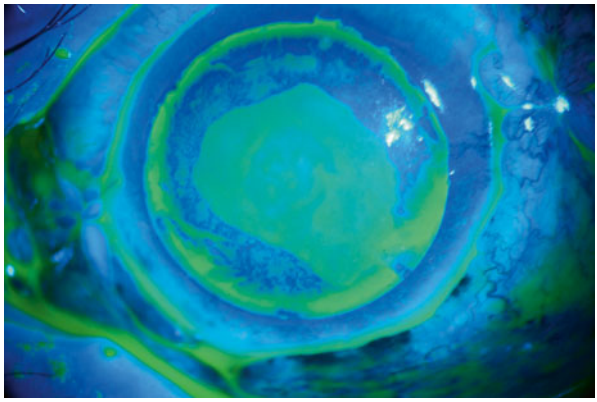

Fig. 8.2 Circular limbal stem cell allograft stained with fluorescein. The centripetal growth of the donor epithelium is easily demonstrated with the stain. Integrity of recipient Bowman's membrane facilitates the epithelial cell sliding towards the corneal apex

has been proposed for the treatment of limbal dysfunction [23]. Many other studies reported short-term success with limbal stem cell allografts combined with amniotic membrane transplant in aniridic patients [24–26]. Autograft is not applicable to patients with bilateral LSCD where there are no remaining limbal stem cells. The disadvantages of limbal allografting are the risk of graft rejection and side effects of chronic systemic immunosuppression compared to limbal autografts [19, 27]. There is a recently published study, only on one patient, where the use of combined HLA-matched limbal stem cells allograft (LAT) with amniotic membrane transplantation (AMT) as a prophylactic surgical procedure to prevent corneal graft rejection was performed. They concluded that combining this with penetrating keratoplasty, may result in a better prognosis of graft survival and improved visual function in these eyes [28]. To validate this finding, more work needs to be done to address these important concerns and make stem cell-based therapy for treating LSCD more successful.

Penetrating Keratoplasty: Results

One of the causes of progressive loss of vision and morbidity in aniridia patients is keratopathy derived from the dysfunction of limbal stem cells. Until many years ago, the approach to treating ARK was based on supporting treatment with topical lubricants, therapeutic contact lenses, amniotic membrane transplantation or tarsorrhaphy [29, 30]. In patients with moderate and severe stages of the keratopathy, medical topical treatment provides only temporary results. No convincing opinion exists as to which surgical procedure is the treatment of choice for aniridic keratopathy. Penetrating keratoplasty may be indicated for corneas opacified from pannus or if the cornea becomes sufficiently opaque. However, surgical results of penetrating keratoplasty are quite poor because (Fig. 8.3) of the recurrence of the same pre-graft corneal changes, followed by subsequent failure of the graft [31]. This is most likely caused by the primary abnormality in the limbal stem cells and highly vascularized host. Therefore, visual outcomes are minimal and the prognosis

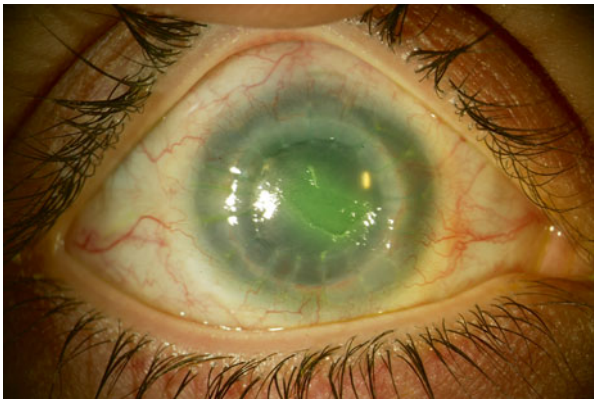

Fig. 8.3 Corneal epithelial central erosion with graft opacification in a penetrating keratoplasty performed 1 year before in a patient with congenital aniridia. Mersilene 11-0 stitches are still in place. Notice the superficial neovascularization in all four quadrants and the opacification of the anterior central stroma

is guarded because of rejection and underlying foveal hypoplasia or other structural defects. Many studies have shown that PK is ineffective for a long-term treatment because it does not address the stem cell deficiency that is the primary etiological factor [32]. The results of penetrating keratoplasty in 11 eyes with congenital aniridia, and found a 64 % risk of rejection. Afterwards, other authors have agreed, postulating an ineffectiveness of PK alone, without treating the primary problem, which is the stem cell insufficiency [29]. Graft failure in 100 % of patients requiring repeat penetrating keratoplasty for recurrent aniridic epithelial disease· They reviewed clinical and histopathological cases of aniridia in order to investigate the features of graft failure in those patients. Similar histopathological findings were observed in all cases, confirming the clinical impression that the keratopathy recurred in all the grafts. Descemet's membrane and endothelium were affected only in one patient, who developed corneal endothelial graft rejection. In a published article by our group, we observed that the mean endothelial cell count was normal in all eyes after performing specular microscopy. Peripheral endothelial cell density and morphology were normal [31]. This was quite an unexpected finding since, embryologically, the endothelium is closely related to the iris, lens and angle structures, which are, in general, affected in patients with congenital aniridia. We are at present performing a study of aniridic corneal buttons to correlate our clinical findings with histopathological evidence. In addition, in our aniridic patients, we also observed that there is no significant difference between limbal transplant and PK in terms of long-term visual prognosis, but we have noted that there is slight improvement in the ocular surface of the limbal allograft group versus the PK group over a period of 1–5 years. So, in conclusion, penetrating keratoplasty as a single procedure should be avoided if there is not a concomitant or previous treatment of the LSCD. Both procedures can be performed sequentially, with an interval of a minimum time of about 6 months, enough to achieve a stable ocular corneal epithelium. A combined procedure has been described (Fig. 8.4) when performing a large diameter penetrating keratoplasty (>9 mm) eccentrically trephined in the donor to include a limbal area, which, theoretically can supply some healthy limbal epithelial cells. In our experience this procedure also ends up with a long-term epithelium failure despite initial good results has been published [33].

The Boston type 1 keratoprosthesis (Fig. 8.5) may provide a more effective approach in the management of ARK [34–37], but it can also have complications, especially related with the anterior segment progressive fibrosis syndrome that typically develops in young aniridia patients.

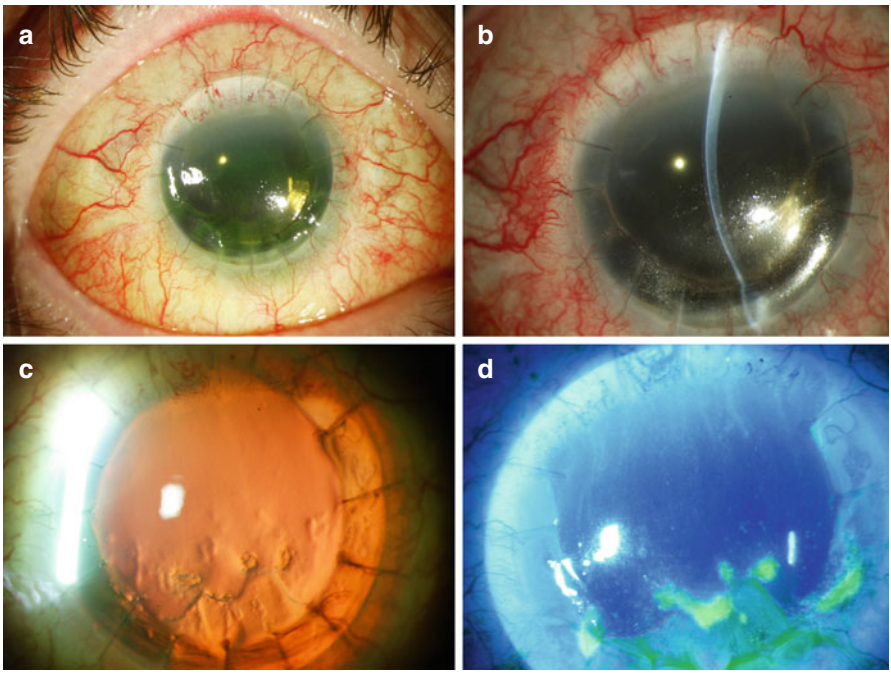

Fig. 8.4 Kerato-limbal allograft. A large diameter graft (9.5 mm. of diameter) was eccentrically obtained from the donor to include limbal area (**a**, **b**) After 16 months, recurrence of epithelial failure with epithelial erosions was seen in the area in which no limbal epithelial barrier was present (**c**, **d**)

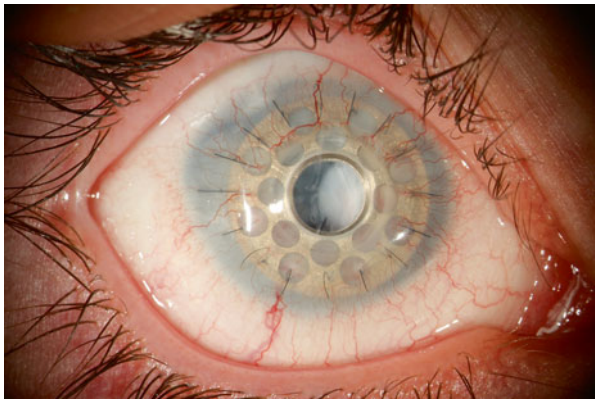

Fig. 8.5 Boston K-pro implanted in a 5-years old girl with congenital aniridia previously operated for glaucoma and cataract. Despite the use of a titanium back-plate, absence of IOL and iris, anterior segment fibrosis developed forming a thick retro-prosthetic membrane, which required a new surgical removal via pars plana

The current treatments for ARK are to replace the limbal stem cells through keratolimbal allograft (KLAL) with or without subsequent keratoplasty for visual rehabilitation, or to implant a Boston type 1 keratoprosthesis. Further research is necessary to find better ways to treat keratopathy in the aniridic patients in the future.

Lamellar Keratoplasty in Congenital Aniridia

Lamellar keratoplasty (LK) surgery consists of placing a partial thickness donor corneal graft in a recipient corneal bed that is prepared by a lamellar dissection of the diseased anterior stromal corneal tissue. The recipient bed consists of a thin posterior stromal layer, Descemet's membrane and endothelium. The indications for LK can be optical, tectonic or therapeutical. Lamellar keratoplasty is useful to remove stromal opacities that usually appear in severe and advanced stages of the keratopathy (Fig. 8.6). However, it does not treat LSCD; so if a limbal transplant does not accompany or precede the keratoplasty, usually it ends up in a recurrent graft failure as described in penetrating grafts [31].

Isolated central lamellar keratoplasty does not have good results in long-term in the majority of the cases of ARK. Homologous lamellar limbo-keratoplasty to transplant limbal stem cells appears to be more effective [11]. Anyway, aniridic patients who have serious risks to take oral immunosuppressants and LT is not recommended, another option is to combine a central LK with a therapeutic contact lens and autologous serum eye-drops in order to protect the corneal surface and stabilize the epithelium.

Based on our experience, we have observed a regular unbroken Descemet's membrane and normal endothelial cell count in most of our aniridic patients. The cases with lower number of cells were in older patients with morphological changes such as cornea guttata or decreased endothelial cell count with pleomorphism and polymeghetism because of a long history of topical glaucoma treatment or previous

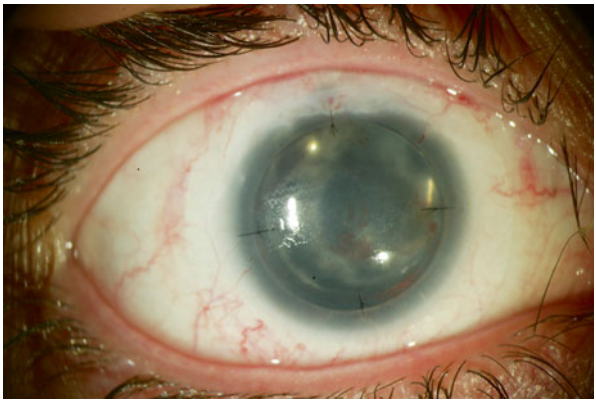

Fig. 8.6 Lamellar anterior keratoplasty performed with femtosecond laser in a patient previously operated with a limbal allograft. Several years after stability of corneal epithelium, lamellar keratoplasty was performed to improve the visual function

ocular surgery like cataract extraction. For that reason, we propose that patients with advanced ARK could be candidates for superficial (SALK) or deep anterior lamellar keratoplasty (DALK) as a primary procedure (preceded by a limbal allograft) instead of PK. Fewer incidences of immune rejection and less keratometric astigmatism may be achieved with this surgical option. Further long-term studies of this surgical technique have to be carried out to increase the knowledge of its long-term results in this rare disease.

Conclusions

Congenital aniridia is a rare, bilateral, genetic disorder affecting the cornea and the ocular surface. Aniridia related keratopathy (ARK) is a multifactorial disease, due to micro-environmental changes and genetic defects, producing slow decline in the limbal stem cell population thru time, causing severe corneal pathologies. The characteristics and anatomical structure of the limbal stem cell niche are still incompletely defined and the specific markers for transplanted limbal stem cells remain uncertain. It is important to meticulously evaluate the ocular surface to determine the effective treatment for each individual case. The establishment of a properly functioning limbus is essential for the survival and function of the corneal graft after keratoplasty in patients with a congenital limbal deficiency. There is currently insufficient evidence to determine which technique may offer better overall outcomes, final visual acuity, risk of rejection, failure or risk of other adverse events. Large randomized trials comparing the outcomes of KP, SALK or DALK in ARK are needed. On the other hand, aniridic patients have to cope with the knowledge that the prognosis for long-term visual acuity is not favorable, despite surgical treatment. Lastly, we must not forget that in many cases aniridia is associated to other systemic disorders that also require our full attention.

References

1. Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L (1984) Aniridia: a review. Surv Ophthalmol 28:621–642
2. Lee H, Khan R, O'Keefe M (2008) Aniridia: current pathology and management. Acta Ophthalmol 86:708–715
3. Gronskov K, Olsen JH, Sand A et al (2001) Population-based risk estimates of Wilms tumor in sporadic aniridia. A comprehensive mutation screening procedure of PAX6 identifies 80% of mutations in aniridia. Hum Genet 109:11–18
4. Ayuso C, Trujillo MJ, Villaverde C, Vallespin E, Lorda I (eds) (2008) Implicaciones genéticas en la aniridia. Protocolo de actuación en pacientes con aniridia. ENE Ediciones S.A. Madrid, pp 28–33
5. Glaser T, Ton C, Mueller R et al (1964) Absence of PAX6 gene mutations in Gillespie syndrome (partial aniridia, cerebellar ataxia, and mental retardation. Genomics 19:145–148
6. Gillespie FD (1965) Aniridia, cerebellar ataxia, and oligophrenia. Arch Ophthalmol 13:338–341

7. Baulmann DC, Ohlmann A, Flugel-Koch C et al (2002) PAX6 heterozygous eyes show defects in the chamber angle differentiation that are associated with a wide spectrum of other anterior eye segment abnormalities. Mech Dev 118:3–17
8. Brandt JD, Casuso LA, Budenz DL (2004) Markedly increased central corneal thickness: an undiagnosed finding in congenital aniridia. Am J Ophthalmol 137:348–350
9. Whitson JT, Liang C, Godfrey DG et al (2005) Central corneal thickness in patients with congenital aniridia. Eye Contact Lens 31:221–224
10. Edén U, Beijar C, Riise R et al (2008) Epidemiology of aniridia in Sweden and Norway. Acta Ophthalmol 86:727–729
11. Holland EJ, Djalilian AR, Schwartz GS (2003) Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. Ophthalmology 110:125–130
12. Macedo K, Fideliz de la Paz M, Álvarez de Toledo J et al (2012) Characteristics of the cornea and the ocular surface in a population of patients with congenital aniridia. J Emmetropia 3(3):130–139
13. Edén U, Riise R, Tornqvist K (2010) Corneal involvement in congenital aniridia. Cornea 29(10):1096–1102
14. Ramaesh K, Ramaesh T, Dutton GN et al (2005) Evolving concepts on the pathogenetic mechanisms of aniridia related keratopathy. Int J Biochem Cell Biol 37:547–557
15. Lagali N, Edén U, Utheim TP, Chen X, Riise R, Dellby A, Fagerholm P (2013) In vivo morphology of the limbal palisades of Vogt correlates with progressive stem cell deficiency in aniridia-related keratopathy. Invest Ophthalmol 54(8):5333–5342
16. Miri A, Alomar T, Nubile M et al (2012) In vivo confocal microscopic findings in patients with limbal stem cell deficiency. Br J Ophthalmol 96:523–529
17. Schermer A, Galvin S, Sun TT (1986) Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. J Cell Biol 103(1):49–62
18. Jastaneiah S, Al-Rajhi AA (2005) Association of aniridia and dry eyes. Ophthalmology 112(9):1535–1540
19. López-García JS, Rivas L, García-Lozano I (2005) Tratamiento de la insuficiencia limbal grave mediante cirugía combinada de trasplante de limbo y trasplante de membrana amniótica. Arch Soc Esp Oftalmol 80:405–412
20. Gris O, Pérez-Santoja JJ, Güell JL (ed) (2008) Alteraciones del limbo esclero-corneal y aniridia. Protocolo de actuación en pacientes con aniridia. ENE Ediciones S.A. Madrid, pp 28–35
21. Mohan R, Chintala SK, Jung JC et al (2002) Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epitelial regeneration. J Biol Chem 277:2065–2072
22. Nubile M, Lanzini M, Miri A et al (2013) In vivo confocal microscopy in diagnosis of limbal stem cell deficiency. Am J Ophthalmol 155:220–232
23. Dua HS, Azuara-Blanco A (2000) Limbal stem cells of the corneal epithelium. Surv Ophthalmol 44:415–425
24. Koizumi N, Inatomi T, Quantock AJ, Fullwood NJ, Dota A, Kinoshita S (2000) Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. Cornea 19:65–71
25. Schwab IR, Reyes M, Isseroff RR (2000) Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. Cornea 19:421–426
26. Tsai RJ, Li LM, Chen J (2000) Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 343:86–93
27. Tseng SC, Prabhasawat P, Barton K, Gray T, Meller D (1998) Amniotic membrane transplantation with or without allografts for corneal surface reconstruction in patients with limbal stem cells deficiency. Arch Ophthalmol 116:431–441
28. Capozzi P, Petroni S, Buzzonetti L (2014) Combined HLA matched limbal stem cells allograft with amniotic membrane transplantation as a prophylactic surgical procedure to prevent corneal graft rejection after penetrating keratoplasty: case report. Ann Ist Super Sanita 50(3):298–300

29. Gomes JA, Eagle RC Jr, Gomes AK et al (1996) Recurrent keratopathy after penetrating keratoplasty for aniridia. Cornea 15:457–462
30. Tiller AM, Odenthal MT, Verbraak FD, Gortzak-Moorstein N (2003) The influence of keratoplasty on visual prognosis in aniridia: a historical review of one large family. Cornea 22:105–110
31. Fideliz de la Paz M, Álvarez de Toledo J, Barraquer RI et al (2008) Long-term visual prognosis of corneal and ocular surgery in patients with congential aniridia. Arch Ophthalmol 186(7):735–740
32. Kremer I, Rajpal RK, Rapuano CJ et al (1993) Results of penetrating keratoplasty in aniridia. Am J Ophthalmol 115:317–320
33. Eberwein PL, Böhringer D, Schwartzkopff J, Birnbaum F, Reinhard T (2012) Allogenic limbokeratoplasty with conjunctivoplasty, mitomycin C, and amniotic membrane for bilateral limbal stem cell deficiency. Ophthalmology 119(5):930–937
34. Akpek EK, Harissi-Dagher M, Petrarca R et al (2007) Outcomes of Boston keratoprosthesis in aniridia: a retrospective multicenter study. Am J Ophthalmol 144:227–231
35. Bakhtiari P, Chan C, Welder JD et al (2012) Surgical and visual outcomes of the type I Boston keratophrosthesis for the management of aniridic fibrosis syndrome in congenital aniridia. Am J Ophthalmol 153:967–971
36. Greiner MA, Li JY, Mannis MJ (2011) Longer-term vision outcomes and complications with the Boston type I keratoprosthesis at the University of California, Davis. Ophthalmology 118:1543–1550
37. Kang JJ, De la Cruz J, Cortina MS (2012) Visual outcomes of Boston keratoprosthesis implantation as the primary penetrating corneal procedure. Cornea 31(12):1436–1440

Chapter 9
Boston Kpro Type I as a Viable Alternative to Visual Rehabilitation in Aniridia Patients: Advances and Limitations

Samantha Williamson, Kimberly Hsu, and Jose de la Cruz

Abstract The purpose of this chapter is to describe the experience of Boston Type 1 Keratoprosthesis in Aniridia. Aniridia-associated keratopathy (AAK) affects up to 90 % of aniridic patients and may significantly decrease best-corrected acuity. Poor outcomes of penetrating keratoplasty in aniridia have been reported for over 20 years. Limbal stem cell transplantation can help restore a healthy ocular surface, but requires long term systemic immunosuppression. Boston type 1 Keratoprosthesis implantation represents a promising alternative for visual rehabilitation in AAK patients. The central PMMA optic is unaffected by corneal graft vascularization or conjunctivalization, and may be customized for aphakia or pseudophakia. Multiple studies have reported improved visual outcomes after Kpro implantation with device retention rates of 70 % or greater. Complications include retroprosthetic membranes, corneal melt, device extrusion, and glaucoma.

Keywords Aniridia • Aniridia-associated keratopathy • Keratoprosthesis

Visual acuity in aniridia may be limited by optic nerve or foveal hypoplasia, cataracts, glaucoma, and aniridia-associated keratopathy (AAK). The keratopathy associated with aniridia affects up to 90 % of patients, and follows a progressive course with early manifestations appearing within the first decade of life [1–4]. Historically, vision loss associated with corneal changes has been difficult to manage, as the recurrence of pathology following penetrating keratoplasty is nearly universal. The Boston keratoprosthesis represents a promising alternative for visual rehabilitation in these challenging patients.

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Several mechanisms contribute to the development and progression of aniridia-associated keratopathy. A deficiency of limbal stem cells underlies the corneal changes, and PAX6 mutations affect epithelial cell differentiation and proliferation. Clinical findings of limbal stem cell dysfunction include peripheral corneal vascularization and the presence of goblet cells on the corneal surface [2]. Imaging and impression cytology have revealed a lack of normal limbal palisades in aniridic corneas, with invasion of the limbal region by vessels, leukocytes, and opaque conjunctival tissue [4, 5]. The ocular surface is further compromised by the effects of PAX6 mutations on corneal wound healing, extracellular matrix remodeling, and cell-to-cell adhesion, increasing the susceptibility of patients to recurrent corneal erosions and ulceration [1, 2, 6]. An acceleration of corneal changes may be observed in aniridics following trauma or surgical interventions, including corneal transplantation [2, 3, 6].

Poor outcomes of penetrating keratoplasty in patients with aniridia have been reported for over 20 years. In 1993, Kremer et al reviewed 11 patients with AAK who underwent penetrating keratoplasty (PKP) [7]. Invasion of the peripheral pannus into the corneal graft occurred in all patients, abnormal epithelial growth from the limbus replaced graft epithelium in 91 %, and central sub-epithelial scarring was seen in 82 % of patients. In addition to the early post-operative return of keratopathy, patients experienced persistent epithelial defects and delayed healing. Graft rejection occurred in nearly two-thirds of patients, and graft failure in roughly one-third. All patients received HLA-matched tissue and remained on topical corticosteroids throughout the study. The authors cited an abnormal healing response and increased graft vascularity as risk factors for graft rejection and failure [7]. Gomes et al described the histologic characteristics of failed PKP grafts in aniridics as similar to those changes seen in corneal specimens prior to transplantation. Failed grafts obtained from patients undergoing repeat keratoplasty revealed recurrent pannus with an inflammatory infiltrate, goblet cells within the graft, stromal vascularization, and a fibrotic membrane between the epithelium and remnants of Bowman's [3]. Similarly, Tiller et al found only a short-lived improvement in visual acuity after transplantation for AAK, with recurrence of keratopathy in all grafts and no sustained visual improvement in long-term follow-up as compared to observation [8]. Together, these studies argue against penetrating keratoplasty alone as management for aniridic keratopathy.

Subsequent surgical interventions, such as keratolimbal allografts (KLAL), have aimed to address the limbal stem cell deficiency presumed responsible for the rapid recurrence of corneal changes in aniridics following transplantation. Holland et al. reported outcomes in aniridia of KLAL, in which a donor corneosclerallimbal ring is transplanted as a source of stem cells. In those patients on systemic immunosuppression, the majority who underwent KLAL alone or KLAL plus penetrating keratoplasty achieved ocular surface stability and improved visual acuity. Only 40 % of KLAL patients maintained on topical immunosuppression alone, however, achieved stability of the ocular surface. In eyes that underwent transplantation following KLAL, a 30 % graft failure rate was seen during follow up [4]. Another series quoted an overall PKP failure rate after KLAL at their institution as 57.1 % [9]. Evaluating long-term

outcomes of KLAL with or without keratoplasty in patients with total limbal stem cell deficiency, Solomon et al described a steady decline in visual acuity and survival of both grafts. At 3 years after surgery, survival rates measured 47.4 % for KLAL and a dismal 13.7 % for penetrating keratoplasty. Of note, no donor cells could be found on the ocular surface in patients on examination 3–5 years after keratolimbal allografting [10]. The authors recommend indefinite systemic immunosuppression given the high rates of rejection and failure [4, 10].

Multiple studies describe disappointing outcomes in patients who underwent simultaneous central corneal and limbal allografts [10–12]. In a series by Tseng et al, 64 % of patients developed corneal graft rejection despite oral cyclosporine administration [11]. Shimazaki et al report an endothelial graft rejection rate of 35.6 % in patients on topical and systemic cyclosporine, and 62.5 % of these patients developed subsequent endothelial decompensation [12]. Simultaneous grafting may accelerate rejection due to increased host exposure to donor corneal antigens at the limbus, and a more exuberant inflammatory and healing response [10, 13].

Thus, although limbal stem cell transplantation followed by penetrating keratoplasty can help restore a stable ocular surface in aniridic patients, this technique requires long-term systemic immunosuppression that is not without risk. The Boston keratoprosthesis (KPro) has been used in patients in whom traditional keratoplasty is prone to failure, and represents a promising alternative. Briefly, the type I Boston keratoprosthesis is a "collar-button" device that consists of a front and back plate that is joined by an optical stem. The corneal graft is sandwiched between the plates and a titanium locking c-ring is used to secure the device. The plates are made from polymethyl methacrylate (PMMA), which is inert and clear, or titanium. The back plate contains holes that allow aqueous to provide nutrition to and hydration of the corneal graft. A bandage contact lens is usually placed indefinitely to protect the ocular surface (Figs. 9.1 and 9.2). The type I KPro is more commonly implanted; the type II KPro is utilized only in end-stage dry eye conditions and requires a permanent tarsorrhaphy through which an anterior nub protrudes. There are several qualities that make the KPro an attractive choice for patients with extensive neovascularization, repeated failed grafts, limbal stem cell deficiency, or other indications for which traditional keratoplasty is likely to fail. As the central optic is made of PMMA, it is unaffected by conjunctivalization or failure of the donor cornea (Fig. 9.3). In addition, it can be customized for pseudophakia or aphakia, which is useful in eyes with complicated ocular statuses such as those with aniridia. Also, as the front plate provides a spherical anterior curvature, there is no significant astigmatism as seen in traditional keratoplasty [14, 15].

There have been several reports of successful implantation of KPro devices in patients with aniridia. Akpek et al. reported on 16 eyes of 15 patients in a multicenter study who underwent type 1 KPro placement for aniridia. Eleven patients had prior keratoplasty. Pre-operative vision ranged from light perception (LP) to 20/300 (median counting fingers). Concurrent glaucoma shunt placement, vitrectomy, cataract extraction or intraocular lens removal was performed in ten patients. Vision improved in all but one patient, and it was felt that this patient's eye was in a prephthisical state. Postoperative visual acuity ranged from hand motion (HM) to

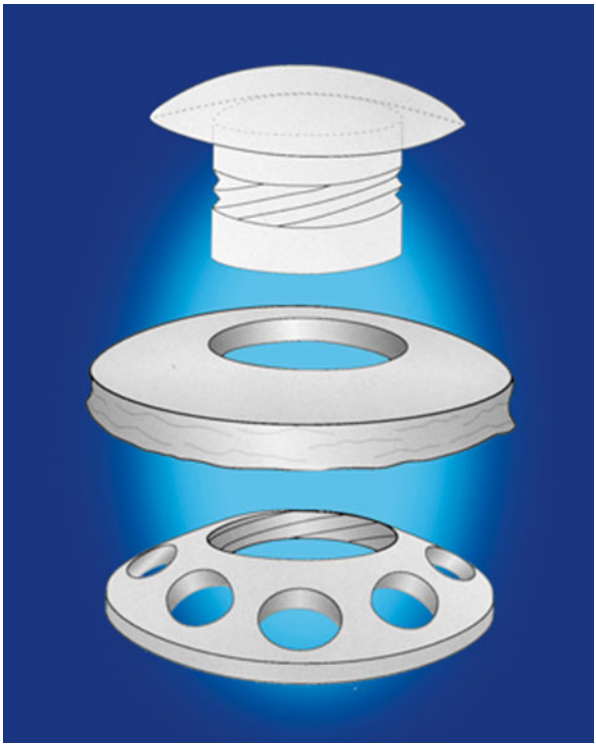

Fig. 9.1 Boston keratoprosthesis type 1 assembly

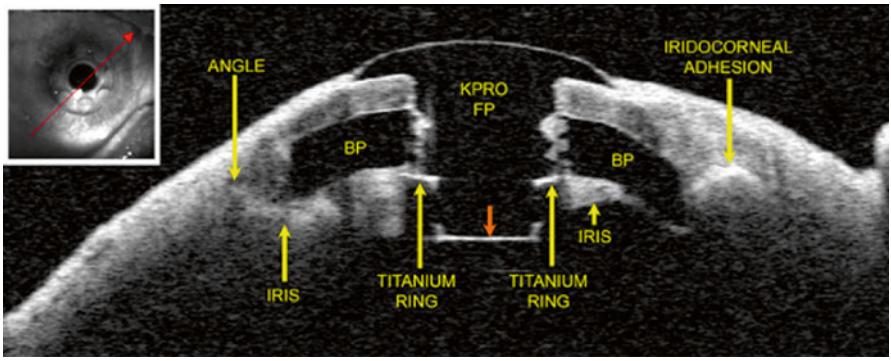

Fig. 9.2 Anterior-segment OCT of an implanted Boston Kpro type 1

20/60 (median 20/200). The follow up period was 2–85 months (median 17 months). One case of tissue melt occurred, which was repaired with a scleral patch graft. Visually significant retroprosthetic membrane (RPM) occurred in two patients and required Nd:YAG treatment. Three patients had choroidal detachments, one of which progressed to retinal detachment with LP vision. One patient developed worsening of glaucoma requiring diode laser. No cases of endophthalmitis were reported. All devices were retained [15].

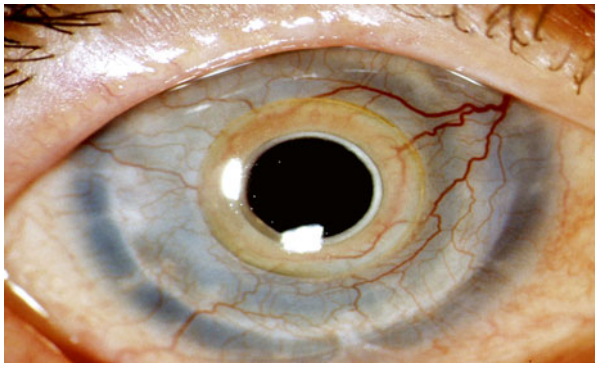

Fig. 9.3 Central optic clarity is unaffected by vascularization of donor cornea

Rixen et al. reported on a series of seven eyes of seven patients with aniridia who underwent KPro at the University of Iowa. Two patients had prior keratolimbal allograft and keratoplasty. Preoperative visual acuity was 20/1,600 to HM (median 20/1,600). In this series, glaucoma shunts were placed in eyes without prior shunts. All eyes had improved best corrected visual acuity postoperatively; at the latest follow up (median 18 months) vision ranged from 20/100 to LP (median 20/125). The patient with LP vision was felt to have vision loss from an occipital stroke. RPM was found in 3 (42.9 %) eyes, although none required treatment. One eye developed wound dehiscence. All devices were retained [16].

Hassanaly et al. reviewed 26 eyes of 19 patients with aniridia who received KPros in a study from Montreal. Seven patients (27 %) had prior failed keratoplasty and keratolimbalallografting. Preoperative visual acuity ranged from LP to 20/300. Postoperative visual acuity was 20/200 or better in 54 % of patients at a mean follow up of 28.7 months. Final visual acuity improved in 65 % of eyes. Retroprosthetic membranes occurred in 15 (58 %) of eyes. Eighty-eight percent of eyes were diagnosed with glaucoma postoperatively; the surgeon chose not to primary place glaucoma shunts, in contrast to other studies. Subsequently, three patients underwent Ahmed valve placement. Retention rate of the initial Kpro was 77 %, with six eyes requiring a replacement due to infectious keratitis, interpseudophakic vascular membrane, trans-prosthetic leakage, corneal melt, and device extrusion. Seventy-three percent of eyes in this study received primary KPro surgery, and the authors comment that there was a non-statistically significant trend toward better BCVA and fewer severe complications in this group [17]. Additional information can be gained from other KPro studies that have not directly addressed aniridia but in which aniridic patients were included in the dataset. Many of these patients did well with KPro placement, although Greiner et al. found that patients with aniridia did worse than others [18–22].

Our own experience at the Illinois Eye and Ear Infirmary supports keratoprosthesis as a useful option in the treatment of aniridia-associated keratopathy. In our experience of Kpro implantation between 2008 and 2014, 18 eyes of 17 aniridic patients were reviewed with a mean follow-up of 33 months. Eleven

eyes had prior failed keratolimbalallografting. Visual acuity preoperatively measured 20/400 or worse in all eyes. Mean implant survival was 1,635 days. Last recorded post-operative BCVA measured 20/250 or better in 55 % of patients, 20/300 or better in 61 %, and 20/400 or worse in 39 %. Sixteen percent of patients had a decline in post-op vision compared to pre-op, which was related to hypotony, retinal detachment, and post-surgical choroidal hemorrhage. Retroprosthetic membranes developed in 67 % of patients. Sterile keratolysis was seen in 16 % of patients with subsequent implant extrusion. As has been suggested in other studies, formation of RPM may play a role in corneal melt [23]. Our three patients developed sterile keratolysis less than 7 months following RPM formation. Interestingly, functional vision was preserved after repeat Kpro implantation, with all three patients experiencing BCVA of 20/250 or better.

The KPro device is not without complications. Retroprosthetic membranes are common, and can typically be treated with YAG laser. In the literature, RPM formation in aniridics ranges from 12.5 % to 67 % [15–18, 20–22]. Glaucoma is often an issue in these patients as both aniridia and KPro placement increase the risk of elevated intraocular pressure. Placement of a tube shunt at the time of surgery can help to control intraocular pressure. We recommend frequent follow-up with automated visual fields and optic nerve head ocular coherence tomography (OCT). As the ocular surface of aniridic patients can be unstable, these patients should be monitored for sterile melts and infectious keratitis despite the central optic remaining clear. Risk factors for sterile keratolysis include concomitant autoimmune disease, exposure of the keratoprosthesis, and retroprosthetic membranes. Retroprosthetic membranes that cover the backplatemay occlude aqueous flow through the backplateholes, preventing nutrient delivery to the donor corneal graft [23]. Anterior segment OCT may be used to both evaluate RPM formation and monitor angle anatomy and the development of iridocorneal adhesions. In one study, the presence of a retro-backplate RPM on anterior segment OCT conferred a risk ratio of 2.9 (95 % CI 1.9–.4) for developing a subsequent melt [23] (Fig. 9.4). Glare and photophobia are common side effects in aniridic patients with keratoprostheses, and may be managed with colored pupil-control contact lenses [24, 25] (Fig. 9.5). Endophthalmitis is the most devastating complication of the KPro, and is an inherent risk of the hardware.

Aniridia is a complex ocular disease associated with limbal stem cell deficiency and progressive keratopathy. The Boston keratoprosthesis is a viable option in these patients who would otherwise require limbal stem cell transplantation with systemic immunosuppression followed by penetrating keratoplasty. Although the KPro has been traditionally reserved for cases that previously failed keratoplasty, placement of the KPro primarily has been associated with good outcomes and may be a reasonable option in aniridic patients [19]. Despite the risks of keratoprosthesis, these studies suggest that KPro is a good option for improving vision in patients with aniridia who have difficulty maintaining a healthy ocular surface.

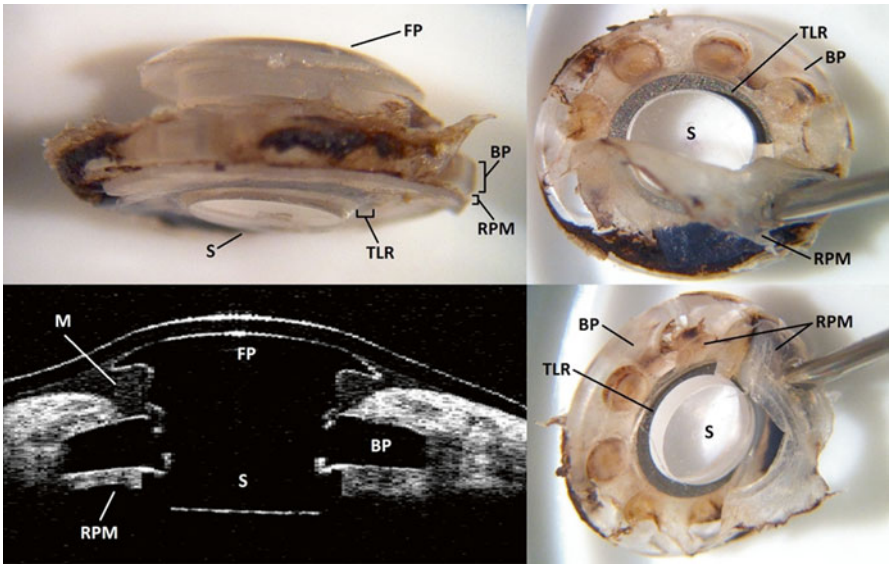

Fig. 9.4 Photographs of an explanted KPro (formalin fixed) from an 85 year old man (*top left*, *top right*, *bottom right*). Shown is a thick retroprosthetic membrane (*RPM*). Note: the donor cornea has been removed. The RPM develops a concave contour posteriorly as it extends over the titanium locking ring and slopes up against the KPro stem (*top left*), consistent with findings seen on AS-OCT obtained from the same eye prior to explantation (*bottom left*) (*FP* front plate, *BP* back plate, *S* stem, *TLR* titanium locking ring, *M* melt)

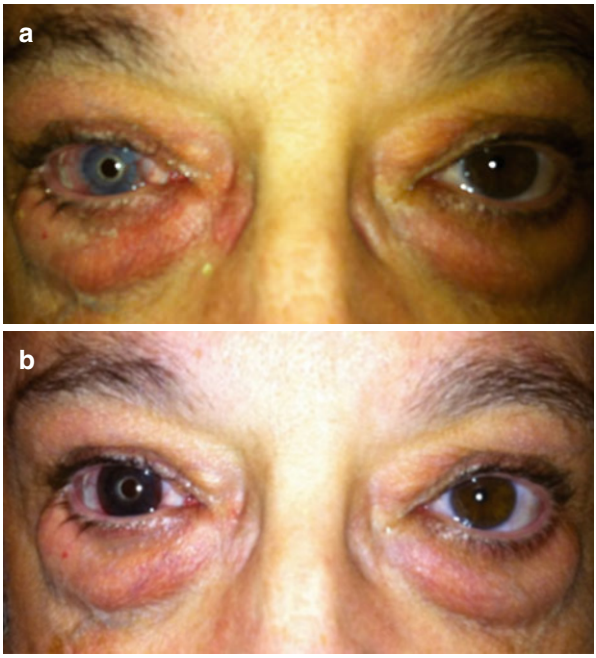

Fig. 9.5 Case study of two patients (**a**) and (**b**) respectively demonstrating clear Kontur bandage lens on the left and tinted Kontur lens on the right to match the patient's fellow eye

References

1. Lee H, Khan R, O'Keefe M (2008) Aniridia: current pathology and management. Acta Ophthalmol 86:708–715
2. Ramaesh K, Ramaesh T, Dutton GN, Dhillon B (2005) Evolving concepts on the pathogenic mechanisms of aniridia related keratopathy. Int J Biochem Cell Biol 37:547–557
3. Gomes J, Eagle R, Gomes A, Rapuano C, Cohen E, Laibson P (1996) Recurrent keratopathy after penetrating keratoplasty for aniridia. Cornea 15:457–462
4. Holland E, Djalilian A, Schwartz G (2003) Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. Ophthalmology 110:125–130
5. Edén U, Fagerholm P, Danyali R, Lagali N (2012) Pathologic epithelial and anterior corneal nerve morphology in early-stage congenital aniridic keratopathy. Ophthalmology 119:1803–1810
6. Lopez-Garcia J, Garcia-Lozano I, Rivas L, Martinez-Garchitorena J (2006) Congenital aniridia keratopathy treatment. Arch Soc Esp Oftalmol 81:435–444
7. Kremer I, Rajpal RK, Rapuano CJ, Cohen EJ, Laibson PR (1993) Results of penetrating keratoplaty in aniridia. Am J Ophthalmol 115:317–320
8. Tiller AM, Odenthal MT, Verbraak FD, Gortzak-Moorstein N (2003) The influence of keratoplasty on visual prognosis in aniridia: a historical review of one large family. Cornea 22:105–110
9. Hou JH, de la Cruz J, Djalilian AR (2012) Outcomes of Boston keratoprosthesis implantation for failed keratoplasty after keratolimbal allograft. Cornea 31(12):1432–1435
10. Solomon A, Ellies P, Anderson DF, Touhami A, Grueterich M, Espana EM, Ti SE, Goto E, Feuer WJ, Tseng SC (2002) Long-term outcome of keratolimbal allograft with or without penetrating keratoplasty for total limbal stem cell deficiency. Ophthalmology 109:1159–1166
11. Tseng S, Prabhasawat P, Barton K, Gray T, Meller D (1998) Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. Arch Ophthalmol 16(4):431–441
12. Shimazaki J, Maruyama F, Shimmura S, Fujishima H, Tsubota K (2001) Immunologic rejection of the central graft after limbal allograft transplantation combined with penetrating keratoplasty. Cornea 20:149–152
13. Ilari L, Daya SM (2002) Long-term outcomes of keratolimbal allograft for the treatment of severe ocular surface disorders. Ophthalmology 109:1278–1284
14. Dohlman CH, Harissi-Dagher M, Khan BF, Sippel K, Aquavella JV, Graney JM (2006) Introduction to the use of the Boston keratoprosthesis. Expert Rev Ophthalmol 1:41–48
15. Akpek EK, Harissi-Dagher M, Petrarca R, Butrus SI, Pineda R, Aquavella JV, Dohlman CH (2007) Outcomes of Boston keratoprosthesis in aniridia: a retrospective multicenter study. Am J Ophthalmol 144(2):227–231
16. Rixen JJ, Cohen AW, Kitzmann AS, Wagoner MD, Goins KM (2013) Treatment of aniridia with Boston type I keratoprosthesis. Cornea 32(7):947–950
17. Hassanaly SI, Talajic JC, Harissi-Dagher M (2014) Outcomes following Boston type 1 keratoprosthesis implantation in aniridia patients at the University of Montreal. Am J Ophthalmol 158(2):270–276
18. Greiner MA, Li JY, Mannis MJ (2011) Longer-term vision outcomes and complications with the Boston type 1 keratoprosthesis at the University of California, Davis. Ophthalmology 118(8):1543–1550
19. Kang JJ, de la Cruz J, Cortina MS (2012) Visual outcomes of Boston keratoprosthesis implantation as the primary penetrating corneal procedure. Cornea 31(12):1436–1440
20. Patel AP, Wu EI, Ritterband DC, Seedor JA (2012) Boston type 1 keratoprosthesis: the New York eye and ear experience. Eye 26(3):418–425
21. Robert MC, Harissi-Dagher M (2011) Boston type 1 keratoprosthesis: the CHUM experience. Can J Ophthalmol 46(2):164–168

22. Srikumaran D, Munoz B, Aldave AJ, Aquavella JV, Hannush SB, Schultze R, Belin M, Akpek EK (2014) Long-term outcomes of Boston type 1 keratoprosthesis implantation: a retrospective multicenter cohort. Ophthalmology. doi:10.1016/j.ophtha.2014.05.030
23. Sivaraman KR, Hou JH, Allemann N, de la Cruz J, Cortina MS (2013) Retroprosthetic membrane and risk of sterile keratolysis in patients with type I Boston keratoprosthesis. Am J Ophthalmol 155(5):814–822
24. Sonsino J, Ewald MD (2010) Corrective options for irregular corneas. CL Spectr 180:26
25. Sayegh RR, Avena Diaz L, Vargas-Martín F, Webb RH, Dohlman CH, Peli E (2010) Optical functional properties of the Boston keratoprosthesis. Invest Ophthalmol Vis Sci 51(2): 857–863

Chapter 10
Cell Therapy for Regeneration of the Corneal Epithelium in Aniridic Patients

Julie T. Daniels, S.J. Tuft, and A.J. Shortt

Abstract Stem cell therapy may in the future become a routine treatment for aspects of aniridia. In this chapter we will discuss how one such corneal stem cell therapy approach is already in use in the clinic as an unlicensed experimental medicine, the results achieved so far and the likely direction of future research to improve therapy efficacy.

Keywords Cornea • Aniridia • Stem cell therapy • Tissue engineering

The Cornea

The cornea is the transparent window on the front surface of the eye. It is comprised of a series of highly organized layers of tissue including the outermost epithelium, the collagenous stroma and the innermost endothelium. The epithelium is maintained during homeostasis and repaired following injury by the division of stem cells and subsequent differentiation of their daughters [1–3]. The precise location of these stem cells is still debated [4, 5]. However, a population of limbal epithelial stem cells (LESC) is known to reside within anatomical structures in the region of the palisades of Vogt at the periphery of the cornea [6–8]. If the LESC population is damaged by injury or disease the normal process of tissue maintenance is compromised and blinding ocular surface failure can occur. This has relevance to one sequela of aniridia, namely aniridia-related keratopathy (ARK) [9].

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Ocular Surface Failure in the Context of ARK

ARK is progressive condition, affecting the ocular surface of a great majority of patients with aniridia [10], although not always with visually significant consequences. Mutations in the PAX6 gene are thought to pre-dispose to LESC deficiency (LESCD) in aniridia [11]. However, diagnosis of LESCD is challenging and mainly relies upon clinical signs including inflammation, vascularization, wound healing impairment, pain, reduction in visual acuity and infiltration of conjunctival goblet cells detected by impression cytology [12]. This is because identification of LESC in a normal eye is also difficult owing to a lack of definitive markers.

The onset of ARK can be accelerated by surgery (e.g. cataract removal or glaucoma filtration surgery) and exacerbated by factors such as dry eye. It can begin as early as the first decade with peripheral vascularization gradually progressing to pan-corneal vascularization, keratinization and opacification. Interestingly, the limbal palisades of Vogt, which provide a niche for LESC, cannot be detected by in vivo confocal microscopy in patients with severe ARK [13–15], suggesting a gradual loss of LESC and/or their supporting environment with disease progression.

Treatment Options for ARK

Management of mild ARK affecting the visual axis may include amniotic membrane application and instillation of preservative-free eye drops or autologous serum [16] to preserve LESC function for as long as possible. However, more severe cases of ARK require LESC transplantation to restore the ocular surface and vision which has been successfully achieved using a combination of keratolimbal allografts and systemic immunosuppression [12]. Meanwhile, the prospect of using a less invasive surgical technique (which may carry reduced risk for ARK patients) was realized in 1997 when Pellegrini et al. described the first use of cultured autologous LESC therapy in two patients with corneal chemical burn injury [17]. Since this landmark paper was published, a variety of culture methods and techniques for transplanting LESC onto the surface of the cornea have been attempted and reviewed elsewhere [18]. Here we will describe a method we have previously used for the culture and transplantation of allogeneic LESC and our experience of using it in patients with ARK.

Cultured LESC Therapy

Amniotic membrane is a familiar material to ophthalmic surgeons and enjoys properties reported to reduce corneal inflammation and vascularization [19, 20]. It was therefore a good candidate substrate to evaluate for efficacy in the culture and

transplantation of human LESC in patients. Indeed, Tsai et al. [21] were able to confirm this for a small number of patients with chemical burn injury and also Stevens Johnson syndrome and ocular cicatricial pemphigoid. Their technique involved taking a biopsy from the patient's healthy eye and attaching it to the surface of amniotic membrane. From this explanted biopsy, limbal epithelial cells grew out to cover the entire surface of the amnion prior to patient grafting. In five out of six eyes transplanted, a stable ocular surface and improved visual acuity was achieved for around 15 months at the time of publication. However, since ARK is a bilateral condition, an alternative source of tissue would-be required for stem cell culture using this method.

We have previously reported a technique for utilizing human donor corneas as a source of LESC for culture on amniotic membrane [22]. Briefly, LESC were isolated and pre-expanded on a growth-arrested feeder cell layer (to provide sufficient cells for patient transplantation as well as for regulatory authority quality control assays) before transfer onto intact amniotic membrane. The second step is where some difficulties were experienced. Even with consistent protocols not every population of donor cells, which had all been successfully expanded on feeders, were able to thrive once transferred onto amniotic membrane. We later discovered that the method of clinical amnion preparation in the UK was not, at that time anyway, ideal for LESC culture [22]. Nevertheless, we were able to obtain LESC cultures suitable for transplantation 70 % of the time. In the first cohort of patients undergoing allogeneic LESC transplantation, three had ARK. The clinical outcome observed between 10 and 13 months showed that two out of three ARK patients experienced improved visual acuity [13]. However, our 3-year follow-up study, including a cohort of ten eyes of nine patients with ARK treated with cultured allogeneic LESC on amniotic membrane showed an eventual decline in therapeutic benefit in most cases [23]. These results are in stark contrast to the success achieved with the use of cultured autologous LESC therapy in patients with unilateral corneal chemical burns [24–27]. In our allogeneic LESC study the patients were treated with systemic immunosuppression for 6 months. Long-term systemic immunosuppression using cyclosporin or mycophenylate was not considered to be appropriate given the lack of evidence of long-term donor cell survival on the ocular surface [28]. However, a study by Paulkin et al. was able to show restoration of the corneal surface at 36 months following application of cultured allogeneic LESC in three out of six aniridic eyes treated [27]. In this study the patients were systemically immunosuppressed for up to 15 months with cyclosporine or mycophenylate, 6–9 months longer than in our study. Hence the role of immunosuppression in cultured allogeneic LESC therapy has yet to be fully evaluated.

Interestingly, we also observed an example of apparent long-term benefit to a PAX6 haploinsufficient patient cornea following cultured allogeneic LESC therapy (Fig. 10.1). This begs the question of the role of cultured LESC in ARK?

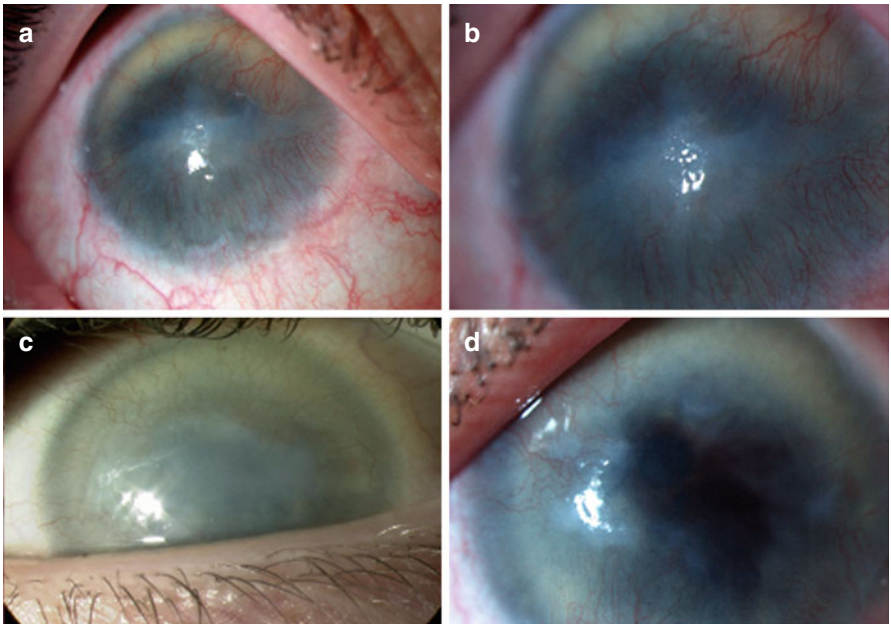

Fig. 10.1 Results of cultured allogeneic LESC transplantation in a patient with PAX6 haploinsufficiency. (**a**, **c**) show the right and left eye, respectively, of a 32 year old female patient with PAX6 haploinsufficiency. This individual has Reigers anomaly, and whilst not phenotypically aniridia, suffered recurrent epithelial breakdown due to LESCD. Both eyes were treated on separate occasions with cultured allogeneic LESC. The right eye received no benefit by 12 months post-transplantation (**b**) while the left eye was able to maintain a transparent window for at least 4 years (**d**)

Lessons Learned from Cultured LESC Therapy

The result shown in Fig. 10.1 highlights the complexity of treating ocular surface failure caused by PAX6 haploinsufficiency (in this case a patient with Reigers anomaly) even between the eyes of one patient, yet hints at the possibility of long-term therapeutic efficacy of cultured stem cell therapy. The maintenance of a transparent area of central cornea in one eye of this patient, over at least a 4-year period, suggests a positive influence of the grafted cells. However, as previously stated, since there is no evidence that allogeneic donor cells survive for very long on the ocular surface post-transplantation [28] the mode of therapeutic efficacy in this patient is unknown. Our hypothesis, which needs to be tested, is that reduced dosage of PAX6 may be related to host LESC dysfunction rather than deficiency and that if cultured stem cells are applied sufficiently early a trophic effect may rejuvenate host LESC activity. If true, the optimum time for cultured stem cell transplantation would need to be determined. A recent study by Eden et al. may shed light on this. Detailed investigations of both eyes of 16 patients with

congenital ARK, including tear film production, tear break up time, best spectacle-corrected visual acuity, corneal touch sensitivity, intraocular pressure measurement, slit-lamp biomicroscopy, ultrasound pachymetry and laser scanning in vivo confocal microscopy were able to identify the features of early ARK onset [29]. Ideally if these parameters were recorded in multiple centres and correlated with the outcome of cultured stem cell therapy (allogeneic LESC or another approach) a possible indicator of the optimal time to treat ARK could be established.

Alternative Cells for Therapy?

For patients with ARK, alternative sources of autologous stem cells could be beneficial since use of systemic immunosuppression is not trivial and best avoided where possible. Cultured oral mucosal epithelial transplantation (COMET) has been used to treat patients with corneal chemical and thermal burn injury, Stevens-Johnson syndrome, mucous membrane pemphigoid (ocular cicatricial pemphigoid) and idiopathic ocular surface disorder [30]. Three-year clinical follow up showed that visual acuity was improved in 50 % of eyes receiving COMET [31]. We are unaware of any published studies using COMET to treat ARK but have plans to try this in the near future. Whilst oral mucosal cells do not normally express PAX6 in the mouth [11], it would be interesting to see if PAX6 gene expression would be induced upon transplantation to the ocular surface and if the cells could have any therapeutic benefit in ARK. There is some precedent for this as it has been shown that following the COMET procedure in an alkali burn induced total LESCD, oral mucosal cells begin to express the corneal markers PAX6 and keratin 12 [32]. However, what is not clear, and would need to be explored, is the potential influence of the mutated PAX6 gene (carried by the cultured oral mucosal epithelial cells) following transplantation.

Other cell types which may be useful for restoration of the ocular surface in ARK include hair follicle epithelial stem cells [33] and olfactory cells [34]. Alternatively, induced pluripotent stem cells (iPSCs) [35] could have a future role in the treatment of ARK. A protocol for generating corneal epithelial cells from adult dermal fibroblasts has been established [36]. Theoretically then, it should be possible to correct the PAX6 gene mutation in iPSC-derived corneal epithelial cells prior to transplantation back to the patient. This is perhaps one of the most exciting prospects for long-term correction of ocular surface failure in ARK. However, in mice it has been shown that heterozygosity of PAX6 (low PAX6 levels which generates in mice a similar phenotype to ARK in humans) does not significantly affect LESC number [37]. Rather PAX6 heterozygosity causes more severe corneal stromal and endothelial defects. Hence, will replacing defective limbal/corneal epithelial cells alone be sufficient or should we also be considering the host environment in a more holistic manner?

Re-creating Elements of the Stem Cell Niche

It has been shown that the architecture of the normal niche environment for LESC is compromised in ARK (Fig. 10.2). We, and others, have learned that cell-cell and cell-matrix interactions are important for LESC survival and function in the laboratory [8, 38, 39] and are therefore also likely to be important in vivo. Our hypothesis is that niche and/or stromal cell support of LESC is compromised in aniridia and that disruption of normal epithelial-stromal cell interactions may contribute to progression of ARK. Studies are currently underway in our laboratory to test this premise.

As mentioned earlier, amniotic membrane has proved to be an unreliable substrate for LESC culture in some circumstances, perhaps due to processing methods [22] and/or inherent biological variability between donor tissues. Yet others have reported amnion to be a suitable surrogate LESC niche [40]. There is, therefore, ongoing research aimed at replacing amnion with alternative substrates including corneal stroma, silk fibroin and a variety of synthetic polymers, reviewed by [41, 42], for the culture and transplantation of human LESC.

We are using a simple approach developed from the original technique of plastic compression of type I collagen hydrogels originally described by Brown et al. [43]. Here a type I collagen hydrogel is prepared (which may contain cells); fluid is extracted onto absorbent papers via the application of weights on the top of the gel until a tissue-like material is formed. Further cell types can then be cultured on the surface. When we first started to use this method the potential for making stem cell-populated corneal tissue equivalents (TE), as an alternative to amnion, quickly became apparent. However, the technique at that time was not sufficiently robust, reproducible or regulatory compliant for clinical application (Daniels et al. 2005, unpublished). Working with Brown and a company (TAP Biosystems, now part of the Sartorius group) we

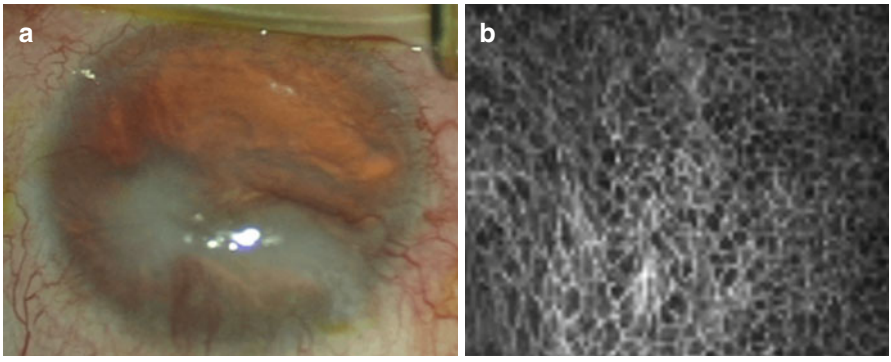

Fig. 10.2 The appearance of the limbus of a patient with ARK. (Image **a**) shows the appearance cornea of a patient with late stage ARK. Whilst epithelial cells could be observed (Image **b**), painstaking in vivo scanning laser confocal imaging (using the Heidelberg HRT II with Rostock corneal module) was unable to detect the typical undulating architecture of the palisades of Vogt in this patient, suggesting significant compromise of the LESC niche

started to develop a new process to produce collagen-based biomimetic tissues. Our first TE was populated with human limbal fibroblasts inside the collagen matrix with a limbal epithelium (containing a sub-population of LESC) on the upper surface [44]. This launched a series of investigations aimed at testing safety and efficacy for use of these TE in ocular surface reconstruction [45, 46]. Following several rounds of innovation **R**eal **A**rchitecture **f**or 3D **T**issues (RAFT) was developed [47] which is radically different from the original method of plastic compression for producing TE. Excitingly we were able to engineer limbal crypts-like features with 3D LESC niche architecture into RAFT [48] which may be of relevance to stem cell transplantation in ARK where the niche architecture has been lost.

Future Research

ARK is a complex condition and its degree of manifestation varies between patients. To fully understand how to treat this sight threatening aspect of aniridia further research is needed. Development of reliable methods for identifying LESC in vivo using non-invasive techniques is challenging but necessary to enable the ophthalmologist to recognize early signs of LESC loss/dysfunction. Currently greater knowledge of the appearance of the limbus in health and disease is being captured using laser scanning in vivo confocal microscopy. Further developments in imaging techniques such as optical coherence tomography, which is capable of imaging single cells at high resolution in the eye, could be valuable in the diagnosis of ARK progression in the future.

The emphasis of this chapter has been on the potential of cultured stem cell therapy to reverse or at least halt progression of ARK. This involves ex vivo expansion of stem cells (LESC or others). Progress has been made in the notoriously difficult field of epithelial stem cell identification. For example, high expression levels of the marker p63α in cultured LESC were correlated with optimal ocular surface reconstruction following transplantation over a 10-year period [24]. This study involved the use of autologous LESC mostly in patients with chemical burn injury. It will be interesting to discover if this correlation will also be a useful indicator of success with other sources of stem cells in the treatment of ARK. Recently a new LESC marker, ABCB5 was identified [49]. This is particularly exciting as limbal epithelial cells expressing this marker could be prospectively isolated and used to successfully reconstruct the ocular surface of LESC deficient mice. Advances such as these will enable us to better understand ARK and monitor efficacy as new therapeutic technologies are developed whether they involve autologous cells such as oral mucosa or genetically altered cells derived from iPSC. Continued progress towards understanding the specific mechanisms controlling limbal/corneal epithelial cell function in ARK will also be informative. Recently it was shown that loss of WNT7A function induces LESC to produce skin-like epithelium, a similar phenotype created by decreased PAX6 gene expression in ARK [50]. Therefore, widening our focus beyond PAX6 mutations in ARK may also be required to optimize future stem cell therapies.

References

1. Mann I (1944) A study of epithelial regeneration in the living eye. Br J Ophthalmol 28:26–40
2. Davanger M, Evenson A (1971) Role of the pericorneal structure in renewal of corneal epithelium. Nature 299:560–561
3. Cotsarelis G, Cheng G, Dong G, Sun TT, Lavker RM (1989) Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. Cell 57:201–209
4. Majo F, Rochat A, Nicolas M, Jacoude GA, Barrandon Y (2008) Oligopotent stem cells are distributed throughout the entire mammalian ocular surface. Nature 456:250–254
5. Sun TT, Tseng SC, Lavker RM (2010) Location of corneal epithelial stem cells. Nature 463:E10–E11
6. Dua HS, Shanmuganathan VA, Powell-Richards AO, Tighe PJ, Joseph A (2005) Limbal epithelial crypts: a novel anatomical structure and a putative limbal stem cell niche. Br J Ophthalmol 89:529–532
7. Shortt AJ, Secker GA, Munro PM et al (2007) Characterisation of the limbal epithelial stem cell niche: novel imaging techniques permit in-vivo observation and targeted biopsy of limbal epithelial stem cells. Stem Cells 5:1402–1409
8. Dziasko MA, Armer HE, Levis HJ, Shortt AJ, Tuft S, Daniels JT (2014) Localisation of epithelial cells capable of holoclone formation in vitro and direct interaction with stromal cells in the native human limbal crypt. PLoS One 9:e94283
9. Mayer KL, Nordlund ML, Schwartz GS, Holland EJ (2003) Keratopathy in congenital aniridia. Ocul Surf 1:74–79
10. Lee H, Khan R, O'Keefe M (2008) Aniridia: current pathology and management. Acta Ophthalmol 86:708–715
11. Li W, Chen YT, Hayashida Y et al (2008) Down-regulation of PAX6 is associated with abnormal differentiation of corneal epithelial cells in severe ocular surface diseases. J Pathol 214:114–122
12. Holland EJ, Djalilian AR, Schwartz GS (2003) Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. Ophthalmology 110:125–130
13. Shortt AJ, Secker GA, Rajan M et al (2008) Ex vivo expansion and transplantation of limbal epithelial stem cells. Ophthalmology 115:1989–1997
14. Lagali N, Eden U, Utheim TP, Chen X, Riise R, Dellby A, Fagerholm P (2013) In vivo morphology of the limbal palisades of vogt correlates with progressive stem cell deficiency in aniridia-related keartopathy. Invest Ophthalmol Vis Sci 54:5333–5342
15. Le Q, Deng SX, Xu J (2013) In vivo confocal microscopy of congenital aniridia-associated keratopathy. Eye (Lond) 27:763–766
16. Lopez-Garcia JS, Rivas L, Garcia-Lozano I, Murube J (2008) Autologous serum eyedrops in the treatment of aniridic keratopathy. Ophthalmology 115:262–267
17. Pellegrini G, Treverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M (1997) Long-term restoration of damaged corneal surfaces with autologous cultivated human epithelium. Lancet 349:990–993
18. Ahmad S, Kolli S, Lako M, Figueiredo F, Daniels J (2010) Stem cell therapies for ocular surface disease. Drug Discov Today 15:306–313
19. Kim JC, Tseng SC (1995) The effects on inhibition of corneal neovascularization after human amniotic membrane transplantation in severely damaged rabbit corneas. Korean J Ophthalmol 9:32–46
20. Tseng SC, Prabhasawat P, Barton K, Gray T, Meller D (1998) Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. Arch Ophthalmol 116:431–441
21. Tsai RJ, Li LM, Chen JK (2000) Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 343:86–93

22. Shortt AJ, Secker GA, Lomas RJ et al (2009) The effect of amniotic membrane preparation method on its ability to serve as a substrate for the ex-vivo expansion of limbal epithelial cells. Biomaterials 30:1056–1065
23. Shortt AJ, Bunce C, Levis HJ et al (2014) Three-year outcomes of cultured limbal epithelial allografts in aniridia and Stevens-Johnson syndrome evaluated using the clinical Outcome Assessment in Surgical Trials assessment tool. Stem Cells Transl Med 3:265–275
24. Rama P, Matuska S, Paganoni G, Spinelli A, De Luca M, Pellegrini G (2010) Limbal stem-cell therapy and long-term corneal regeneration. N Engl J Med 363:147–155
25. Sangwan VS, Basu S, Vemuganti GK et al (2011) Clinical outcomes of xeno-free autologous cultivated limbal epithelial transplantation: a 10-year study. Br J Ophthalmol 95: 1525–1529
26. Sangwan VS, Matalia HP, Vemuganti GK et al (2006) Clinical outcome of autologous cultivated limbal epithelium transplantation. Indian J Ophthalmol 54:29–34
27. Pauklin M, Fuchsluger TA, Westekemper H, Steuhl KP, Meller D (2010) Midterm results of cultivated autologous and allogeneic limbal epithelial transplantation in limbal stem cell deficiency. Dev Ophthalmol 45:57–70
28. Daya SM, Watson A, Sharoe JR, Giledi O, Rowe A, Martin R, James SE (2005) Outcomes and DNA analysis of ex vivo expanded stem cell allograft for ocular surface reconstruction. Ophthalmology 112:470–477
29. Eden U, Fagerholm P, Danyali R, Lagali N (2012) Pathologic epithelial and anterior corneal nerve morphology in early-stage congenital aniridic keratopathy. Ophthalmology 119: 1803–1810
30. Inatomi T, Nakamura M, Koizumi N, Sotozono C, Yokoi N, Kinoshita S (2006) Midterm results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation. Am J Ophthalmol 141:267–275
31. Nakamura T, Takeda K, Inatomi T, Sotozono C, Kinoshita S (2011) Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders. Br J Ophthalmol 95:942–946
32. Gaddipati S, Muralidhar R, Sangwan VS, Mariappan I, Vemuganti GK, Balasubramanian D (2014) Oral epithelial cells transplanted on to corneal surface tend to adapt to the ocular phenotype. Indian J Ophthalmol 62:644–648
33. Blazejekska EA, Schlotzer-Schrehardt U, Zenkel M, Bachmann B, Chankiewitz E, Jacobi C, Kruse FE (2009) Corneal limbal microenvironment can induce transdifferentiation of hair follicle stem cells into corneal-like epithelial cells. Stem Cells 27(3):642–652
34. Collinson JM, Quinn JC, Hill RE, West JD (2003) The roles of *PAX6* in the cornea, retina and olfactory epithelium of the developing mouse embryo. Dev Biol 255:303–312
35. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676
36. Hayashi R, Ishikawa Y, Ito M, Kageyama T et al (2012) Generation of corneal epithelial cells from induced pluripotent stem cells derived from human dermal fibroblast and corneal limbal epithelium. PLoS One 7:e45435
37. Mort RL, Bentley AJ, Martin FL et al (2011) Effects of aberrant PAX6 gene dosage on mouse corneal pathophysiology and corneal epithelial homeostasis. PLoS One 6:e28895
38. Notara M, Shortt AJ, Galatowicz G, Calder V, Daniels JT (2010) IL6 and the human limbal stem cell niche: a mediator of epithelial-stromal interaction. Stem Cell Res 5:188–200
39. Chen SY, Hayashida Y, Chen MY, Xie HT, Tseng SC (2011) A new isolation method of human limbal progenitor cells by maintaining close association with their niche cells. Tissue Eng Part C Methods 17:537–548
40. Tsai RJ, Tsai RY (2014) From stem cell niche environments to engineering of corneal epithelium tissue. Jpn J Ophthalmol 58:111–119
41. Levis H, Daniels JT (2009) New technologies in limbal epithelial stem cell transplantation. Curr Opin Biotechnol 20:593–597
42. Feng Y, Borrelli M, Reichl S, Schrader S, Geerling G (2014) Review of alternative carrier materials for ocular surface reconstruction. Curr Eye Res 39:541–552

43. Brown RA, Wiseman M, Chuo CB, Cheema U, Nazhat SN (2005) Ultra-rapid engineering of biomimetic tissues: a plastic compression fabrication process for nanao-micro structures. Adv Funct Mater 15:1762–1770
44. Levis HJ, Brown RA, Daniels JT (2010) Plastic compressed collagen as a biomimetic substrate for human limbal epithelial cell culture. Biomaterials 31:7726–7737
45. Kureshi AK, Drake RA, Daniels JT (2014) Challenges in the development of a reference standard and potency assay for the clinical production of RAFT tissue equivalents for the cornea. Regen Med 9:167–177
46. Massie I, Levis HJ, Daniels JT (2014) Response of human limbal epithelial cells to wounding on 3D RAFT tissue equivalents: effect of airlifting and human limbal fibroblasts. Exp Eye Res 127:196–205
47. Levis HJ, Menzel-Severing J, Drake RA, Daniels JT (2013) Plastic compressed collagen constructs for ocular cell culture and transplantation: a new and improved technique of confined fluid loss. Curr Eye Res 38:41–52
48. Levis HJ, Massie I, Dziasko MA, Kaasi A, Daniels JT (2013) Rapid tissue engineering of biomimetic human corneal limbal crypts with 3D niche architecture. Biomaterials 34:8860–8868
49. Ksander BR, Kolovou PE, Wilson BJ et al (2014) ABCB5 is a limbal stem cell gene required for corneal development and repair. Nature 511:353–357
50. Ouyang H, Xue Y, Lin Y et al (2014) WNT7A and PAX6 define corneal epithelium homeostasis and pathogenesis. Nature 511:358–361

Chapter 11
Strategies for Success in Limbal Allograft Transplantation for Aniridia

Omar Hassan and Ali R. Djalilian

Abstract Aniridic keratopathy can be seen in up to 90 % of patients. Traditionally, therapy was supportive followed by penetrating keratoplasty when stromal scarring occurred. More recently, keratolimbal allograft (KLAL) has been shown to be an effective treatment. In this chapter we discuss how to maximize the visual outcome of aniridic keratopathy patients. Glaucoma should be addressed by placing shunts in patients prior to KLAL in order to limit topical glaucoma medication in the post operative course. Aniridic keratopathy patients with deteriorating corneal surfaces should be operated on before stromal scarring occurs. Fibrin glue may be used in surgery to reduce operating time and increase patient comfort. Immunosuppression plays a vital role in maintaining the allograft without rejection and should be monitored by a transplant specialist. With correct management, donor cells have been shown to populate the corneal surface years after surgery.

Keywords Aniridic keratopathy • Keratolimbal allograft • KLAL • Limbal stem cell transplantation • Penetrating keratoplasty

Aniridic keratopathy, to various degrees, can be seen in up to 90 % of patients [1]. Signs keratopathy appear as early as the first decade of life, though the median age of diagnosis is at 33 years of age [2]. It begins with conjunctivalization and vascularization of the peripheral cornea with slow progressive advancement into the central cornea. Clinically, patients with aniridic keratopathy experience recurrent erosions, chronic pain, corneal ulceration, and, eventually, loss of vision [3]. Traditionally, the approach was supportive followed by penetrating keratoplasty when stromal scarring occurred. The outcomes for this procedure for aniridic keratopathy are poor [4].

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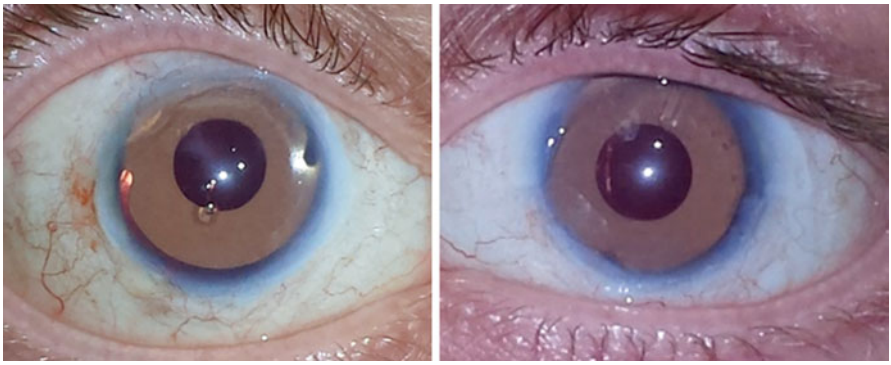

Fig. 11.1 Successful bilateral KLAL in 53 year old aniridia patient, 2.5 years post-op

The role of the PAX6 gene has been shown to be related to many of the features of an aniridic phenotype and is involved in the regulation of corneal epithelium and the limbal stem cell niche [1, 2, 5]. Keratolimbal allograft (KLAL) has been shown to be an effective treatment for aniridic keratopathy, with promising results [3] (Fig. 11.1). As opposed to penetrating keratoplasty, KLAL addresses the underlying etiology of aniridic keratopathy by replenishing the host stem cells that supply the corneal surface.

Over the past decade, this approach has been validated clinically, though close follow up is needed to ensure long term KLAL success. We note that the patients who fared best after limbal allograft transplantation had the following factors in common, listed in descending order of importance:

- Proper immunosuppression
- Early surgical treatment of glaucoma
- Earlier surgical intervention

Surgical Timing

Aniridic Keratopathy

It is important to operate early before stromal scarring occurs [3]. A limbal allograft addresses the primary etiologic factor that results in the keratopathy as opposed to a keratoplasty, which simply treats the outcome and proves a poor long-term treatment option. With early limbal stem cell transplantation and systemic immunosuppression, a better visual outcome is achieved, more patients achieve a stable ocular surface, and the need for subsequent penetrating keratoplasty is decreased.

Glaucoma

Glaucoma develops or progresses in almost all aniridic eyes after limbal stem cell transplant. Management of this disease alongside the transplant presents a problem. Post operative steroid drops are necessary for immunosuppression, yet they increase intraocular pressure. Furthermore, glaucoma drops are toxic to the epithelial layers and stem cells, leading to an increased risk of transplant failure. As such, we have seen that the insertion of a tube shunt prior to limbal allograft transplantation has favorable results due to the limitation of topical glaucoma medication. A tube shunt can be placed through the sulcus, posterior to the graft, in order to achieve the desired effect with lower risk to the corneal epithelium [6].

Surgical Technique

Three options exist for the source of stem cells in limbal stem cell transplantation: keratolimbal allografts (KLAL), living-related conjunctival-limbal allograft (lr-CLAL), and ex vivo expanded epithelial cells. Ex vivo expanded epithelial transplants will be discussed elsewhere.

Keratolimbal Allograft

Tissue containing limbal stem cells can be obtained from cadaver donor between the ages of 5 days and 50 years. Greater than a 3 mm scleral or conjunctival ring can be transplanted either with a full 360° transplant or in three segments of 180° each (Fig. 11.2).

Fibrin glue, such as those used extensively in pterygium and other conjunctival surgeries, can also be used to assist in KLAL. Cyanoacrylate glue can aid in stabilization of tissue during dissection, while fibrin glue can be used to stabilize and secure the graft. By tucking the donor tissue under the host conjunctiva with the aid of fibrin glue, operative times are reduced due to easier techniques and post-operative patient comfort is improved [7, 8].

Living Related Conjunctival-Limbal Allograft

Operative techniques for lr-CLAL are similar to that of KLAL (Fig. 11.3). Tissue source in this case is from a directly related donor such as sibling, parent, or child. ABO as well as HLA A, B, and DR are matched, leading to overall less rejection

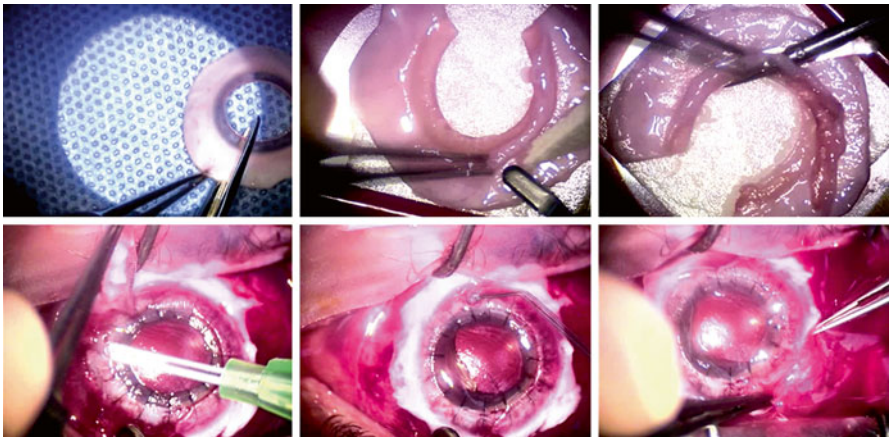

Fig. 11.2 Overview of KLAL surgery

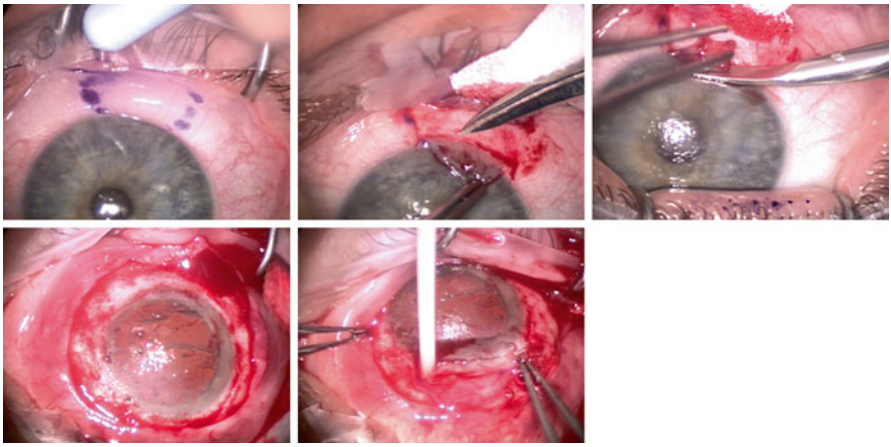

Fig. 11.3 Overview of lr-CLAL surgery

than KLAL. There is some concern whether or not lr-CLAL contains a sufficient number of limbal stem cells, but studies indicate that there is at least enough for effective treatment of aniridic patients [9].

Immunosuppression

Of the factors that are involved in long term ocular surface stability after KLAL, immunosuppression is of the greatest importance (Fig. 11.4) [3]. Many different combinations and durations of systemic and topical immunosuppression exist. We

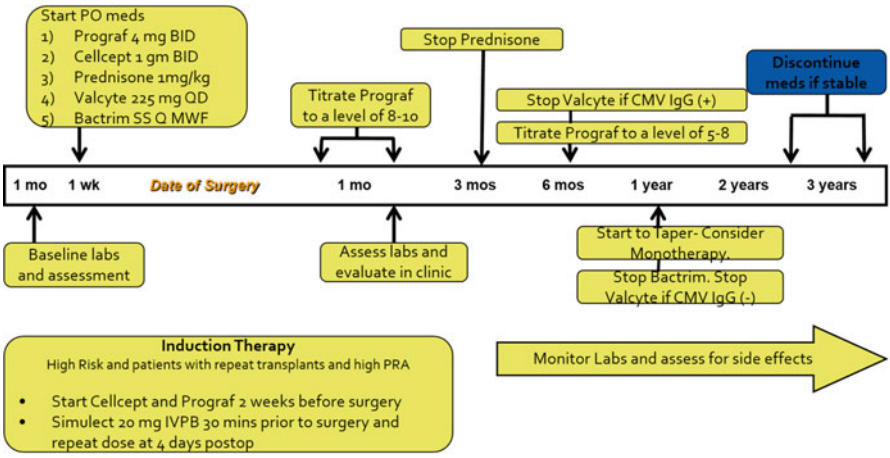

Fig. 11.4 Overview of clinical progression of immunosuppression

present here what has been effective in our experience with transplant patients. It is also necessary to note that immunosuppression should be coordinated by a transplant specialist and should be approached similarly to other organ transplants, such as kidney transplants.

Local

Topically, it is important to use more potent steroids, such as difluprednate or Pred Forte, in the initial 1–2 years following KLAL. Following this period, they can be tapered to weaker steroids along with topical cyclosporine (0.05–1 %) two to four times a day, but should be continued indefinitely. This decision is not a static choice; titrations can be made to counter the level of inflammation observed clinically.

Systemic

Two protocols have been developed for systemic immunosuppression following KLAL. They contain medications from three immunosuppressive families: (1) corticosteroids, (2) T-cell inhibitors, and (3) antimetabolites. An example of our immunosuppression protocol on the post-surgical timeline can be seen in Fig. 11.4. Note that, for high risk patients and those with high panel reactive antibody, we begin T-cell inhibitor and antimetabolite therapy 2 weeks prior to surgery than 1. 20 mg of basiliximab (Simulect) is also administered as IVPB 20 min prior to surgery and again 4 days after operation.

	Protocol 1	Protocol 2
Corticosteroid	Prednisone	Prednisone
	1 mg/kg/day tapered over 3 months	1 mg/kg/day tapered over 2–3 months
T-cell inhibitor	Cyclosporin A	Tacrolimus
	4 mg/kg/day	4 mg twice daily
Antimetabolite	Azathioprine	Mycophenolate mofetil
	1.5 mg/kg/day	1–2 g/day

Steroids

Oral prednisone is used to ensure control of early post-operative inflammation. We begin at a 40–60 mg/day dose for the first week. This is eventually tapered to about 15–20 mg/day by the end of the first month and completely tapered off by 2–3 months.

T-Cell Inhibitors

There are two options for the T-cell inhibitor arm of immunosuppression. We recommend cyclosporin A at 3–5 mg/kg, with a 2 h level of 1,000–1,500 ng/ml in the first 6 months of treatment [10]. In the long term, a 2 h level of 800–900 ng/ml is sufficient. Tacrolimus can also be started at 4 mg twice daily, with an initial 12 h level of 8–10 ng/ml in the first 3 months, then 3–8 ng/ml after that [11].

Anti-proliferative Agents

Three options exist for anti-proliferative agents after KLAL, modeled after the protocol of a local organ transplant team. These options are: azathioprine at 1.5 mg/kg, mycophenolate at 1,000 mg twice daily, and sirolimus 1–2 mg/day.

Individualization of Immunosuppression, Risks and Benefits

Choosing the right immunosuppressive drugs and doses is a delicate balancing act. Too much immunosuppression will save the graft, but carries well-known side effects such as cardiovascular disease, infection, neoplasia, and nephrotoxicity. On the other hand, not enough immunosuppression will cause allograft rejection.

This not only bodes well for the prognosis of the current graft, but, as mentioned previously, will lower the chances of a successful reoperation due to sensitization.

In order to quantify the side effects from systemic immunosuppression in ocular stem cell transplantation, a study of 136 patients with a mean duration of 42.1 months of immunosuppression was performed [12]. This showed only three severe adverse events in two patients (1.5 %) which involved two myocardial infarctions and one pulmonary embolism, though none of these events could be directly attributed to the immunosuppressive medication. There were also 21 minor adverse events in 19 patients (14.0 %) that included diseases such as hypertension, elevated glucose, and liver enzyme changes. These all resolved or where successfully treated, leading the study to conclude that, "with appropriate long-term monitoring, the risk of irreversible toxicity is minimal."

The specific outcomes of immunosuppression after KLAL showed similar results, albeit with more minor adverse events. In a study of 16 patients with greater than 1 year of immunosuppression, 75 % had a minor adverse effect [13]. Eighty-three percent of patients with comorbidities, compared to 25 % of those without comorbidities, experienced an adverse event. None of the adverse events where irreversible. The study also showed that younger patients were associated with significantly less risk than older patients.

Rejection

Despite systemic immunosuppression, there is still a 25–30 % occurrence of acute stem cell rejection. Many of these cases present as either mild or severe within 1–2 years [14]. Severe rejections present with discomfort and characteristic graft edema and intense inflammatory rejection. An epithelial rejection line is often present along with subconjunctival hemorrhage (Fig. 11.5). Mild rejections, on the other hand, are often asymptomatic and present with mild limbal injection and, in some

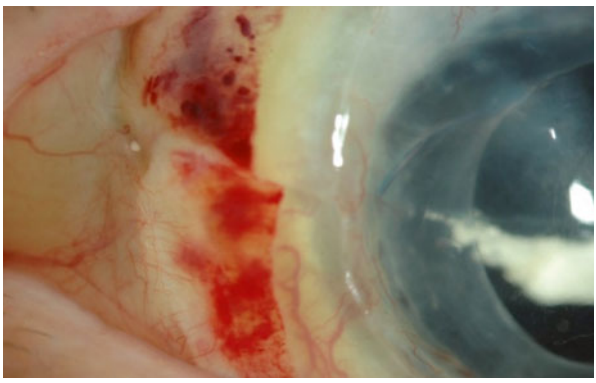

Fig. 11.5 Acute klal graft rejection evident by swollen injected grafts along with conjunctival injection

cases, an epithelial rejection line. Chronic rejection most commonly manifests as chronic injection, which may or may not progress to an epithelial line rejection. It is important to recognize immune rejection in KLAL patients.

Case

A 42 year old aniridic patient status post KLAL 5.5 years prior. She had been off systemic immunosuppression for 2 years and recently decided to stop topical steroids on her own accord. She presents with epithelial rejection. The decision is made to start intense topical steroids as well as restarting mycophenolate.

Surgical Outcomes

The Holland group has demonstrated a 75–80 % success with limbal transplant in aniridia [12]. Healthy corneas, previously untouched by surgery, had a higher success rate than those with previous penetrating keratoplasty or endothelial disease. In patients with previous failed penetrating keratoplasty and/or KLAL, a Boston keratoprosthesis may be preferred to repeat KLAL procedures. We have also noted that surgical treatment at earlier stages of the disease results in higher success. As mentioned above, glaucoma status and the amount of drops in the treatment plan also has an effect on the overall success of treatment.

Donor Tissue Survival in KLAL

Theoretically, limbal stem cell transplant replenishes the supply of host stem cells to counter corneal pathology. In practice, it is important to note whether these cells survive in the long term. In a study of three patients at least 3 months after successful limbal allograft transplant, corneal buttons were removed at the time of penetrating keratoplasty. The epithelium of the button, removed by either scraping or laser capture microdissection, was then analyzed for the presence of donor and recipient cells. The corneal phenotype was verified by ensuring K12 expression. The DNA was isolated and specific microsatellites were amplified with PCR and compared to blood DNA. The presence of non-host polymorphisms were interpreted as being donor derived. One patient, 24 months after KLAL and lr-CLAL showed mixed donor and recipient cells in three out of four quadrants, with only recipient cells in one quadrant [15]. In another case, 3.5 years after KLAL, only donor cells were detected in all quadrants [15].

This suggests that, though the patient had discontinued immunosuppression, immunologic tolerance had occurred and allowed for the persistence of the donor stem cells. It should also be noted that late KLAL rejection, up to 8 years after

surgery, has been reported, suggesting even longer-term persistence of donor cells than that objectively identified. The mix of donor and recipient cells in the cornea of some patients suggests a chimera produced by a recipient limbal niche supported with donor stem cells.

Summary

Maximizing the success of limbal allograft transplant in aniridia depends on a few considerations:

- Operating early in the disease course, before stromal scarring occurs.
- Managing glaucoma pre-operatively with tube shunt placement, even if pressure is controlled with drops.
- lr-CLAL may be preferred over KLAL when available to further reduce the risk of rejection.
- Standard immunosuppression protocols that continue at least 3–4 years, with monitoring by an organ transplant specialist.
- Chronic injection signifies chronic rejection, and must be addressed

References

1. Lee HJ, Colby KA (2013) A review of the clinical and genetic aspects of aniridia. Semin Ophthalmol 28(5–6):306–312
2. Mayer KL, Nordlund ML, Schwartz GS, Holland EJ (2003) Keratopathy in congenital aniridia. Ocul Surf 1(2):74–79
3. Holland EJ, Djalilian AR, Schwartz GS (2003) Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. Ophthalmology 110(1):125–130
4. Kremer I, Rajpal RK, Rapuano CJ et al (1993) Results of penetrating keratoplasty in aniridia. Am J Ophthalmol 115:317–320
5. Lee H, Khan R, O'Keefe M (2008) Aniridia: current pathology and management. Acta Ophthalmol 86(7):708–715
6. Aref AA, Sivaraman KR, Djalilian AR (2013) Glaucoma drainage implant surgery and ocular surface transplant graft preservation. Semin Ophthalmol 30(3):210–3
7. Nassiri N, Pandya HK, Djalilian AR (2011) Limbal allograft transplantation using fibrin glue. Arch Ophthalmol 129(2):218–222
8. Welder JD, Pandya HK, Nassiri N, Djalilian AR (2012) Conjunctival limbal autograft and allograft transplantation using fibrin glue. Ophthalmic Surg Lasers Imaging 43(4):323–327
9. Biber JM, Skeens HM, Neff KD, Holland EJ (2011) The Cincinnati procedure: technique and outcomes of combined living-related conjunctival limbal allografts and keratolimbal allografts in severe ocular surface failure. Cornea 30(7):765–771
10. Cole E, Midtvedt K, Johnston A, Pattison J, O'Grady C (2002) Recommendations for the implementation of neoral C(2) monitoring in clinical practice. Transplantation 73(9 Suppl):S19
11. Anglicheau D, Verstuyft C, Laurent-Puig P, Becquemont L, Schlageter MH, Cassinat B, Beaune P, Legendre C, Thervet E (2003) Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. Am Soc Nephrol 14(7):1889

12. Holland EJ, Mogilishetty G, Skeens HM, Hair DB, Neff KD, Biber JM, Chan CC (2012) Systemic immunosuppression in ocular surface stem cell transplantation: results of a 10-year experience. Cornea 31(6):655–661
13. Krakauer M, Welder JD, Pandya HK, Nassiri N, Djalilian AR (2012) Adverse effects of systemic immunosuppression in keratolimbal allograft. J Ophthalmol 2012:576712
14. Baradaran-Rafii A, Eslani M, Djalillian AR (2013) Complications of keratolimbal allograft surgery. Cornea 32(5):561–566
15. Djalilian AR, Mahesh SP, Koch CA, Nussenblatt RB, Shen D, Zhuang Z, Holland EJ, Chan CC (2005) Survival of donor epithelial cells after limbal stem cell transplantation. Invest Ophthalmol Vis Sci 46(3):803–807

Chapter 12
The Paediatric Patient: Identifying Congenital Aniridia as Soon as Possible

Elena Piozzi and Davide Allegrini

Abstract Aniridia is a congenital panocular condition affecting iris, cornea, anterior chamber angle, lens, retina and optic nerve. It is rare but it can progressively impair vision in multiple causes including keratopathy, cataract, glaucoma, foveal hypoplasia, nystagmus. Aniridia is a genetic haplo-insufficiency expression of the PAX6 gene located on the chromosome 11p13. Aniridia, genital anomalies, retardation and Wilms tumor are called WAGR Syndrome. In this chapter we emphasize the importance of a thorough ophthalmologic evaluation of the anterior and posterior segment, and orthoptic for the evaluation of strabismus, nystagmus and ocular motility. The assessment of visual acuity for distance and near must take into account the age of the patient, in order to use more appropriate methods. There are various forms, w hich are different for clinical manifestations and visual acuity. It is important an early diagnosis and an early treatment of complications, to save visual ability and the visual field, in order to reduce the damage and to maintain a better quality of life in aniridic patients.

Keywords Aniridia • PAX 6 • WAGR syndrome • Aniridic keratopathy • Foveal hypoplasia

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Introduction

Aniridia is a congenital panocular conditioning affecting iris, cornea, anterior chamber angle, lens, retina and optic nerve [1]. It has an incidence of between 1/64.000 and 1: 100.000 and it may be found in isolation or in association with other syndromes characterized by partial or complete absence of the iris and iris hypoplasia [2].

Aniridia can occur in a sporadic or a familiar form. About two-thirds of children with aniridia have an affected parent, with the disorder being inherited as an autosomal-dominant trait, which is expressed with high frequencies in the offspring [3]. One-third of cases occur in sporadic form, and about one-third of these individuals also develop Wilms tumor. Aniridia, genital anomalies, retardation, Wilms tumor are called WAGR Syndrome [4]. Familiar forms are more easy to diagnose, because the pediatrician routinely requires ophthalmologic evaluation.

Aniridia is a disorder affecting tissues of the eye, in addition to the iris abnormalities for which it is named [2, 5, 6]. Affected individuals characteristically have absent or altered iris tissue and foveal hypoplasia, which generally leads to nystagmus and depressed visual acuity (usually 20/110–20/200). Most cases present within 6 weeks of birth with an iris or pupillary abnormality or nystagmus [2, 5–7]. Later onset cataracts, glaucoma and corneal opacification are responsible for progressive visual reduction.

Early diagnosis is very important to recognize the disease and consequently to carry out a correct management of pediatric patients. In familiar form the pediatrician recommends an eye exam in the first months, but in a sporadic form is more difficult for him evaluates alterations of the anterior segment early, especially in case of dark iris. Instead nystagmus and anisocoria are signs which are easier to be evaluated.

The purpose of this chapter is to provide an update on ocular manifestations and perform some information to pediatrician which permits to refer quickly the patient to pediatric ophthalmologist. The application of a standard ophthalmic evaluation protocol may serve as an important diagnostic and disease monitoring tool in patients.

Ocular Manifestations

Iris

The Iris deficiency is connected with decreased visual acuity, glare and photophobia [5]. This defect is the first sign, it is different, in some cases, the defect can to be limited or partial, regarding a portion of iris, similar to an atypical coloboma (Figs. 12.1 and 12.2). In some cases asymmetrical involvement of iris is present. Two principal hypotheses for the pathogenesis of hypoplastic iris development (“aniridia”) have been proposed: (1) the ectodermal theory, positing incomplete

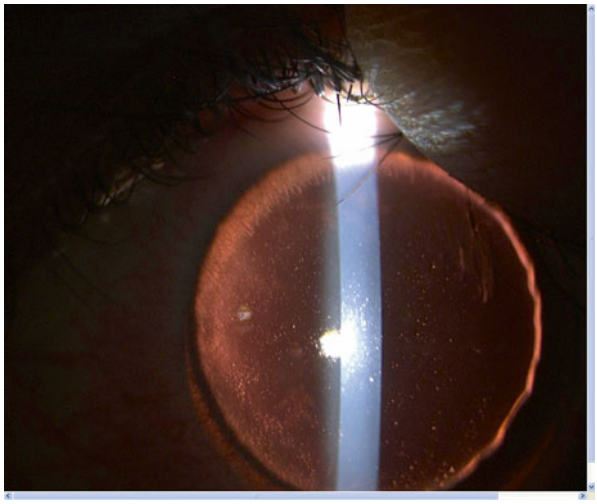

Fig. 12.1 Complete aniridia

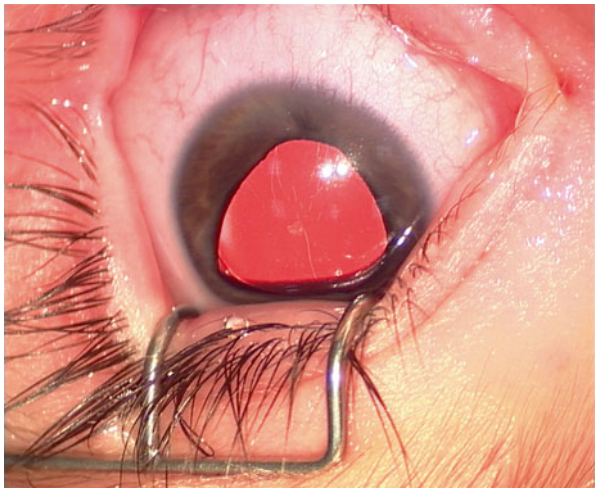

Fig. 12.2 Partial aniridia. Similar to an atypical coloboma

elaboration of the cup, resulting in an absence of framework for further development; and (2) the "mesodermal" theory, wherein inadequate migration or proliferation of mesenchymal elements is proposed. Finally, it is also possible that aniridia results from excessive remodeling, in that portions of the iris may form and then regress inappropriately [8]. Some Authors reported stromal hypoplasia, full-thickness iris holes and radial stromal defects in patients who had the additional ophthalmic findings consistent with what we define as classic congenital aniridia (poor visual acuity, fovea hypoplasia, nystagmus, cataract and glaucoma). Aniridia and its variants may cause diagnostic difficulty especially when the iris is not wholly or partially absent [9, 10]. In congenital iris ectropion (also called ectropionuveae), the posterior pigment epithelium of the iris extends onto the anterior iris surface causing

darkening around the pupil. Willcock et al. report a man and his infant son with variant aniridia and a mutation in the PAX6 gene, where the major anterior segment finding was iris ectropion [11].

Lens

Congenital lens opacity (especially polar) are common [2, 5, 12, 13]. Occasionally there are remnants of foetal vascularization of the anterior lens capsule (tunica vasculosalentis) or a persistent pupillary membrane. Cataracts are rare in infancy, but visually significant lens opacities eventually develop in 50–85 % of aniridics [2], often in the teens [14]. Histological studies performed on the anterior capsule of aniridia cataracts have found them to be very fragile [15, 16]. Other lens abnormalities are subluxation, coloboma, posterior lenticonus and microsferophachia. It is very important before the rimotion or extraction a detailed evaluation of visual function. In fact the extraction is connected with decreased visual function. The surgery requires particular attention and the modality of operation is important to avoid or reduce the limbus damage. The stem cell deficiency worse after the surgery.

Glaucoma

There is an incidence of glaucoma of approximately 6–75 % in aniridia [2]. Glaucoma in aniridia is linked to developmental abnormalities in the drainage angle of the eye, which obstructs the outflow of the aqueous humour through Schelemm's canal. Generally, although these abnormalities are present at birth, but the ocular pressure is normal. Monitoring the pressure is imperative every 6 months. Central pachymetry is thicker than in the general population and overestimates ocular pressure if a corrective coefficient is not used. Glaucoma usually develops in later childhood or adulthood but may be present in infancy with a large corneal diameter and corneal oedema (buphthalmos) [2, 5, 6, 12, 13]. Margo did a histopathologic study of the anterior segment in seven enucleated eyes of children with congenital aniridia. Besides iridic and ciliary body hypoplasia three congenital abnormalities of the anterior segment were noticed: anomalous development of the anterior chamber angle, incomplete cleavage of the anterior chamber angle, and attenuation of Bowman's membrane. Three acquired abnormalities of the anterior segment were identified: corneal pannus, peripheral anterior synechiae, and lenticular degeneration. The two cases showing anomalous development of the anterior chamber angle occurred in children with a partial deletion of the short arm of chromosome 11 [17]. Aniridia is a genetic haploinsufficiency expression of the PAX6 gene located on chromosome 11p13, this causes insufficient differentiation and schlemn's canal is absent [18]. It has been well recognized in the past that there are gonioscopic and histologic differences between nonglaucomatous and glaucomatous aniridic eyes, and it has been recognized clinically in

aniridia in the past that buphthalmos is rare and glaucoma in infancy is unusual, but that glaucoma commonly develops later in childhood. Grant and Walton observed that in most of the children who developed glaucoma the anterior stromal layer of the stump gradually extended further anteriorly over the trabecular meshwork, and the intraocular pressure became elevated as the filtration area became covered by the extension of abnormal iris tissue. They believe that in congenital aniridia there is a progressive degeneration of the corneo-scleral angle, with the development of a contractile membrane between the surface of the iris and the angle wall playing a role in the gradual obstruction or closing of the angle [19].

Cornea

Aniridia associated keratopathy (AK) occurs secondary to limbal stem cell deficiency, and is thought to have an incidence of 20 % [20]. The cornea in aniridic patients appears normal and transparent during infancy and childhood [2, 21]. However, during early teens the cornea begins to show changes. The early changes are marked by the ingrowth of blood vessels from the limbal region into the peripheral cornea (Fig. 12.3). Subsequently, goblet cells appear in the corneal epithelium [2, 21]. These changes can eventually culminate in opacification of the corneal stroma, which leads to visual loss [2, 21]. There is a large increase in central corneal thickness [22, 23]. AAK is often worse after surgery that involves excessive manipulation of the limbus, or after the application of topical antimetabolites in order to treat the aniridia-associated glaucoma [2]. This stimulus appears to be enough to disrupt the fragile balance that maintains the corneal epithelium in aniridia [5]. Histological changes in the aniridic cornea include superficial stromal neovascularisation, stromal infiltration with inflammatory cells, destruction of Bowman's layer, the presence of goblet cells

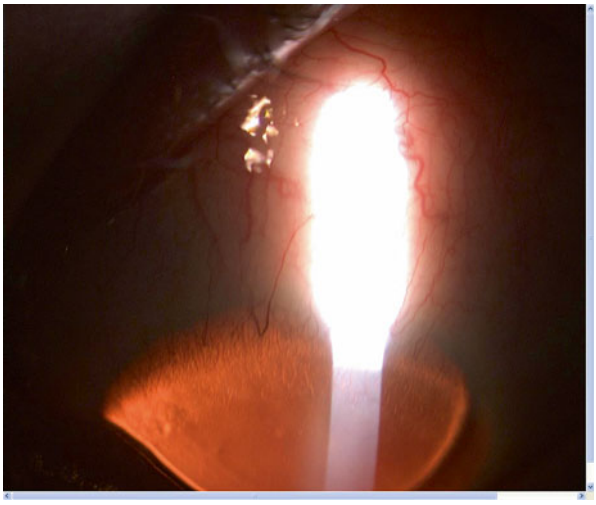

Fig. 12.3 Blood vessels from the limbal region into the peripheral cornea

and conjunctival cells on the corneal surface [17, 24]. Heather et al. described a subset of patients referred for undiagnosed cornea pathology who presented with signs and symptoms of AK. Because iris findings were generally mild and nystagmus or other findings of classic aniridia, including foveal hypoplasia, were mild or not present, the diagnosis of AK was not previously entertained by the referring physicians. Nonetheless, they demonstrated that a subset of these patients has defined mutations in PAX6 and that all patients responded well to keratolimbal allograft (KLAL). It is important to identify that the cornea changes in these patients are associated with AK because without limbal stem cell transplantation, routine penetrating keratoplasty is destined to fail [24]. The corneal changes in aniridia may be related to an abnormality within the limbal stem cell niche. The mechanisms underlying progressive corneal pathology in aniridia appear multi-factorial and include: (1) abnormal corneal healing responses secondary to anomalous extracellular matrix metabolism; (2) abnormal corneal epithelial differentiation leading to fragility of epithelial cells; (3) reduction in cell adhesion molecules in the PAX6 heterozygous state, rendering the cells susceptible to natural shearing forces; and (4) conjunctival and corneal changes leading to the presence of cells derived from conjunctiva on the corneal surface.

Optic Nerve

In the aniridia abnormalities may involve any portion of the anterior segment; additionally, abnormalities of posterior ocular structures, namely foveal and optic nerve hypoplasia, may occur and in part or entirely be responsible for visual impairment [2, 25–30]. Optic nerve hypoplasia occurred in roughly 10 % of patients with aniridia and foveal hypoplasia has been suggested as a possible cause [2, 26–28]. Consistent previous reports observed the simultaneous occurrence of optic nerve and foveal hypoplasia in several patients. Based on this observation, have proposed a causal relationship between foveal and optic nerve hypoplasia. McCulley et al. study data suggest an alternate aetiology in some if not all patients, as 50 % of optic nerve hypoplasia cases occurred independent of foveal hypoplasia. Although foveal hypoplasia might, in some instances, contribute to optic nerve hypoplasia, given that PAX6 mutations have been reported to result in both isolated nerve and foveal hypoplasia, our observation of nerve hypoplasia occurring independent of marked foveal hypoplasia suggests that its occurrence in patients with aniridia is at least in part a direct result of the PAX6 mutation [31].

Retina

Foveal aplasia or hypoplasia, directly due to the PAX6 mutation, and phototoxicity, a result of the poorly developed iris, both likely occur and to varying degrees account for retinal dysfunction [31]. Although the occurrence of retinal dysfunction is generally accepted, its aetiology is a source of debate. The foveal

hypoplasia is suspected during the fundus examination: The lack of macular reflex is a sign of macular hypoplasia confirmed by ulterior deepening examination with OCT (Figs. 12.4 and 12.5). Electroretinogram alterations were observed in the majority of aniridic patients, in 100 % (11/11) by Tremblay et al. [28] and 74 % (14/19) of patients by Wu et al. [29]. Thus, retinal dysfunction, as quantified by electroretinography, should be considered a cardinal feature of the aniridia phenotype; the visual impairment that aniridic patients suffer from may not be the exclusive consequence of an anterior segment dysplasia. Electroretinogram results varied from almost normal to severely affected, suggesting heterogeneity in the retinal function of aniridic patients [28]. Mc Culley et al. believed that ERG testing was not routinely performed and the proportion of patients with retinal phototoxicity or subtle hypoplasia, not resulting in a complete loss of the foveal depression, cannot be accurately estimated. However, severe foveal hypoplasia, complete absence of a foveal depression and reflex,

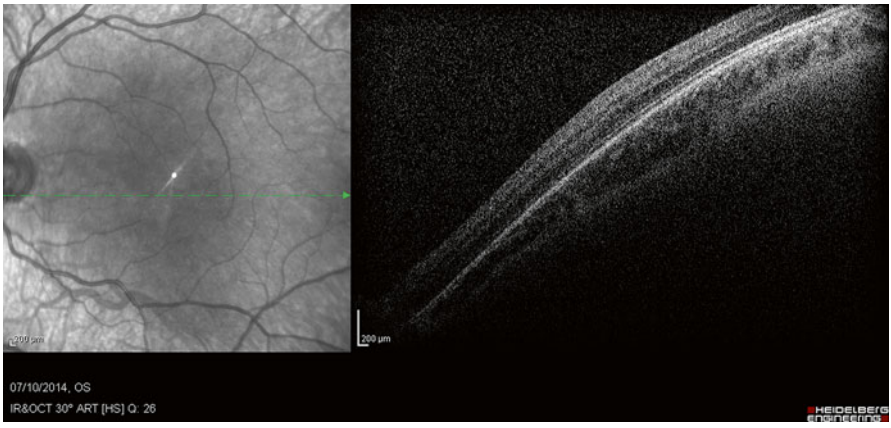

Fig. 12.4 Complete foveal hypoplasia

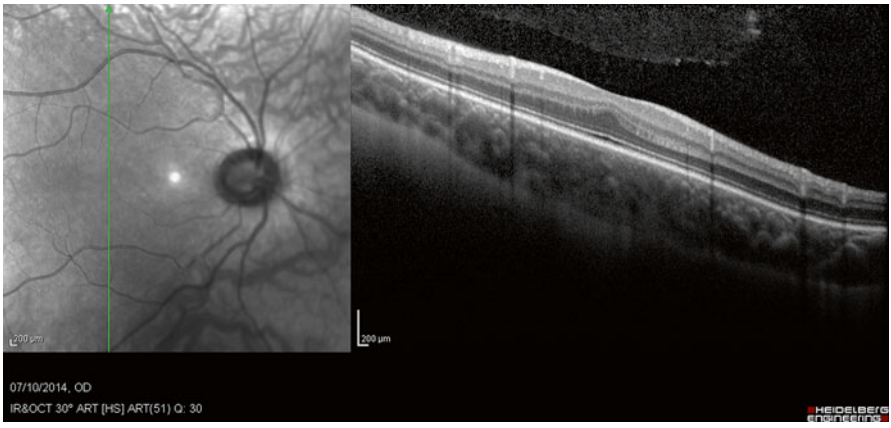

Fig. 12.5 Partial foveal hypoplasia

was observed in 10.7 % of aniridic patients [31]. Pendular nystagmus is often present related to macular hypoplasia. In some cases, poor acuity (and nystagmus secondarily) might be due to causes such as light toxicity or amblyopia. Another explanation is that in some cases nystagmus is not due to a sensory deficit but occurs independently. Finally aniridia may also be associated with retinal tears and detachments [32].

Refractive Error, Strabismus and Ptosis

Myopia, hypermetropia and astigmatism, are commonly seen in aniridics as well as squint. Up to 10 % of patients may have ptosis [6, 13, 33].

Diagnosis

The diagnosis is performed in different ways depending on the age of the patient. It is needed a complete framework for ***newborns and children under 2 years*** of age with a suspect of aniridia. This framework requires venous sedation and topic anesthetic (benoxinate eye drop), for a correct evaluation of the anterior and posterior segment.

Corneal diameter is useful to evaluate the presence of micro and megalo cornea that may be associated with aniridia. As for the case of the megalo cornea, it can be a consequence of intraocular high pressure.

Cycloplegic refraction (after administration of one drop of cyclopentolate twice, the exam is possible after 30 min) intraocular high pressure can cause the high myopia. It is used handheld refractometer and traditional Skiascopic refraction by streak retinoscopy. The autorefractometry is not easy and limited for associated nystagmus.

Ultrasound examination is important to evaluate the change of axial length, because in some cases the glaucoma in the child is not associated with high pressure but with increased axial length of the eye.

Pachymetry corneal thickness is often greater. It is important to consider this aspect in the relevation of eye pressure. Anyway, since there aren't any tables of conversation for children, the interpretation of the pressure is not clear.

Tonometry (I Care Pod, Perkins) with this kind of tonometer is possible to measure the eye pressure on bed during sedation. It is very important to monitor the pressure every 6 months. Glaucoma in infancy is unusual, in fact that glaucoma commonly develops later in childhood.

Gonioscopy (Ret Cam 130) with a microscope and Ret cam's lens (130) it is possible to observe if the angular structures are present or completely developed and if it is present a contractile membrane between the surface of the iris and the angle wall.

Lens exam if it is present fetal vascularization of the anterior lens capsule (tunica vasculosalentis) or a persistent pupillary membrane. Cataracts are rare in infancy and more common in the teens.

Anterior synechiae it is possible to observe them between iris and lens.

Fundus exam (Ret cam, 20 or 28 lenses) is used to evaluate optic nerve hypoplasia, lack of macular reflex and retinal periphery.

Optical coherence tomography (OCT) The Handheld OCT is the only method that allows us to study the retinal morphology during sedation examination [34, 35] (Figs. 12.4 and 12.5).

Children after 2 years of age can be put through more tests, not in sedation, but using some tricks that can make the patient more cooperative. It is very useful visiting patients after meals, reducing waiting times, explaining in advance what you are doing.

Photo of Anterior segment ingrowth of blood vessels from the limbal region to the peripheral cornea and beyond, it is possible to observe the opacification of the corneal stroma. The Iris deficiency can be limited, partial or asymmetrical, regarding a portion of iris, similar to an atypical coloboma (Figs. 12.1, 12.2, and 12.3).

Confocal microscopy (HRT – Heidelberg) this exam is not possible in the case of nystagmus, because the instrument is in direct contact with the eye, and the cornea may be damaged due to continuous movements. It is very important to assess a deficiency of limbal stem cells, stromal neovascularisation, stromal infiltration with inflammatory cells, destruction of Bowman's layer, the presence of goblet cells and conjunctival cells on the corneal surface.

Corneal topography (Pentacam) to assess changes in the surface and corneal thickness.

Optical coherence tomography (OCT) HRA – Heidelberg to evaluate the reduction or absence of foveal depression has been used by us. In the case of nystagmus, you can capture images in a "null point" (where the eye movements are less amplified), tilting the instrument with horizontal and vertical displacements. Anyway the Handheld OCT (Bioptigen Inc.) in young children with nystagmus [34] is highly sensitive and specific to investigate fovea morphology and abnormalities; also it provides reliable measurements in children with and without nystagmus [35] (Figs. 12.4 and 12.5).

Orthoptic Evaluation

Nystagmus: it is a rhythmic oscillation of the eye(s), simply a sign. It is important to describe direction, waveform, frequency and amplitude. In children the patterns of nystagmus are often quite variable and therefore have limited localizing and diagnostic value. Pendular nystagmus is often present related to macular hypoplasia. In some cases, poor acuity might be due to causes such as light toxicity, amblyopia or it might occur independently.

Cover test, convergence, ocular motility: squint is common [6, 13, 33].

Stereopsis (Lang, Titmus): amblyopia is common and the stereopsis may be reduced or absent.

Importance of Early Visual Assessment

It is very important to assess early the child with aniridia, although there is no treatment for many problems such as foveal hypoplasia and the corneal disorder and glaucoma occur later in childhood or adulthood. The early refractive examination and application of glasses with protective filters can increase the quality and the visual potential of the aniridic patient and reduce the risk of amblyopia and the damage to retinal photoreceptors.

Distance Vision

Visual acuity is measured clinically using several subjective techniques such as preferential-looking task or letter charts. In patients with nystagmus it is important not to occlude the contralateral eye, but it is better to use a "fogging sfere" (+6,00sf) instead.

Between birth and 2 year of age. Preferential looking techniques rely on the observation that infants will fixate patterned surface more than featureless surface. We use the Teller acuity cards, which is a behavioral test, that may be obtained in sets featuring a series of gratings spaced in intervals. Testing distance of about 38 and 55 cm are used for infants and young children respectively. The acuity estimate is supposed to be the finest grating the child is believed to see. The procedure has been proven useful for assessing neurologically impaired individuals of any age and provides sufficient information about an acuity estimate in patients between birth and 18 months. Generally after 12 months the attention to the test is reduced.

Infant between 2.5 and 4 years of age. Picture optotype visual acuity tests usually depend on the child being able to correctly name familiar objects depicted on chart or flip cards. One problem resulting from the use of a picture chart is the confusion caused by having two objects of completely different size appearing on the same line. We use generally Pigassou or Pesando pictures, sometimes Kay picture tests. They use pictures constructed on Snellen principles. The chart is designed for 6 m, but could be used at shorter distance.

Infants between 4 and 6 years of age. We use the tumbling or illiterate E tests predate the Stycar method. The test use Snellen E letter optotypes constructed on a 5 by 5 grid. In clinical use the open side of the E is presented either facing up, down, right or left. The success of the test depends on the patient being able to accurately communicate the orientation of the symbol to the examiner. Unfortunately, the knowledge of spatial orientation is not well developed in young children, particularly in distinguishing between right and left. Testing distance of about 4 m.

School age child (over 6 years old). We use Snellen letters, that are high-contrast letter, using 5 by 4 or 5 by 5 grid stroke widths of 1 unit. Testing distance of about 4 m. Letter optotype is presented either as a single target on a flip card or the face of a cube or as multiple targets on a chart or flip card.

Acuity assessment using Vep's. Vep's provide an alternative and objective measurement in some patients and this enables to perform these assessments.

Near Vision

It is very important to study reading acuity in children. It is always advisable to assess visual acuity for near (Testing distance of about 30 cm) using cards with letters or symbols, depending on the patient's age. We use generally symbol test for pre-verbal children or **MNREAD** test (Fig. 12.6) for scholastic children, these charts are continuous-text reading acuity charts suitable for measuring reading acuity and reading speed of normal or low vision patients, developed at the Minnesota laboratory for low-vision Research. The Italian version of charts is commercially available. The assessment of visual acuity in aniridic patients and generally in children require more time. The **LEA** Vision Test System (Fig. 12.7) is a series of

Fig. 12.6 MNREAD chart for near vision

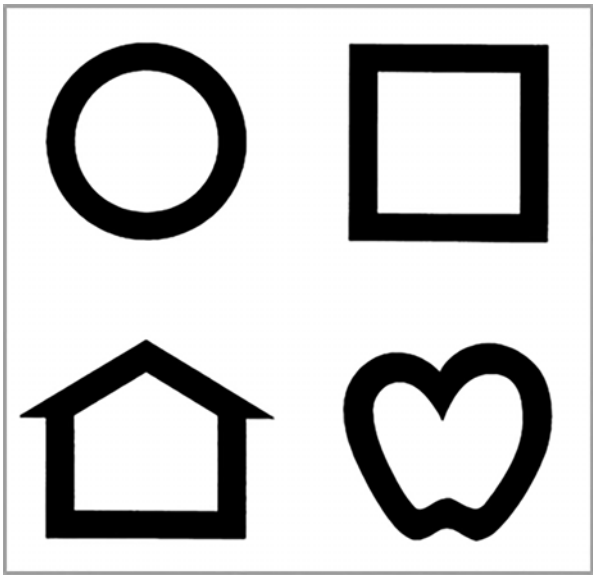

Fig. 12.7 LEA chart for near vision

pediatric vision tests designed specifically for children who do not know how to read the letters of the alphabet that are typically used in eye charts. There are numerous variants of the LEA test which can be used to assess the visual capabilities of near vision, as well as several other aspects of occupational health, such as contrast sensitivity, visual field, color vision, visual adaptation, motion perception, and ocular function and accommodation. Tests must be performed from the same operator and is important to repeat the test every check for monitoring the changes of visual acuity.

The monitoring of visual function must to repeat every 6 months during the first years.

Conclusions

Aniridia is rare but can progressively impair vision in multiple causes including keratopathy, cataract, glaucoma, foveal hypoplasia, nystagmus. It requires close collaboration with the geneticist for the early diagnosis and a close collaboration with the urologist and pediatrician to exclude WAGR Syndrome. There are various forms, which are different for clinical manifestations and visual acuity. It is important an early diagnosis and an early treatment of complications, to save visual sharpness and the visual field, in order to reduce the damage and to maintain the better quality of life in aniridic patients.

References

1. Mackman G, Brightbill FS, Optiz JM (1979) Corneal changes in aniridia. Am J Ophthalmol 87:497–502
2. Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L (1984) Aniridia. A review. Surv Ophthalmol 28:621–642
3. Shaw MW, Falls HF, Neel JV (1960) Congenital aniridia. Am J Hum Genet 12(4 Pt 1): 389–415
4. Miller RW, Fraumeni JF, Manning MD (1964) Association of Wilms' tumour with aniridia, hemihypertrophy and other congenital malformations. N Engl J Med 270:922–927
5. Lee H, Khan R, O'Keefe M (2008) Aniridia: current pathology and management. Acta Ophthalmol 86:708–715
6. Valenzuela A, Cline RA (2004) Ocular and nonocular findings in patients with aniridia. Can J Ophthalmol 39:632–638
7. Khaw PT (2002) Aniridia. J Glaucoma 11:164–168
8. Beauchamp GR, Meisler DM (1986) An alternative hypothesis for iris maldevelopment (aniridia). J Pediatr Ophthalmol Strabismus 23:281–283
9. Mintz-Hittner HA, Ferrell RE, Lyons LA et al (1992) Criteria to detect minimal expressivity within families with autosomal dominant aniridia. Am J Ophthalmol 114:700–707
10. Sonoda S, Isashiki Y, Tabata Y et al (2000) A novel PAX6 gene mutation (P118R) in a family with congenital nystagmus associated with a variant form of aniridia. Graefes Arch Clin Exp Ophthalmol 238:552–558
11. Willcock C, Grigg J, Wilson M, Tam P, Billson F, Jamieson R (2006) Congenital iris ectropion as an indicator of variant aniridia. Br J Ophthalmol 90(5):658–569
12. Gronskov K, Rosenberg T, Sand A, Brondum-Nielsen K (1999) Mutational analysis of PAX6:16 novel mutations including 5 missense mutations with a mild aniridia phenotype. Eur J Hum Genet 7:274–286
13. Hingorani M, Williamson KA, Moore AT, Van Heyningen V (2009) Detailed ophthalmologic evaluation of 43 individuals with PAX6 mutations. Invest Ophthalmol Vis Sci 50:2581–2590
14. Hingorani M, Hanson I, Van Heyningen V (2012) Aniridia. Eur J Human Genet 20:1011–1017
15. Schneider S, Osher RH, Burk SE, Lutz TB, Montione R (2003) Thinning of the anterior capsule associated with congenital aniridia. J Cataract Refract Surg 29:523–525
16. Hou ZQ, Hao YS, Wang W, Ma ZZ, Zhong YF, Song SJ (2005) Clinical pathological study of the anterior lens capsule abnormalities in familial congenital aniridia with cataract. Beijing Da Xue Xue Bao 37:494–497
17. Margo CE (1983) Congenital aniridia: a histopathologic study of the anterior segment in children. J Pediatr Ophthalmol Strabismus 20:192–198
18. Bremond-Gignac D, Crolla JA, Copin H et al (2005) Combination of WAGR and potocki-snaffer contiguous deletion syndromes in a patient with an 11p11.2–p14 deletion. Eur J Hum Genet 13:409–413
19. Grant WM, Walton DS (1974) Progressive changes in the angle in congenital aniridia, with development of glaucoma. Trans Am Ophthalmol Soc 72:207–228
20. Tseng SC, Li DQ (1996) Comparison of protein kinase C subtype expression between normal and aniridic human ocular surfaces: implications for limbal stem cell dysfunction in aniridia. Cornea 15:168–178
21. Nishida K, Kinoshita S, Ohashi Y, Kuwayama Y, Yamamoto S (1995) Ocular surface abnormalities in aniridia. Am J Ophthalmol 120:368–375
22. Brandt JD, Casuso LA, Budenz DL (2004) Markedly increased central corneal thickness: an unrecognized finding in congenital aniridia. Am J Ophthalmol 137:348–350
23. Whitson JT, Liang C, Godfrey DG, Petroll WM, Cavanagh HD, Patel D, Fellman RL, Starita RJ (2005) Central corneal thickness in patients with congenital aniridia. Eye Contact Lens 31:221–224

24. Skeens HM, Brooks BP, Holland EJ (2011) Congenital aniridia variant: minimally abnormal irides with severe limbal stem cell deficiency. Ophthalmology 118(7):1260–1264
25. Layman PR, Anderson DR, Flynn JT (1974) Frequent occurrence of hypoplastic optic disks in patients with aniridia. Am J Ophthalmol 77:513–516
26. Ivanov I, Shuper A, Shohat M, Snir M, Weitz R (1995) Aniridia: recent achievements in paediatric practice. Eur J Pediatr 154:795–800
27. Hittner HM, Riccardi VM, Ferrell RE, Borda RR, Justice J Jr (1980) Variable expressivity in autosomal dominant aniridia byclinical electrophysiological and angiographic criteria. Am J Ophthalmol 89:531–539
28. Tremblay F, Gupta SK, De Becker I, Guernsey DL, Neumann PE (1998) Effects of PAX6 mutations on retinal function: an electroretinographic study. Am J Ophthalmol 126:211–218
29. Wu L, Ma Q, Wu DZ, Luo T (1991) Abnormalities of ERG incongenital aniridia. Yan Ke Xue Bao 7:151–152
30. Ginsberg J, Bove KE, Cuesta MG (1980) Aplasia of the optic nerve with aniridia. Ann Ophthalmol 12:433–439
31. McCulley TJ, Mayer K, Dahr SS, Simpson J, Holland EJ (2005) Aniridia and optic nerve hypoplasia. Eye 19:762–764
32. Dowler JG, Lyons CJ, Cooling RJ (1995) Retinal detachment and giant retinal tears in aniridia. Eye 9:268–270
33. Hewitt AW, Kearns LS, Jamieson RV, Williamson KA, van Heyningen V, Mackey DA (2007) PAX6 mutations may be associated with high myopia. Ophthalmic Genet 28:179–182
34. Lee H, Sheth V, Bibi M, Maconachie G, Patel A, McLean RJ, Michaelides M, Thomas MG, Proudlock FA, Gottlob I (2013) Potential of handheld optical coherence tomography to determine cause of infantile nystagmus in children by using foveal morphology. Ophthalmology 120(12):2714–2724
35. Lee H, Proudlock F, Gottlob I (2013) Is handheld optical coherence tomography reliable in infants and young children with and without nystagmus? Invest Ophthalmol Vis Sci 54(13):8152–8159

Chapter 13
Aniridia: Early Diagnosis: The Key Roles of Neonatologists, Paediatricians and Paediatric Ophthalmologists

Kristina Tornqvist

Abstract Aniridia is a rare, genetic disorder involving several structures of the eye. The disorder itself causes visual impairment which will most probably be worsened by the complications associated with the disease such as glaucoma, corneal clouding and cataract. The genetics include a mutation in the PAX6-gene on chromosome 11p13. More extensive alterations on the chromosome 11p may include the WT1-gene (Wilms tumor gene) which may cause WAGR-syndrome (Wilms tumor, anirida, genitouritary abnormalities and mental retardation). The ophthalmological signs include a partial or nearly total absence of the iris, foveal hypoplasia and nystagmus. Eventually severe complications such as glaucoma, corneal clouding which may be severe and cataract may worsen the situation.

Keywords **A**niridia-congenital eye diseases-PAX6 gene-glaucoma-corneal clouding

Introduction

Aniridia a rare, genetic disorder with a prevalence of 1:64,000–1:96,000 [1]. In a study of a Swedish-Norwegian population the prevalence was found to be 1: 72,000 in the entire group studied [2] and in the younger age-group 1:47,000 [3]. The disorder is caused by a mutation in the PAX6-gene on chromosome 11p [4, 5]. It may occur in systemic disorders such as WAGR-syndrome [6–8] or Gillespie syndrome (aniridia, cerebellar ataxia and mental retardation) [9, 10]. This syndrome is autosomal recessive and genetically distinct from autosomal dominant anirida [10].

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The relative infrequency of aniridia will entail that many ophthalmologists see only one or a few cases during their professional life. In the case aniridia is diagnosed by someone without experience of the disorder it is wise to refer the patient to a centre where such an experience is available. Furthermore the future care of the patient will be a teamwork involving e.g. ophthalmologist, paediatrician and low vision clinic.

Diagnosis

The Role of the Neonatologist

In a case with classical aniridia with a complete or nearly complete absence of the iris the diagnosis may be easy to establish. However the phenotype may vary considerably with more or less iris remnants present giving the impression of other anterior segment dysgenesis such as e.g. Rieger anomaly. In Gillespie syndrome the aniridia may also be partial and remnants of the pupillary membrane may be seen as thin strands in the pupil.

If an eye exam or screening for congenital cataract is included in the paediatric examination at the maternity ward the diagnosis may well be established by a neonatologist/paediatrician who should refer the child to an ophthalmologist as soon as possible. Even a suspicion of an eye abnormality should result in a referral. If anything unusual is seen at the paediatric examination it is also wise to ask the parents about eye disorders in the family as two-third of aniridia cases are hereditary [1]. If it is a non-hereditary case of aniridia the paediatrician may well take steps for a more thorough paediatric examination of the child considering the risk for WAGR or Gillespie syndrome in sporadic cases.

If no eye exam is performed by the paediatrician the unusual appearance of the eyes or other symptoms such as nystagmus, lack of development of fixation, lack of social smile or unwillingness to open the eyes/light-sensitivity will be noted by the parents and result in a consultation with an ophthalmologist.

The Role of the Paediatric Ophthalmologist

The suspicion of anirida ought to prompt a visit without substantial delay. A meticulous family history is advised as well as an eye examination of the parents and if possible also of siblings.

A complete ophthalmological examination should be done including evaluation of the anterior segment and lens, ophthalmoscopy to evaluate the optic nerve as well as the fovea as foveal hypoplasia is present in aniridia. Eye movements should be assessed and possible strabismus checked. Intraocular pressure (IOP) should be measured as well corneal diameter which if later increasing may indicate elevated

intraocular pressure. Cycloplegic refraction should be checked early but not necessarily at the first visit. Visual acuity can be checked with Teller acuity cards [11, 12] as early as at 3–4 weeks of age and should be examined as early as possible taken into account that delayed visual maturation (DVM) may be present. As individuals with aniridia have an abnormal tear film [13] preservative-free lubricants can be prescribed.

If a sufficiently good examination is not achieved without sedation examination under anaesthesia (EUA) becomes necessary.

Intraocular pressure should be monitored carefully even in the small child. EUA may be necessary bur every effort to manage IOP measurements without anaesthesia should be made.

If the parents are not familiar with the disorder it is important to explain to them what kind of disorder this is and our plan for the future care and follow-up of the child.

Contact with the local low vision clinic should be established early not only because of their ability to endow the child with appropriate glasses (both optically and with adequate light protection) and visual aids but also due to the fact that they often have psychologists or social workers employed who can support the parents in a possible crisis as well as with advice about allowances for children with disabilities and with help to solve a number of practical problems which may arise in this situation.

The Role of the Paediatrician

In every case of aniridia genetic testing and genetic counselling to the parents has to be done. This can be initiated by the ophthalmologist or by the paediatrician, the important thing is that it is done.

Furthermore the paediatrician should evaluate whether there is anything indicating WAGR- or Gillespie syndromes. If WAGR-syndrome is suspected the result of genetic analysis gets even more important. A FISH-test can be an alternative to mutation analysis. A confirmed WAGR-syndrome means continuous and long-time paediatric follow up.

The patient with Gillespie syndrome should also be subject to further paediatric contact.

As hearing may be affected in aniridia a hearing test may be valuable.

Conclusion

The child with aniridia, syndromic or not, will have many contacts within the health system and as this is an eye disorder and as the eyes need frequent check-up due to the serious complications that may occur the ophthalmologist should be the coordinator of all these efforts.

References

1. Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L (1984) Aniridia. A review. Surv Ophthalmol 28(6):621–642
2. Edén U, Iggman D, Riise R, Tornqvist K (2008) Epidemiology of aniridia in Sweden and Norway. Acta Ophthalmol 86(7):727–729
3. Edén U, Beijar C, Riise R, Tornqvist K (2008) Aniridia among children and teenagers in Sweden and Norway. Acta Ophthalmol 86(7):730–734
4. Churchill A, Booth A (1996) Genetics of aniridia and anterior segment dysgenesis. Br J Ophthalmol 80(7):669–673
5. Prosser J, van Heyningen V (1998) PAX6 mutations reviewed. Hum Mutat 11(2):93–108
6. Breslow NE, Norris R, Norkool PA, Kang T, Beckwith JB, Perlman EJ, Ritchey ML, Green DM, Nichols KE, National Wilms Tumor Study Group (2003) Characteristics and outcomes of children with the Wilms tumor-Aniridia syndrome: a report from the National Wilms Tumor Study Group. J Clin Oncol 21(24):4579–4585
7. Grønskov K, Olsen JH, Sand A, Pedersen W, Carlsen N, Bak Jylling AM, Lyngbye T, Brøndum-Nielsen K, Rosenberg T (2001) Population-based risk estimates of Wilms tumor in sporadic aniridia. A comprehensive mutation screening procedure of PAX6 identifies 80% of mutations in aniridia. Hum Genet 109(1):11–18
8. Muto R, Yamamori S, Ohashi H, Osawa M (2002) Prediction by FISH analysis of the occurrence of Wilmstumor in aniridia patients. Am J Med Genet 108(4):285–289
9. Gillespie FD (1965) Aniridia, cerebellar ataxia and oligophrenia in siblings. Arch Ophthalmol 73:338–341
10. Glaser T, Ton CC, Mueller R, Petzl-Erler ML, Oliver C, Nevin NC, Housman DE, Maas RL (1994) Absence of PAX6 gene mutations in Gillespie syndrome (partial aniridia, cerebellar ataxia, and mental retardation). Genomics 19(1):145–148
11. Preston KL, McDonald M, Sebris SL, Dobson V, Teller DY (1987) Validation of the acuity card procedure for assessment of infants with ocular disorders. Ophthalmology 94(6):644–653
12. Teller DY, McDonald MA, Preston K, Sebris SL, Dobson V (1986) Assessment of visual acuity in infants and children: the acuity card procedure. Dev Med Child Neurol 28(6):779–789
13. Edén U, Fagerholm P, Danyali R, Lagali N (2012) Pathologic epithelial and anterior corneal nerve morphology in early-stage congenital aniridic keratopathy. Ophthalmology 119(9): 1803–1810

Chapter 14
Aniridia Guides and Aniridia-Syndrome (PAX6-Syndrome): Do's and Dont's in Clinical Care

Implementation of Supra-Regional "Aniridia Guides" Can Delay Progressive Vision Loss and Improve Comprehensive and Individualized Medical Care

Barbara Käsmann-Kellner, Arne Viestenz, and Berthold Seitz

Abstract Congenital aniridia manifests in different forms: it can be transmitted in an autosomal dominant way, as sporadic aniridia, and as part of several syndromes including WAGR- and WAGRO-syndrome and other syndromes with intellectual impairment. Furthermore, recent research shows that aniridia associated with alterations in the PAX6 gene often shows further systemic implications (endocrine, metabolic and neurological pathologies). Therefore, PAX6-related aniridia is more and more thought of and described as "Aniridia Syndrome" or "PAX6-Syndrome".

Keywords Congenital aniridia • Aniridia-Syndrome • PAX6-Syndrome • Aniridia associated keratopathy • Aniridic glaucoma • Aniridia fibrosis syndrome • Low vision • Aniridia guide

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Purpose

We present a group of 130 patients with congenital aniridia to enhance awareness of the ocular complexity and the systemic implications of the inborn ocular malformation. Different to other congenital visual impairments aniridia is characterized by many ocular complications arising during life which may lead to total blindness (cataract, aniridic keratopathy, secondary glaucoma). Furthermore, there is a specific surgical risk entity: Aniridia fibrosis syndrome or Anterior Segment Fibrosis Syndrome (ASFS) which leads to a non-infectious fibrous scarring and membrane formation of the anterior segment, often followed by hypotonia and phthisis. Aniridic glaucoma presents yet another severe complication which often is diagnosed late due to diagnostic problems and which may lead to irreversible optic nerve damage.

Our main aim is to point out that complications in aniridia involve several ophthalmosurgical subspecialties (cornea, cataract, glaucoma, anterior and posterior segment surgery) and that aniridia patients may encounter problems concerning a comprehensive treatment of all possible complications plus concerning low vision support, academic and professional aid and compensation strategies if they are treated by one subspecialty surgeon.

Aniridia Guide: A Proposal to Improve the Care for Aniridia Patients

We suggest a model of "aniridia pilotage" or "aniridia guide" where **one** ophthalmologist, preferably a paediatric and low vision specialist with a thorough knowledge of aniridia and a functional network to ophthalmosurgical subspecialists, follows the patient over years, thus caring for his low vision needs, supporting inclusion and observing possible complications – and if those arise, send the patient guiding ophthalmologist to a subspecialty surgeon to treat the complications. This "guiding ophthalmologist" will care for the patient following any surgical procedure, he will adapt low vision support according to changes in visual acuity and will ensure that no aniridia patient gets lost between the different subspecialty surgeons.

In addition, the guiding ophthalmologist should inform the aniridic patients about possible systemic manifestation of PAX6-Syndrome concerning metabolic and neurologic implications and should initiate appropriate investigations if applicable.

Other Possible Aspects to Improve the Care of Aniridia Patients

The following approaches might help to improve the lifelong care of aniridia patients and might benefit the aim to lessen the impact of complications in aniridia:

- Topical prophylaxis of aniridic corneal epitheliopathy from very early age onwards
- Early support of young aniridia children:
  - Early correction of refractive errors
  - Introduction to low vision support services and Early Intervention measurements
  - Alternating patching to promote visual development in each eye (depending on the presence or absence of strabismus)
  - Glare reducing filter glasses without change of colour perception
- Regular measurement of intraocular pressure starting in young children
- Yearly VEP measurements, routine visual field testing as soon as possible
- Comprehensive optimization of surgical care independent of department location (only the very best surgeons within their subspecialty should treat the different complications) while one experienced paediatric and low vision ophthalmologist should follow the patient continuously ("aniridia guide" for the patient), thus monitoring the disease and stages of complications and advising the patient where to go for surgical treatment.
- This low vision ophthalmologist continuously follows the patient's course including adaptation of low vision aids according to the course of the disease, helping the patient concerning integration at school and at the place of work and advises about social and legal compensation possibilities.

Introduction

– OMIM Entries for Aniridia
  – 106210: ANIRIDIA; AN
  – 194072: WILMS TUMOR, ANIRIDIA, GENITOURINARY ANOMALIES, AND MENTAL RETARDATION SYNDROME; WAGR
  – 206700: ANIRIDIA, CEREBELLAR ATAXIA, AND MENTAL RETARDATION (GILLESPIE SYNDROME)
  – 607108: PAIRED BOX GENE 6; PAX6

"Aniridia" is actually a misnomer, because the "absence" of the iris is the most obvious sign in childhood. However, there always is a small iris stump visible on gonioscopy. In addition, the lack of iris itself is not the reason for the progressive visual loss during life. Congenital Aniridia is a severe pan-ocular congenital eye malformation including (possibly profibrotic) changes in the anterior and posterior segments of the eye and systemic findings. Most cases are associated with dominantly inherited mutations or deletions of the PAX6 gene.

Children with aniridia characteristically have a variable degree of iris hypoplasia and foveal hypoplasia, which leads to sensory defect nystagmus (SDN) and congenitally impaired visual acuity (usually 0,1 best corrected). Other congenital features may include corneal opacification, glaucoma, cataract, lens subluxation, strabismus, optic nerve coloboma and hypoplasia.

List of abbreviations

Abbreviation	Stands for	Describes
AFS/ASFS	Aniridia fibrosis syndrome resp. Anterior segment fibrosis syndrome	Profibrotic intraocular progressive fibrotic scar formation hitherto only described in aniridia following intraocular surgery. Scar formation leads to a firm fibrotic plate (Tsai 2005, [27])
AN	Aniridia	Formerly AN2 was used in OMIM to describe PAX6-relatd aniridia, this has been changed to PAX6 related aniridia
AAK	Aniridia-associated keratopathy	Typical LSCI (see below) caused progressive keratopathy in aniridia
CYP1B1	Cytochrome P450 Family 1 Subfamily B Polypeptide 1 – Gen	Aniridia without relation to PAX6, further manifestations in CYP1B1-deficit: Buphthalmia, ASD Anterior Segment Dysgenesis, Peters Anomaly [14]
FOXC1	Forkhead box C1 Gen	Aniridia without PAX6 association in ididogonodysgenesis
LSCI	Limbal stem cell insufficiency	Limbal stem cell insufficiency is the main cause for all three major complications in aniridia: namely keratopathy, but glaucoma and cataract formation seem to be related to LSCI as well
PAX	PAired-BoX-Gene	Developmental old genes which code for transcription factors which are crucial for embryonal tissue differentiation and for embryonal organ formation. In addition, postnatally they are important for physiological function of specific cell types [13, 24]
PAX6	PAired-Box-6-Gene	Master-Gene of embryonal ocular differentiation. PAX6 is in addition important in the embryonal differentiation of the central nervous system, the olfactory bulbs, the pancreas. Up to now, over 350 mutations associated with aniridia have been described Further ocular manifestations of PAX6 insufficiency can be coloboma of iris, choroid, and retina, morning glory anomaly (coloboma) of the optic nerve head and Peters Anomaly [13, 24]
PITX2	Paired-like Homeodomain Transcription – factor 2	Aniridia without relation to PAX6 in Axenfeld-Rieger-Syndrome Type1 RIEG1, Iridogoniodysgenesis Type II IRID2, Peters Anomaly
WAGR(O)	Wilms-Tumor, aniridia, genitourethral anomalies, retardation (plus obesity)	Nephroblastoma in over 50 % of affected children, occuring mostly between 1st and 4th year of life. Higher risk of renal insufficiency in adult age. Genito-urethral anomalies may lead to ambiguous morphology of the sexual organs. Mental retardation, in cases of microdeletion 11p14-p12 obesity is frequent [13, 28, 29]

List of used aniridia related abbreviations

Later during life, progressive sight-threatening complications include cataracts, glaucoma, corneal opacification due to limbal stem cell insufficiency and a high risk of aniridia fibrosis syndrome following intraocular surgery.

Furthermore there are often systemic, mostly metabolic abnormalities in PAX6 gene associated aniridia. It would therefore be more precise to call PAX6-related

Fig. 14.1 Homburg (Saar), Germany, Aniridia Meeting June 2013 with representatives of Aniridia Europe and Aniridie-WAGR e.V Germany German support group

aniridia "Aniridia Syndrome" or "PAX6 Syndrome".

With this chapter, we want to propose a change in the standard life-long ophthalmological care of patients with aniridia, who often need several ophthalmosurgical subspecialists to address their different complications such as glaucoma, cataract and corneal scarring. Changing subspecialists or remaining with just one subspecialist may promote a belated diagnosis of other complications. This might, as in delayed diagnosis of glaucoma, lead to irreversible visual loss.

In our opinion, one surveying low vision and paediatric ophthalmologist should "guide" the aniridia patient, searching the best subspecialists for any complication arising, and caring for the patient postoperatively. This is why we propose the AGOs – Aniridia Guides in Ophthalmology, hoping that this new approach can perhaps improve the care of many patients. Figure 14.1 shows a picture from the first Aniridia Germany Meeting in Homburg(Saar), where as well the representatives of Aniridia Europe were present and where the AGO topic was first introduced.

PAX6-Related Aniridia (PAX6-Syndrome) and Other Forms of Aniridia

One can distinguish PAX6-gene-related aniridia and other forms of aniridia without changes in PAX6. PAX6-related aniridia, however, occurs much more frequently, and these present the typical clinical complications more often than in aniridia without PAX6 association (Table 14.1).

PAX6 Related Aniridia (Aniridia Syndrome, PAX6-Syndrome)

PAX6-gene related aniridia can be categorized into the autosomal dominant and the sporadic types. In addition, rare syndromes can be found, which are also PAX6 linked and inherited in an autosomal recessive way: Gillespie syndrome (Aniridia, cerebellar ataxia, mental retardation). See Table 14.2 for references according to ICD, OMIM and others.

Table 14.1 Ocular associations of congenital aniridia

Glaucoma	
Cornea	Tear Film instability and epithelial defects
	Limbal stem cell deficiency and pannus
	Opacity
	Dermoids
	Microcornea
	Sclerocornea
	Keratolenticular adhesions
Lens	Absence, Spherophakia
	Anterior polar cataract
	Subluxation
	Persistent pupillary membranes
Fundus	Foveal hypoplasia
	Disc hypoplasia
	Coloboma
Nystagmus	

Table 14.2 References to PAX6 aniridia

References	Meaning	Aniridia PAX6-associated (11p13)
ICD-9	Internat. classific. of disease Vers. 9	743.45
ICD-10	Internat. classific. of disease Vers. 10	Q13.1
OMIM	Online Mendelian Inheritance in Man	106210 – AN
		194072 – WAGR
		612469 – WAGRO
		206700 – Gillespie-Syndrome
		136520 – Foveal Hypoplasia Type I FVH1 with or without anterior segment anomalies
DiseaseDB	Disease database	723
MeSH	Medical subject headings	D015783

In former years PAX6-linked aniridia was divided into so-called "isolated" aniridia and "syndromatic" aniridia. During the last years, however, it became more and more evident that many types of PAX6-linked aniridia often show systemic manifestations and comorbidities. Therefore, PAX6-linked aniridia should rather be called Aniridia Syndrome or PAX6 Syndrome [1–4]. Table 14.3 gives an overview of PAX6 gene linked types of aniridia with possible syndromes and accompanying disorders.

Caveat: PAX6-associated aniridia is a pan-ocular profound developmental disorder of the eyes, the consequences of which can lead to blindness in the course of life. Also, there are often systemic manifestations in PAX6 Syndrome with metabolic and neurological alterations. Aniridia patients may as well have other sensory deficits including reduced olfaction (hyposmia) and hearing problems.

Homozygous PAX6 mutations are not compatible with life. Missense mutations of PAX6 often are accompanied by atypical phenotypes (small iris anomalies up to Peters anomaly) and microphthalmia. There are some PAX6 mutations where

Table 14.3 Subtypes of PAX6 related aniridia

OMIM	Details
106210	**AN** – "**Isolated**" **aniridia** (**Haploinsufficiency in intragenic mutation**):
	Possible metabolic findings:
	Diabetes
	Obesity
	Disturbances of melatonin metabolism (Epiphyseal gland)
	Possible anatomical and neuroanatomical findings
	Hyposmia, anosmia (hypoplasia of the bulbi olfactorii)
	Hypoplasia oder aplasia of the epiphyseal gland
	Unilateral disturbances of gyration
	Hypoplasia of corpus callosum
	Hypoplasia of the anterior commissura
	Possible nephrologic findings
	WAGR or WAGRO-Syndrome
	Not depending on WAGR(O): renal insufficiency may present in middle adult age
106210	**Syndromatic aniridia – subtypes**:
	Aniridia + mental retardation
	Aniridia + ptosis + mental retardation
	Aniridia + ptosis + mental retardation+ obesity – sporadic
	Aniridia + ptosis + mental retardation + obesity – dominant
	Aniridia + missing patella
	Aniridia + uni- or bilateral renal agenesis + mental retardation
	Aniridia + progressive renal insufficiency
194072	WAGR – Syndrome (Miller-Syndrome, 11p- -Syndrome)
612469	WAGRO – Syndrome
206700	Gillespie-Syndrome: Aniridia + cerebellar ataxia + mental retardation
136520	Foveal hypoplasia Type I FVHI with or without anterior segment anomalies

patients achieved visual acuities far above the aniridia average and showed a lower rate of complications [5, 6, ADD1, ADD2]. Figure 14.2 illustrates the frequent problem of limbal stem cell insufficiency (LCSI) in aniridia, which can lead to a progressive pannus formation with subepithelial corneal fibrosis and centripetal corneal neovascularization in the course of life.

WAGR-Syndrome, WAGRO-Syndrome

Larger deletions of PAX6 gene, affecting the adjacent WT1 (Wilmstumor) gene are the underlying cause of the WAGR/O syndrome (Wilmstumor, Aniridia, Genitourinary anomalies, and mental retardation/obesity) [7–10]. Patients with sporadic aniridia have a risk of about 30 % of developing Wilmstumor and if there is contiguous gene deletion of PAX6 and WT1 patients show a risk of 50 % of developing this tumor. Contiguous gene syndrome means that the affected DNA segment encompasses several neighbouring genes, and a phenotype results with involvement of several body

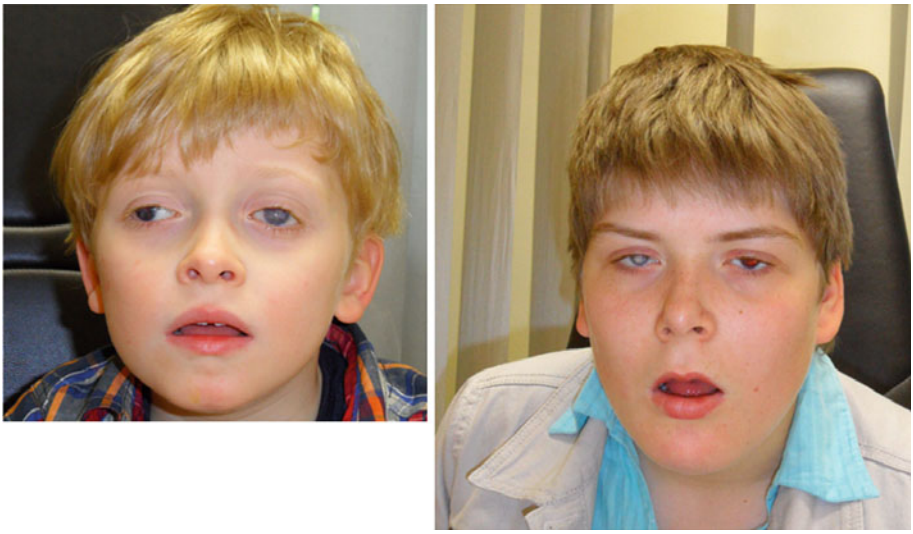

Fig. 14.2 WAGR children often show slight syndromatic signs such as hypotonia, anteverted nares and slightly low-set ears

and cellular subsystems. Wilmstumor occurs at 80 % between the first and fifth year of life. Figure 14.2 shows the syndromatic faces of two unrelated WAGR patients.

Aniridia Unrelated to Alterations in PAX6 Gene

There are a lot of other loci and genes whose alterations can be associated with an incomplete or complete aniridia. Table 14.4 shows a survey on aniridia manifestations without linkage to PAX6. It has to be remembered that any case of microphthalmia can be associated with incomplete or complete aniridia [11–14]. Aniridia unlinked to PAX6 show significantly less the typical complications of corneal pannus formation and vascularisations due to limbal stem insufficiency (LSCI) [3, 15] – this again promotes the nomenclature PAX6 Syndrome.

Figures 14.3 and 14.4 show the distribution of clinical types of aniridia in our 130 patients in Homburg/Saar. Within the higher proportion of (supposedly) sporadic aniridia, patients where no PAX6 mutation was detected are summarized as well (in particular patients with above-average good visual acuity or associated microphthalmia). In addition, this group includes patients in whom up to now no molecular genetics has been carried out (patients from abroad European or Asian ($n=17$) or patients with a migration background ($n=18$). Similarly, one has to bear in mind that germline mutations can lead to apparently sporadic aniridia, but will then be inherited and transmitted in a dominant pattern. The greater percentage of presumably sporadic aniridia is also frequently mentioned in the literature [7, 11, 12]. In our patients there is a higher frequency of female patients in the group of dominant familiar aniridia as compared to (apparently) sporadic aniridia which was not described in literature before [ADD1-3].

Table 14.4 Clinical manifestations of complete or incomplete aniridia without association to PAX6 gene defects (selection)

Chromosome	Gene	Clinical manifestationen (described with and without aniridia)	Heredity
6p25.3	Forkhead box C1 Gen (FOXC1)	Iridogoniodysgenesis Type I IRID1	AD
4q25	Paired-like Homeodomain Transcriptionsfaktor 2 (PITX2)	Rieger-Syndrome Type 1 RIEG1 Iridogoniodysgenesis Type II IRID2 Peters Anomaly	AD AD AR >> AD
2p22.2	Cytochrome P450 Family 1 Subfamily B Polypeptide 1 – Gen (CYP1B)	Congenital Glaucoma Type 3A Juvenile Glaucoma Peters Anomaly [14]	AR AR AR >> AD
Mikrophthalmiae	SOX2, OTX2, PAX2, CHD7, POMT1		AR

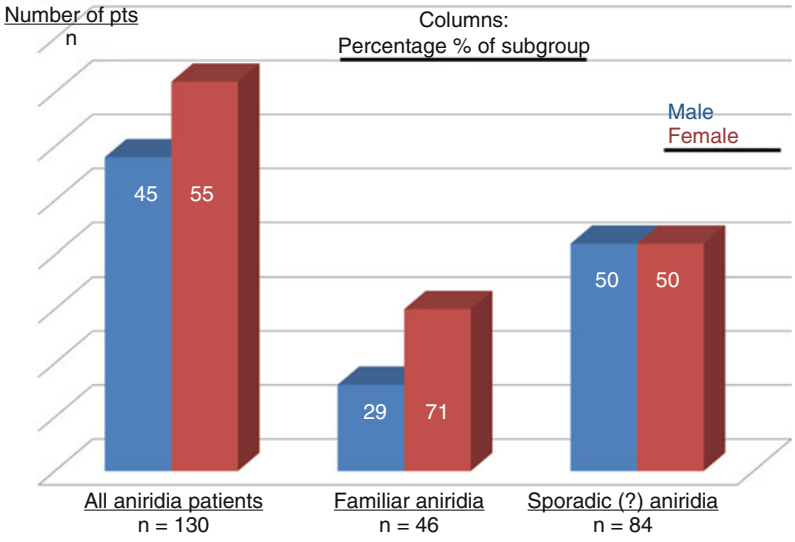

Fig. 14.3 The distribution of clinical types of aniridia in our 130 patients in Homburg/Saar

Ocular and Systemic Findings in Aniridia, Treatment Specifics

Ocular Findings at Birth and During Childhood

Children with manifest "an-iridia" are usually diagnosed early by the paediatrician, while children with only moderate iris pathology may go undiagnosed for many years.

Variations in iris pathology range from almost total absence to only mild hypoplasia of the iris. In the less severe cases the pupil size may be normal, but there

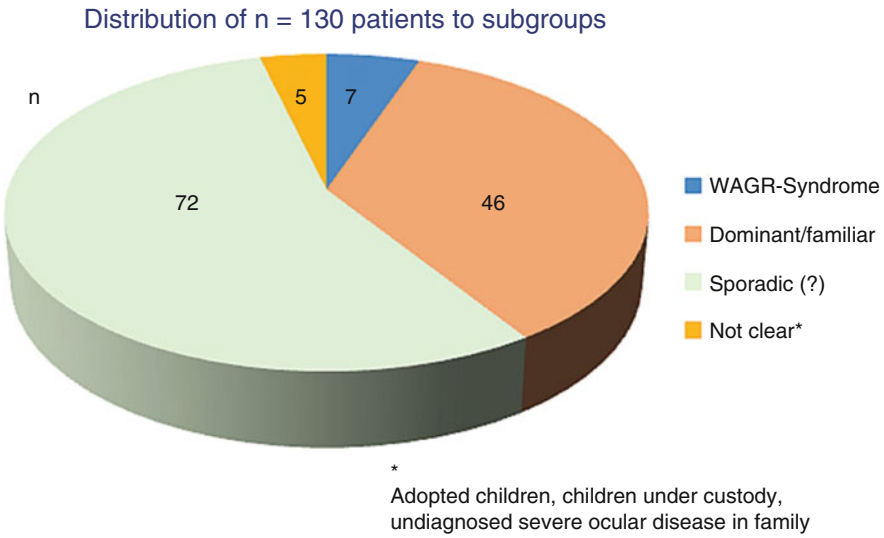

Fig. 14.4 The distribution of clinical types of aniridia in our 130 patients in Homburg/Saar

may be loss of the iris surface architecture or transillumination defects. Other iris changes include partial iris defects as atypical coloboma (not directed to the 6 o'clock position), eccentric pupils, polycoria or iris ectropion. Iris pathology can show different manifestations in severity between the two eyes of one individual (Figs. 14.5 and 14.6).

Central small lens opacifications of the anterior or posterior capsule are often present at birth and often remain unchanged. Premature and progressive cataract formation, however, is a frequent sign in aniridia especially in children aged 5–15 years (Fig. 14.7).

Characteristically babies have a variable degree of iris hypoplasia and foveal hypoplasia, which leads to sensory defect nystagmus (SDN) and congenitally impaired visual acuity (usually 0,1 best corrected). Aniridia is in one third of all patients accompanied by delayed visual maturation DVM – a delayed development of fixation capabilities during the first months of life which is characteristic for many inborn visual impairments. Other congenital features may include corneal opacification, glaucoma, cataract, lens subluxation, strabismus, optic nerve coloboma and hypoplasia.

There is a high intra-individual variability of ocular findings even in familiar aniridia. However, there usually are only few differences between the two eyes of an individual patient.

The following list gives a survey on possible ocular findings in Aniridia Syndrome.

Overview of the Possible Ocular Findings in Aniridia Syndrome

- Globe
  - Microphthalmia

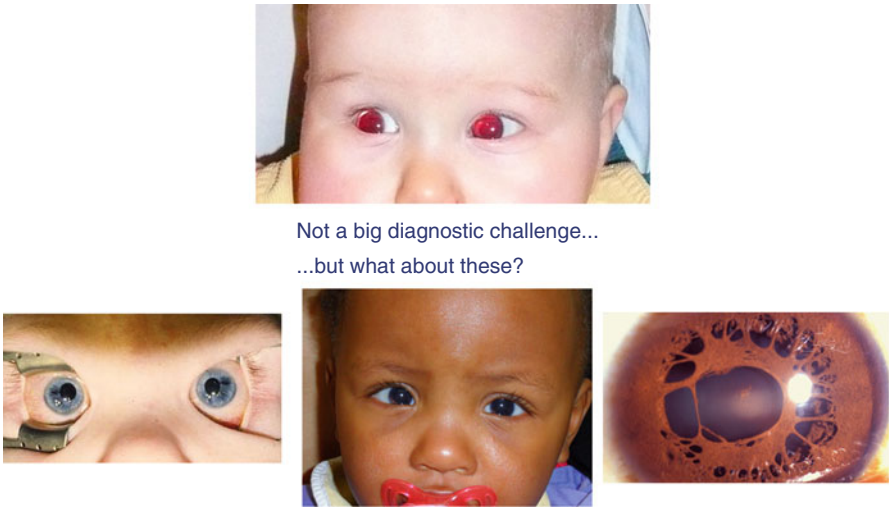

Fig. 14.5 First visit is usually because the patient is diagnosed with (typical) anirida, nystagmus in baby or delayed visual maturation that occurs in approximately 25 % of aniridic babies. Diagnosis of aniridia in cases of atypical presentation is important at the first visit

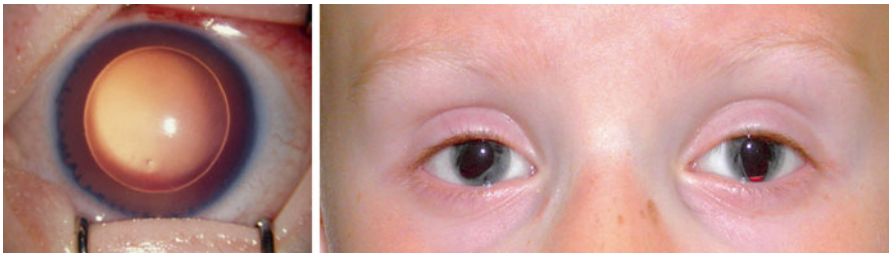

Fig. 14.6 First visit is usually because the patient is diagnosed with (typical) anirida, nystagmus in baby or delayed visual maturation that occurs in approximately 25 % of aniridic babies. Diagnosis of aniridia in cases of atypical presentation is important at the first visit

- Cornea
  - Microcornea
  - LSCI with pannus formation: circular limbal grayish opacification extending to the corneal center, first changes are avascular, then vessels follow the pannus centripetally
  - Corneal epithelium can contain ectopic conjuctival goblet cells
  - Corneal neovascularizations often start at the 12 and 6 position, later progress circumferentially
- Iris – see Figs. 14.5 and 14.6
  - "Complete aniridia"–gonioscopic examination always reveals a rudimentary iris stump
  - Incomplete aniridia

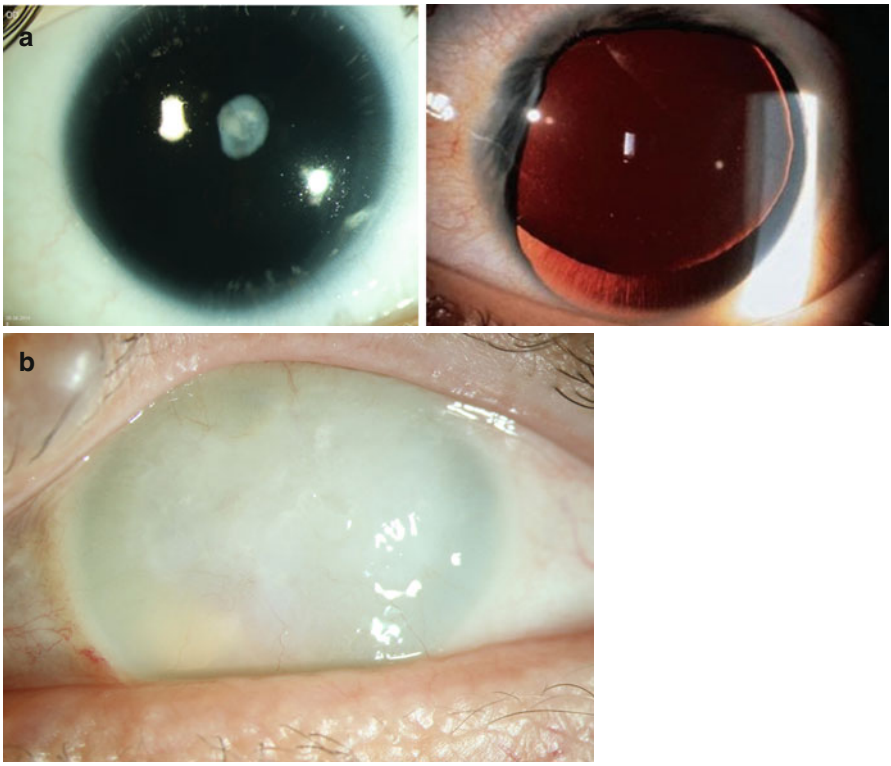

Fig. 14.7 (**a**) Retinoscopy is very valuable to estimate degree of visual disturbance. *Left* – Frequent finding in aniridia Polar anterior or posterior. *Right* – Lenses in aniridia may be luxated and present refractive problems. (**b**) Aniridic eye having suffered from AFS. Anterior chamber filled with fibrotic scars, corneal decompensation (female patient, aged 64)

  - Atypical coloboma
  - Ectropium uveae
- Crystalline lens
  - Congenital: frequent central cataracta polaris anterior or posterior without any signs of progression Fig. 14.7
  - Premature cataract formation → Cataract surgery often needed in childhood or youth
  - Subluxation or letal extopia caused by insufficient zonular fibers stability
- Intraocular pressure
  - Aniridia related secondary glaucoma often starts in childhood
  - Mechanism/pathophysiology: there is a contractile membrane over the anterior chamber angle which causes an increasing displacement of the iris stump towards the corneal endothelium, thus causing progressive blockage of the anterior chamber angle.
  - CAVE: Undiagnosed secondary glaucoma is the highest risk factor for persistant vision loss and blindness in aniridia.

- Macula and fovea
  - Hypoplasia of the macular structures
  - Foveal and foveolar hypoplasia
- Optic nerve head
  - Optic nerve head often very small
  - Hypoplasia of optic nerve
  - Dysplasia of optic nerve
- Peripheral retina
  - Hypopigmentation of the peripheral retina (i.e. the pigment epithelium) is frequent
- Changes of the sensory system
  - Congenitally underdeveloped visual acuity – inborn low vision
  - Atypical visual development
  - Delayed visual maturation (DVM)
  - Nystagmus (up to 90 % of all patients)
  - Strabismus is frequent (over three fourth of patients)

Ocular Findings and Aniridia Related Complications in Young and Adult Patients

Aniridia Related Complications: General Aspects

Aniridia is characterized by numerous ocular complications possibly developing during life. This makes aniridia very different from other congenital ocular abnormalities like albinism, as patients with aniridia cannot count on their visual acuity to remain stable during school and professional life. Therefore, a patient with aniridia may be much more exposed to difficulties during academic and professional life, including long sick leaves and changing needs of adaptive technology.

The main reasons for complications with visual loss are ocular surface disease OSD due to LSCI and associated AAK (aniridia associated keratopathy: AAK, Figs. 14.8 and 14.10) [16, 17]. Premature cataract development and insufficiently managed intraocular pressure in aniridic glaucoma are further reasons for complications associated with vision loss – especially aniridia glaucoma can ultimately lead to blindness by late-diagnosed glaucomatous optic atrophy [17–19].

Anterior Segment Fibrosis Syndrome ASFS = Aniridia Fibrosis Syndrome

One further visually devastating complication has up to now only been described in aniridia: aniridia-fibrosis syndrome (AFS). This represents a non-inflammatory intraocular fibrotic scar formation, often associated with hypotension and phthisis [20] following intraocular surgery. This challenging postoperative course was first

described by Tsai and colleagues in 2005 as "anterior segment fibrosis syndrome ASFS" [17].

Complications Related to the Crystalline Lens

At birth, there often is a cataracta polaris anterior or posterior (see Fig. 14.7) usually showing no progression. Depending on the severity of aniridia and of the anomaly of the zonularfibers an early a subluxation of the lens can result, usually upwards (Fig. 14.8). Cyclodestructive procedures may worsen the tendency of dislocation and premature cataract formation and should therefore be avoided.

Premature cataract formation often renders cataract surgery necessary in young patients. In order to avoid AAFS/AFS or late complications like chronic uveitis, we suggest to perform the smallest possible incision, foldable untinted lenses and the strict exclusion of iris replacements, iris lenses or artificial diaphragmas, ring segments and large incisions.

Secondary Glaucoma (Aniridic Glaucoma)

Secondary glaucoma is the most threatening complication for permanent visual loss in aniridia [18, 19]. Progressive angle closure is caused by iridocorneal adhesions: a tractive membranous process pulling the rudimentary iris tissue over the trabecular meshwork. In aniridia gonioscopic examination shows find strands which contract and close the anterior chamber angle, while Axenfeld-Rieger syndrome shows broad tissue strands.

Figure 14.9 shows the optic nerve heads of an aniridic boy with distinct glaucomatous changes in the optic nerve OD. He has had a successful trabeculotomy 1,5 years ago which lead to a slight reduction in glaucomatous excavation.

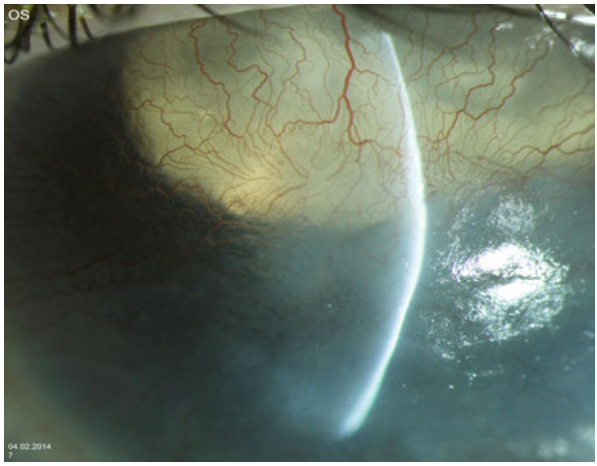

Fig. 14.8 14 year old girl with IOP 40+. Severe finding of keratopathy plus mature and luxated cataract. Visual acuity at first presentation: no light perception

Potential hazards to vision are numerous and lie within the diagnosis, follow-ups, conservative treatment and the complications of glaucoma surgery and are summarized as follows:

- Problems in diagnosing aniridic glaucoma and in the follow-up of glaucoma
  - Development of glaucoma may occur already in childhood and might be overlooked due to:
    - Reduced compliance during
    - Visual field measurements, laser scanning of the optic nerve rim, photographic documentation and other glaucoma examinations are either not possible or difficult and unreliable in young children and in patients with high amplitude nystagmus
  - Evaluation of the optic nerve head is more challenging due to:
    - Cataract (see Figs. 14.8 and 14.9)
    - Progressive corneal opacification
    - Nystagmus
    - Inborn form anomalies of the optic nerve head which render he evaluation of glaucomatous cup-disc-relation difficult: optic nerve hypoplasia and optic nerve dysplasia
    - In pre-perimetric children:
      - Use VEP (preferably pattern VEP) as a baseline for glaucoma follow up
      - Evaluate monocular colour vision and colour comparison OS/OS
  - Estimation of the validity of intraocular pressure measurement is difficult due to:
    - Corneal thickness usually is higher in aniridia.
    - Changes during the course of disease may however change this:
      - Progressive fibrotic scarring may lead to thinner cornea.
      - Secondary endothelial decompensation with edema would render an addition to the measured IOP necessary
    - Regular corneal parameter measurements are therefore necessary in aniridia patients.
- Problems in conservative, non-surgical treatment of aniridic glaucoma
  - Severity of ocular surface disease due to LSCI in aniridia
    - Topical medications are not tolerated as well as in non-aniridic patients
    - Compliance may therefore be lower in children and in adults
    - Local pain, foreign body sensations may lead to reduced treatment adherence
    - The more local medications are needed, the more corneal problems can arise including punctate keratopathy and recurring erosions, leading to increased neovascularizations
  - Systemic side effects of topical antiglaucomatous drugs
    - This affects mainly children, but may limit the spectrum of drug subgroups decidedly
    - Beta blockers often lead to tiredness and loss of physical strength in children
  - Systemic side effects of systemic antiglaucomatous medication

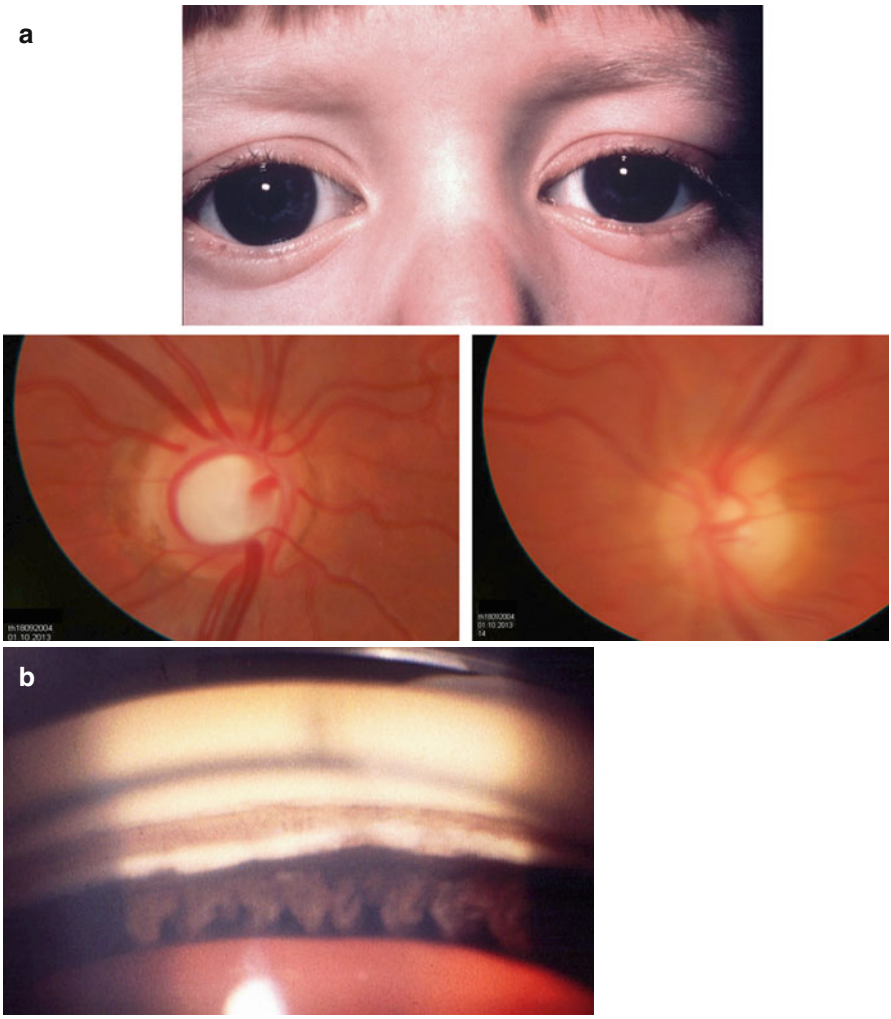

Fig. 14.9 (**a**) 4 year old Aniridic boy, cataract left eye > right eye OD successfully treated by trabeculotomy 1.5 years ago shows buphthalmia OD > OS. (**b**) Gonioscopy reveals iris stump and closed anterior chamber angle due to synechiae

    - Sometimes a temporary use of dorzolamide is necessary to regulate IOP. This may lead to
        - Lab electrolyte changes (low potassium)
        - Sickness, vomiting
        - Reduced physical fitness

- <u>Problems in surgical treatment of aniridic glaucoma leading to further reduction of vision</u>
    - Spectrum of indications of antiglaucomatous surgeries differs from non-aniridic glaucoma patients

- Gonioscopic laser surgery to reduce IOP is neither possible nor indicated in aniridia
- Cyclophotocoagulation
  - Cyclophotocoagulation is more difficult due to:
    - Anatomic anomalies in the position of the ciliary body and the angle
  - Cyclopotocoagulation shows more postoperative complications:
    - Severe intraocular inflammation
    - Destruction of zonulafibers with consequent subluxation of the lens
    - Worsening of presenile cataract formation
  - Cyclophotocoagulation should be avoided
- Trabeculotomy should be preferred to tabeculectomy
  - Reduced risk of Aniridia Fibrosis Syndrome
  - Repeatable if necessary
  - May be difficult due to
    - Anatomical malposition of Schlemm's Canal
    - Limbal stem cell insufficiency and reduced visualisation
- Glaucoma surgery involving valves (Ahmed, Baervaldt)
  - May be needed earlier than in non-aniridic patients

– Prognosis of surgery is lower and intra-/postoperative risks of glaucoma surgeries are higher:
  - Intraoperative risks:
    - Higher bleeding tendency
    - Higher prevalence of inflammatory response
    - Less predictability of surgery
  - Postoperative risks:
    - Less predictability of stability of IOP lowering
    - Higher scarring risk
    - Higher risk of recurrence of elevated IOP
    - Risk of permanent low pressure, choroideal detachment without normalization of IOP and consequent atrophy and phthisis formation
    - Risk of developing Aniridia Fibrosis Syndrome is directly related to the extent of trauma during intraocular surgery
  - Repeat surgery is needed more frequently than in non-aniridia glaucoma surgery
    - This should be explained to the patient
    - Better do a careful surgery with less risk of AFS and secondary scar formation even if that means doing a repeat surgery than doing a glaucoma surgery too forcefully
  - Anatomy of Schlemm's Canal and trabecular meshwork is different
  - High rate of scarring and of consequently insufficient lowering of IOP
  - CAVE: risk of Aniridia Fibrosis Syndrome
  - Valve surgery is needed more often than in non-aniridicglaucoma

- Summary: Points to remember when treating aniridia patients and treating aniridic glaucoma

- Diagnosis of glaucoma
  - Glaucoma may occur *early* in childhood
  - Each visit of any aniridia patient *has* to include IOP measurement, irrespective of age
  - Lack of cooperation or visibility must *not* lead to undiagnosed glaucoma!
  - If in doubt: perform exam under general anaesthesia
  - Take corneal thickness into account
- Follow-up in aniridic glaucoma
  - Check corneal parameters at least yearly
  - Try perimetry as soon as possible
  - Do a baseline VEP (pattern)
  - Low IOP is the best neuroprotective treatment – IOP should not exceed 16 mmHg
  - If in doubt → treat!
- Conservative treatment of glaucoma
  - Take corneal surface disease and LSCI into account
  - Monitor OSD and LSCI
  - Limit of topical drugs should be three to avoid further strain to the cornea
  - Always use preservative free eye drops (!)
  - Add corneal epithelial support (dexpanthenone, hyaluronic acid) if a patient constantly needs antiglaucomatous treatment
- Surgical treatment of glaucoma
  - Repeat surgery is needed more frequently than in non-aniridia glaucoma surgery
    - This should be explained to the patient
    - Better do a careful surgery with less risk of AFS and secondary scar formation even if that means doing a repeat surgery than doing a glaucoma surgery too forcefully
  - Anatomy of Schlemm's Canal and trabecular meshwork is different
  - High rate of scarring
  - CAVE: risk of Aniridia Fibrosis Syndrome
  - Valve surgery is needed more often than in non-aniridic glauccoma
  - See chapter of Peter Netland for further details!

Corneal Complications: LSCI: Pannus Formation, Vascularized Corneal Scars, AAK Aniridia Associated Keratopathy, Secondary Nodular Degeneration of Salzmann

While secondary glaucoma massively endangers aniridia patients visually, patients with AAK Aniridia Associated Keratopathy commonly suffer from recurring and sometimes severe OSD related pain and changes in visual acuity, both of which significantly affect everyday life.

PAX6-related aniridia shows a congenital anomaly of the stem cell niche and consequently leads to severe LSCI limbal stem cell insufficiency, thus impairing epithelial cell integrity, epithelial regeneration and healing.

The first sign of LSCI is a grayish avascular pannus formation in the corneal periphery, starting at the 6 and 12 o'clock position (Fig. 14.10), then involving the whole limbal circumference.

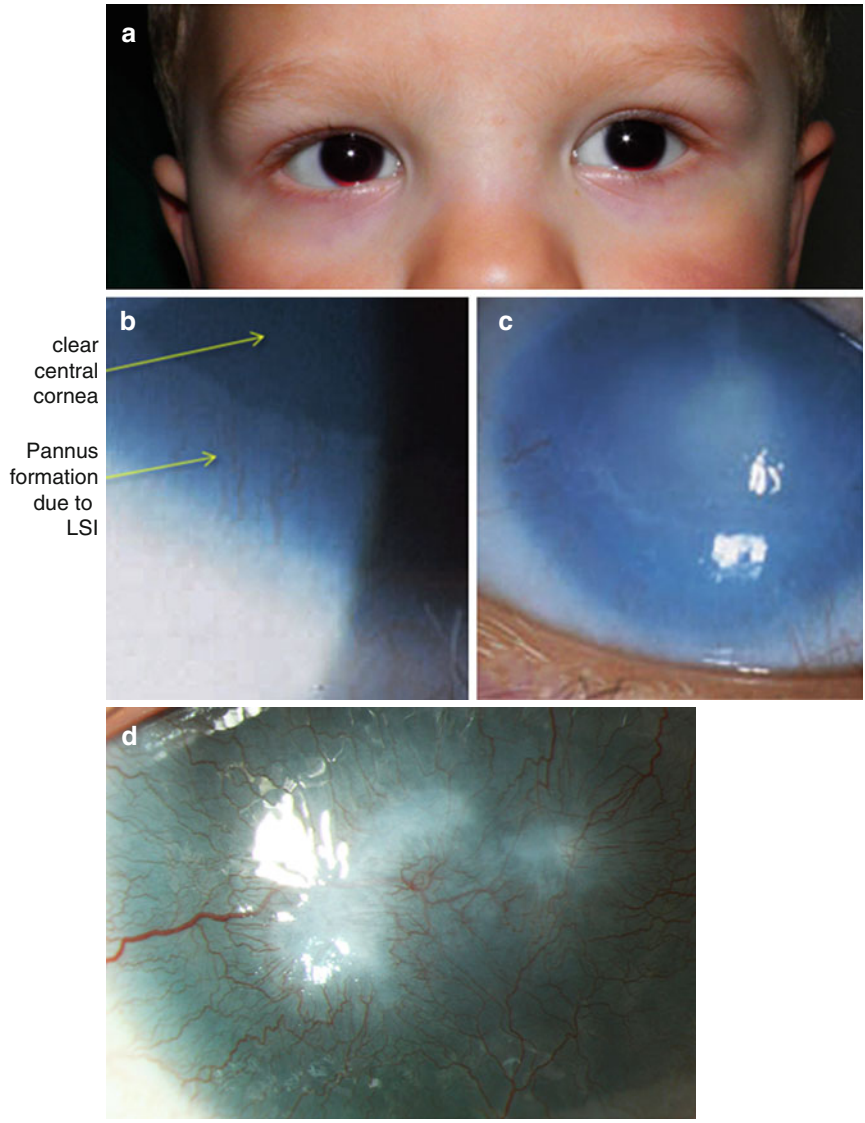

Fig. 14.10 Demonstrates in three youngsters with PAX6-related aniridia how different the clinical course in LSCI and AAK may be in individuals. 4 different children with PAX6-related aniridia: Age of children: (**a**) 22 Months; (**b**) 7 years; (**c**) 9 years; (**d**) 14 years

With progressing conjuctivalization of the cornea and immigration of goblet cells, neovascularizations start to invade the cornea and progress centripetally [3, 16, 17]. This development is accompanied by increasing loss of epithelial integrity and epithelial wound healing problems, recurrent erosions and the risk to develop corneal ulcerations.

In some patients, in addition to the vascularized corneal opacities a Salzmann's secondary nodular degeneration develops. This, however, can be treated more easily than the vascularized corneal scars: Eximer laser assisted phototherapeutic keratectomy (PTK) and pannectomy can notably improve the corneal surface and provide the patient with a slightly better visual acuity at least for some months or years.

Extensive cyclophotocoagulation in aniridia glaucoma can massively speed up the process of corneal decompensation and vascularized scar formation (Fig. 14.11) and should therefore be avoided (Figs. 14.12, 14.13, and 14.14).

Prevention Respectively Delay of Corneal Complications?

Up to now, there is no evidence-based prevention of AAK. In our opinion the most efficient preventive treatment of LSCI-related AAK is the prophylaxis of chronic nutritional disorders of the cornea, as these usually result in epithelial disintegration and ocular surface disease. A continuous local therapy started in early childhood

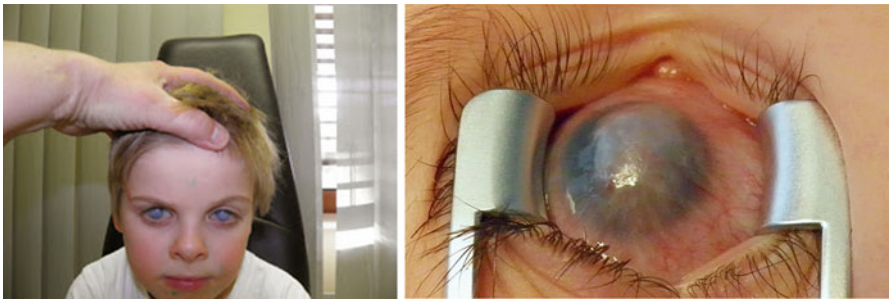

Fig. 14.11 6 year old boy. Aniridia glaucoma from 1st year of life, had multiple cyclophototreatments elsewhere, now continuous pain (reason for first presentation), ulcus formation, IOP not regulated

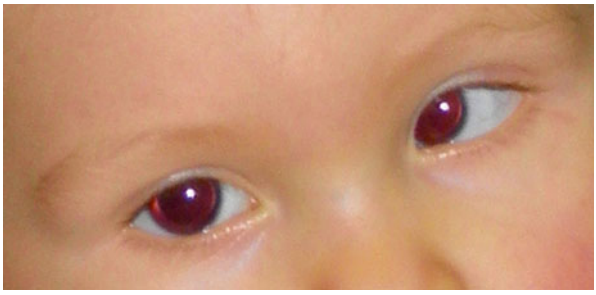

Fig. 14.12 Strabismus and amblyopia in aniridia

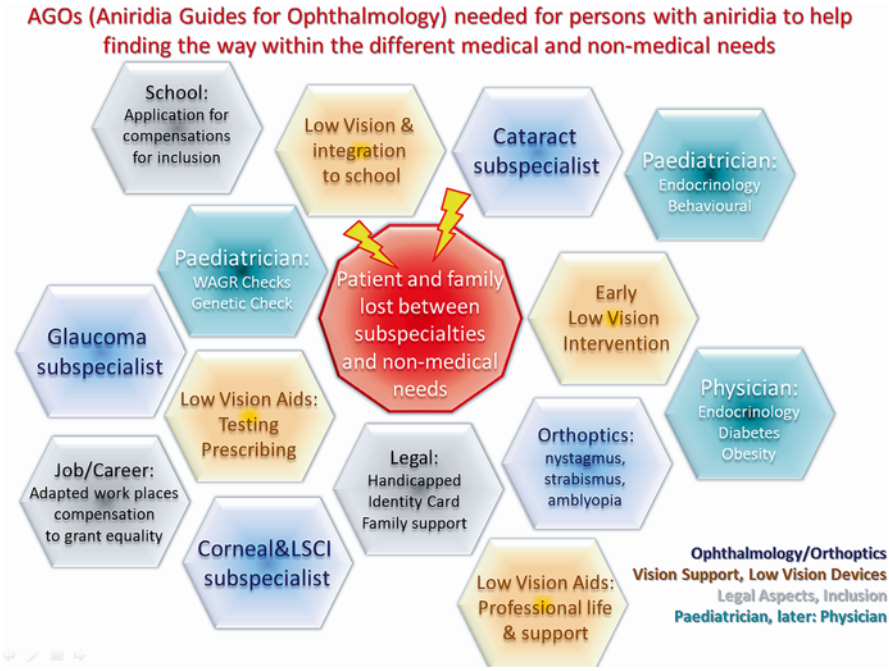

Fig. 14.13 Graphical representation of all requirements and medical and non-medical needs an aniridia patient faces during life and demonstrating the need of an Aniridia Guide Ophthalmology (*AGO*)

with preservative-free gels, artificial tear drops or ointments, e.g. medications containing hyaluronic acid, according to our limited experience may slow down at least a quick progression of AAK.

New Therapeutic Approach to Aniridia Related Complications by Molecular Genetics

A new approach to the treatment of LSCI and AAK was recently presented by Gregory-Evans et al. and – following successful rodent trials – now is administered to the first selected patients in a first clinical study [21, 22] since 2014. The approach can be used in patients who show anin-frame nonsense mutation with premature stop codon resulting in absent translation and lack of protein. In the mouse model a mutation independent suppression of the pathological stop codon could modify postnatal PAX6 activity. The authors showed a deceleration of complications and even some reversal of pathological corneal findings in rodents.

Patients with PAX6 gene haploinsufficiency receive a topical formulation containing a suppressor of the pathological stop codon and thus increases ocular PAX6 gene activity. The aim is to slow down the consequences of aniridia complications or even reverse present corneal involvement. Should this approach prove to be

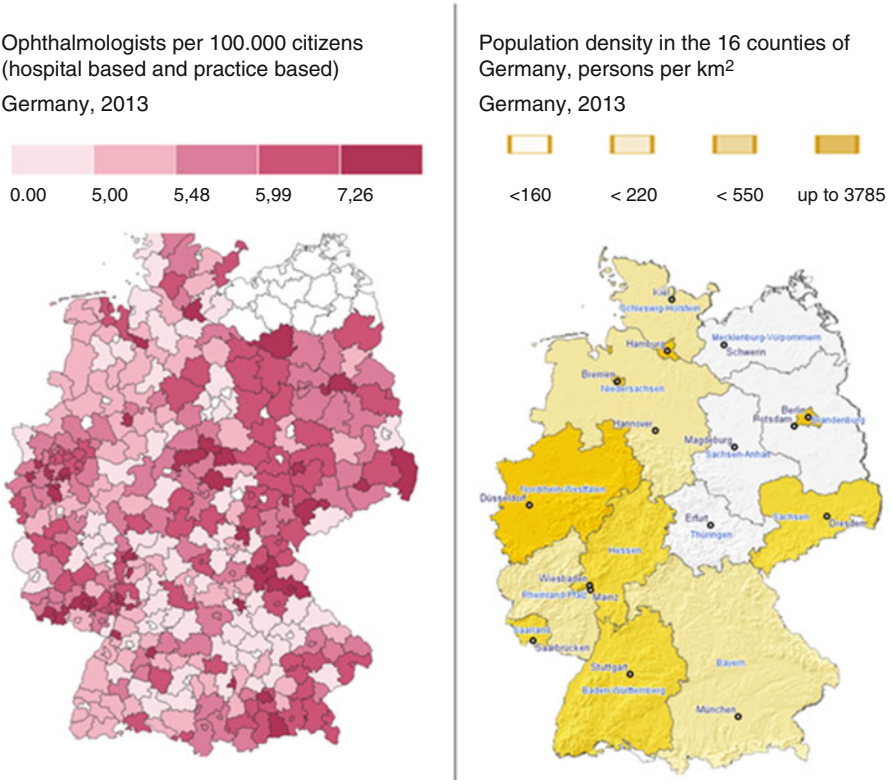

Fig. 14.14 Density of ophthalmologist compared to density of inhabitation/citizens per km² (Source: Destatis, Database GENESIS, Health 2014, Data: IGES and Bertelsmann Foundation; own choice of parameters and compilation (2014))

successful, this sure will change treatment paradigms in those patients who show the appropriate molecular genetic variation (in-frame nonsense mutation with premature stop codon). The authors also discuss whether this treatment might also be used to reduce the neurological findings some patients show.

Systemic Findings in Patients with PAX6 Related Aniridia

Additional systemic abnormalities have been described for many years in PAX6-related aniridia (formerly called: "syndromatic aniridia") [23–25]. Since decades an association with (congenital, not secondary) ptosis, obesity and learning disabilities has been shown in sporadic and familiar cases of airidia.

Another entity is the Gillespie syndrome (Aniridia, cerebellar ataxia and mental disability), as well as aniridia with missing patellae. Also, renal insufficiencies have been described in adulthood.

In recent years, more systemic abnormalities were also revealed in patients with PAX6-related aniridia, emanating from the other anatomical areas where PAX6 acts

as important control gene of embryonic development [3, 6, 26]. The systemic features are summarized in Table 14.3 [3, 6, 26, 27].

The knowledge about these PAX6 gene related developmental influences is especially important when caring for aniridic children who show developmental delays – not everything might be due to inborn low vision, but could be directly related to PAX6 syndrome. We therefore suggest to examine any aniridia child exhibiting a suspended development according to the findings listed in Table 14.3.

Routine Examinations and Complication Management: Proposal to Implement Supra-Regional "Aniridia Guides" (AGOs)

Proposal of Implementation of Regional "Aniridia Guides": AGO: Aniridia Guide Ophthalmology

Congenital aniridia is a disease which involves nearly all ophthalmological subspecialties: during the first years a paediatric ophthalmologist with experience in congenital low vision and nystagmus is needed, plus examinations and treatment for strabismus and amblyopia are necessary (too often amblyopia is missed in visually handicapped children!). Later, other subdisciplines are needed as aniridia related complications arise: anterior segment surgery (cataract), glaucoma specialist, corneal specialist, in case of Aniridia Fibrosis syndrome or retinal detachment (higher prevalence in aniridia) a vitreoretinal surgeon is needed.

Livelong the patient will need a dedicated low vision ophthalmologist who, apart from trying and prescribing low vision devices, can as well support the patient with many socio-legal aspects (application for handicap-related state benefits, requesting a specialized handicapped identity badge, claiming the benefit of time extension for written exams and many more) where professional expertise and written expert opinions are mandatory.

Our Experience

Many patients of our aniridia center had been seen for many years by one single ophthalmologist. Usually, that was in a hospital setting, not in one-physician health insurance covered practices which usually deal with approx. 80 % of all ophthalmological patients in Germany. Often, the regional university or municipal hospital and the treating ophthalmologist had been chosen when a first complication had manifested – therefore, the patients were either with an anterior segment surgeon, a glaucoma specialist or with a corneal consultant and surgeon.

Usually the patients remained there for further examinations.

From our patients we learned that very often the subspecialist would mainly concentrate on his specialty. This could lead to progression of other complications and in the worst case could even result in blindness due to permanent optical nerve damage. In addition, the treating ophthalmologist (mostly being a surgeon)

had no or little connection to and knowledge about low vision support services. We often see patients who had up to now not been informed in detail about academic, occupational and social low vision and legal support possibilities. In no way do we want to be the weisenheimer of fault-finder – on the contrary. We just want to point out that nowadays, with techniques progressing in all fields and subspecialty knowledge expanding, it is not possible any more to comprehensively treat a disease as complex as aniridia by just one ophthalmologist/one hospital.

Conclusion: Supraregional AGOs (Aniridia Guides Ophthalmology) for Continuous Care of All Aniridia Related Aspects and if Needed: Only the Best Subspecialists in Case of Complications

AGOs (Aniridia Guides for Ophthalmology) should possess the following standards in order to successfully support the aniridia patient:

- Main consultancy as low vision and/or paediatric ophthalmologist
- Interest in the demanding task of following up aniridia patients
- Technical requirements for a comprehensive follow-up should be in-house:
  - Glaucoma services (computer assisted perimetry, laser scanning of the optic nerve, OCT)
  - Corneal services including topography, anterior segment OCT
  - Ocular surface disease clinics
- Broad experience in re/habilitative care and socio-legal aspects of low vision patients of all ages, including
  - A low vision department in his/her clinics
  - Functional contact members:
    - Early visual support teams
    - Schools for visually handicapped
    - Inclusion/integration support offices
    - Social benefit/welfare offices
    - Manufacturers of adaptive, optical and electronical low vision devices
- Profound ophthalmological and ophthalmopathological knowledge, especially in:
  - Glaucoma
  - Limbal stem cell insufficiency and sequelae
  - Ocular surface disease
  - Postoperative care for corneal transplants and glaucoma surgery patients
- Having or creating a functionally effective network to experienced surgeons of all subspecialties (!)
  - Arranging cooperation and quick referral options for the patient

From this short list, two facts seem evident:

- The AGO usually works in a hospital setting, mostly at a University Hospital
- Usually, not all subspecialists work at the same place→ the AGO uses his subspecialists network to send the patient for optimal surgical treatment and sees the patient postoperatively.

In our opinion, a country of the size of Germany (~370.000 km^2) and a population of nearly 81 million people should at least have four, better six AGOs evenly distributed in Germany depending on population densities.

As the following image shows, population density is very variable in Germany (as in most European countries) and contrasts with the density of ophthalmologists.

There are areas of high population density and high ophthalmologist density – especially counties like Nordrhein-Westfalen have many Universities, many confluent cities and thus a high rate of ophthalmologists per 100.000 citizens.

There are regions in Germany, however where the ophthalmologist density seems to be sufficient (Sachsen-Anhalt, Thüringen, Mecklenburg) but where the population density is so low that individual patients have to travel long ways to reach an ophthalmologist.

All these aspects have to be taken into account if one wants to implement supraregional AGOs for Germany who will have equal shares and demands to work up with the aniridic patients.

Mismanagement or delayed treatment in each of the subspecialties can potentially lead to blindness, which might be irreversible (fibrosis syndrome, glaucoma). In addition, as the patients reach school age and later needs to find and follow an occupational career, low vision support has to be flexible to changing visual acuities due to complications and their treatment. In aniridia, vision can never be expected to be stable and reliable as in other congenital visual impairments – e.g. albinism.

The following conclusive enumeration summarizes the "to dos" of routine checkups and complication management in aniridia patients.

Non-surgical Care

Early Improvement of Retinal Image and Promoting Visual Development

The young aniridic child should be treated and supported as early as possible to promote the development of visual acuity and to prevent or delay the occurrence of complications.

Early visual support helps the brain to learn to use (reduced) visual inputs as well as possible – in spite of the morphological changes of the eyes.

The following schedules should be followed for children with aniridia as soon as the diagnosis is confirmed:

- **Protection from glare and optimization of the visual input and the retinal image**

(a) Cycloplegic refraction

  (i) In aniridia there is often a myopia or hyperopia and astigmatism – these are optical aberrations of the eye which can and should be corrected to improve the visual quality the child perceives

(b) Prescription of two pairs of spectacles

(i) Both pairs of spectacles need to block UVA and UVB light, they need a blocking filter at 400 nm
(ii) One pair should have a light dampening of 20 % (for inside and cloudy days outside)
(iii) The other pair of glasses should have a tint of 80 % (outside, sunny days)

Early Intervention for Low Vision Children

The paediatrician and the ophthalmologist should not underestimate the benefits of early low vision intervention for the very young aniridics: of course, one cannot actually "treat" foveal hypoplasia and optic nerve dysplasia. But in spite of them being present, one can promote and improve visual perception and therefore enhance visual development during the first 6 years. Neither anterior segment changes nor foveal and optic nerve hypoplasia imply a "given" or "static" low visual acuity.

Vision Is a Learned Function!

One can train visual development, and the difference between 0,05 and 0,2 may be very important for later life! Muscles, for example, can be trained life-long. But with visual development and the plasticity of the brain, one is confined to the first 6–8 years. Chances not used then are lost forever.

Therefore any aniridic child should be correctly refracted, should be given spectacles and should be connected to Early Low Vision Intervention services as soon as possible. In addition, parents should be encouraged to support visual development in a playful manner at home.

- **Support of early visual development**

(a) Getting into contact with early low vision intervention services

(i) The services can often be located at the regional schools for visually handicapped children.

(b) Tell parents to start early support at home by playfully inspiring the child to look at high contrast objects, promoting the eye-hand-contact and the child's interest to visually explore the surroundings – this as well helps the general development concerning motor and social and cognitive development.

Early Prevention of Possible Complications

Details for prevention and possible delay of aniridia related complications see above. The following list summarizes the most important aspects.

- **Prevention respectively delay of later complications**

(a) Early start of protective cornea and tear film treatment →supports corneal epithelium

(i) Artificial tear drops over the day
(ii) Gel or nourishing ointment to be applied every night
(iii) No preservation additives!

(b) Regular measurements of IOP → early detection of glaucoma
(c) Wearing the UV-blocking glasses →may delay cataract formation, beneficial for OSD

Low Vision Aids Prescription

Low vision control strategies

- Establish the cause of visual loss
- Surgical interventions if appropriate
- Assessment of the child's various visual functions (distance vision, near vision, contrast sensitivity, and visual field)
- Contrast sensitivity testing
- Glare testing
- Color vision testing
- Refraction and provision of spectacles
- Examine and improve contrast sensitivity and contrast vision (important!)
- Low vision devices (magnifiers)
- Non-optical low vision devices (reading stands)
- Training in the use of devices with follow-up
- Monitor stability or progression of disease and changes in visual abilities as rehabilitation progresses
- Assess eccentric viewing postures and skills
- Assess scanning ability (for patients with restricted fields)
- Assess patient motivation
- Teach basic concepts and skills (i.e., to eccentrically view) relevant to the rehabilitation process.

The goal of a low vision exam is to help maximize the use of remaining vision. Contrast sensitivity has emerged as a valuable measure of visual glare sensitivity, amount of light needed. Reduced contrast sensitivity can affect reading ability, ability to navigate through the environment, and risk for falls.

Surgical Care

Just the Best Subspecialists for the Aniridia Patient!

As said above, in our opinion only the best surgical subspecialists should treat aniridia patients. This will ensure that "the best" subspecialists will have more exposure to aniridia patients which helps for future treatments.

This may include sending the patient away from the hospital where the AGO works – but good networking should ascertain that the patient returns for postoperative and future checks.

In our opinion this might be the only way to reduce vision threatening complications like aniridia fibrosis syndrome.

Other Ophthalmosurgery in Aniridia

Often aniridia patients need additional ophthalmic surgery, as for example:

- Ptosis
- Nystagmus
- Strabismus

Social, Academic and Legal Support Given by the "Aniridia Guides" and Low Vision Consultants

Support Group

All families afflicted by aniridia should be brought into contact with the regional and national Aniridia Support group. For Germany, the following contact details apply:

Internet:	www.aniridie-wagr.de
	www.aniridieforum.de
Postal	DeniceToews-Hennig,
	President
	Georg Friedrich-Händel-Str. 7
	96247 Michelau/Oberfranken
Tel:	09571-9738575
Mail:	info@aniridie-wagr.de

Help for Integration and Inclusion

Inclusion refers to the integration of students with special needs learning alongside students without special needs in regular schools and classes with appropriate supportive services. Inclusion helps blind/low vision children go to schools within their own localities and interact with children within their own communities and adopt norms and values of their own communities.

Medical and educational assessment provides the opportunity for parents of blind and low vision children to work with them. It helps equip them with skills on how to motivate them to explore the wider world and engage in daily living and survival skills.

Low Vision and assessment centres are ideal for early clinical identification, diagnosis, appropriate intervention and placement for blind and low vision children. There is however, the need to make kindergarten and schools more inclusive with the availability of accessible schools, support services teaching and learning materials.

Vision Is Not Defined by the Eyes Only!

A person with low vision has severely reduced visual acuity and/or has significantly obstructed field of vision that cannot be corrected by glasses, medicine or surgery. Persons with visual impairments face a variety of challenges on a day-to-day basis. These difficulties often lead persons with visual impairments to suffer from loneliness, social and peer isolation, and depression with behavioural challenges.

Congenital low vision may cause a lifelong reduction in a child's visual performance. Reading is one of the main avenues for education and educational achievement. If visual impairment affects the child's ability to read, it could be a great impediment of his/her educational success.

Reading is a first step in education and is a predictor of good academic success. Children with low vision usually need some form of magnification to resolve letters that are lower than their threshold. With a detailed low vision examination and an accurate visual correction, children might achieve a better reading performance. There are other examinations than visual acuity that should be included in the low vision examination. Acuity reserve and contrast reserve are good predictors of reading performance and are important in children. The optimum magnification, acuity reserve and contrast reserve tend to lead to the optimum possible reading fluency.

Conclusions

Table 14.5 summarizes the conclusions, and lists the points the supervising ophthalmologist needs to think about when caring for an aniridia patient, depending on age and course of disease. The aim should be to prevent a rapid progression and reduce complications as much as possible. Table 14.5 is a modification of a table developed for the Aniridia Brochure recently presented by the European Aniridia support group Aniridia Europe. The author was one of the authors of the brochure and developed the "What to think of" table. Therefore there is no copyright problem present – and physicians should know as well as our patients what the important points in treating aniridia are!

Table 14.5 Important things to think about depending on age

	Examinations at the ophthal-mologist	Treatment to improve vision quality	Prevention of complications	Is any surgery planned?	Additional things which help to live with aniridia
0–2 years	Every 3–4 months	Refraction UV-Blocking glasses for inside and outside Early visual support activities	Each visit: measurement of the eye pressure Daily application of artificial tear drops and nourishing ointment for the cornea	**Think twice** Make sure you understand every aspect your ophthalmologist explains Do not hesitate to ask questions Do not hesitate to consult another specialist for a second opinion Ask your national aniridia group or ask Aniridia Europe forum	Get in touch with your national Aniridia Support Group Get attached to Aniridia Europe Share experiences with other parents and learn from parents with older children Ask about the regulations of your country for allowances for handicapped children and apply for them
2–8 years	Every 6 months	Keep dioptric values up to date See above	See above First low vision aids can be used from age 5		Start to be a contact person for parents with younger children and give back help and advice you received when your child was very young
8–18 years	Every 6–8 months	See above Low vision aids for school	Treatment of elevated eye pressure should be started as early as it is detected No laser surgery if eye drops are not sufficient		Young persons can participate in aniridia meetings to get to know other youngsters with similar persons Sports with other visually challenged persons
Adult persons	Yearly, in case of upcoming problems shorter time spans	See above Check-ups depend on the kind of complications	Often more than one specialist is needed! See a corneal specialist See a glaucoma specialist		
Always beware of or take second opinions if the following things are suggested	Wearing contact lenses				
	Wearing contact lenses with iris prints				
	Implantation of an artificial iris – this sounds good but might destroy the eye and any residual vision				
	Use of eye drops which include conservation agents				
	Always check and do not use eye drops containing phosphates				
	No laser surgery for glaucoma if eye drops cannot control eye pressure sufficiently				

References

1. Bamiou DE, Campbell NG, Musiek FE, Taylor R, Chong WK, Moore A, van Heyningen V, Free S, Sisodiya S, Luxon LM (2007) Auditory and verbal working memory deficits in a child with congenital aniridia due to a PAX6 mutation. Int J Audiol 46(4):196–202
2. Bamiou DE, Free SL, Sisodiya SM, Chong WK, Musiek F, Williamson KA, van Heyningen V, Moore AT, Gadian D, Luxon LM (2007) Auditory interhemispheric transfer deficits, hearing difficulties, and brain magnetic resonance imaging abnormalities in children with congenital aniridia due to PAX6 mutations. Arch Pediatr Adolesc Med 161(5):463–469
3. Hingorani M, Hanson I, van Heyningen V (2012) Aniridia. Eur J Hum Genet 20(10): 1011–1017
4. Peter NM, Leyland M, Mudhar HS, Lowndes J, Owen KR, Stewart H (2013) PAX6 mutation in association with ptosis, cataract, iris hypoplasia, corneal opacification and diabetes: a new variant of familial aniridia? Clin Exp Ophthalmol 41(9):835–841
5. Beby F, Dieterich K, Calvas P (2011) A [c.566-2A>G] heterozygous mutation in the PAX6 gene causes aniridia with mild visual impairment. Eye (Lond) 25(5):657–658
6. Netland PA, Scott ML, Boyle JW, Lauderdale JD (2011) Ocular and systemic findings in a survey of aniridia subjects. J AAPOS 15(6):562–566
7. Grønskov K, Olsen JH, Sand A, Pedersen W, Carlsen N, BakJylling AM, Lyngbye T, Brøndum-Nielsen K, Rosenberg T (2001) Population-based risk estimates of Wilmstumor in sporadic aniridia. A comprehensive mutation screening procedure of PAX6 identifies 80% of mutations in aniridia. Hum Genet 109(1):11–18
8. Lee HJ, Colby KA (2013) A review of the clinical and genetic aspects of aniridia. Semin Ophthalmol 28(5–6):306–312
9. vanHeyningen V, Boyd PA, Seawright A, Fletcher JM, Fantes JA, Buckton KE, Spowart G, Porteous DJ, Hill RE, Newton MS (1985) Molecular analysis of chromosome 11 deletions in aniridia-Wilmstumor syndrome. Proc Natl Acad Sci US A82(24):8592–8596
10. vanHeyningen V, Hoovers JM, de Kraker J, Crolla JA (2007) Raised risk of Wilms tumour in patients with aniridia and submicroscopic WT1 deletion. J Med Genet 44(12):787–790
11. Brémond-Gignac D, Bitoun P, Reis LM, Copin H, Murray JC, Semina EV (2010) Identification of dominant FOXE3 and PAX6 mutations in patients with congenital cataract and aniridia. Mol Vis 16:1705–1711
12. Henderson RA, Williamson K, Cumming S, Clarke MP, Lynch SA, Hanson IM, FitzPatrick DR, Sisodiya S, van Heyningen V (2007) Inherited PAX6, NF1 and OTX2 mutations in a child with microphthalmia and aniridia. Eur J Hum Genet 15(8):898–901
13. Khan AO, Aldahmesh MA, Mohamed JY, Hijazi H, Alkuraya FS (2014) Complete aniridia with central keratopathy and congenital glaucoma is a CYP1B1-related phenotype. Ophthalmic Genet 35(3):187–189
14. Law SK, Sami M, Piri N, Coleman AL, Caprioli J (2011) Asymmetric phenotype of Axenfeld-Rieger anomaly and aniridia associated with a novel PITX2 mutation. Mol Vis 17:1231–1238
15. Lim HT, Seo EJ, Kim GH, Ahn H, Lee HJ, Shin KH, Lee JK, Yoo HW (2012) Comparison between aniridia with and without PAX6 mutations: clinical and molecular analysis in 14 Korean patients with aniridia. Ophthalmology 119(6):1258–1264
16. Gregory-Evans K, Cheong-Leen R, George SM, Xie J, Moosajee M, Colapinto P, Gregory-Evans CY (2011) Non-invasive anterior segment and posterior segment optical coherence tomography and phenotypic characterization of aniridia. Can J Ophthalmol 46(4):337–344
17. Le Q, Deng SX, Xu J (2013) In vivo confocal microscopy of congenital aniridia-associated keratopathy. Eye (Lond) 27(6):763–766
18. Almousa R, Lake DB (2014) Intraocular pressure control with Ahmed glaucoma drainage device in patients with cicatricial ocular surface disease-associated or aniridia-related glaucoma. Int Ophthalmol 34(4):753–760
19. Gramer E, Reiter C, Gramer G (2012) Glaucoma and frequency of ocular and general diseases in 30 patients with aniridia: a clinical study. Eur J Ophthalmol 22(1):104–110

20. Kothari M, Rao K, Moolani S (2014) Recurrent progressive anterior segment fibrosis syndrome following a descemet-stripping endothelial keratoplasty in an infant with congenital aniridia. Indian J Ophthalmol 62(2):246–248
21. Gregory-Evans CY, Wang X, Wasan KM, Zhao J, Metcalfe AL, Gregory-Evans K (2014) Postnatal manipulation of Pax6 dosage reverses congenital tissue malformation defects. J Clin Invest 124(1):111–116
22. Gregory-Evans CY, Williams MJ, Halford S, Gregory-Evans K (2004) Ocular coloboma: a reassessment in the age of molecular neuroscience. J Med Genet 41(12):881–891
23. Crolla JA, van Heyningen V (2002) Frequent chromosome aberrations revealed by molecular cytogenetic studies in patients with aniridia. Am J Hum Genet 71(5):1138–1149
24. Malandrini A, Mari F, Palmeri S, Gambelli S, Berti G, Bruttini M, Bardelli AM, Williamson K, van Heyningen V, Renieri A (2001) PAX6 mutation in a family with aniridia, congenital ptosis, and mental retardation. Clin Genet 60(2):151–154
25. Simioni M, Vieira TP, Sgardioli IC, Freitas EL, Rosenberg C, Maurer-Morelli CV, Lopes-Cendes I, Fett-Conte AC, Gil-da-Silva-Lopes VL (2012) Insertional translocation of 15q25-q26 into 11p13 and duplication at 8p23.1 characterized by high resolution arrays in a boy with congenital malformations and aniridia. Am J Med Genet A 158A(11):2905–2910
26. Shaham O, Menuchin Y, Farhy C, Ashery-Padan R (2012) Pax6: a multi-level regulator of ocular development. Prog Retin Eye Res 31(5):351–376
27. Shimo N, Yasuda T, Kitamura T, Matsushita K, Osawa S, Yamamoto Y, Kozawa J, Otsuki M, Funahashi T, Imagawa A, Kaneto H, Nishida K, Shimomura I (2014) Aniridia with a heterozygous PAX6 mutation in which the pituitary function was partially impaired. Intern Med 53(1):39–42

Addenda

ADD1 – Käsmann-Kellner B, Seitz B (2014) Das Aniridie-Syndrom. Klinische Befunde, problematische Verläufe und Vorschlag zur Betreuungsoptimierung (“Aniridie-Lotse”). Ophthalmologe. doi:10.1007/s00347-014-3058-4, in press

ADD2- Käsmann-Kellner B, Seitz B (2014) Kongenitale Aniridie oder PAX6-Syndrom? Ophthalmologe. doi:10.1007/s00347-014-3058-4, in press

ADD3- Seitz B,Käsmann-Kellner B, Viestenz A (2014) Stadiengerechte Therapie der kongenitalen Aniridie. Ophthalmologe. doi:10.1007/s00347-014-3058-4, in press

Chapter 15
Assessing the Visual Function in Congenital Aniridia and Following the Child During Daily Life

Luisa Pinello

Abstract Congenital aniridia frequently causes severe visual impairment that is usually evident early in life. A precocious management of ocular complications is essential to prevent or limit low vision in these children. For planning rehabilitation treatment a complete opthalmological examination includes a careful visual assessment, using tests appropriate for the child's age and ability to cooperate. Measurement of visual acuity is challenging in children, especially in infants or in patients with mental retardation (WAGR or Gillespie Syndrome). In such patients visual acuity can be evaluated with preferential looking test (Teller Acuity Cards). Starting from the 3 years of age, visual acuity can be evaluated with ETDRS charts. Management of children with aniridia and low vision is problematic: glare, reduced distance vision, reduced near vision due to foveal hypoplasia, fatigue, accommodation spasms, blurry vision, difficulty in distinguishing colors, anomalous head posture (compensatory positioning), nystagmus and the absence of stereopsis. The aim of rehabilitation is to improve visual performance though the correction of refractive errors, specific strategies and low vision aids, to reduce or relieve symptoms (glare, photophobia), and to promote learning, communication and daily living safety skills, and to foster social and scholastic participation and the child's development and overall well-being.

Keywords Children • Visual assessment • Low vision • Congenital aniridia • Low vision aids

Congenital aniridia is a potentially vision – threatening problem. Severe visual impairment is usually evident early in life in children with aniridia. In Western Countries aniridia causes 20 % of paediatric low vision in the group of ocular

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malformations. Numerous factors contribute to low vision: greater amount of light rays entering the eye, corneal and lens opacities, glaucoma, foveal and optic nerve hypoplasia [1, 2] photophobia, nystagmus, and high myopia [3]. Best-corrected visual acuity (BCVA) is generally low: in a study involving 124 adults the mean BCVA was 0.2 (<0.3 in 80 % and <0.1 % in 18 % of cases) [1]. Another study in 12 children reported a BCVA ranged from 0,7 to light perception (>0,3 in 38 %) [4].

A precocious approach of ocular complications at the onset in congenital aniridia is important to limit low vision or to manage it, but low vision is usually present.

The visual assessment starts with elements from case history and is based on accurate assessment of visual function. In addition to family history and findings from physical and diagnostic examinations, the case history will include information on visual function. For example, parents will note glare and photophobia in very young children unable to describe these phenomena, which may be caused by exposure to outdoor light (mild photophobia), indoor light (moderate photophobia), or even dimly lit environments (severe photophobia). Other essential information concerns near, intermediate, and distance vision, mobility and orientation outdoors, communication, environmental problems at home and at school, and alterations or delays in development [5].

For planning rehabilitation therapy, a complete ophthalmologic evaluation will necessary include assessment of visual function by measuring BCVA using tests appropriate for the child's age and ability to cooperate [6, 7], near visual acuity and by evaluating fixation, eye movement, nystagmus, head positioning, cover test to detect strabismus, photophobia, refraction in cycloplegia (to correct high myopia and aphakia), visual field (Goldmann perimeter, arc perimeter or confrontation technique), electrophysiology (ERG and VEP) and low vision aids.

Measurement of visual acuity is challenging in children, especially in infants or in patients with mental retardation (WAGR or Gillespie Syndrome). In such patients visual acuity can be evaluated with preferential looking test (BCVA examination by Teller Acuity Cards), even if this test is not comparable to Snellen acuity, but it can provide some informations [1].

Starting from the 3 years of age visual acuity can be evaluated with ETDRS logMAR charts:in toddlers (3–4 years), it can be evaluated with LEA tests; in preschoolers (4–6 years) visual acuity is tested using Snellen letter E charts or letter charts in children aged 6 years and older. Eye examinations should be performed every 6 months, more often in the 0–2 years of age or if complications are present (corneal or crystalline lens disorders or glaucoma), depending on the individual case and related problems.

Children with aniridia should undergo lifelong follow-up by an ophthalmologist to detect glaucoma and other complications as early as possible and to treat or limit visual problems.

The aim of treatment is to manage the eye disorders that impair visual function, improve visual performance though rehabilitation strategies and aids, reduce or relieve symptoms, and, in patients with low vision, to promote learning, communication and daily living safety skills, and to foster social and scholastic participation and the child's development and overall well-being.

Management of children with aniridia and low vision is problematic: glare, reduced distance vision, reduced near vision due to foveal hypoplasia, fatigue, accommodation spasms, blurry vision, difficulty in distinguishing colors, anomalous head posture (compensatory positioning), anomalous eye movement (nystagmus) and the absence of stereopsis.

Aniridia usually causes photophobia and glare. Treatment for glare that impairs vision involves adopting precautions such as: avoiding bright light; providing for adequate room illumination with indirect or suffuse light; not viewing video screens unless environmental illumination is adequate; not placing light sources at eye level; using shaded lamps; not using spotlights for reading; wearing sunglasses. Treatment for glare from sunlight that produces eye irritation and discomfort includes: wearing a wide-brimmed hat or cap, avoiding bright or reflecting surfaces, avoiding abnormal reflections, reducing the glare from foliage, book pages, desks and blackboards.

An early prescription of UV filter glasses is necessary for outside and inside. The use of sunglasses or photochromic glasses helps to reduce the intensity of light reflecting off windows, mirrors, and smooth white or brightly colored surfaces. Sunglasses with UV filters serve a dual purpose: to reduce glare and photophobia and to protect against the harmful effects of increased UV light rays entering the eyes. Also recommended is the use of spectral filters (511–585 nm) [8] though, because of the rarity of aniridia, there is little published evidence supporting their use. Nonetheless, they should be prescribed as needed for outdoor and indoor use according to wearer comfort. In younger children, they should be used depending on how the child reacts on exposure to bright light.

Children with aniridia often present with severe refraction defects, particularly elevated myopia in up to 64 % of cases.

Prescription eyeglasses or contact lenses should be used to correct refraction defects as measured with fixed or preferably portable autorefraction under cycloplegia. The objective of the prescription is to improve BCVA, even in persons with severely low vision, to increase depth perception and reduce visual impairment, if present, within the first year of life. One might think that cycloplegia is not useful because the iris is either absent or reduced in size, however, a certain degree of accommodation persists. Optical treatment of high myopia in aniridia should be meticulous. Amblyopia, if present, should be treated with eye patching: children with structural asymmetries often experience improvement in visual acuity after treatment for amblyopia.

The use of contact lenses for nystagmus, photophobia and morphofunctional or cosmetic purpose should be evaluated case by case, weighing the risks and the benefits. Consensus is lacking as to whether contact lenses should be preferred for optical correction of refraction defects; however, they are indicated in the treatment of elevated or anisiometric defects (hydrogel contact lenses), as they provide for a better visual field and are highly recommended in aphakia surgery (silicone elastomere contact lenses). The distinct advantages to contact lenses reside in their morphofunctional and cosmetic aspects (38 % hydrogel HEMA contact lenses) and nontoxic tints. Furthermore, contact lenses form an artificial pupil (5 mm) that attenuates photophobia, glare from above, and nystagmus, protect against UVA and

UVB rays, facilitate the use of monocular magnifying aids for distance vision, and improve vision, comfort and quality of life.

The disadvantages of contact lenses include the increased risk of infections and corneal damage in patients with aniridic keratopathy due to altered stem cell production, resulting in longer time to healing of infection or corneal scarring. Cosmetic contact lenses may also cause vision problems in dim light or at night because the pupil does not change size to accommodate to darkness. They require extra care and attention, which parents will need to tend to in small children. They also require more frequent monitoring than eyeglasses and are not as effective as eyeglasses in correcting astigmatism, particularly if severe.

Treatment of aniridia-related problems and rehabilitation of visual function oriented to improving the child's quality of life all have a positive effect on learning, communication and activities of daily living, thus facilitating insertion and participation in community life and enhancing the child's development and overall well-being [5].

Low vision examination is performed by evaluating:

- Color test
- Contrast sensitivity test
- Low vision device
- Instruction in the use of low vision devices
- Ergonomic strategies in the use of sight, particularly for ambient facilities and school requirements and needs (inclined school desk, lap desk, ergonomic chair, lamps, large print for books, bold line paper, specific notebooks, etc.

Evaluation of low vision devices (Table 15.1) is necessary to provide:

- Magnification aids for distance visual tasks (hand held telescope)
- Magnification aids for near visual tasks, in children with extremely low vision due to foveal or macular hypoplasia (spectacle magnifiers, hand magnifiers, CCTV, magnifying software)

Table 15.1 Types of adaptive/assistive devices

Optic devices for distance vision	Contact lenses (for aniridia, aphakia due to cataracts), spectral filter lens to protect against harmful light radiation or reduce glare, telescopic magnifiers for distance vision (Galilean, Keplerian), monocular magnifier, portable eyeglass- or head-mounted telecamera with portable monitor or LCD
Systems for nearvision	Magnifying lenses; aplanatic bifocal magnifying lenses; Galilean and Keplerian telescopes; monocular or binocular hypercorrection eyeglasses (prisms); prismatic binocular eyeglasses for myopia
Aids for the blind	Braille printer; text-to-speech reader; Braille writing tablet; Braille display; Braille typewriter
Other aids	Inclined desktop; reading lectern; ergonomic chair; overhead lighting
Electronic devices and technologies	Fixed video magnifier or CCTV systems; portable video magnifier; magnification software for PC; text recognition and reading system with scanner and OCR-ICR application software

- Optical correction to improve visual acuity
- Light filtration (spectral filters or photocromic lenses) to reduce glare and to protect lens and retina from UV damages for outside and inside and cap (baseball cap).
- Contrast enhancement

Visual rehabilitation encompasses the use of electronic/optic and nonoptic devices according to the person's cognitive development. Choices from among the vast range of available devices will follow on from decisions of how best to provide an efficacious and personalized response to the child's needs, appropriate for age and cognitive development, and remaining or potential visual ability [4]. Prescription of a device will be based on accurate diagnostic assessment of organic abnormalities and visual function, after having corrected the underlying refraction defect and after a trial phase and training in the use of the device.

Rehabilitation aids and devices are aimed at reducing glare, improving optic correction, and facilitating environmental skills, together with modifying the home environment, adjusting contrast (to minimize problems with stereoptic vision), providing for adequate lighting and ergonomic devices to correct posture and cope with nystagmus, will all enhance the child's independent mobility at home and at school.

Also recommended skills are learning in orientation and daily living safety, mobility coaching, and personal independence or other personalized interventions. Furthermore, psychological support is recommended to assist children with delayed development and behaviour disorders, resulting from the psychological effects of low vision on self image and self esteem, appearance-related dissatisfaction with assistive/adaptive devices or other causes, psychiatric problems and mental retardation (WAGR and Gillespie syndromes).

Parental support is fundamental, which can be enlisted starting from a frank discussion of the diagnosis, the child's visual prognosis, and treatment and rehabilitation options, and must be sustained through support provided to the parents directly or parent groups.

While a generalized scheme of preferences and practices in rehabilitation may respond to the needs of the majority children with aniridia, it cannot cover all circumstances. This means that rehabilitation must be personalized to the individual child who will have different needs that require specific responses.

The ophthalmologist provides the school and educational agencies with a learning pathway of the child with low vision, specifying the strategies, methodologies, materials and aids most appropriate for learning activities, with description of visual status, print size recommendations, print media recommendations, recommendations for optical devices and adaptive technology and environmental modifications.

Environmental modifications are recommended such as seating close to board, away from windows and the classroom should be fitted with a blackboard that provides strong contrast without abnormal reflection, shaded windows, and indirect lighting. Because children with aniridia and low vision often develop fatigue and accommodation spasms or blurry vision, frequent rest periods are necessary.

In conclusion an early diagnosis, a careful assessment, treatment of complications and a specific rehabilitation approach can improve visual functional outcome to ensure a better quality of life for children with congenital aniridia.

References

1. Edén U, Beijar C et al (2008) Aniridia among children and teenagers in Sweden and Norway. Acta Ophthalmol 86(7):730–734
2. Edén U, Riise R, Tornqvist K (2010) Corneal involvement in congenital aniridia. Cornea 29(10):1096–1102
3. Valenzuela A, Cline RA (2004) Ocular and nonocular findings in patients with aniridia. Can J Ophthalmol 39(6):632–638
4. Pinello L, Mazzarolo M (2012) Visual outcome and rehabilitation in 12 children with congenital aniridia: a clinical study. In Abstracts of the 1 st European Conference on Aniridia, Oslo, 8th–10th June 2012
5. Hyvärinen L (1995) Considerations in evaluation and treatment of the child with low vision. Am J Occup Ther 49(9):891–897
6. Battistin T, Lanners J, Vinciati M, Pinello L, Caldironi P (2005) Visual assessment in multidisabled infants. In: Elsevier (ed) International Congress Series. 1282 p. 2–5
7. Clifford – Donaldson CE, Haynes BM, Dobson V (2006) Teller acuity cards norms with and without use of a testing stage. J AAPOS 10(6):547–551
8. Rosenblum YZ, Zak PP et al (2000) Spectral filters in low-vision correction. Ophthalmic Physiol Opt 20(4):335–341

Chapter 16
Children with Aniridia and Healthcare Systems: From Needs Assessment to a Comprehensive Program of Care and Assistance

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Abstract Aniridia is a paradigm of the challenges posed to healthcare systems by childhood-onset rare diseases. The care of children with rare diseases presents peculiar aspects of complexity, due to the chronicity of these conditions and their disability spectrum, with different types of impairments and different severity levels, both within the same disease and the same patient across life. Recently, the provision of more comprehensive and effective care has been the aim of health policies specifically addressed to rare disease patients. The experience carried out in this field by the Veneto region (4.9 million inhabitants, north-east of Italy) is presented. An information system, accessed by all the different health professionals involved in patients' care, has been developed. The system, adopted so far in other seven Italian Regions, allows patients' recording, treatments' prescription and provision and the formulation of care plans, according to the individual health care needs' profile. The process of information sharing can effectively reduce the fragmentation of the care provided to these children and their families by a multiplicity of actors, medical and non-medical ones. Furthermore, it can ease the transition from paediatric to adult care, an emerging crucial issue in the care of children presenting special care needs, as children with aniridia.

Keywords Aniridia • Rare diseases • Health-care needs' definition • Individual care plan • Information system

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Rare diseases, given their considerable impact both at individual and community level, have imposed themselves as a major public health issue. Patients with rare diseases can experience challenging medical problems, as lack of available information, diagnostic delay, scarcity of therapies able to modify the natural history of the disease. Furthermore, they can experience an extra-burden, as all life dimensions of the individual are strongly influenced by the disease process. These aspects of complexity are amplified when a rare disease has a childhood onset. According to recently reported data, a non-negligible part of rare diseases patients are children; in this age group, nearly half of them are diagnosed with congenital malformations or complex genetic syndromes [1]. All these patients require lifelong and multidisciplinary care. Aniridia is a paradigm of the challenges posed by childhood-onset rare diseases. A population-based study performed in Sweden and Norway reported an aniridia prevalence of 1:47,000 in patients under the age of 20 and outlined that the condition was responsible of the 1.8 % of cases of low vision in children. Furthermore, the study documented that many eye complications can appear early in life, influencing the severity of the visual impairment [2]. Despite new insights in genetics and pathogenic mechanisms at the basis of the disease and progresses in early diagnosis and management, treatments remain partially effective in modifying the long-term visual prognosis. This problem is common in rare diseases, where we face a general paucity of etiologic treatments able to re-establish the original structures and functions. The rarity per se implies little research and limited amount of evidence-based knowledge. Even when available, delays may occur in the process of transferring new knowledge into the clinical practice. Besides some impressive successful cases, only a minority of rare diseases patients can actually benefit from therapies able to modify significantly the natural history of their disease. The importance for clinicians to focus on the global management of the disease has been advocated, especially in the case of rare diseases leading to childhood-onset disabilities [3]. This is also the case of patients diagnosed with aniridia [4]. Especially in these cases, the disease burden has been reported to overcome the individual dimension, affecting the functioning of the whole family [5]. This justifies why increasing attention is deserved to interventions able to support the global well-being of the person and the family. This process, called global "*prise en charge*", is always possible to be carried out, in every disease and during every disease phase, in a very specific way, according to who is the child and the family experiencing a disease. The final aim is to support the maximum development and the best quality of life the child can achieve, taking into account the health profile, possible limitations, but also potentials, personal attitudes and preferences, and the context in which the child lives, first of all the family. The dignity of the person, his/her role and inclusion in society, non-discrimination, and the individual's rights recognition in the educational and work context, according to age, are all principles that must orient and define each action carried out in the context of a global care plan.

The actions and the interventions which define a comprehensive *prise en charge* are based on the care needs' profile. This profile is time depending and greatly differs according to who is the child we are caring for, his/her family and the com-

munity context. The prerequisite for this process to start is the health-care needs' definition, which is the result of a complex pathway, which includes, but is not limited to, a diagnosis definition. Following the diagnosis, an assessment of the structural and functional impairment should be performed, both considering actual and developmental harms. As an example, patients with aniridia, despite having the same diagnosis, with described PAX6 mutations, can actually present very different impairment profiles. In this disease, a spectrum of ocular anomalies has been described: from isolated iris involvement to pan-ocular manifestations, including corneal opacities, cataracts, nystagmus, foveal and optic nerve hypoplasia [6]. In addition, uncommon ocular manifestations and systemic findings have been reported in patients [7]. Consequently, the impact of the disease on the visual function can vary a lot, depending not only on the anomalies' combination, but also on the time in which they occur, on the presence of possible treatments complications and on other factors, influencing, for example, the pro-fibrotic nature of the disease. Therefore, individuals sharing the same diagnosis of aniridia can actually present various degrees of vision impairment. Apart from ocular manifestations, brain-imaging studies performed in patients with *PAX6* mutations have revealed various malformations and alterations of the cortical tracts, for example of the inter-hemispheric fibers, potentially affecting the hearing function in some individuals [8]. These alterations can modify in time, defining not only an inter-individual variability, but also a dynamic profile of harms occurring in the same person. Given the complexity of the development processes and its influence on the modeling of the brain structures and their connections, even a partial differentiation due to the functional alterations caused by the primary alteration can determine a subsequent more complex developmental harm, with consequent impairment. We define all this as "developmental harm". A further element of complexity is that in infants several factors can influence the type and entity of harm. Initial similar structural alterations can have completely different outcomes, according to the presence of appropriate environmental interactions and of timely interventions, due to the complex and only partially known genes-environment interactions. Innovative imaging studies, as cortical tractographies can reveal and quantify the entity of these harms. Brain imaging studies performed in children with aniridia due to *PAX6* mutations have documented a reduction in the volumes of the corpus callosum and structural abnormalities of the hearing inter-hemispheric pathway [9]. These children have been diagnosed with auditory processing test deficits, with associated hearing difficulties, despite normal audiograms. Therefore, in children with aniridia, besides the visual function, it can be very important to evaluate other functions, as the cognitive and the hearing one. This is an example of how important is, especially in children, a comprehensive functional assessment, following the impairments' profile definition. This assessment contributes to the definition of the activities, that is to say what a specific child can do in his/her daily life, what he/she prefers to do, what he/she is expected to do. In general, activities, dealing with actions and performances that every one of us carries out in daily life, depend on multiple functional axes and are influenced by limitations potentially present in each individual, combined with his/her potential, besides presenting a certain disease. Potentials, impairments and limi-

tations ultimately constitute the combination that defines every person, and that is strongly influenced by the life context, and, above all, by the quality and complexity of the family and social relations. Only at the very end of this complex pathway, it is possible to define the health care needs' profile and, consequently, which are the treatments and the actions that have to be included in a therapeutic and care plan, tailor-made for the person. In this way, the care plan includes medical interventions, but it is also formulated taking into account the multiple dimensions of the person and the daily-life context. This approach implies that a multiplicity of actors, belonging to different backgrounds, sometimes working in physically separate settings, have to contribute harmoniously to the same project, in which the child and the family are not passive receivers of the interventions, but active contributors. The question is how can we translate this theoretical and cultural approach into the clinical practice and, concretely, into the daily lives of patients? This crucial issue depends on two conditions to be satisfied: the first one deals with the transfer from available scientific evidence to enforceable rights of persons with rare diseases. The second one regards the transition process from theoretical guidelines to actually accessible interventions and benefits for patients.

These points are critical in the health planning of interventions addressed to children experiencing complex health care needs, as children diagnosed with aniridia and, more in general, rare diseases patients. As well as other rare diseases, aniridia is challenging from the point of view of patient care, involving different health professionals, operating in highly specialized Centres of expertise, in the primary care setting, as well as in other services/institutions. The role of patients' associations has been pivotal in boosting specific public health policies addressed to rare diseases patients. Furthermore, the increasing attention posed in the care of these complex patients has been associated with the development of informative systems, which have been designed to respond to multiple needs. First, while facilitating the collection of previously scattered data, they represent a very powerful research tool. At the same time, using information as a binding agent, they foster the collaboration and the real-time interaction between all the different health professionals involved in patients' care. The Veneto Region (4.9 million inhabitants, north-east of Italy) experience, developed in the broader context of specific rare diseases health policies put in place since 2002, is presented. A web-based system, combining aspects of a population-based registry, useful for the collection of epidemiological data, as well as aspects of a clinical registry, collecting data supporting the clinical decision process, has been set up. The system can be defined as informative, since it goes beyond mere patients' registration purposes, collecting data able to orient the clinical decision process on patients, individually considered. A prerequisite is the definition, through a transparent and objective procedure, of a network of Centres, entitled to the diagnosis and care of groups of rare diseases patients, according to their health care needs. In this context, the information represents the tool, which realizes the connection between labelled Centres for rare diseases, other hospitals and other services, entitled to tackle rare diseases patients' health and social needs. The informative system developed in the Veneto Region allows the diagnosis' definition and certification, the issue of an exemption leading to benefits' entitlement and the

registration of rare diseases cases, followed by Centres of expertise active in the area. According to the system architecture, the electronic clinical record of the patient is accessed and filled in by all the different professionals, taking care of the person. The sharing of the same information tool assures a high level of standardization in the clinical practice. The informative system has been progressively extended, according to a modular approach, to other Regions (Trentino Alto-Adige, Emilia-Romagna, Liguria, Puglia, Campania, and recently to Umbria and Sardinia). System's users are 5,493 working in 1110 services, and nearly 96,400 patients with a rare disease have been registered in the whole area so far.

Beyond being essential for patients' management, the informative system is a very powerful tool for both improving knowledge and for the monitoring of the concrete implementation of the regional health policies and health services' organization put in place.

The data derived from this health information system produce a more comprehensive knowledge about the rare disease phenomenon. In fact, clinicians working in Centres of expertise usually are familiar with the collection of data on clinical manifestations and prescribed treatments, whilst they can be more unfamiliar with the collection of information related to other domains as disability, rehabilitation programmes, autonomy, level of social inclusion, learning and/or working abilities, etc. Partial knowledge about these aspects goes together with the scarce interest of research on these issues. Therefore, modern information systems should be designed not only for the collection of data on diagnosis and prescriptions, but primarily should allow the formulation of these more comprehensive health care plans.

This approach can also serve another important purpose in the care of children with special health care needs, as children diagnosed with aniridia. The drawing of health care plans, performed by professionals working in different settings, shared through the same information system, can be instrumental in assuring an efficient transition process from pediatric to adult care. This process is increasingly perceived as a crucial event in the life course of many children with chronic rare diseases and disabilities. We believe that a patient-centered information is a prerequisite for an effective transition process, as well as for the moving of the patient across different care settings and institutions during the entire disease course. Further research is needed to assess the entity of the positive returns on patients and families, in terms of improved disease management, reduced disengagement from services and better quality of the care received.

References

1. Mazzucato M, Visonà Dalla Pozza L, Manea S, Minichiello C, Facchin P (2014) A population-based registry as a source of health indicators for rare diseases: the ten-year experience of the Veneto Region's rare diseases registry. Orphanet J Rare Dis 9:37
2. Edén U, Iggman D, Riise R, Tornqvist K (2008) Epidemiology of aniridia in Sweden and Norway. Acta Ophthalmol (Copenh) 86(7):727–729

3. Zurynski Y, Reeve K, Leonard H, Elliott E (2008) Rare childhood diseases: how should we respond? Arch Dis Child 93(12):1071–1074
4. Lee H, Khan R, O'Keefe M (2008) Aniridia: current pathology and management. Acta Ophthalmol (Copenh) 86(7):708–715
5. Dellve L, Samuelsson L, Tallborn A, Fasth, Hallberg LR (2006) Stress and well-being among parents of children with rare diseases: a prospective intervention study. J Adv Nurs 53(4):392–402
6. Hingorani M, Hanson I, van Heyningen V (2012) Aniridia. Eur J Hum Genet 20(10): 1011–1017
7. Netland PA, Scott ML, Boyle JW 4th, Lauderdale JD (2011) Ocular and systemic findings in a survey of aniridia subjects. J Am Assoc Pediatr Ophthalmol Strabismus 15(6):562–566
8. Chien YH, Huang HP, Hwu WL, Chien YH, Chang TC, Lee NC (2009) Eye anomalies and neurological manifestations in patients with PAX6 mutations. Mol Vis 15:2139–2145
9. Bamiou DE, Free SL, Sisodiya SM, Chong WK, Musiek F, Williamson KA, van Heyningen V, Moore AT, Gadian D, Luxon LM (2007) Auditory interhemispheric transfer deficits, hearing difficulties, and brain magnetic resonance imaging abnormalities in children with congenital aniridia due to PAX6 mutations. Arch Pediatr Adolesc Med 161(5):463–469

Chapter 17
European/International Guidelines on Aniridia: The Patients' Point of View

Barbara Poli, Rosa Sánchez de Vega, and Corrado Teofili

Abstract Aniridia is a rare disorder and knowledge about it is insufficient and dispersed. Clinical practice guidelines are therefore a fundamental tool to ensure clinical appropriateness, equity of care and a comprehensive approach to the complexity of the disease. Guidelines development requires scientifically grounded methodologies, a multidisciplinary panel of strongly committed experts, involvement of public health authorities. As these conditions are hardly ever recurrent in the field of rare diseases, the role of patients' organizations is crucial, because they can promote and collaborate to this process as a reliable and active partner together with physicians, researchers and public health institutions. Guidelines do not only define what must or must not be recommended: they also determine what issues remain undecided or controversial, thus helping in establishing priorities for confrontation among professionals and for research projects. Aniridia Europe, the federation of aniridia associations in Europe, as a partner of the RareBestPractices project led by Eurordis, will cooperate in collecting and disseminating the already existing documents on aniridia (the Spanish Protocol and the Italian Guidelines) and will promote the development of European/international guidelines based on a shared consensus among the professionals involved and the patients' communities.

Keywords Aniridia • Clinical practice guidelines • Patients' organizations

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Aniridia is a very rare disorder and as such it requires an appropriate approach to face the diagnosis, to manage its clinical consequences and to find a conscious and intelligent adaptation to the fact that the disease will accompany the person during his/her whole life. Aniridia patients quite often experience economic and social vulnerability due to the medical challenges that they face, as well as difficulties in taking action for their own cases. As the needs of rare diseases are great, not only patients' associations but also professionals play an essential role in advocating the rights of these patients, as well as in raising awareness and spreading knowledge of the disorders.

After experiencing it personally, and meeting other parents and patients, the authors, along with a group of patients and families, decided to establish an association in their own countries and later to federate with the other similar associations in Europe in order to turn out different experiences into common knowledge which could lead to an improvement of the quality of life and care for all.

We realized that the communication of the diagnosis is a crucial step in the life of a family affected by any rare disease and that factors such as how the information is communicated, the attention to the accuracy and the comprehensibility of the information provided, the willingness not to leave parents alone with the diagnosis are understood to significantly affect the impact of the diagnosis on the family.

We saw that the knowledge about aniridia, as it usually happens with the rare disorders, was insufficient and dispersed and consequently it was difficult to find centres of expertise and to get equal and correct treatment. Moreover, we had many examples in which wrong treatments had often lead to worsening the condition.

We therefore understood that there was a need to gather all the available medical knowledge and evaluate it on scientific grounds in order to build some kind of document, hopefully guidelines, that could be considered reliable by doctors and disseminated as much as possible.

These guidelines would not only describe the "state of the art" on the disease, but would also be the first stone on which it would be possible to build new knowledge.

But developing clinical practice guidelines on a rare disease is a challenge for many reasons:

- the adopted methodology must be scientifically grounded "Trustworthy guidelines should be based on a systematic evidence review, developed by panel of multidisciplinary experts, provide a clear explanation of the logical relationships between alternative care options and health outcomes, and provide ratings of both the quality of evidence and the strength of the recommendations", NIH-NHLBI [1];
- the working group involved in its definition must be formed by a panel of multidisciplinary experts with a strong commitment to the project;
- public health institutions should be involved to ensure the document the greatest possible reliability, strength and implementation.

All these conditions are hardly ever recurrent in the field of rare diseases, and their same low incidence makes it inherently difficult to conduct epidemiological and clinical trials sufficiently powered to provide strong evidence to support clinical recommendations. Moreover, why should a physician or a health care institution

devote expertise, resources and commitment on a specific disorder that is only one among the thousands of existing rare diseases?

From this perspective it is clear why the role of patients' associations is crucial. They can advocate for appropriate care for every patient in every country, promote the dialogue with doctors and help them understand the real needs of patients, collaborate with the healthcare institutions, and support the whole development process of guidelines. In other words, patients' representatives, particularly the associations, can be a reliable and active partner in building a system in which all the component parts (healthcare institutions, physicians, researchers, patients) work together, each one playing its own role, with the aim of ensuring clinical appropriateness and equity of care.

As far as aniridia is concerned, we can relate the two existing experiences: the Spanish *Protocolo de actuación en pacientes con aniridia* AEA [4] and the Italian guidelines *Gestione dell'aniridia congenita*.

The structure of the two documents is different ISS-CNMR [5] and the history of their development shows that, starting from the same needs and having the same objectives, they followed quite different paths. The differences came mostly from the context of healthcare systems that determined the methodologies adopted.

The Spanish protocol was developed under the supervision of a group of doctors that decided the topics, invited other experts as coordinators and authors for each chapter and revised the document before publication. The Spanish association played the key role of promoting and coordinating the whole process.

Instead, the Italian guidelines were developed under the guidance of the National Centre for Rare Diseases following the methodology developed by the National System for Guidelines http://www.iss.it/cnmr/?lang=2, with the implementation of various procedural steps and the involvement of different specialties ISS – SNLG [2]: (i) creation of a multidisciplinary panel, (ii) definition of clinical questions, (iii) selection and critical evaluation of clinical studies, (iv) synthesis of evidence in narrative form, (v) formulation of recommendations, (vi) consensus achievement and (vii) revision by external referees. In this case, the national association promoted the process, was part of the multidisciplinary panel and wrote a chapter on the information and assistance procedures for patients and their families.

These two experiences allow defining some general features these kinds of documents should have in Other national associations have developed guides to support the treatment and care of aniridia, but these cannot be described as clinical practice guidelines. Nonetheless, they are important tools for both patients and professionals; see Nerby and Otis [6] and AN-OU [7] addition to the ones stated above.

A key issue is the definition of the target for recommendations: ophthalmologists and low vision experts are undoubtedly the most direct targets, but it is important to remember that a multidisciplinary approach is requested by the complexity of pathology and that the concept of care should include both the strictly clinical as well as the social aids. EURORDIS [3], pp 3–4.

Other professional figures should therefore be included as potential recipients for guidelines: geneticists, general practitioners, pediatricians, child psychiatrists who oversee the neurological development of the child, psychologists, physiotherapists,

class teachers, special needs teachers and school managers, educational assistants, health policy makers and social workers.

Moreover, guidelines should also be directed to patients and their families who must take collaborative decisions and adopt a conscious competent approach in the difficult challenge that they face.

Recommendations directed to these figures will certainly promote a comprehensive approach to the patient and will potentially result in an improvement of his/her quality of life.

Once this tool is published, it is very important to disseminate it in order to:

- promote the knowledge on the disease and hence ensure better treatment and care;
- create interest and confrontation among the professionals, which can lead to the development of new knowledge;
- make this knowledge available also to those countries where a national protocol seems more difficult to achieve.

A regular update should also be scheduled within an appropriate timeframe.

While examining all the advantages connected to the development of clinical practice guidelines on aniridia, we should also be aware that this tool does not give all the answers: as a matter of fact, it is difficult to develop very strong recommendations simply because strong evidence is rarely available for rare diseases. More often, questions remain undecided or controversial.

Far from considering it a weakness, this confers an important additional value to guidelines.

In fact, apart from determining what is already sure and must (or must not) be recommended, they help in identifying which issues remain unknown or controversial and require further investigation.

This offers guidance in orienting priorities and choices for research.

The cooperation that Aniridia Europe is establishing, with the constitution of a Scientific Committee and the creation of a network of European physicians and researchers that includes also their colleagues from USA and Canada, is the prerequisite for any research project on aniridia at a wider than national level.

In this context, the development of European or international clinical practice guidelines that clearly result from the critical evaluation of the existing bibliography and from a shared consensus among a multidisciplinary panel of experts would be a very useful tool in prioritizing resources and efforts for research.

Conclusions

In conclusion, guidelines must always be seen as a multipurpose work in progress.

On a first level, they provide an immediately available tool to improve knowledge of the disease and to address the choices on specific diagnostic procedures, alternative therapeutic strategies or social and health care interventions.

On a second level, by identifying areas of uncertainty, they promote confrontation among professionals and suggest a careful approach on controversial issues.

On a third level, they help in determine which topics require further investigation, thus orienting research projects and resources allocation.

Aniridia Europe, as a partner in the RARE-Best-practices project (one of whose aims is the collection of rare diseases guidelines in a European database), led by Eurordis, will promote the dissemination of all the existing protocols and guidelines and the development of European/international guidelines as a new tool, based on a shared consensus and on a regular update, that we believe would provide great benefits for all the professionals involved and for the patients' communities.

References

1. NIH-NHLBI (2013) About systematic evidence reviews and clinical practice guidelines. [Online] Available from: http://www.nhlbi.nih.gov/health-pro/guidelines/about.htm. Accessed 24 Oct 2014
2. ISS – SNLG (2002) Methodological handbook – how to produce, disseminate and update clinical practice recommendations. [Online] Available from: http://www.snlg-iss.it/en_SNLG_methodological_handbook. Accessed 24 Oct 2014
3. EURORDIS (2008) Position paper on Centres of expertise and European reference networks for rare diseases. [Online] Available from: http://www.eurordis.org/sites/default/files/publications/position-paper-EURORDIS-centres-excellence-networksFeb08.pdf. Accessed 24 Oct 2014
4. AEA (2008) Protocolo de actuación en pacientes con aniridia. Asociación Española de Aniridia, Madrid. English translation available from: http://www.aniridia.eu/. Accessed 24 Oct 2014
5. ISS – CNMR (2013) Gestione dell'aniridia congenita. Istituto Superiore di Sanità, Roma. Available from: http://www.snlg-iss.it/lgmr_aniridia_congenita. Accessed 24 Oct 2014. English translation available from: http://www.aniridia.eu/. Accessed 24 Oct 2014
6. Nerby JA, Otis JJ (eds) (2010) Aniridia and WAGR syndrome. A guide for patients and families. Oxford University Press, Oxford
7. AN-OU (2010) Aniridi Norge and Oslo Universitetssykehus (eds), Om å leve med aniridi [Living with aniridia]. [Online] Available from: http://www.aniridi.no/handbok/. Accessed 24 Oct 2014

Chapter 18
What to Do When Diagnosed with Aniridia: The Role of Patients' Associations – Bringing Together Support, Education, and Research to Find the Aniridia Solution

Jill A. Nerby

Abstract Aniridia has been known for decades as a genetic eye disorder most commonly caused by a mutation in the PAX6 gene. However the "true scope" of this disorder was not fully understood until recently. Today the disorder is known as "aniridia syndrome" since research has shown that the PAX6 gene is responsible for more than just development of the eyes. It has been found that the role of PAX6 can have systemic effects as well; although more research is necessary in the areas of the brain, pancreas, olfactory and central nervous system. The Aniridia Foundation International (AFI), a 501(c)3 nonprofit, collects data to assist in research, provides educational conferences and support to those with aniridia syndrome. AFI is headquartered in the Department of Ophthalmology at the University of Virginia (UVa). Several of the AFI programs will be incorporated into the UVa Ophthalmology's new Congenital Eye Disorder program. Progress has been made in the understanding of aniridia syndrome through this "team effort model" involving physicians, basic science researchers and those affected with aniridia syndrome. The benefit of these collaborations are that those with genetic syndromes like aniridia or congenital eye disorders can receive specialized ophthalmic and medical care, education, patient support and assist with research advancement through studies and clinical trials all in one place. The information gained from this unique collaboration, and the programs discussed here will benefit those with congenital aniridia syndrome today and in future generations.

Keywords Aniridia • Genetic syndrome • Fibrotic scarring or AFS • Glaucoma • Corneal pannus

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Clinical care by physicians with extensive experience in ***successfully*** treating those with aniridia syndrome and overall research advancement are important things to those born with aniridia syndrome, their parents, and families. However, many people not affected by vision loss themselves may not realize that just as important as the medical care and research is the quality of support received by these individuals and their families from organizations such as Aniridia Foundation International (AFI).

"Aniridia" was named many years ago likely due to the most phenotypic feature that physicians observed. In Latin, "aniridia" means "lack of iris" and those with aniridia had dark eyes with no iris color noticeable. Interestingly, with what we know today about aniridia, it may have been called something else if the true scope of this genetic disorder had been realized. In fact, some people today do not understand why it was named after the LEAST important aspect of the condition. The iris is uninvolved with the degenerative loss of vision, the most prevalent problem. Also, in rare cases, some people with aniridia actually have some visible iris, although lacking the ability to dilate. This is frustrating to those affected with visual disability, as the public sees the definition "a lack of iris" as more cosmetic in nature. Through the efforts of organizations such as AFI, public awareness has been raised, and greater understanding of the seriousness and true scope of aniridia has been achieved. However, it is vital for those with aniridia to form connections with others who have a personal experience and sensitivity of the implications of the condition.

With the internet, people can share internationally; however, having local support groups are important for two main reasons. First, government laws and health care systems are sometimes very different in many countries. It would be difficult and time consuming for an international support group to know or advocate for all the laws or create protocols among all countries in the world. This is where the local groups can advocate for their community better on a local level.

Secondly, personal interaction in a local area opposed to sharing via the internet is sometimes preferred. Personal interaction can have a greater impact than virtual communication. Thus, it is beneficial that organized aniridia support groups have grown throughout Europe and in the United States of America. The majority of these groups are run by people who actually have aniridia syndrome themselves or are a parent of an afflicted child. Organization leaders, who have the technical, medical and scientific knowledge about this syndrome, are essential. By having experienced life with aniridia themselves, they add a valuable special dimension to the support aspect that counselors without aniridia may not have.

Aniridia Foundation International: Bringing a Team Effort

Aniridia Foundation International (AFI) began in 2001 as the group USA Aniridia Network and was renamed the Aniridia Foundation International in 2006. This is a non-profit organization with a membership of those who were born with aniridia,

their parents, their families, interested physicians, researchers, and low vision professionals. The initial goal was to unite those with aniridia and their families to provide peer support and education, as is the goal of many support groups. However, we had plans to take our mission deeper than just education and providing support in the future. For this reason, our nonprofit organization started out with a selectively chosen Medical and Scientific Board of physicians and researchers. In addition, AFI started their network with 17 families who were seeking information, answers to their questions, and support. They also wanted to encourage the medical community to find better treatments for the conditions found in aniridia, which are responsible for loss of vision: difficult to control glaucoma, corneal pannus (scarring), childhood cataract and retinal detachment. They wanted to inspire researchers to help them find answers.

The growth of the program to over 400 registered families demonstrates the need the organization is fulfilling. These families are made up of sporadic cases, families with multiple people affected in the immediate family, and some families recording the inheritance through many generations. Many parents who remember the days of rearing their child without this valuable help can truly see the all the advances and accomplishments made over the last decade. Many of them had wished for this form of support and education when their children were younger; therefore, some have become active AFI volunteers in helping new parents walk through the "aniridia journey" with less fear and anxiety.

In addition to providing patient support, educational programs for physicians are a high priority for our Board of Directors. AFI attends meetings such as the American Academy of Ophthalmology (AAO) and Association of Research in Vision and Ophthalmology (ARVO) to share the information with the medical and scientific communities, as well as inform physicians treating patients with aniridia (Fig. 18.1).

Additionally, a recently published book, *Aniridia and WAGR syndrome*: *A guide for Patients and Their Families* [1] assists many, especially new parents who did not know what to expect for their child's future. Chapters written by the AFI Medical and Scientific board advisors explain the medical conditions and personal experiences are described by those who have "lived" with aniridia.

Lastly, our most successful educational program has been our unique conferences which bring together our AFI Medical and Scientific advisors, other physicians, researchers, low vision professionals, with patients with aniridia syndrome and their families (Fig. 18.2).

AFI leaders felt that if we could educate those affected with aniridia and their families then they would be able to understand better the disorder of aniridia and would be able to make better educated decisions about their personal eye care according to the latest research and clinical findings.

For years, only a small percentage of ophthalmologists knew the latest information about the special care required for those with aniridia. For example, patients with aniridia are now screened early for glaucoma, to recognize and treat vision threatening elevations in pressure. More recently, in the last decade and a half, it has been discovered that those with aniridia heal differently due to limbal stem cell deficiency (LSCD) when they experience eye surgery or corneal abrasions.

Fig. 18.1 Attending and exhibiting at conferences, volunteers gave their time to share the latest information with the medical and scientific communities

Educating both physicians and patients to the latest information regarding the unique needs of the aniridic patient has improved both diagnosis and treatment, with better visual results for the patient.

The first AFI educational and support conference was held in 2002. Today, these conferences are held biennially and are known as the AFI "Make a Miracle" conferences derived from the AFI slogan "Take our Hands, Walk with Us, Share our Dreams and Help us Make a Miracle!" (Fig. 18.3).

These unique AFI conferences are strategically designed with the idea of working the problem from all sides: medically (physicians), scientifically (researchers), and personally (families with aniridia). Typically, physicians attend medical meetings and researchers attend scientific meetings to share among their own communities. AFI felt if the medical and scientific community could work as a team and share at conferences we could make more progress. From the beginning, the AFI conferences brought together both clinical and basic science researchers on aniridia to share and work together to address aniridia issues. We felt this was important in promoting translational research. AFI also included the individuals with aniridia and their families during certain presentations as part of the "team" for discussion

Fig. 18.2 Peter Netland, M.D., PhD. presents the latest glaucoma information and treatments for those with aniridia to physicians, researchers and those affected by aniridia at the Aniridia Foundation International conference

Fig. 18.3 A few of the Make a Miracle attendees with aniridia syndrome

and education. The aniridia community learned about their disorder from the experts and the experts often learned from the aniridia families. This often resulted in new avenues for basic research or improved approaches to clinical care.

As stated earlier, one of our goals was to be more than a support group. Therefore, in 2006 with the help of our medical and scientific advisors, AFI created a research program called The Medical Registry. This registry collects human data from those with aniridia to help advance research. Data collection ranges from the demographic to the clinical, i.e., questionnaires to blood draws for genetic analysis. The comparison of the characteristics of aniridic patients with the unaffected population has led to several published papers from this data.

The biggest change as far as understanding this congenital eye disorder is the re-definition of "aniridia" as "aniridia syndrome". A syndrome is a group of signs and symptoms that together are characteristic of a particular disease or disorder. Relabeling the condition as a syndrome is supported by research data showing that the PAX6 mutation is more involved than previously thought. In addition to causing underdevelopment problems in the eye structures, it is also responsible for the development and maintenance of the pancreas, parts of the brain, and the central nervous system. Current ongoing research is showing significant data that systemic conditions may be a result of the PAX6 mutation as well.

Returning to ophthalmic manifestations of aniridia, Aniridia Fibrosis Syndrome (AFS) was initially described by investigators who were also AFI Medical Board advisors [2]. This cement-like scarring *inside* the eye, opposed to the known aniridic keratopathy (outside scarring of the cornea) can obstruct glaucoma drainage implant tubes, destroy the ciliary body causing lowered or non-existent aqueous production (hypotony) and even, in later stages, cause retinal detachment (Fig. 18.4).

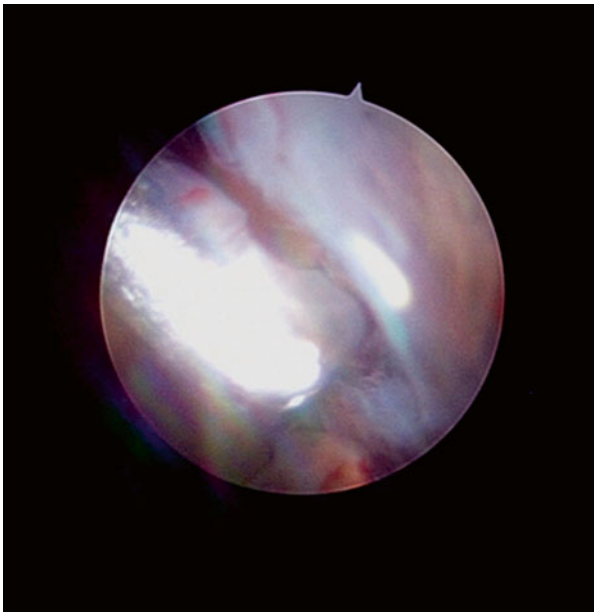

Fig. 18.4 Advanced Aniridia Fibrosis Syndrome (AFS) (Photo courtesy of Christopher Riemann, M.D., Cincinnati Eye Institute)

It also has been known to displace intraocular lenses or iris implants causing damage from corneal touch. AFS is an area of important investigation, as its etiology, mechanism and natural history is unknown, despite its potentially devastating implications for visual and globe preservation. Publications on non-ophthalmic manifestations of the syndrome have resulted from the data collected by the AFI Medical Registry notably the finding that metabolic issues are very likely a part of this syndrome [3]. And that the glucose intolerance and diabetes incidence are related to aniridia's PAX6 genetic mutation [4]. The AFI Medical Registry will also be adding new data from all those with aniridia who wish to help advance research through participation in this program.

Collaborating with the Medical and Scientific University Systems

The year 2006 was an exciting year for us. USA Aniridia Network became Aniridia Foundation International, we created the AFI Medical Registry research program, and the Hamilton Eye Institute at the University of Tennessee invited us to move our offices there. By this time, individuals and families affected by aniridia had already attended four AFI conferences where they received education by the experts, support from their peers, and we were ready to expand the research aspect of our mission.

Then in 2011, we were invited to relocate our offices to the Department of Ophthalmology at the University of Virginia. Here AFI would collaborate with clinicians and basic researchers to create a unique Congenital Eye Disorder program in which those with aniridia receive invaluable education, ongoing support, clinical care by ophthalmologists experienced in the care of aniridia patients, medical care for systemic issues, and can participate in various research and data collection opportunities. For example, the Department of Ophthalmology has created a gene and tissue bank in which many samples are from those with aniridia. Future clinical trials are in development for those with a special kind of mutation causing aniridia. Currently, in basic research laboratories, research is being done on metabolism issues related to aniridia.

Research on eye development has been going on for many years, but has been hampered by lack of an animal model for aniridia. However with the development of a frog with aniridia [5], studies can now advance. It is easier to study eye development in the frog's translucent eggs and because the eyes of a frog are fully formed after just 2 days. The Congenital Eye Disorder program will be a "one stop shop" for those seeking experienced care in aniridia syndrome, opportunities for research advancement, and will continue to grow with other congenital disorders.

From a home office to a top university, Aniridia Foundation International has grown and helped many people with aniridia syndrome over the years since its inception in 2001. AFI's collaboration with the medical and scientific communities,

and other aniridia organizations, such as Aniridia Europe, has expanded the number of patients and families affected by this disorder. The combined approach of scientific education and patient support is vitally important both in motivating people to want to be a part of the solution and emotionally supporting those who have often felt "different" for all their lives. Continuing to use this "aniridia team" interactive model will benefit those with aniridia today with the latest medical care, benefit those affected tomorrow with the advancement of research, and make a difference in many lives and future generations (Fig. 18.5). If you are not involved with AFI, on behalf of those affected by aniridia, we ask you to "Take our Hands, Walk with Us, Share our Dreams and Help us Make a Miracle!"

Fig. 18.5 Child with aniridia wearing AFI slogan t-shirt

References

1. Nerby J, Otis J (2010) Aniridia and WAGR syndrome: a guide for patients and their families. Oxford University Press, University of Oxford, UK
2. Tsai JH, Freeman JM, Chan CC, Schwartz GS, Derby EA, Petersen MR, Holland EJ (2005) A progressive anterior fibrosis syndrome in patients with postsurgical congenital aniridia. Am J Ophthalmol 140(6):1075–1079
3. Netland PA, Scott ML, Boyle JW 4th, Lauderdale JD (2011) Ocular and systemic findings in a survey of aniridia subjects. J AAPOS 15(6):562–566
4. Yasuda T, Kajimoto Y, Fujitani Y et al (2002) PAX6 mutation as a genetic factor common to aniridia and glucose intolerance. Diabetes 51(1):224–230
5. Nakayama T, Fisher M, Nakajima K et al (2014) Xenopus pax6 mutants affect eye development and other organ systems, and have phenotypic similarities to human aniridia patients. Elsevier Dev Biol (submitted for publication)

Chapter 19
Future Avenues of Research in Aniridia

Tor Paaske Utheim

Abstract Aniridia is a rare, sight-threatening disorder that affects the iris, retina, optic nerve, lens, and cornea. Aniridia most often occurs as an isolated ocular abnormality without obvious systemic involvement, but may also be part of certain syndromes. Absence or hypoplasia of the iris and foveal hypoplasia are characteristic features that present from birth in patients with aniridia, usually resulting in photophobia, reduced visual acuity (normally 20/100–20/200) and nystagmus. Cataract, glaucoma, and aniridickeratopathy are frequently associated progressive ocular disorders with typically later onset. The prevalence of aniridia is about 1:80,000 with no known race or gender effect [1]. Approximately two-thirds of cases are inherited in an autosomal dominant fashion and one-third are sporadic. Aniridia is associated with PAX6 gene mutations. There is considerable phenotypic heterogeneity, but usually little difference between the two eyes.

Keywords Aniridia • Genotype-phenotype correlations • Future perspectives

Aniridia in Brief

Aniridia is a rare, sight-threatening disorder that affects the iris, retina, optic nerve, lens, and cornea. Aniridia most often occurs as an isolated ocular abnormality without obvious systemic involvement, but may also be part of certain syndromes. Absence or hypoplasia of the iris and foveal hypoplasia are characteristic features that present from birth in patients with aniridia, usually resulting in photophobia, reduced visual acuity (normally 20/100–20/200) and nystagmus. Cataract, glaucoma, and aniridickeratopathy are frequently associated progressive ocular

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disorders with typically later onset. The prevalence of aniridia is about 1:80,000 with no known race or gender effect [1]. Approximately two-thirds of cases are inherited in an autosomal dominant fashion and one-third are sporadic. Aniridia is associated with PAX6 gene mutations. There is considerable phenotypic heterogeneity, but usually little difference between the two eyes.

Summary of Main Clinical Features in Aniridia

Iris

Iris hypoplasia is the most commonly seen ocular abnormality. Normally, only residual iris tissue is left in patients with aniridia. However, PAX6 mutation with no clinical evidence of defects in irides has been described [2].

Cornea

Aniridickeratopathy is thought to be caused by limbal stem cell deficiency secondary to PAX6 gene mutation [3]. It is a common feature, but often presents relatively late in the disease [4]. Corneal changes vary from mild peripheral vascularisation to pancorneal vascularisation, ulceration, keratinization, and opacification. Aniridickeratopathy can be painful and ultimately result in blindness [4]. Aniridickeratopathy may be triggered by surgical intervention for glaucoma or cataract [4].

Lens

The prevalence of cataract in aniridia varies from 50 % to over 90 % [1, 5]. Significant lens opacities typically develop from the teens. Lens subluxation or dislocation may also occur occasionally.

Glaucoma

Glaucoma develops in about half of patients with aniridia with mean onset in the teens [6]. Initially, patients are usually treated with intraocular pressure-lowering medications, but most eventually require surgery to control intraocular pressure.

Retina

Foveal hypoplasia is seen in about four out of five patients, whereas optic nerve hypoplasia is less common (about one in five cases) [7].

Dry Eyes

Dry eye disease is frequently observed in aniridia [8]. It is often caused by Meibomian gland dysfunction with stenosed Meibomian orifices [9]. Dry eye disease exacerbates the ocular surface problems associated with aniridic keratopathy [4].

Vision

Visual acuity is often between 20/100 and 20/200 [4], and nystagmus has been described in 95 % of the cases [7].

Systemic Features

Aniridia may be part of syndromes such as WAGR (Wilmstumour-Aniridia-Genital anomalies-Retardation) or Gillespie syndrome (cerebellar ataxia, partial aniridia, and developmental delay). In sporadic aniridia there is an increased risk of involvement of both the PAX6 and the WT1 gene, which strongly predisposes patients to developing Wilmstumour, a paediatric nephroblastoma. There is increasing awareness that even 'isolated' aniridia may have characteristic systemic features, such as reduced olfaction and auditory deficits [10, 11].

Future Avenues for Research: Examples

Genotype: Phenotype Correlations

Greater awareness of the huge variations in severity of aniridia is important to avoid misdiagnosed or undiagnosed mild presentations. The range of phenotypes described, with which PAX6 mutation is associated, is steadily increasing. The total number of unique DNA variants reported in the PAX6 locus-specific database is also increasing (359 at present). Studies correlating genotype with phenotype will

facilitate diagnostics and make it easier to estimate a prognosis. The use of optical coherence tomography (OCT) for phenotypic characterization should be encouraged for three reasons: (1) its non-invasive nature; (2) few studies are hitherto performed; and (3) it gives valuable and quantifiable morphological information of the eye.

With recent advances involving nonsense mutation suppression drugs for certain types of aniridia [12], precise knowledge about the individual's genotype may become therapeutically more important than ever. As pharmaceutical therapy is associated with adverse side effects, knowledge about the association between the genotype and phenotype will help guide the clinician as to when a possible intervention is advisable. However, the highest value in genetic testing is to determine whether there is an underlying WAGR deletion (i.e. deletion of PAX6 and WT1) due to the increased risk of cancer.

Aniridic Fibrosis Syndrome

Intraocular fibrosis has been noted after ocular surgery in 8 % of patients [13]. A characteristic feature of aniridic fibrosis syndrome is the development of fibrosis in the absence of clinically observable inflammation [13]. Activation of immature vessels in the rudimentary iris in patients with aniridia, as a consequence of the surgery, has been put forward as a potential mechanism. However, the syndrome is poorly understood. More research is needed to explore the underlying mechanisms, thereby paving the way for improvements in the clinical outcome of surgery for cataract, glaucoma, and aniridickeratopathy.

AniridicKeratopathy

There is undoubtedly room for improvement in the treatment of progressive ocular disorders such as glaucoma and cataract in patients with aniridia. For severe aniridickeratopathy caused by limbal stem cell deficiency, the potential for improvement may be significant. It is unclear whether limbal stem cell deficiency is primarily caused by reduced number of stem cells or unfavorable alterations in their microenvironment. Basic research on the interaction between limbal stem cells and their niche structures may provide valuable insight into the best therapeutic approach. Limbal stem cell deficiency may be treated by transplantation of either non-cultured [14] or ex vivo cultured tissue [15].

The use of cultured cells, rather than non-cultured cells, for treating limbal stem cell deficiency has some advantages [16]. If the cells are cultured, only a small biopsy is needed for producing a sufficiently large transplant [17], thus minimizing the risk of inducing stem cell failure in the donor eye [18]. The high rate of rejection

following transplantation of non-cultured foreign tissue (i.e. limbal allograft) is associated with the presence of antigen-presenting cells, vessels, and lymphatics in the limbal region. If cultured cells are used, no or very few antigen-presenting cells are contained in the transplants, which reduces the risk of provoking an immune response [16, 19].

As limbal stem cell deficiency in aniridia is almost invariably bilateral, there is a need for a non-limbal cell source for ex vivo culture and transplantation. This avoids the need for immunosuppression and its many known adverse effects. Recently, several non-limbal autologous cell sources have shown promising results in treatment of limbal stem cell deficiency. These cells are derived from various locations, including oral mucosa, conjunctiva, epidermis, dental pulp, and hair follicles [16]. This field remains largely unexplored for patients with aniridia, apart from the transplantation of cultured oral mucosal cell sheets in four patients with aniridia [20, 21].

The costs related to the establishment and maintenance of a stem cell laboratory is a disadvantage of ex vivo based cell therapy. However, such facilities open up the possibility for genetic manipulation of cultured cells before they are transplanted to the patients. This is particularly relevant for patients with aniridia where PAX6 mutations are the cause of their limbal stem cell deficiency.

Recently, several non-cell based approaches to treat mild or moderate forms of limbal stem cell deficiency that do not require surgery have emerged. These include electro-stimulation, oxygen therapy, amniotic membrane extract, and limbal fibroblast conditioned medium [16]. These alternative methods of treating limbal stem cell deficiency have not yet been evaluated in clinical trials in aniridia. Such approaches may prove particularly useful for patients with aniridia as surgical intervention is associated with more complications in these patients compared to the general population.

Dry Eye Disease

There are very few studies on dry eye disease in patients with aniridia despite its high prevalence [8]. It is generally believed that increased lubrication has a beneficial effect on the development of aniridickeratopathy. Therefore, research on dry eye disease may serve a dual purpose: lessening dry eye disease symptoms and reducing the severity of aniridickeratopathy. Autologous serum may be effective in the treatment of some types of dry eye disease, but it does not address. Meibomian gland dysfunction, which is the most common form of dry eye disease. Meibomian gland dysfunction, if left untreated, may result of atrophy of the glands. Research on the many possible new strategies to treat Meibomian gland dysfunction, such as the use of Blephasteam (LaboratoiresThéa) and LipiFlow (TearScience), should be prioritized in patients with aniridia.

A Mutation-Independent Nonsense Mutation Suppression Strategy

Some mutations of PAX6 belong to the larger category entitled nonsense mutations, for which a novel therapeutic approach has recently been tested in a mouse model of aniridia [12]. The most successful results in this model were achieved through topical application of the drug formulation START (0.9 % sodium chloride, 1 % Tween 80, 1 % powdered ataluren, 1 % carboxymethylcellulose). Topical application has the benefit of reducing the risk of systemic adverse effects. Gregory-Evans and co-workers demonstrated that nonsense mutation suppression inhibited disease progression and, more remarkably, reversed retinal, lens, and corneal malformations [12]. It also restored electrical and behavioral responses of the retina. These findings suggest that START was able to suppress the nonsense mutation in order for full-length PAX6 protein to be synthesized [12]. More research, including additional animal studies, is warranted to fully explore the mechanism of action of this drug, in which ataluren is known to have nonsense suppression effect. Such efforts may lead to initiation of clinical trials applying nonsense mutation suppression strategies, which, if successful, may represent a paradigm shift in the therapy of aniridia.

Conclusion

Gene therapy and pharmaceutical therapy, such as nonsense suppressiondrugs, open up new exciting possibilities for research in aniridia. These advances, coupled with the emergence of strong international networks of clinicians, scientists, and patients, may pave the way for large, well-coordinated studies to significantly advance our knowledge and treatment of aniridia in the future.

Conflict of Interest Tor Paaske Utheim is co-founder of the Norwegian Dry Eye Clinic, Oslo, Norway. The clinic is sponsored by Abbott Medical Optics, Abigo, Alcon, Allergan, BolPharma, Santen, TearScience, and Thea Laboratories. Utheim also holds patent applications on storage of cultured epithelial cells for treating limbal stem cell deficiency and age-related macular degeneration.

References

1. Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L (1984) Aniridia. A review. Surv Ophthalmol 28:621–642
2. Thomas S, Thomas MG, Andrews C et al (2014) Autosomal-dominant nystagmus, foveal hypoplasia and presenile cataract associated with a novel PAX6 mutation. Eur J Hum Genet EJHG 22:344–349
3. Nishida K, Kinoshita S, Ohashi Y, Kuwayama Y, Yamamoto S (1995) Ocular surface abnormalities in aniridia. Am J Ophthalmol 120:368–375

4. Hingorani M, Hanson I, van Heyningen V (2012) Aniridia. Eur J Hum Genet EJHG 20:1011–1017
5. Eden U, Lagali N, Dellby A et al (2014) Cataract development in Norwegian patients with congenital aniridia. Acta Ophthalmol 92:e165–e167
6. Netland PA, Scott ML, Boyle JW, Laudedale JD (2011) Ocular and systemic findings in a survey of aniridia subjects. J AAPOS Off Publ Am Assoc Pediatr Ophthalmol Strabismus Am Assoc Pediatr Ophthalmol Strabismus 15:562–566
7. Hingorani M, Williamson KA, Moore AT, van Heyningen V (2009) Detailed ophthalmologic evaluation of 43 individuals with PAX6 mutations. Invest Ophthalmol Vis Sci 50:2581–2590
8. Rivas L, Murube J, Rivas A, Murube E (2003) Impression cytology study of dry eyes in patients with congenital aniridia. Arch Soc Esp Oftalmol 78:615–622
9. Jastaneiah S, Al-Rajhi AA (2005) Association of aniridia and dry eyes. Ophthalmology 112:1535–1540
10. Sisodiya SM, Free SL, Williamson KA et al (2001) PAX6 haploinsufficiency causes cerebral malformation and olfactory dysfunction in humans. Nat Genet 28:214–216
11. Bamiou DE, Free SL, Sisodiya SM et al (2007) Auditory interhemispheric transfer deficits, hearing difficulties, and brain magnetic resonance imaging abnormalities in children with congenital aniridia due to PAX6 mutations. Arch Pediatr Adolesc Med 161:463–469
12. Gregory-Evans CY, Wang X, Wasan KM, Zhao J, Metcalfe AL, Gregory-Evans K (2014) Postnatal manipulation of Pax6 dosage reverses congenital tissue malformation defects. J Clin Invest 124:111–116
13. Tsai JH, Freeman JM, Chan CC et al (2005) A progressive anterior fibrosis syndrome in patients with postsurgical congenital aniridia. Am J Ophthalmol 140:1075–1079
14. Holland EJ, Djalilian AR, Schwartz GS (2003) Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. Ophthalmology 110:125–130
15. Shortt AJ, Bunce C, Levis HJ et al (2014) Three-year outcomes of cultured limbal epithelial allografts in aniridia and Stevens-Johnson syndrome evaluated using the Clinical Outcome Assessment in Surgical Trials assessment tool. Stem Cells Trans Med 3:265–275
16. Utheim TP (2013) Limbal epithelial cell therapy: past, present, and future. Methods Mol Biology (Clifton NJ) 1014:3–43
17. Utheim TP, Lyberg T, Raeder S (2013) The culture of limbal epithelial cells. Methods Mol Biology (Clifton NJ) 1014:103–129
18. Jenkins C, Tuft S, Liu C, Buckley R (1933) Limbal transplantation in the management of chronic contact-lens-associated epitheliopathy. Eye (Lond) 7(Pt 5):629–633
19. Espana EM, Di Pascuale M, Grueterich M, Solomon A, Tseng SC (2004) Keratolimbal allograft in corneal reconstruction. Eye (Lond) 18:406–417
20. Burillon C, Huot L, Justin V et al (2012) Cultured autologous oral mucosal epithelial cell sheet (CAOMECS) transplantation for the treatment of corneal limbal epithelial stem cell deficiency. Invest Ophthalmol Vis Sci 53:1325–1331
21. Sotozono C, Inatomi T, Nakamura T et al (2013) Visual improvement after cultivated oral mucosal epithelial transplantation. Ophthalmology 120:193–200

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