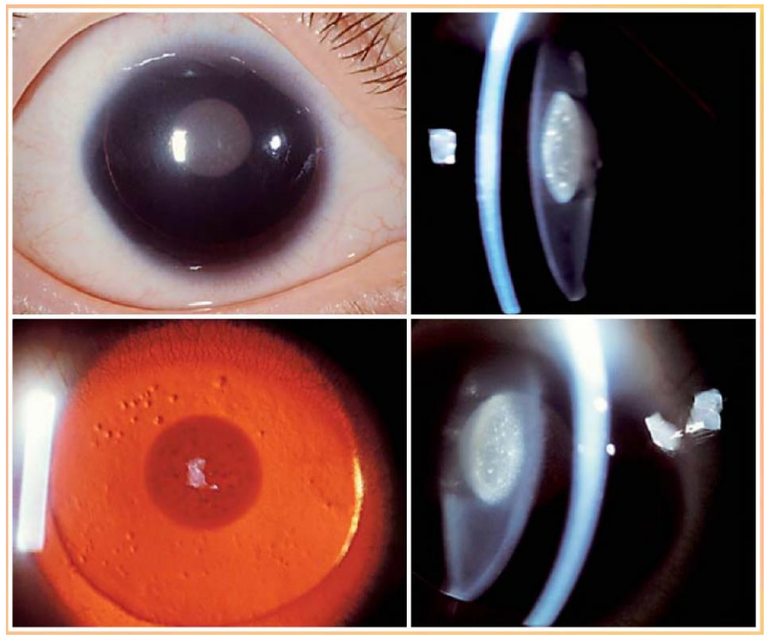

SPANISH GUIDELINES FOR THE MANAGEMENT OF CONGENITAL ANIRIDIA

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PROLOGUE

I appreciate the invitation of the Spanish Association of Aniridia to write some words as an introduction to the "Spanish Guidelines for the Management of Congeniltal Aniridia", as fundraiser and coordinator. A publication where some of the most relevant Spanish ophthalmologists participate and that means a milestone in aniridia healthcare.

I appreciation is doublé: On one hand, as the current Minister of Health, responsible to guarantee the right to healthcare for all citizens, it gives me the opportunity to show our activities in the field and also because it allows me to renew my personal committment with a group of diseases – "rare diseases", the society is more and more aware of, thanks to the effort made by platients´ organizations.

Aniridia, that according to estimations affects 1 person per 90.000 born children, fully fits in this group of low prevalence disorders, but together affect a wide number of people, since rare diseases could be around 6000. In fact, the figure of people with rare diseases at European leve lis estimated around 30 million people, this explains to be a priority in the Public Health Frame Programme 2003-2008 of the European Union and in Health Policies in Spain.

This is why, we have pushed forrward initiatives to improve the knowledge, care and treatment of patients with these diseases. Then, one of the first Biomedical Research Network Centre, we have developed, is focused on them. 535 researchers work in this consortium in 61 research groups, this means a great effort that we are sure will contribute drastically to improve the epidemiological knowledge, translational research and therapeutic strategies.

This is a very important aspect, since because rare disorders face difficulties specially to develop studies, mainly clinical trials, and innovative pharmaceutical products research is not financially attractive enough. This is why, the Ministry of Health is funding independent research, which means to be among the first countries in Europe regarding public funding of orphan drugs research.

But the relevance of the problems requires to go further. A Nationa agreement is needed to coordinate initiatives developed by each of the different Administrations in the field of their competences.

Then, based in the scientific evidence analysis, in collaboration with Patients Organisations and Scientific Societies, and with the autonomous Communities consent, a National Strategy for Rare Diseases is being developed, we are sure that will very much contribute to give a right answer to the challenge meant by such rare diseases as Aniridia.

To reach this goal, a common effort of the society is required. This is why I would like to appreciate and support such a needed initiative as this Guidelines. There, the knowledge is updated, experiences are shared and all relevant questions are analysed, from the basic aspects of the disease to the medical and surgical therapeutic strategies. I congratulate, then, both the authors and coordinators of this publication and the Spanish Association of Aniridia that make it possible and I am pleased to have such a useful tool for a shared goal: to improve citizens´healtcare.

Bernat Soria

Minister of Health.

SECTION I

CHAPTER 1
INTRODUCTION TO ANIRIDIA

Juan Murube del Castillo, MD, PhD.

Congenital aniridia is usually an autosomic dominant hereditary disease affecting one of about 60,000 to 100,000 newborns. It frequently evolves along their life to visual impairment. Nevertheless it was not identified until very recently by Barratta in 1818 (**Ref 1**). Its late description is surprising, mainly because this disease usually affects several members of a family.

1.1. Origin of the name

Iris (Ιρις, -ιδος) was the name given in ancient Greek mythology to the messenger of the goddess Hera (under whose throne she lived and slept, always shoed, ready to journey at any moment). She may also have been messenger of Zeus. (**Ref 2**).

The word Iris was also used in classical ancient Greece to name the rainbow –which was believed to be the trail of the messenger Iris-, the colored or luminous circle, the iris of the eye, and the botanic lily. It was regarded as of femenine gender.

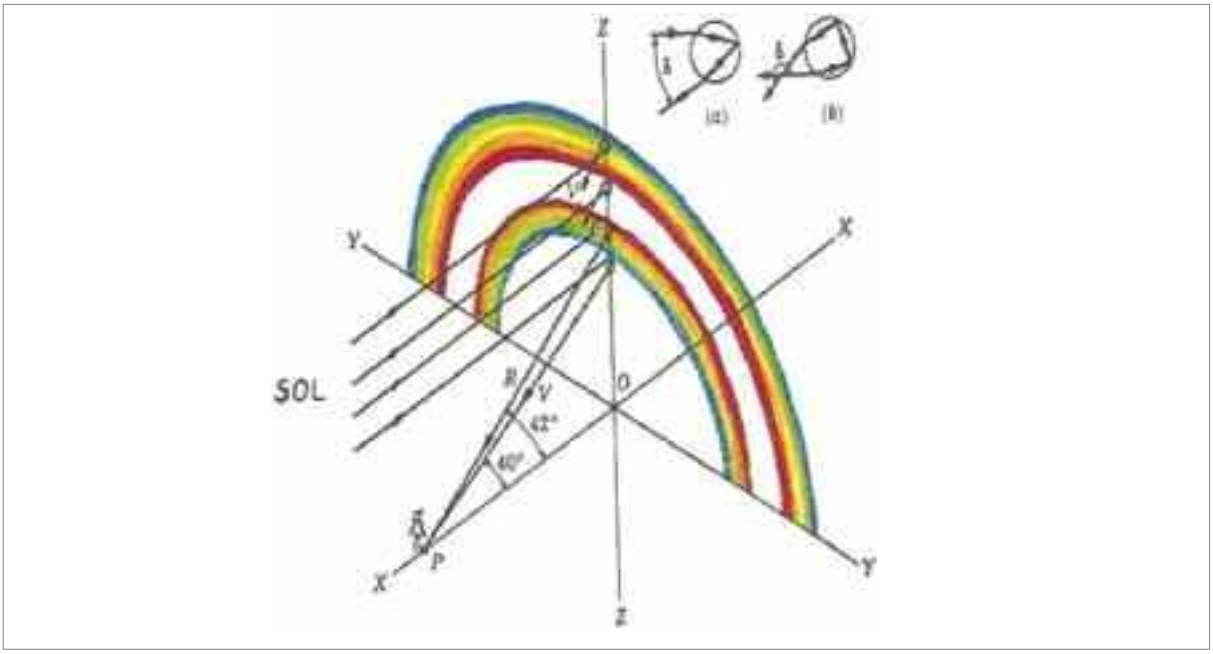

Figure 1.1. Rainbow. The rainbow is seen in front of the spectators when water drops in the atmosphere are in front of them, and when the sun illuminates from behind. This is due to the refraction of the sun light in the anterior surface of the atmospheric water drops, and the reflection of the light in the posterior surface of the drops.

Ancient classic Rome incorporated a large part of the Greek mythology. Iris continued being the messenger of the goddess Juno. Pliny the Elder (**Ref 3**) maintained the name "iris" for the atmospheric rainbow, the colored disc of the anterior sector of the eyeball, and the gladiolus, using the femenine gender; and for a river of the Asiatic South-West that flows into the Black Sea, using the masculine gender.

In the modern Romanic Indo-European languages, the term iris used for the meaning of rainbow was complemented or substituted with the anteposition of the substantive arcus. So, in French this evolved to "arc-en-ciel" (arc in the firmament), in Italian to "arcobaléno" (baléno = lightning), and in Spanish to "arco iris". In Teutonic Indo-European languages, as German, rainbow is "Regenbogen" (arc of the rain). In English, which is mainly a mixture of Germanic and Latin roots, the Teutonic term "rainbow" has been maintained. In Greek and Latin languages the gender of the term was femenine, maybe because of its relation with the goddess Iris. In Romanic languages the gender of the term is masculine, because its relation with the goddess Iris was forgotten, and the masculine arc prevailed. In German the term Regenbogen is also masculine

In the medical books the total or partial lack of iris received the term "irideremia" or "aniridia" because although this disease has many other manifestations, the most evident from birth is the total or partial lack of iris (**Fig. 2**). This occurred about two centuries ago, when the

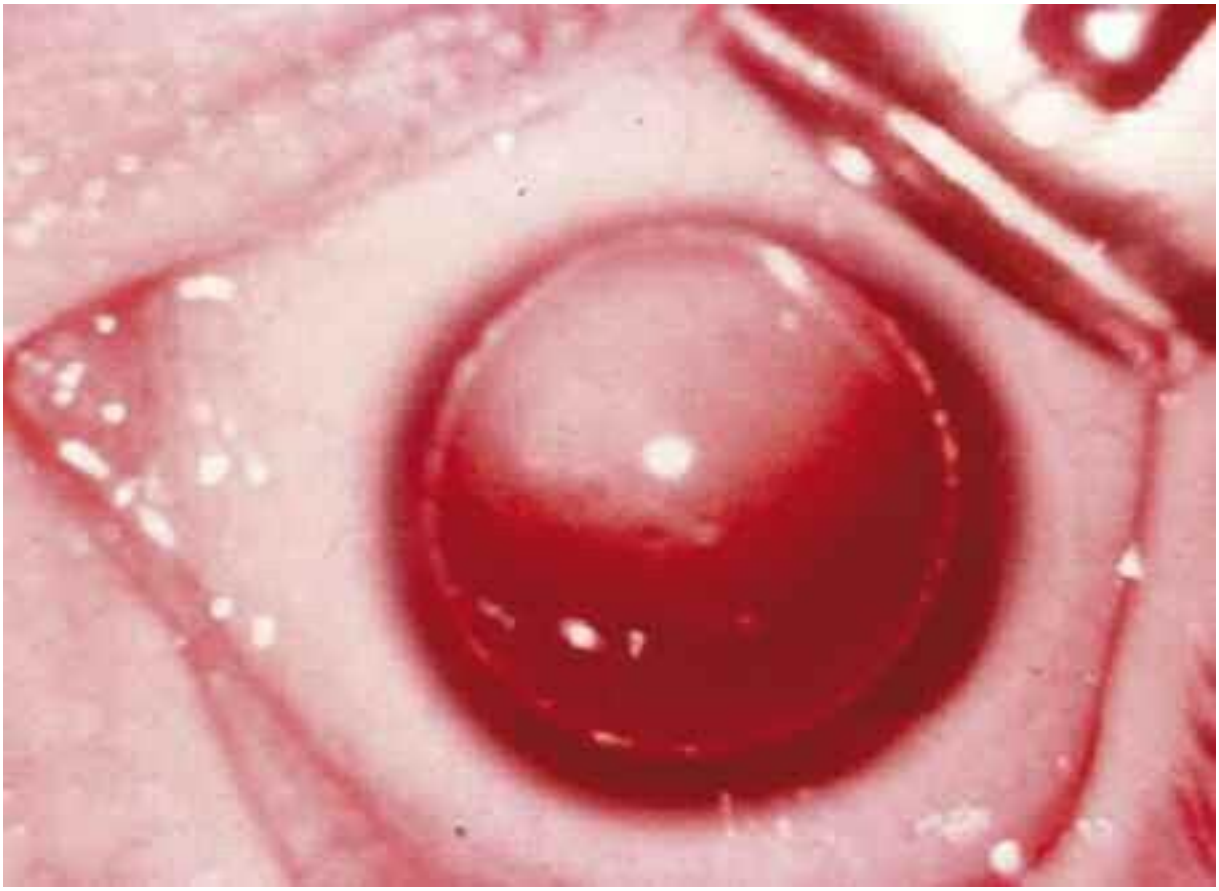

Figure 1.2. Congenital aniridia.

pathologic entities were mainly named by their external physical manifestations, more than by their etiopathogeny, which was almost always unknown. An older example of this is the term glaucoma, derived from Greek *glaukos*, which means marine bluish color, because the cloudy color of the cornea in the painful acute glaucoma was the only intraocular hypertension diagnosed in old times; currently, the painless chronic intraocular hypertension maintains the term glaucoma despite the fact that the cornea keeps a normal transparence. The terms irideremia and aniridia are descriptively very exact, because the affected patients do have an iris, although with diverse grades of hypoplasia. Irideremia, the term most used in the XIXth century, comes from Greek iris (ιρις, -ιδος = iris) and eremia (έρημία = short, incomplete, desert). Aniridia, the term most used in the XXth century and currently, also comes from Greek *an-*, a negative prefix, and *iris, -idos*, iris.

1.2. The problem: Inheritance and clinics

The first genodendrons or genealogical trees of patients with aniridia were published in 1834 by Gutbier (**Ref 4**) in a tree of four generations, and in 1845 by Cunier (**Ref. 5**) in a tree of three generations. Risley (1915) (**Ref. 6**) reported 221 eyes with aniridia in a genodendron of 119 persons. These genealogical trees showed that the syndromes of aniridia had no preference in relation to sex. Later on it was also observed that they did not have significative racial differences.

The genetic upset that causes the aniridia is a mutation or a deletion of the chromosome 11p13.3, affecting the PAX6 gene. At present 130 different mutation have been identified in this gene. (**Ref 7**).

The PAX6 gene may activate the transcription of a cascade of approximately 2,500 genes (**Ref. 8**), as the WT1, and others (**Refs. 9, 10, 11, 12, 13**). The expresion of this gene increases when the plaque of the crystalline lens is developing (**Ref 14**), and these alterations produce ocular malformations, the most gaudy being the hypoplasia of the iris.

The disease, almost always heterozygote, is transmitted in autosomic dominant form. When the patient has a homozygote alteration of the PAX6 genes, this rarely occurs because both father and mother are affected (**Ref 15**), but usually because a homozygote mutation has occurred (**Ref 16**). These homozygote cases have frequently the addition of anophthalmia, cerebral abnormalities, and high rate of mortality. (**Ref 18**).

Along the approximately 180 years of the diagnosis of aniridia many different associations with hypoplasia iridis have been described. At the beginning it was thought they were casual coincidences, but gradually there were syndromic associations, as surfocular damage and corneal opacities (**Refs 5, 7**), ectopia lentis[4,6,17,18,19,20], cataract[5,17,18,19,21,22,23], hypoplasia of the ciliary body (**Refs 17, 18, 24**) , glaucoma (**Refs 17, 18, 20, 21**), hypoplasia of the macula and optic nerve (**Refs. 19, 25, 26**), retinal detachment (**Ref 18**), opacities of the vitreous body (**Refs 6, 18**), microphthalmos (**Refs 5, 27, 28, 29**), strabismus (**Refs 19, 30**), nystagmus **(Refs 5, 19, 25, 31, 32**), and blepharoptosis congenita (**Ref 8**). Many of these additions produce symptoms along the life of the patients, such as photophobia, refractive defects, amblyopia and low vision.

More recently extraocular associations were determined, as the WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and mental delay) (**Refs 33, 34**), Gillespie syndrome (aniridia, cerebellar ataxia and mental delay) (**Ref 35**), and Peters' anomaly (dysgenesis of the

anterior segment and surf-oculus) (**Ref 8**). In WAGR syndrome, the nephroblastoma has no direct relation with the ocular manifestations of the PAX6gene (**Refs 8, 36**). In Gillespie syndrome mutations of the PAX6 gene have not been detected, and the responsible gene remains unknown (**Ref 8**). Some abnormalities of Peters' anomaly plus (opening in the lip, short body stature) have been sometimes associated with PAX6 mutations. (**Ref 37**).

The damage and opacification of the cornea was described in the first publication of congenital aniridia (**Ref 1**). Later on some keratopathies associated with congenital aniridia, such as malformations, opacifications and vascularization were sometimes cited (**Refs. 5, 17, 19, 27, 38, 39, 40**). Lagrange (1905) (**Ref 17**) reported that the corneal malformations and alterations associated with congenital aniridia are the most frequently published, and Mackman et allii (1979) (**Ref 40**) classified their different types.

For more than one century it has been well known that congenital aniridia is frequently associated with diffuse limits between cornea and sclero-conjunctiva (**Ref 5**), and for more than two decades the scarcity or lack of limbal palisades of Vogt has been well known (**Refs 41, 42**). Along the 70s and 80s of the last century it was determined that the niche of the stem cells of the corneal epithelium was in the limbal surfocular ring (**Refs 43, 44, 45**), and that it is frequently altered in aniridic patients. It was also verified that the anatomical tissues behind the limbal ring, such as Schlemm canal, irido-corneal angle, ciliary body, and cilio-crystalline zonule, are frequently abnormal.

There are three basic types of dacryoglands (ie., aqueoserous, lipid and mucinic). In my clinical experience I have not detected statistical difference in the quantity of aqueoserous tear in the aqueoserous glands (main and accessory lacrimal glands) in persons with aniridia and without aniridia. The lipid glands (meibomian glands, Zeis glands) develop an age-related and hormonal gland dysfunction in all aging people, but without differences between aniridic and non-aniridicindividuals. It has been reported that aniridic patients develop a narrower lid aperture and more lid wrinkles (**Ref 46**), but this does not modify the lipid tear component. The mucinic glands (mainly the goblet/caliciform cells dispersed over the conjunctiva) are the only dacryoglands deficitary in the congenital aniridia. This was cited to my knowledge for the first time by Bietti (1963) (**Ref 47**) , who published five cases of dysgenesia mesodermalis, iridopupilar malformation, and conjunctival xerosis with Bitot's spots due to the deficitary number of caliciform conjunctival cells. Later on new cases were published (**Ref 48**). Nevertheless, two authors (**Refs 41, 42**) who only studied the lower bulbar conjunctiva found more goblet cells in this area. But other authors (**Ref. 49**) who analysed the four quadrants of the bulbar conjunctiva determined that 56% of aniridic patients have a moderate deficit of goblet cells in the four quadrants of the surfocular conjunctiva.

The limbal epithelium has two main functions: to produce the epithelial cells of the cornea, and to prevent that the conjunctival epithelial cells invading the corneal surface. When the limbal cells are deficitary and pathologic they can not impede the invasion of the corneal epithelium by cells of conjuntival epithelium of non-secretory or mucinsecretory type. This pathologic phenomenon is quite frequent in patients with congenital aniridia. Binder (1853) was the first author to report the lack of mucinsecretory goblet cells in the perilimbal conjunctival ring (**Ref 50**) in normal people. Barraquer (1980) was the first author to publish that when the limbal epithelium is damaged and deficitary, the conjunctival epithelium invades the surfocular layers of the cornea (**Ref 51**). This invasive phenomenon was later on named

"conjunctivalization of the cornea". The conjunctivalized corneal epithelium maintains many of its normal functions, but not others.

At present the most evident manifestation of the conjunctivalization of the corneal epithelium is the presence of mucinsecretory goblet cells in the surfocular cornea, and the metabolic alterations of the subjacent corneal stroma induced by the conjunctival migrated epithelium. The permeability of the conjunctival epithelium to the fluorescein is higher than that of the corneal epithelium (**Ref 52**), and therefore the conjunctivalized corneas dye with fluorescein more than normal corneas. Margo (**Ref 53**) published that the cornea of aniridic patients is easily invaded by fibrovascular pannus. And Tseng et al. (**Refs 54, 55**) associated the limbal insufficiency of the congenital aniridia with its corneal opacification and vascularization.

1.3. The fight against aniridia

The medical society have been trying to ease or to resolve the problems of the aniridic patients since the disease became known (**Refs 56, 57**). First, already in the XIXth century, an attempt was made to diminish the excess of light that enters in the eye of aniridic patients by the use of spectacles with dark glass (**Ref 5**), Serres' panoptic spectacles (**Ref 28**), Donders pinhole stenopeic glasses (**Ref 17**), and Königshöfer diaphragmatic spectacles (**Refs 17, 58**) (**Fig 3**). Later on, in the XXth century, new optic treatments appeared, such as surfocular contact lenses, opaque or colored in their periphery (**Ref 59**), intracamerular diaphragmed artificial iris, and pseudophakic diaphragmed lenses (**Refs. 60, 61, 62, 63**).

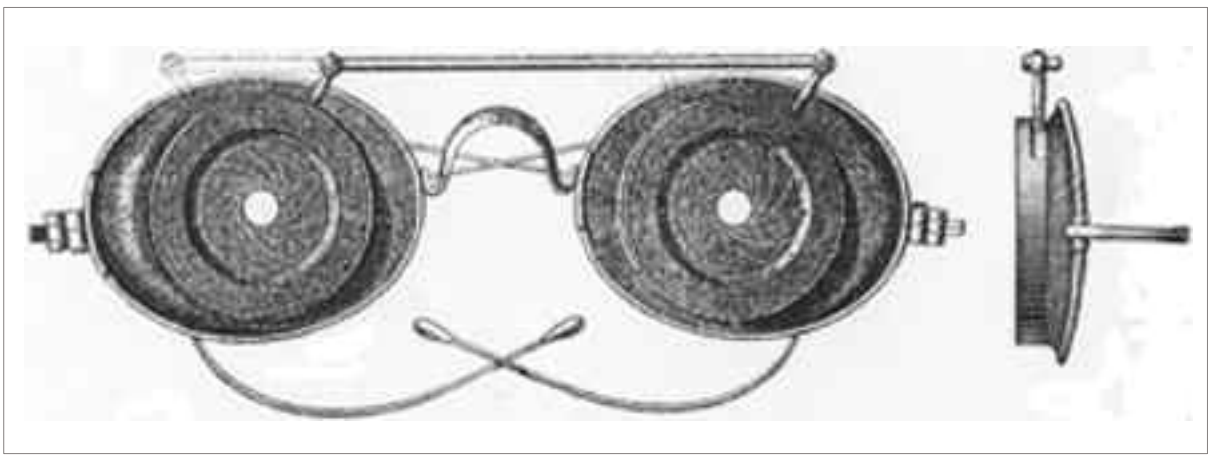

Figure 1.3. Königshöfer stenopeic diaphragmatic spectacles (Encycl. Franç. Ophtalmol, Vol II, pages 388-9. 1905)

The medical and surgical treatment of the glaucoma in patients with genetic aniridia has gradually improved. The limbal transplant with limbal stem cells (**Ref 62**), and the cultures of stem cells (**Refs 63, 64, 65**) prevent the damage that its defect may produce (**Ref 66**).

The genetic information and advice was based from the beginning of the identification of the genetic aniridia by making the family's genodendron (**Ref 67**), and for these patients to avoid having descendants. Later on, the prenatal diagnosis by amniocentesis and analytic study of the extracted DNA was introduced. More recently, the advances in molecular biology and genetic medicine have improved the approach to this disease.

1.4. Medical and patients associations

The health organizations and the patients groups with their current knowledge and objectives are intent on finding the best option for patients with genetic aniridia.

The health organizations are aware that any disease requires the collaboration of many health specialities, which today are named proto-specialities. Centuries ago proto-specialities appeared, such as parturition and oculist, followed by dermatology, oto-rhino-laryngology, cardiology, genetic, etc.

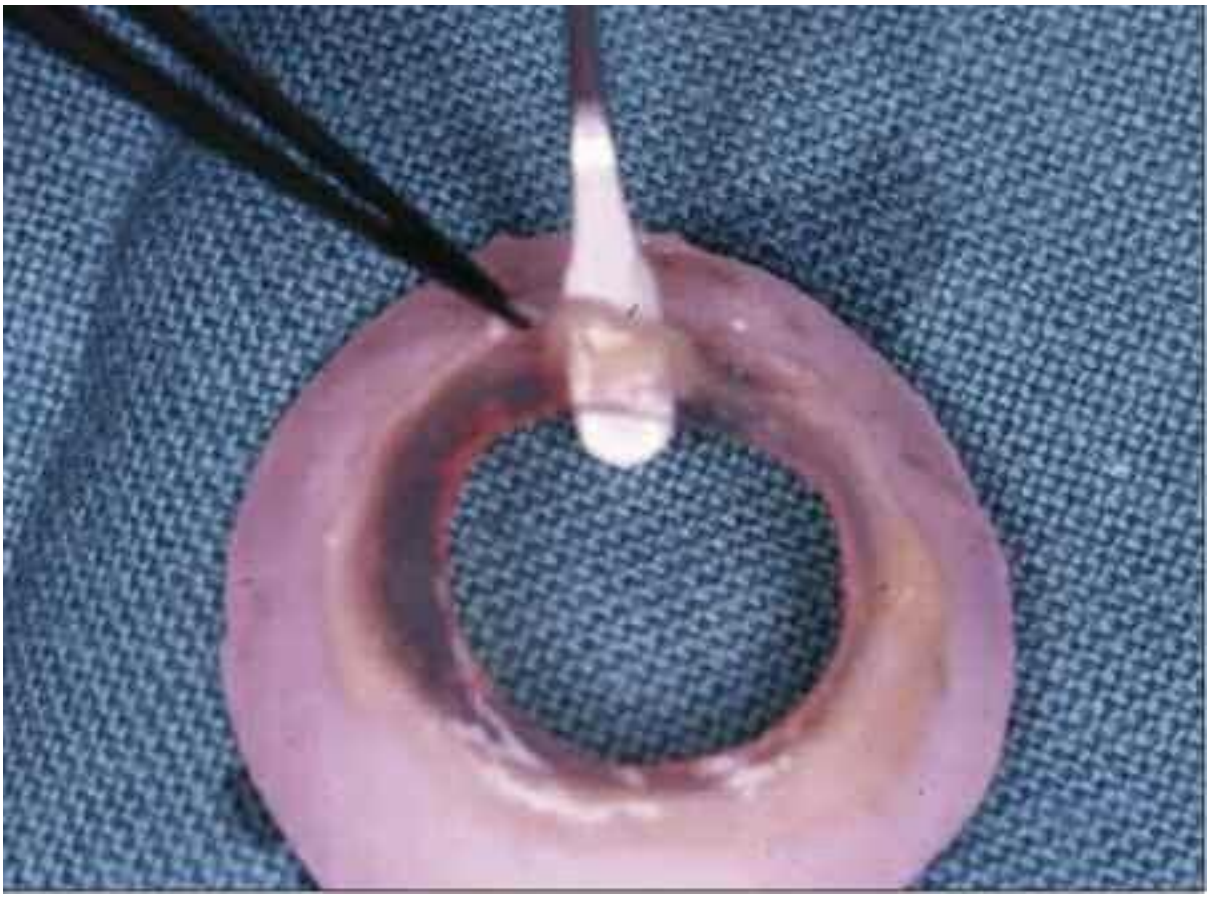

Figura 1.4. Extracción de un anillo de superficie limbal de ojo de cadáver para transplante limbal heterólogo.

After those proto-specialities, branches and combinations of them appeared and are still appearing. They are named deutero-specialities or specialities of second generation, which are also coined sub-specialities or super-specialities. New deutero-specilities of ophthalmology are cataractology, retinology, glaucomatoloy, strabismology, dacryology, etc. Trito-specialities or specialities of third generation are slowly being stablished. They treat vey specific problems, that usually need the concourse and cooperation of various branches of deutero-specialities. The benefit of this evolution is evident for doctors and patients, as the objective is to resolve the medical problems as nearly as possible in 100% of the cases. This does not mean a reduction in the knowledge of the doctors, but only a reduction in the knowledge that does not benefit the patient of their trito-speciality. However there is an increase in the very diverse knowledge that resolves the maximum of the diseases they treat. The patient obtains the most benefit of this evolution.

The patients with congenital genetic aniridia were until very recently passive protagonists of their disease. But little by little the social evolution and the easier intercommunication is making them active participants in the search of a solution for aniridia and its associated problems. The most important step was the foundation of societies of self-help, directed by patients with aniridia, which seek social help for activating and stimulating research in this field.

The first Society of self-help in Aniridia was the Asociación Española de Aniridia (AEA, Spanish Association of Aniridia), founded in 1996 by Rosa Sánchez-de-Vega. After directing it for more than ten years, organizing meetings with patients and doctors, conferences, awards for research in aniridia, she was substituted by Yolanda Asenjo, who has maintained the same high activity, aided by her Administrative Secretaries Marta Gaitán and Maru Cruz. The AEA organized an International Symposium of Aniridia, in Madrid in 2002, that increased the interest in aniridia in medical, laboratory, sanitary and social ambients (**Ref 68**). Later a National Prize in Aniridia, open to any researchers on Aniridia was created and awarded at every annual congress of the Spanish Society of Ophthalmology; in 2005 the award was given to "Mörcher lenses in Aniridia" (by Drs Brandao, Toledo, Barberán & Álvarez-de-Toledo), in 2006 to "Surfocular Treatment in Aniridia" (by Drs. López-Gª, Rivas & Gª-Lozano), in 2007 to glaucoma and Aniridia.

After the creation of the Asociación Española de Aniridia other societies were founded in several European countries that eventually developed the European Federation of Aniridia, Aniridia Europe AE www.aniridia.eu
There, you can see the different European Aniridia Associations

There are other Associations, not specifically devoted to aniridia, but to rare disorders, among which aniridia is included. In 1983 the National Organization for Rare Disorders (NORD) was founded in USA (web: www.rarediseases.org , email: orphan@rarediaseases.org), and in 1997 the European Organization of Rare Diseases (ERURORDIS) (web: www.eurordis.org) was founded in France. In 1999 the Federación Española de Enfermedades Raras (FEDER) (web: www.enfermedades-raras.org) was founded in Spain, It is to be hoped that the political and financial powers will collaborate in the near future with patients, medical, doctors, genetists, sociologists, educators, pedagogues and in the fight for an answer to aniridia. As a result of promotion in research and therapeutical application, the complex diseases included in the so named congenital aniridia may well reach a solution.

This future will be reached within a very few generations, and later on, as at the end of a storm, the sun will come again, and a beautiful atmospheric rainbow will appear.

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CHAPTER 2
EPIDEMIOLOGY OF CONGENITAL ANIRIDIA: REVIEW OF THE LITERATURE AND ANALYSIS OF DATA OF ECEMC (SPANISH COLLABORATIVE STUDY OF CONGENITAL MALFORMATIONS)

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2.1. Introduction: The importance of Epidemiology in the research on congenital defects.

In general, it can be said that in spite of the great advances in the field of Medicine in the last century, congenital defects continue being widely unknown, and only a few exceptions run away from this generalization. In ancient times, it was considered that the birth of a baby with anomalies was surrounded by bad omens or malign influences, and its origin was often looked for in both deities' fury and glory.1 It is true that birth defects prompted some interest and curiosity, but mainly due to that supernatural halo that encompassed them. From the health's point of view, congenital defects have generally lacked interest, due to the fact that they have classically been considered as unpredictable, incurable, unavoidable, possibly hereditary, and luckily not very frequent.2 In the past century, there were two facts that exerted a catalytic action on the research about causes of congenital defects: first, the description by Gregg3, in 1941, of congenital cataract as one of the most characteristic defects of the clinical pattern produced by the prenatal exposure to the rubella virus; and second, the suspicion in 19614 and further recognition in 1962,5 by Dr. Widukind Lenz, of thalidomide as the agent that caused diverse types of alterations of prenatal development, mainly limb reduction defects, in thousands of infants worldwide. Since that moment, there has been a general raise of awareness on the need for research on the causes of congenital defects. Partly due to that reason, epidemiological surveillance systems were created in many countries. These were specially aimed at detecting variations in the frequency of congenital defects, that can help in identifying their causes and to prevent them. On the other hand, the great advances in the field of Genetics in the last decades have completed a scenario in which it is intended to dig into knowledge on congenital defects to find the most adequate approach in each case and, additionally, seek their prevention and favor that prenatal development goes by the most propitious conditions, thus preventing its alteration. In this

sense, Epidemiology has a pivotal role because experiments are not possible in humans and the results from animal experiments can not be extrapolated to our species. For this reason, it must be tried to obtain the maximum yield from observation of our reality, to stab to infer the causes of congenital defects.

It is frequent to assimilate Epidemiology to the mere study of frequencies. However, this is a much wider discipline that allows carrying out analytical studies, specifically addressed to the causal investigation. Therefore, the epidemiological studies are a fundamental pillar in which such investigation is based. But, in order to get valid conclusions from the epidemiological studies, these must be based on data obtained from unselected series of cases, as well as an appropriate reference group (for comparisons), and the size of the samples must be sufficient. These must not be biased, neither in the phase of collection of the information nor in the phase of analysis. In this way, from the comparison of the characteristics of the individuals presenting with the pathology under study, against healthy individuals, some conclusions can be obtained about the causes that give rise to such alteration of prenatal development. However, to secure the accomplishment of all these conditions is neither easy nor always possible. Therefore, this kind of studies implies great difficulties, and the conclusions from them must have considered all the possible limitations of each study due to unfulfilling of one or several of those some requirements.

2.1.1. Epidemiology of congenital Aniridia

The diagnosis of many congenital eye anomalies can be difficult at birth. Therefore, this one is one added difficulty to get big adequate samples for epidemiological studies on these pathologies. This is probably the reason by which there are very scarce studies on the epidemiology of congenital ocular defects.6-23 On the other hand, many of those studies approached only partial aspects or they grouped very diverse pathologies, and therefore only very limited information can be obtained regarding the epidemiology of specific defects. In the case of aniridia the situation is even more severe due to its very low frequency,15 and its rather complex detection in newborn infants. In addition, the clinical variability observed gives rise also to difficulties for the diagnosis,24,25 being difficult to differentiate it from some colobomas, remaining undetected in certain cases presenting with good vision, or being uncorrectly diagnosed in those with a thin iris.

Following the current usual procedures for medical literature review, it is easy to confirm the shortage of papers on the epidemiology of aniridia, since if we establish “aniridia” and “epidemiology”, as search criteria, the PubMed.gov database26 (in the search performed on February 9, 2014) gives us 55 results, but only one27 approached specifically the epidemiology of aniridia as the main issue of the article. This means that the review of the epidemiological aspects of congenital aniridia must be done by analyzing the results of papers that also include data related to aniridia among other information.

2.1.2. Frequency of congenital Aniridia.

According to Nelson et al.,[28] the frequency of *aniridia* varies between 1 in 64,000 and 1 in 96,000 individuals. In the Chinese population, Hu et al.[29] established its frequency in 0.75 per 100,000 individuals, or 1 in 133,931. More recent population data in Denmark,[30] give a frequency of 2.5 per 100,000 live born infants, or 1 in 40,000, while in Sweden it was 1 in

70,000 (95% confidence interval: 1:59,000-1:85,000), and in Norway it was 1 in 76,000 (95% confidence interval: 1:61,000-1:103,000).[27] The generally accepted figure is 1 in 50,000-100,000 individuals.[30] In Spain, the minimal estimation of its frequency, obtained by the Spanish Collaborative Study of Congenital Malformations (ECEMC) was 0.42 per 100,000 newborn infants.[15]

Regarding its sex distribution, although Okamoto et al.[31] observed a slight excess of males, given that the analyzed sample was very small (6 males and 4 females), these data have a limited value. In Denmark, the study by Grønskov et al.,[30] performed on data gathered between the years 1875 and 1999, both sexes were equally affected, after studying 87 females and 83 males. A slight predominance of females was observed in Sweden (male/female ratio was 0.85) and the contrary was registered in Norway ((male/female ratio was 1.2),[27] after studying a total of 181 patients from both countries.

2.1.3. Types of presentation of congenital Aniridia.

Aniridia can be uni or bilateral, and can present clinically isolated or associated to other ocular or extraocular defects.[30,32] It can be familial[30,32] or sporadic (in up to a third of cases[28,33]). Valenzuela and Cline's conclusion[34] was similar, indicating that 70% of aniridia cases are familial. Grønskov et al.[30] established that the overall proportion of familial/sporadic cases was 100/44. In familial cases, aniridia is transmitted as an autosomal dominant trait (with high penetrance but variable expressivity),[35] although there are also some cases compatible with autosomal recessive intheritance.[30,32]

2.1.4. Association of Aniridia with other congenital defects.

As expressed in the previous paragraph, aniridia can present as an isolated defect or associated to other anomalies. [30,32] Chapter 4 provides an exhaustive review of the systemic pathology to which aniridia can be associated. However, only to approach all the aspects of the epidemiology of aniridia, we provide here some figures on the most frequent clinical pattern in which it appears: approximately one third of the sporadic cases of patients with aniridia develop Wilms tumor in association with genitourinary defects and mental retardation[36,37] and are therefore diagnosed as WAGR (acronym for *Wilms tumor, Aniridia, Genital abnormalities and Retardation*) syndrome.

2.1.5. Morbidity and mortality associated to Aniridia.

While the *morbidity* in cases with aniridia is relatively high, due to the considerable reduction of sight and the associated alterations, especially the Wilms tumor, the *mortality* of patients presenting only with aniridia is very low, since this is not a lethal pathology.

2.1.6. Teratogens related to Aniridia.

At present there is no evidence of aniridia being caused by any teratogen.

2.2. Epidemiology of congenital Aniridia in ECEMC's data.

ECEMC (Spanish Collaborative Study of Congenital Malformations), as defined in its Operating Manual, is "a research program on the *clinical* and *epidemiological* aspects of congenital defects in humans", and it is based on a permanent system of registry of newborn infants with congenital anomalies.[38,39]

2.2.1. Study population.

ECEMC was created and has been operating since 1976. Until 1979, only data on liveborn infants were registered. In January 1980 gathering of data on stillborn infants was initiated. Therefore, since then, data on the total newborn infants (wheather live or still born) are available. Thus, in this chapter, the data registered by ECEMC since January 1980 to December 2012 (the most recent available data at the time this chapter was translated from its original in Spanish, published in 2008) have been analyzed in order to refer figures to total births. In that period, ECEMC surveyed a total of 2,819,321 births, among which 41,179 (1.46%) had congenital defects detected during the first 3 days of life, which is the detection period at the ECEMC's registry.

2.2.2. ECEMC's methodology.

ECEMC's methodology includes the exploration of every newborn infant and the detailed description of each defect, either major or minor/mild, in every case with anomalies. The results of such exploration and the description are documented by images and complementary studies needed for the diagnosis of each case, and are made by the physicians integrating the *ECEMC's Peripheral Group*. They are in charge of the detection of cases, the selection of controls and the collection of the corresponding information for the cases as well as for the controls, which is gathered in the specific ECEMC's protocols. A total of about 312 data per infant are stored (demographic data, family history, obstetrical history and any type of exposures during pregnancy and even previous to it). All that information is sent to the *ECEMC's Coordinating Group*, which processes it, including coding of all the anomalies and the high resolution and molecular cytogenetic study, apart from the clinical analysis specifically designed at ECEMC, taking into account both the etiological and pathogenetic aspects. For that purpose, a system developed by this group is used, and it is based on a modified and extended version (to increase its specificity) of the International Classification of Diseases (ICD). Once all the defects have been coded, the ECEMC Coordinating group uses its own system for coding the global pattern of defects in each child, in three levels,[38-41] and that is the starting point of ECEMC's clinical and dysmorphologic analyses, which are performed in several steps, and are the base for further epidemiological analyses:

1. First, to establish possible pathogenetic relationships between the defects observed in each child, trying to identify the different altered processes during the prenatal development. It is intended to define the diverse dysmorphogenetic patterns, taking into account the most modern concepts of the errors of morphogenesis[42,43] and, if some defects have originated others in a sequential manner, to define the primary malformation(s) and the secondary defects derived from the primary defect(s). Once this has been done, the corresponding codes for those dysmorphogenetic patterns are assigned, in order to enter them into the ECEMC's database.
2. Second, taking into account all that, each child is classified into one of the three big groups of clinical presentation: Isolated, Multiple Congenital Anomalies (MCA) patterns, and Syndromes. These groups are also respectively coded.
3. Third, the corresponding subgroups within those three main groups of clinical presentation are also conveniently coded.

Once the clinical and dysmorphological analyses have been performed following the ECEMC's methodology, and once the different clinical groups have been established, the data are ready to perform the epidemiological analysis in the pertinent clinical groups, according to each study's objectives.

2.2.3. Congenital Aniridia cases registered by ECEMC.

During the period comprised between January 1980 and December 2012, among a total of 2,819,321 births surveyed, 17 had congenital aniridia, for a birth prevalence of 0.6 per 100,000 newborn infants (95% Confidence Interval [CI]: 0.35-0.97). This figure registered by ECEMC is close to the one described by some authors,[29] and lower than the one offered by others.[28,30] It must be said that the detection period at ECEMC comprises the first three days after delivery, and due to the difficulties for the diagnosis of aniridia, that were already commented at the beginning of this chapter (although the physicians who participate in the ECEMC program have a long experience in the detection of birth defects in newborn infants, it is also possible that in some cases this ocular malformation could have not been detected. On the other hand, although aniridia can not be prenatally detected, it is possible that a certain proportion of gestations of fetuses in which other defects associated to aniridia are prenatally diagnosed could have been interrupted. For these reasons, the figure registered by ECEMC must be considered as a minimal estimation of the real frequency of aniridia in the Spanish population.

Table 1 shows the distribution of the 17 cases registered with aniridia, by clinical presentation of the defect. Among the 17 cases, seven (41.2%) were isolated, and the remaining 10 (58.8%) had other anomalies associated. Among the isolated, four (57.1%) were sporadic and three (42.9%) were familial, compatible with an autosomal dominant pattern of inheritance. The percentage of sporadic cases at ECEMC is higher than the 30% described by other authors.[28,33,34]

Table 1. Distribution of cases with congenital Aniridia registered by ECEMC in 1980-2012, by type of clinical presentation

Type of clinical presentation	Number of cases
Isolated	- 4 sporadic cases - 3 familial cases (dominant inheritance)
Asociated to other defects	- 3 WAGR syndrome with 11p13 deletion - 2 Aniridia-plus syndrome [described by Hamming et al.[56]] - 1 Trisomy 13 - 4 with aniridia associated to other ocular defects

Regarding the sex distribution, there is a clear the predominance of males in the ECEMC's series, since 12 out of the total of 17 were males and only 5 were females.

With respect to the presence of deletions in the critical region for aniridia, among the 17 cases registered, 11 were cytogenetically studied: six had normal karyotypes, one had an extra Y chromosome, one had trisomy 13, and three had deletions at 11p13.

The laterality of the defect was specified in 16 of the 17 cases registered, being bilateral in 10 and unilateral in a total of six (three for each side).

Table 2 shows the values for a series of variables in the group of sporadic cases with isolated aniridia registered by ECEMC in the mentioned period. The mean and standard deviation for the quantitative variables are expressed in the table for the cases and controls. Nevertheless, since the number of cases is small, it is difficult that the conclusions can be made extensible to all the cases with aniridia. The objective of detailing those data in the table is that these can be available for other researchers analyzing comparable series of cases. Regarding the obtained results, we have observed a statistically significant ($p<0.001$) reduction of the gestational age of cases, being the gestation of patients with sporadic aniridia more than four weeks shorter than

Tabla 2.2. Distribución de los casos esporádicos de Aniridia congénita aislada registrados por el ECEMC entre 1980 y 2005, para una serie de variables.

	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5	CASES		CONTROLS				
VARIABLES	1	2	3	4	5	Mean	SD	Nr.	Mean	SD	t	p
Gestational age (weeks + days)	41+1	41+3	38+3	23+5	38+6	36+5	7+2,7	30.372	39+5,3	1,80	39+5,3	<0,001
Survival	LB	LB	LB		LB	-	-	-	-	-	-	
Gender	M	F	F	M	F	-	-	-	-	-	-	-
Birth weight (g.)	3.185	2.880	2.295	780	2.920	2.412	968,5	32.106	3.279,43	482,57	4,02	<0,001
Birth length (cm.)	51	-	46	-	-	48,5	3,5	14.880	49,65	2,26	0,72	NS
OFC at birth (cm.)	35	-	31		-	33	2,8	14.839	34,22	1,53	1,12	NS
Maternal age	21	29	29	32	30	28,2	4,2	32.114	28,38	5,34	0,08	NS
Paternal age	27	39	31	36	38	34,2	5,1	31.535	31,14	5,72	1,20	NS
Paternal age difference	6	10	2	4	8	6	3,16	31.521	2,73	3,66	2,00	<0,05
Number of pregnancies	1	2	1	5	1	2	1,7	31,598	1,96	1,17	0,08	NS
Consanguinity	No	No	No	No	No	-	-	-	-	-	-	-
Ethnic group	White	White	White	White	White	-	-	-	-	-	-	-

LB: liveborn infant; M: Male; F: Female; OFC: occipito-frontal circumference; SD: Standard deviation; NS: Statistically not significant.

the ones of the controls. There is also a statistically significant reduction of the birth weight ($p<0.001$), that is not observed for the birth length and occipito-frontal circumference (OFC) at birth. Therefore, taking into account the mean gestational age of our cases, their mean birth weight falls between the 3rd and 25th centile, being the birth length and OFC between the 75th and 90th centiles. Regarding the parental ages, although there are no statistically significant differences between cases and controls, the paternal age seems to be slightly higher among the cases, what could be indicating a higher mutation rate.

2.3. Epidemiology of WAGR (Wilms tumor, Aniridia, Genital abnormalities and Retardation) syndrome.

After a review of the literature, it is clear the importance of WAGR syndrome when studying aniridia, because it is the most frequent clinical pattern in which aniridia presents associated to other anomalies. In the ECEMC's series, three out of the total 17 registered, had WAGR syndrome with chromosomal microdeletions at chromosome 11p13. Since the detection period at ECEMC includes the first three days after delivery, the follow up of cases can reveal that the real frequency of WAGR syndrome is higher than ours. In fact, some babies with isolated aniridia at birth will develop Wilms tumor and mental retardation, thus being considered as WAGR syndrome. Therefore, its epidemiology deserves to be specifically approached, as some authors have already done so far.

Initially, it was considered that 30% of the individuals with aniridia and no family history of the defect develop Wilms tumor in the first 5 years of live,[44] although posteriorly it was observed that the risk must be lower.[30]

In patients with WAGR syndrome there is a deletion of both *PAX6* gene (responsible for aniridia) and *WT1* (responsible for Wilms tumor),[33] that are adjacent genes located at the p13 region of chromosome 11 (11p13). This syndrome is, therefore, associated to deletions in the region 11p13,[36,37] and this will be explained in detail in chapter 3. It has been observed that 70% of cases with deletions in the region 11p13 develop Wilms tumor,[45] although this estimation is based on small samples.[44,46] According to Grønskov et al.,[30] 40% of the individuals with the deletion of the Wilms tumor gene developed the tumor. So, after the detection of a deletion of *WT1* gene, an ultrasonographic follow-up every three months up to the age of eight years is recommended, while in absence or the deletion the risk for Wilms tumor is very low and such follow-up is not required.[30,47]

Actually, there are very few studies on the WAGR syndrome including a considerable number of patients. In the greatest published study,[48] an evaluation of the clinical-pathological characteristics of a group of 64 patients was made, and it was based on data from the archives of the National Wilms Tumor Study Group (NWTSG), from the USA. When that study was performed, such archives contained information on a total of 8,533 children with renal tumors that represented between 70 and 80% of the North-American children with this type of tumors. A further study[49] included data from 54 patients aged 7 months to 42 years, with a mean age of 9.2 years.

- **Risk for Wilms tumor in patients with sporadic aniridia:** According to Breslow et al.,[48] if we accept that the prevalence of sporadic aniridia is 1.2 per 100,000, if the cumulative incidence of Wilms tumor is 1 in 10,000 births, and the prevalence of sporadic aniridia

among patients with Wilms tumor is 7.5 per 1,000 (as those authors calculated[48]), then the risk for Wilms tumor among patients with sporadic aniridia can be estimated in 6.3% (0.0075 x 0.0001/0.000012). Although this figure is lower than the one provided by Ivanov et al.[32] (who indicated that approximately one third of the patients with sporadic aniridia would have WAGR syndrome), is very close to the risk of 2 in 44 (4.5%; with 95% confidence interval [IC]: 0.6-15.5%) observed in the Danish populational study by Grønskov et al.[30] According to these last authors, patients with sporadic aniridia have a 67-fold higher risk (CI: 8.1-241) than the general population to develop Wilms tumor. Among patients with molecular study, two out of five individuals with a deletion of the Wilms tumor gene (*WT1*) developed the tumor,[30] although the patients with small deletions or intragenic mutations did not develop the tumor. It can be said that the risk for Wilms tumor in the sporadic cases (intended as first occurrence in a family) is 40-50% if the individual has the deletion in 11p13. Grønskov et al.[30] detected mutations in 68% of the sporadic cases and in 89% of the familial cases. If no deletion in *WT1* gene is detected, the risk for Wilms tumor is low.[30,47] Absence of one *WT1* allele in the germline leads to a high risk (~45%) of Wilms tumor occurring through somatic mutation that results in loss of heterozygosity (LOH) in a single differentiating kidney cell.

- **Frequency of the syndrome:** The frequency of Wilms tumor in the population is 1 in 10,000 individuals younger than 15 years, and its incidence seems to be higher in Afro-americans and African blacks, being reduced to a half in Asians. [50,51] The prevalence of WAGR syndrome among patients with Wilms tumor was 0.81% in a French hospital series in which four cases with the syndrome were detected among 501 patients with Wilms tumor, between 1982 and 1989.[52] However, such prevalence went up to 2.19% in a British populational-based series (12 affected with the syndrome among a total of 549 individuals with Wilms tumor).[53] According to data from the NWTSG,[48] from the USA, 0.75% of the registered cases with Wilms tumor had the WAGR syndrome; the prevalence of the syndrome remained stable along the period 1969-2002, without any statistically significant tendency. The prevalence of WAGR syndrome is approximately 1:500,000.
- **Sex distribution:** Twenty-seven (42%) patients from the NWTSG[48] with WAGR syndrome were females, while in the group with Wilms tumor without WAGR syndrome it was observed a slight preponderance of female patients (54%).[48] In the study by Fischbach et al.,[49] twenty-three (42.59%) patients with WAGR syndrome were females and 31 were males, being these figures totally concordant with those described by Breslow et al.[48]
- **Somatometry:** These patients seem to have lower birth weight and length than the healthy individuals.[50,54,55] According to data from the NWTSG,[48] the mean birth weight was 2,940 g., while in the group with Wilms tumor without WAGR syndrome the mean birth weight was 500 g. higher ($p<0.00001$). Nevertheless, since the NWTSG did not have any data on the gestational age, it was not possible to figure out whether this observation could be due to shorter gestations. At the time of diagnosis the length was 4.5 cm. shorter than among patients with WAGR syndrome ($p<0.00001$), what was not observed for the weight, that was only slightly lower than that of patients with the tumor but without the syndrome, although the difference was not statistically significant.
- **Mean age at diagnosis:** Wilms tumor in patients with WAGR syndrome is diagnosed at earlier ages than in patients without the syndrome.[48,50,54,55] According to data from the

NWTSG,[48] the mean age at diagnosis of the tumor was 22 months, compared to 39 months in the group without WAGR syndrome; only 16% of the patients with WAGR syndrome were diagnosed after the age of four years, compared to 38% in the other group.

- **Histology and laterality of the tumor:** In patients with WAGR syndrome, Wilms tumor is more frequently bilateral.[50,54,55] Anaplasia was not observed in any of the 64 patients with WAGR syndrome and studied in the NWTSG.[48] However, it was identified in 8% of the patients without the syndrome (p=0.05), the histology being favorable in 100% of the patients with WAGR syndrome, and in 91.9% of the cases without it (p=0.0004). In such study,[48] fourteen percent of the patients with WAGR syndrome had bilateral affectation regarding the Wilms tumor; in 48% of the patients with unilateral tumor this was stage I, and only one patient (2%) had metastasis (stage IV) at the time of diagnosis (p=0.002 for the trend test). Most (81%) of the kidney specimens from patients with the syndrome had nephrogenic rests, that were only observed in 42% of the patients with tumors without WAGR syndrome.
- **Clinical evolution of patients with WAGR syndrome:** The initial clinical evolution of patients is favorable. According to data from the NWTSG,[48] relapses in the first four years reached 0.99% (CI 95%: 0.51-1.89), and it has been observed that in spite of the good response to the treatment of the Wilms tumor, patients with WAGR syndrome have a high risk for renal disease in an advanced stage as they reach the adult age. The cumulative risk for renal failure was estimated in 52.8% (CI 95%: 24.4-70.6%) at 20 years of age.
- **Survival:** The survival of patients with WAGR syndrome in the NWTSG[48] was 95% ± 3.0% four years after the diagnosis (compared to 92% ± 0.3% in patients without the syndrome), and 47.8% ± 17% twenty-seven years after the diagnosis (compared to 85.8% ± 1.0% among cases without the syndrome).

In summary, as it can be observed in the epidemiological data included in Table 3, from Breslow et al.,[48] the tumor is diagnosed earlier in patients with WAGR than in any other type of patients, they have mostly bilateral affectation, have a high incidence of intralobar nephrogenic rests, their tumors have a favorably histology, with general good response to the treatment and their initial survival is comparable to that of the patients with tumor but not having WAGR syndrome. However, they frequently evolve to an advanced stage of renal disease at the adolescence, what results in a lower survival rate at the adult age.

2.4. Final comments.

The epidemiological data available in relation to aniridia allow outlining several guidelines of clinical utility in affected individuals. On one hand, given its frequent hereditary nature, it is advisable to rule out minimal affectations in parents and sibs of a patient in order to provide an accurate recurrence risk assessment to the family. It is also important to perform a detailed examination to confirm or rule out the presence of other alterations of the prenatal development and, of course, a thorough ophthalmological study, since aniridia can present associated to other ocular defects. Genetic studies are recommended to look for alterations at chromosome region 11p13. Moreover, the follow-up of these patients is important, given their risk to develop Wilms tumor.

Tabla 2.3. Características epidemiológicas de los pacientes con síndrome de WAGR, en relación con el tumor de Wilms (tomado de Breslow et al.[47])

	Patients with Wilms tumor	
	With WAGR syndrome	Without WAGR syndrome
Frequency among individuals with Wilms tumor	0,75%	99,25%
Mean birth weight	2.940 g.	3.450 g
Mean age at diagnosis of the tumor	22 meses	39 meses
Bilateral tumor	17%	6%
Metastasis	2%	13%
Favorable histology of tumor	100%	92%
Intralobar nephrogenic rests	77%	22%
Survival at 4 years from the diagnosis	95 ± 3%	92 ± 0,3%
Survival at 27 years*	48 ± 17%	86 ± 1%
Unilateral renal disease in advanced stage*	11/37 (29,7%)	44/5.489 (0,8%)
Bilateral renal disease in advanced stage*	5/10 (50%)	55/440 (12,5%)

* After a mean follow-up of 12.6 years after the diagnosis of Wilms tumor

Finally, given the scarcity of epidemiological studies, whether descriptive or analytic, regarding aniridia, we consider of great importance to highlight the need to study unselected series of cases that are big enough and providing data to identify possible risk factors for this defect of ocular development that can bear so determining consequences for affected people.

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CHAPTER 3
GENETIC IMPLICATIONS OF ANIRIDIA

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3.1. ANIRIDIA and PAX6 GENE

Aniridia is a panocular disorder (MIM 106210) caused by mutations in the developmental *PAX6* gene. This gene encodes a transcriptional regulator which interacts with DNA regulating other genes function and is involved in oculogenesis and other developmental processes.

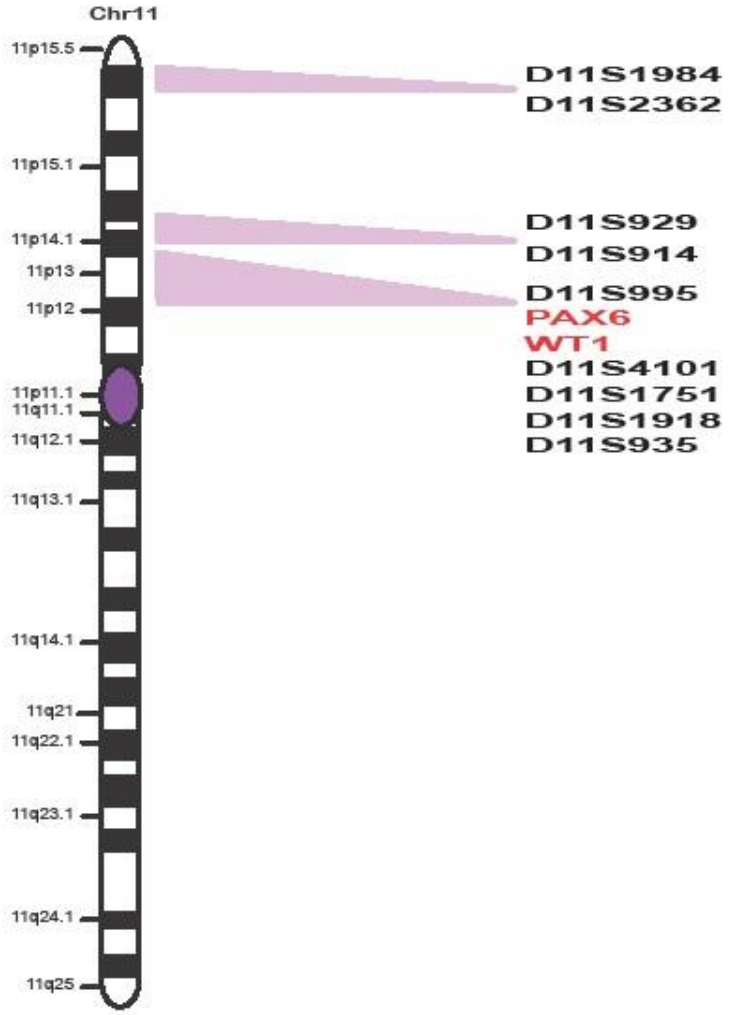

Figure 1. Chromosome 11outline showing *PAX6* gene and Wilms' tumour locus at 11p13, flanked by the microsatellite markers used for indirect or familial genetic analysis of aniridia and related phenotypes.

PAX6 gene is involved in eye embryonic development in several organisms from invertebrates as flies (*Drosophila melanogaster*)[1] to humans[2], and both its sequence and expression pattern are highly conserved along evolution, thus demonstrating its crucial biological role[3, 4]. Human *PAX6* gene maps on the short arm of chromosome 11 (11p13), close to other genes like the tumour suppressor *WT1* gene (related with urogenital development and whose mutations are responsible for Wilms' tumour) and other genes related with mental retardation (Figure 1). The *PAX6* gene encompasses 16 exons spanning 28 kb and encodes several different transcripts either due to selection of different promoters or through post-transcriptional alternative splicing. There are two major PAX6 isoforms, the "canonical PAX6"of 422 amino-acids and the isoform PAX6(5a) of 436 amino-acids. The 422-amino acids protein contains two DNA-binding domains: a bipartite paired domain (PD) of 128 amino acids, which is shared by all of the PAX proteins, and a homeodomain (HD) of 61 amino acids, both separated by a glycine-rich linker of 78 amino acids. The C-terminal domain is enriched with proline-serine-threonine (PST) residues and plays a role in the transactivation activity of PAX6 (Figure 2). The transcriptional variant PAX6(5a), produced by alternative splicing of exon 5a, contains an insert of 14 amino acids in the PD domain[5].

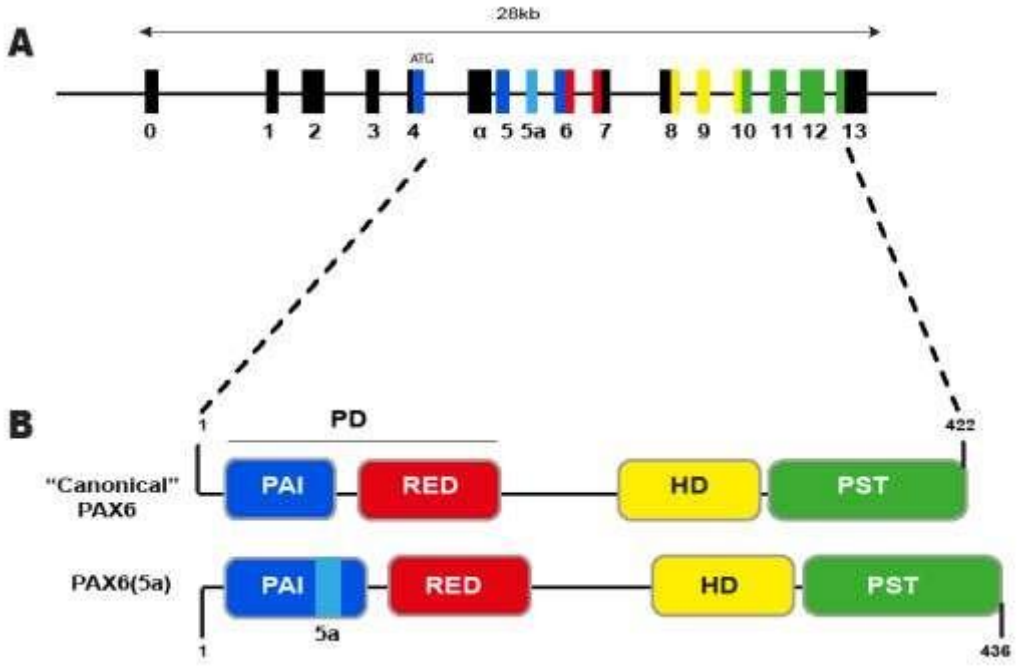

Figure 2. Gene and protein structure of PAX6. **A.** Genomic structure of the *PAX6* gene encompassing 28kb of the chromosomal 11p13 region is showed. The coding exons are coloured, the non-coding exons are in black. **B.** Schematic representation of the structure of two proteic PAX6 isoforms, the canonical PAX6 and the PAX6(5a) proteins of 422 and 436 amino- acids, respectively. The three main domains are represented. The Paired domain (PD) is subdivided in PAI (blue) and RED (in red) subdomains. The homeodomain (HD) and the proline-serine-threonine (PST) enriched domain

are represented in yellow and green, respectively. In PAX6(5a) isoforms, the oligopeptide of 14 amino-acid (in light blue), encoded by exon 5a, disrupts the PD domain (Modified of Chauban et al, 2004)[24].

Dominguez *et al*. 2004 demonstrated in *Drosophila* that both Pax6 isoforms are needed for the eye development. Pax6(5a) induces proliferation without differentiation, whereas canonical Pax6 induces differentiation in the primordial eye without ocular growth[1,6]. *PAX6* expression mainly occurs at cell nucleus in the fetal eye, brain, spine and olfactory epithelia, with an important role in their morphogenesis as well as in pancreas where it is required for the α-pancreatic islands differentiation.

During the mouse forebrain neurogenesis, Pax6 regulates other transcriptional factors and its absence affects signal molecules as retinoic acid or Rlbp1[7]. During development of neocortex, Pax6 expression is for the right specification of the main areas and plays a crucial role in the regionalization of the main divisions of telencephalon and diencephalon[8].

In pancreas, it competes with Pax4 binding the Glucagon, insulin and somatostatine promoters. The short Pax6(5a) isoform seems to function as a molecular switch for several specific target genes. During vertebrate development, *Pax6* is expressed in eyes in stem cells of optic vesicle neuroepithelium regulating their proliferations and differentiation (neurogenesis and identity of retinal stem cells)[9]. *Pax6* is expressed in different ocular regions as cornea, lens, camera angle, ciliary body and all the retinal layers. A critical dose of the Pax6 protein is required to initiate the transcription of its downstream target genes for normal eye development[10].

Beyond embryonic development, the Pax6 protein is present during all life in cerebellum, eye, and pancreas. It plays a role in cell proliferation control of corneal epithelium and in maintenance of retinal stem cells present in the ciliary body[11,12]. Pax6 regulates adult cells architecture of the pancreas islands[13].

3.2. Gene mutations in PAX6

Mutations in the *PAX6* gene are known to cause ocular disorders such as aniridia and Peter's anomaly. The ocular malformations caused by *PAX6* mutations vary considerably in pattern and severity. Iris hypoplasia, nystagmus, and foveal hypoplasia are the most common, but also microcornea related to *PAX6* mutations has been also described. Aniridia (OMIM 106210) is a rare, bilateral, congenital ocular disorder causing incomplete formation of the iris. It is phenotypically and genetically heterogeneous. In 85% of cases aniridia appears confined to the eye, as a trait either inherited as an autosomal dominant trait (two thirds) or sporadic (one third). Once the mutation occurs, it can be transmitted from the affected patient to the children in the next generation, as an autosomal dominant trait.

In around 13% of patients, aniridia is part of the autosomal dominant WAGR syndrome that associates Wilms' tumour, Aniridia, Genitourinary abnormalities, and mental Retardation[14,15]. In very few instances (around 2%)[15], aniridia occurs as part of other disorders, including Peters anomaly[16] and Gillespie syndrome[17], in autosomal dominant and recessive inheritances, respectively.

Congenital aniridia is transmitted as an autosomal dominant trait with high penetrance and variable expressivity and it occurs due to decreased dosage of the *PAX6* gene (haploinsufficiency mechanism). Mutations occur heterozygously and are scattered throughout the gene.

Animal models with both *Pax6* gene alleles altered (both copies of the gene) result in individuals with complete absence of eyes (anophthalmia), choanal atresia and severe central nervous system malformations. In humans and animal models, high lethality is observed when both alleles of the gene are mutated[10,18-20].

The *PAX6* locus-specific database currently contains 375 unique sequence changes in the human *PAX6* gene (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6, *PAX6* mutation database, last access 01 March, 2014)[21]. Over 90% of these variants are likely pathological mutations disrupting the protein. The vast majority of those reported so far lead to the introduction of a premature termination codon (PTC), causing a loss of function[22], as frameshift deletions and insertions, "nonsense" mutations, and splicing mutations that are predicted to cause premature truncation of the PAX6 protein. The remainders are probably neutral polymorphisms. Mutant transcripts containing a PTC are degraded by the nonsense-mediated decay (NMD) mechanism that prevents the synthesis of a truncated protein. These mutations that result in truncated or non- functional proteins, usually cause an aniridia phenotype associated or not with extraocular abnormalities. The PTC mutations are distributed throughout the coding region of *PAX6*, with the exception of the 2nd half of exon 12 and the coding region of exon 13. Approximately 10% of the *PAX6* mutations are amino-acid substitutions (missense mutations) that produce a full-length protein[23]. Most of these missense mutations are located in the paired domain and may affect the DNA binding function of PAX6. These mutations can produce a less severe phenotype than the aniridia but this rule is not fulfilled[24].

According to the *PAX6* mutation database[21], the most frequent mutations in aniridia are three stop gain mutations (p.Arg203*), (p.Arg240*), (p.Arg317*) and a stop loss mutation (p.*423Leufsext*108) leading to a C-terminal extension (CTE). There are four mutational hot-spots in four CpG dinucleotides located in exons 8, 9, 10 and 11, [(p.Arg203*), (p.Arg240*), (p.Arg261*), (p.Arg317*)] respectively. Mutations type "transition" at these points account for about half of all aniridia patients having nonsense mutations.

Among “de novo” cases, around 30% may present associated anomalies, usually Wilms’ tumour[25]. These associated anomalies are due to a "contiguous gene deletion syndrome”, caused by heterozygous submicroscopic deletions on chromosome 11 (11p13) affecting not only the *PAX6*gene, but alsoWT1 and other genes[26].

However, it should be noted that *PAX6* gene has regulatory elements located in either intragenic and extragenic regions, up to 165kb beyond its sequence[2]. This could explain the existence of cases of chromosomal rearrangements and mutations which do not disrupt or alter the gene sequence itself, that remains intact, but that affect *PAX6* gene control elements and cause the phenotype[27,28]. This should be taken into account, because it might explain the apparent absence of mutations in some cases of familial aniridia[29].

Tabla 3.1. Tasa de detección de mutaciones en el gen PAX6 en pacientes con aniridia, en diferentes poblaciones.

Casos mutados	Datos Clínicos	Técnica	Población	Autor
5/9 casos familiares *(56%)*	Aniridia	SSCP	INDIA	Neethirajan et al. 2006
5/11 casos familiares *(46%)*	Aniridia	Secuenciación	CHINA	Wang et al. 2006
30/54* casos *(56%)*	Aniridia o relacionados	DGGE (ex 4-13) + SSCP (ex 9 y 10)	EUROPA	Vincent et al, 2003

*13/ 18 casos familiares (16 Aniridia + 1 microftalmia+ 1 nistagmus)
*17/ 36 casos esporádicos (34 Aniridia +2 esporádicos de hipoplasia foveal)

3.3. PAX6 Associated Phenotypes

In Drosophila, *Pax6* mutations lead to *eyeless* phenotype (absence of the eyes). In mice, mutations in the *Pax6* gene are associated both to small eyes and to the lack of eyes. In humans, mutations in *PAX6* gene are mainly associated with aniridia (pan-ocular phenotype) and in some cases with Peters anomaly [MIM: 604229] (central corneal leukoma, absence of posterior corneal stroma and Descemet's membrane, and a variable degree adherence of iris or lenticular remnants to the posterior cornea). Congenital lens opacities are common in aniridia patients[30] and some *PAX6* mutations have been reported to be associated with juvenile cataract (http://www.ncbi.nlm.nih.gov/ books/NBK1360/)[30,31].

There are other possible phenotypes associated with *PAX6* mutations, as ectopia papillae [MIM: 129750), foveal hypoplasia, isolated or associated with presenile cataract [MIM: 136520], the autosomal dominant aniridia-related keratopathy [MIM: 148190]. In addition, it rarely produces ocular coloboma [MIM: 120200], optic nerve coloboma [MIM: 120430], bilateral optic nerve hypoplasia [MIM: 165550] or microphthalmia. Extraocular symptoms, such as cranial or CNS malformations, hypo- or anosmia and glucose intolerance could be also associated with aniridia. Over 50% of patients develop glaucoma and 50-85% develops cataracts[30].

There is no correlation between genotype (location / type of *PAX6* mutation) and a particular phenotype in aniridia cases and great clinical variability and differences in the clinical expression of aniridia may even be seen, as a consequence of the same mutation. Besides, there seems to be a pattern between induced alteration in protein and the ocular phenotype (anirida vs. versus other ocular phenotypes)[16]. Thus, PTC mutations are mainly associated with aniridia; while on the contrary, non-aniridia phenotypes are usually associated with missense mutations[23].

3.4. Genetic Diagnosis of Aniridia and Related Disorders

3.4.1. Mutational Analysis

3.4.1.1. Point mutations detection

Screening techniques (dHPLC, HRM, etc) and Sanger sequencing. The sensitivity for change detection of these screening techniques is variable (between 90 and 99%). Changes detected by these techniques are mostly in the coding sequence, as the regulatory elements are not usually analyzed, and they need a further identification and confirmation by Sanger sequencing.

3.4.1.2. Detection of large deletions

MLPA, FISH and familial haplotype studies (with *PAX6* chromosomal region markers). The MLPA technique detects large deletions and duplications affecting *PAX6* exons. Some commercial kits also include the *WT1* region, being very useful in "de novo" cases or in cases with suspected Wilms' tumour. Usually, point mutations are not detected.

Other techniques for genomic or chromosomal analysis (such as high-resolution karyotyping, FISH or CGH-arrays) are used for the diagnostic of large chromosomal rearrangements in the 11p13 region and therefore may affect the *PAX6* gene or its regulatory regions[32,33]. They are useful in cases of WAGR syndrome, "de novo" aniridia cases, and / or in those cases in which no alterations have been detected with the use of the previously described techniques[33].
It is worth remarking that since the existence of mutations outside the coding sequence is probable, the analysis of the coding region allows for a very variable detection rate of mutations in the *PAX6* gene, detection of 27-91% of familial aniridia cases, depending on the studied population and technique used (Table 1).

Table 1. Mutation detection rate for *PAX6* gene in aniridia patients from different populations.

Mutated cases	Mutation rate	Phenotype	technique	Population	Author
Familial	55.6% (5/9)	Aniridia	Screening	India	Neethirajan G, et al. 2006[37]
Familial	45.5% (5/11)	Aniridia	Sanger Sequencing	China	Wang P, et al. 2006[38]
Familial	70.4% (19/27)	Aniridia	Sanger Sequencing + MLPA	China	Zhang X, et al. 2011[39]
Familial + sporadic	56% (30/54)*	Aniridia + related phenotypes**	Screening	Europe	Vincent MC, et al. 2003[34]
Familial + sporadic	60% (42/70)*	Aniridia+ related phenotypes**	Sanger Sequencing + MLPA	Caucasian	Redeker EJ, et al. 2008[40]
Familial + sporadic	30% (9/30)*	Aniridia	Screening	Mexico	Villarroel CE, et al. 2008[41]
Familial	66.7% (4/60)	Aniridia	Screening	Thailand	Atchaneeyasakul LO, et al. 2006[42]
Familial + sporadic	88.9% (16/18)*	Aniridia	Sanger Sequencing	Korea	Park SH, et al. 2012[43]

-Vincent MC, et al. 2003[34]
*Familial: 72.2% (13/18), Sporadic: 47.2% (17/36).
**Familial: 16 Aniridia +1microphthalmia + 1 nistagmus. Sporadic: 34 Aniridia + 2 foveal hypoplasia
-Redeker EJ, et al. 2008[40]: *Mutated cases: not specified.**Phenotypes: not specified
-Villarroel CE, et al. 2008[41] : *Familial: 27.3% (3/11).Sporadic: 47.2% (17/36).
-Park SH, et al. 2012: *Familial: 90.9% (10/11). Sporadic: 85.5% (6/7).

3.4.1.3. Other techniques

RNA Studies

RT-PCR analysis can be used to analyze the *PAX6* messenger ribonucleic acid (mRNA) level to identify aberrant splicing events due to splicing mutations[34].

Next-generation sequencing (NGS): WES (Whole Exome Sequencing) / WGS (Whole Genome Sequencing)

In the future, the recently developed next-generation sequencing strategies will replace the previously direct screening methods and chromosomal analysis for identifying intragenic point mutations at coding and regulatory regions of *PAX6* and also chromosomal rearrangements.

3.4.2. Guidelines for Genetic Study

3.4.2.1. Familial cases

Inherited isolated aniridia cases can be addressed through direct genetic study involving the screening of point intragenic mutations and/or Sanger sequencing of the coding regions of *PAX6*. If a mutation responsible for aniridia is identified by this study, the familial mutation study may be extended to other individuals in the family. In these instances, early, prenatal or pre -implantation diagnosis is feasible.

When mutation is not identified by this first approach, the analysis can be completed with by MLPA, FISH or CGH-array techniques for the study of large and small deletions / duplications in the *PAX6* locus[4,32-33]. When no point mutation or deletion /duplication has been detected with the previous techniques, the karyotype can be useful to identify chromosomal rearrangements, without genetic material loss, displacing or separating the regulatory regions from the *PAX6* gene coding sequence.

If no mutation is identified with the previous techniques, it is appropriated to perform an indirect genetic study, analyzing extragenic polymorphic markers within the chromosome 11p13 region, in all healthy and affected family members, to confirm if there is linkage between the aniridia locus and the clinical phenotype. This last approach, in the absence of identified mutation, allows for discarding cases of possible genetic heterogeneity (the gene responsible for the phenotype could be other than *PAX6*).

Although not mutation is detected, some cases present high suspicion of extragenic or outside the coding sequence alterations. This way, when co-segregation between linked markers and aniridia exists, and when the haplotype linked to the disease is known, it is possible to perform prenatal genetic diagnosis.

In cases in which mutation has not been identified and the linkage to *PAX6* cannot be excluded, sequencing the entire locus of *PAX6* should be addressed to identify alterations in the regulatory region and for those variants found, functional analyses have to be made to establish their pathogenic role.

Despite it has not yet performed for uncharacterized cases of aniridia, as in any case without genetic diagnosis, analysis by exome or genome sequencing could be useful as a research tool to identify new gene(s) or genetic mechanisms causing aniridia out of *PAX6* gene.

3.4.2.2. “De novo” cases

When facing the diagnostic of a “de novo” aniridia case, whether it is a newborn or child under four years of age, it is urgent to discard large deletions that include not only the *PAX6* gene but the *WT1* gene, due to the high risk of developing Wilms’ tumour[35]. Therefore, deletion analysis in these patients is recommended first using MLPA and/or CGH-array, because of the clinical importance of detecting deletions associated with WARG syndrome.

3.4.3. Indications for Genetic Study

The indications for the genetic study of aniridia are[36]: 1) the presence of isolated or syndromic aniridia (WAGR syndrome), or 2) the presence of any of the possible phenotypes associated with mutations in the *PAX6* gene, such as Peters anomaly, ectopia pupillae, foveal hypoplasia isolated or associated with presenile cataract, autosomal dominant aniridia-related keratopathy. Other less common indications (and more unlikely to have mutational result) are colobomas (ocular, or optic nerve) and bilateral optic nerve hypoplasia.

3.4.4. Reasons for Genetic Study

Genetic Study is useful for[36]:

a) Clinical diagnosis confirmation, when causative genetic defect is identified (clinical diagnosis cannot be discarded in those cases without identified mutation).
b) Genetic assessment: advising of the risk of repetition to affected and unaffected relatives. This can also be predicted based on clinical diagnosis and patients family tree, even before the molecular study.
c) Assessment of prognosis and / or evolution in mutations with known genotype-phenotype correlation.

It is essential to:

a) Know the risk of Wilms' tumor in "de novo" cases of congenital aniridia in infants or very young patients.
b) Perform a preimplantational or prenatal diagnosis.

3.4.5. Protocol for Genetic Study

The protocol for the genetic diagnostic and genetic clinical management of patients with aniridia[36] is reflected in Figure 3.

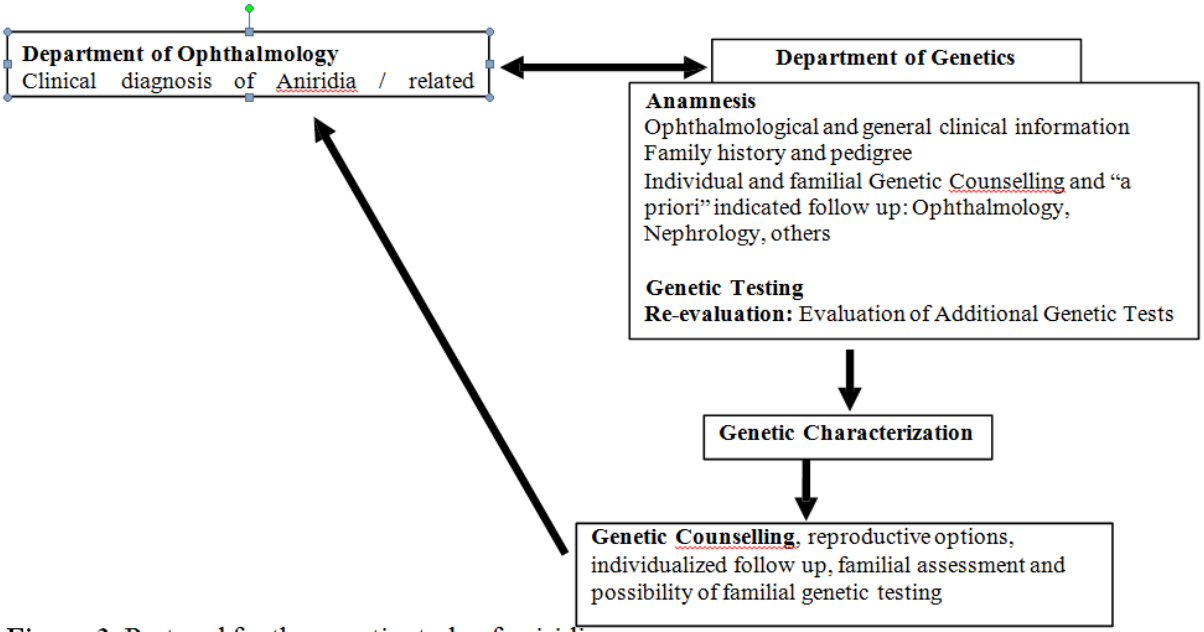

Figure 3. Protocol for the genetic study of aniridia

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CHAPTER 4

MAJOR SYNDROMES ASSOCIATED WITH ANIRIDIA

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4.1. Introduction

Severe aniridia, which often results in blindness, cataracts and congenital glaucoma, can be associated with Wilms tumor (WT) in an isolated way, although it is more commonly associated with several anomalies leading to different syndromes, the best known being called WAGR syndrome (Wilms tumor, Aniridia, Genitourinary anomalies, and mental Retardation). The multiple abnormalities that can accompany this syndrome are caused by a deletion of genetic material from chromosome 111. The FISH2 (fluorescence in situ hybridization) molecular cytogenetic technique must be used to confirm these deletions (see Chapter 3). Mutations of the PAX6 gene, located on chromosome locus 11p13, are responsible for about 80% of cases of aniridia, both sporadic and familial. Between 33 and 50% of patients with WAGR are at risk for WT. WT occurs in association with severe isolated aniridia in around 50% of cases.

4.2. Signs and symptoms of WAGR syndrome (SW)

The diagnostic criteria for *WAGR syndrome* require aniridia to always be present and at least one of the other three anomalies. Some children with WAGR have a low birth weight and most of them have short stature and microcephaly. No other specific signs or problems have been reported in studies involving the analysis of large series of patients although severe obesity has been observed in nearly 20 percent of cases described. In some cases, especially in older children, psychiatric disorders have also been described[3].

Aniridia occurs in 1/50,000 to 1/100,000 individuals. Two thirds of cases involve families with autosomal dominant aniridia caused by PAX6 gene[4] alterations. The remaining one third of cases of aniridia is sporadic and is frequently associated with WAGR syndrome, showing a high risk for WT.

Aniridia, or iris hypoplasia, per se causes photophobia and can be associated with foveal hypoplasia, optic nerve hypoplasia, cataract, lens subluxation and glaucoma. Other clinical manifestations that may be associated with aniridia are nystagmus, amblyopia and strabismus. Blepharophimosis, microphthalmia, palpebral ptosis, anterior segment anomalies and retinal dysplasia occur more rarely but some cases have been reported[5].

The precise risk for Wilms tumor development is unknown, although several surveys estimate it to be between 30 and 50%, with a higher risk for males (62% males, 38% females). In 80% of WT cases, patients are diagnosed before the age of 5, but there have been a few reports of cases in patients over age 20. Deletion of two tumor-suppressor genes for WT have been identified: WT1, which is located at chromosome 11p13 and WT2, which is at

chromosome 11p15. Mutations in these genes contribute directly to alterations in the development of the genitourinary tract[6,7].

WT is the most common malignant kidney tumor in children and at present is one of the best examples of the good results that can be obtained in Pediatric Oncology by using multidisciplinary treatments and protocols. Overall cure rates are close to 80% and in some stages are higher than 90%[8].

In over 70% of cases, WT manifests clinically as an abdominal mass with few, if any, accompanying symptoms which is usually discovered by chance by a parent or the pediatrician during a routine check-up (Fig. 4.1). More rarely, patients may present with abdominal pain, hematuria or hypertension. Prognosis is basically related to the histological type of the tumor, its clinical stage and the age of the patient.

Being an embryonic tumor, the histological type usually contains epithelial tissue that is blastemal or mesenchymal, although in 5% of cases anaplastic or sarcomatous foci may appear. These are called unfavorable histology WT and account for more than 60% of deaths. Patient age also tends to be directly related to prognosis, which is usually more favorable in those cases diagnosed in children under the age of one year. With regard to clinical stage, five stages are described, depending on how much the tumor has spread (Table 4.1), with a

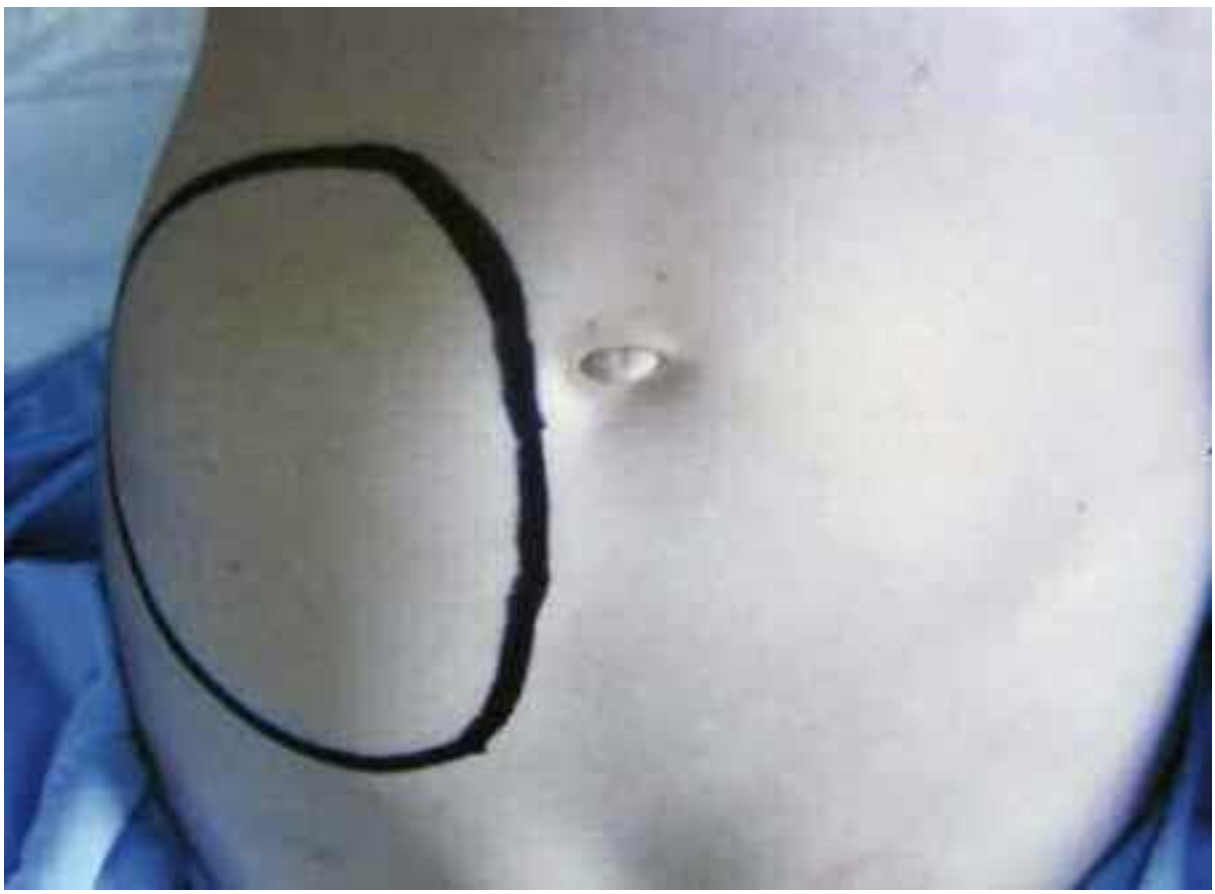

Figure 4.1. Abdominal mass in the right flank that does not cross the midline

Table 4.1. Stages of Wilms Tumor

Stage I.	The tumor is limited to the kidney and is completely resected.
Stage II.	The tumor extends beyond the kidney (fat, pelvis, vein) but is completely resected.
Stage III.	Incomplete resection of the tumor.
Stage IV:	Distant metastases.
Stage V:	Both kidneys are affected.

progressively poorer prognosis the more it has spread. In stage IV, metastases occur almost exclusively in the lung (Fig. 4.2), with bone or brain metastases being very unusual and, in the event, usually related to cases of unfavorable histology.

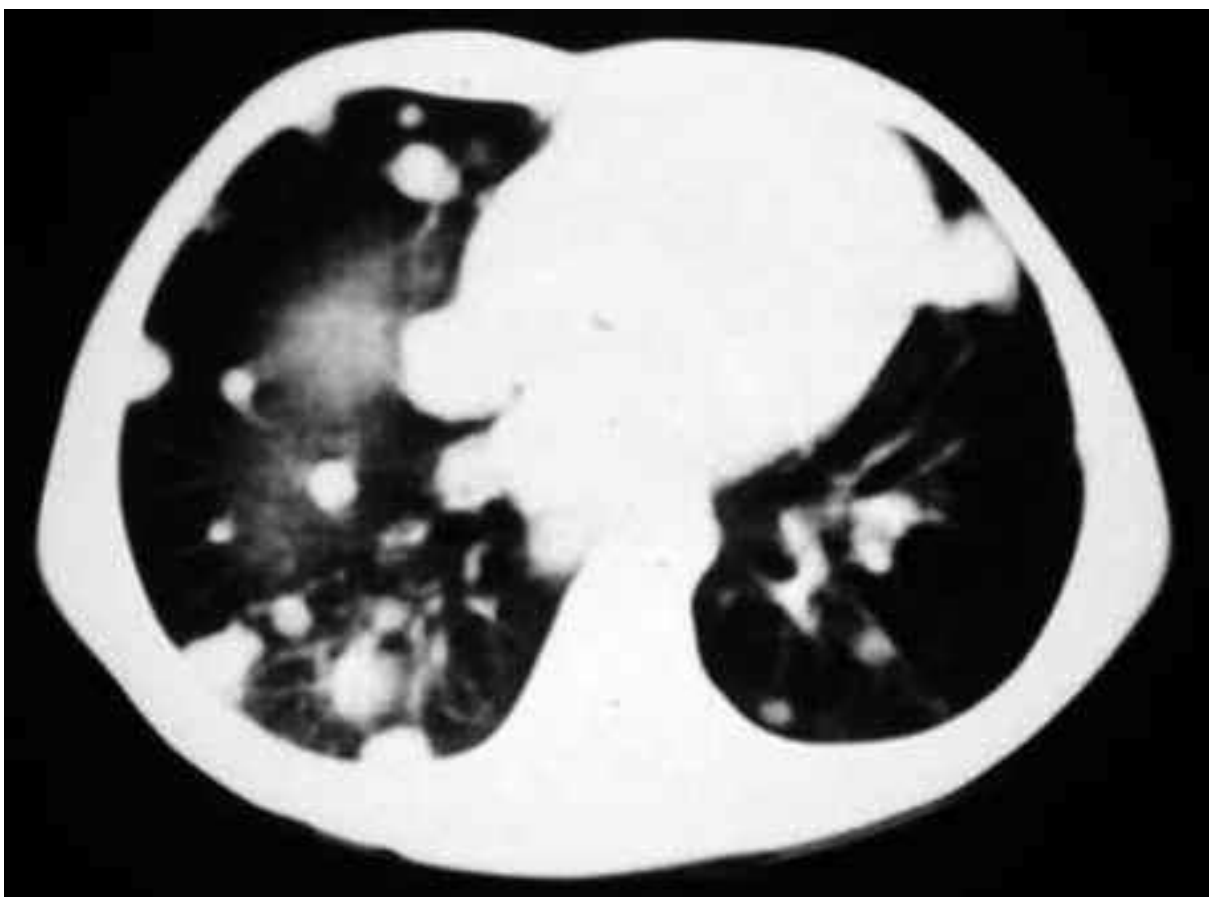

Figure 4.2. Chest Computer Tomography. Scan showing multiple lung metastases

With respect to treatment, there are globally two major trends in therapeutic approaches, one led by the European SIOP (Société Internationale d'Oncologie Pédiatrique), which advocates preoperative chemotherapy (CT) and the approach followed by the NWTSG (American National Wilms Tumor Study Group), which recommends surgery as the first phase of treatment[9].

SIOP protocols are followed in our country (Spain), with preoperative administration of vincristine and actinomycin D during a period of 4-8 weeks. The aim is to reduce the tumor size (which is highly chemosensitive) to make surgery easier and minimize the risks involved, as well as to prevent a possible rupture of the tumor during the procedure, as they are often large tumors (Fig. 4.3). The surgical procedure involves the en bloc removal of the tumor, kidney and ureter (Fig. 4.4) as well as an examination of the other kidney and nearby lymph nodes. Postoperative treatment will consist of more or less aggressive CT (actinomycin D, vincristine, carboplatin and epi-adriamycin) on the basis of favorable or unfavorable histology and clinical stage. Radiation therapy is usually reserved for patients with unfavorable histology.

4.2.1. Genitourinary malformations

Some reviews of WAGR syndrome show that nearly 40% of patients develop kidney failure within about 20 years after a diagnosis of WT and that after 25 years the cumulative risk for renal failure is 60%. Glomerulosclerosis is the most common cause of kidney failure in patients with WAGR, being found in more than 50% of patients over the age of 12 years who are affected by this syndrome. Proteinuria is usually the first sign, so it is advisable to have a urinalysis done every 6 months and, subsequently, refer the patients to an experienced nephrologist for management, as most of them will eventually require a kidney transplant[10].

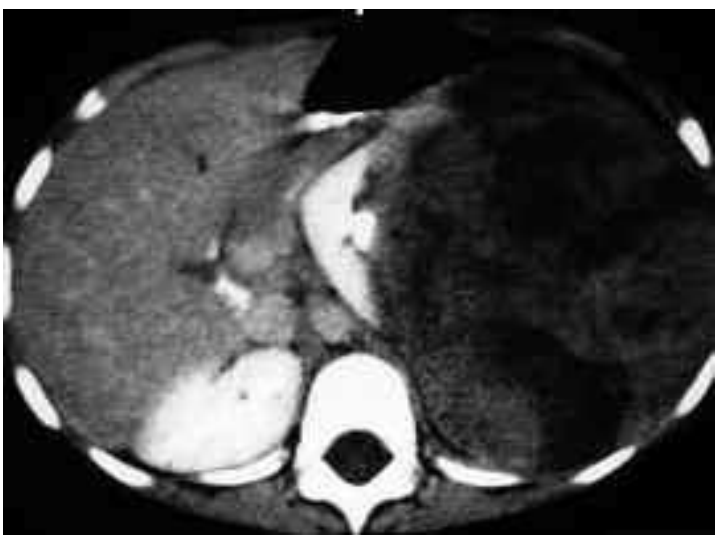

Figure 4.3. Abdominal Computer Tomography with contrast. Large left renal tumor that displaces the kidney

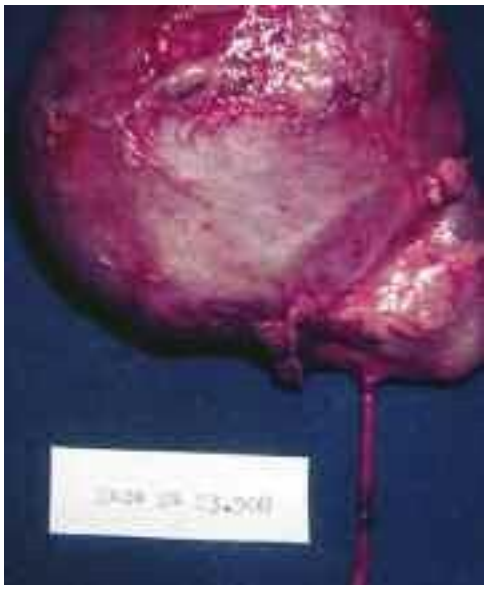

Figura 4.4. Surgical specimen including the tumor, kidney and ureter

The genital abnormalities that are most frequently present in nearly 90% of males are cryptorchidism, hypospadias, micropenis and hypoplastic scrotum. Rarely they may present with severe genital ambiguity, although every newborn with an unexplained severe genital anomaly should be examined by an ophthalmologist to rule out aniridia or iris hypoplasia. No external genital abnormalities have been described in girls but uterine and ovarian anomalies have been reported. Gonadoblastomas have been reported in both sexes so it is advisable for girls to have an annual pelvic ultrasound and for boys to undergo regular testicular examination, and in both cases biopsies should be performed if there is any reasonable doubt.

4.2.2. Neurological manifestations

Mental retardation is the most commonly found neurological disorder in these patients. Approximately 60% of WAGR patients present with motor impairments (hypotonia, hypertonia, incoordination), neuromuscular disorders (kyphosis and scoliosis) and absence crisis, so all of these patients must be examined by a child neurologist. They will require early stimulation or other treatment strategies that are the standard of care for their associated problems.

4.2.3. Other signs and symptoms

Other anomalies that have been described in some series, although with a lower incidence, are E.N.T. infections (ear and sinus) in about 10-12% of patients, reactive airway disease in 14-15% and, more rarely, heart defects, diaphragmatic hernia, cleft palate and exostoses.

While it is important to know that these anomalies may be present in patients with WAGR syndrome, it is also very important to know that 50% of patients will not present with WT, that genital abnormalities may be minimal or non-existent and that a significant number of individuals will have IQs that are normal or even above normal. The diagnosis of WAGR syndrome will be difficult to establish in some of these patients. They should be periodically examined by different pediatric specialists with experience in the different types of abnormalities that may be present. In addition, frequent radiological studies should be performed (Table 4.2).

4.3. Gillespie syndrome

This is an extremely rare genetic disorder that involves eye and brain abnormalities: partial aniridia, cerebellar ataxia and mental retardation. It was first described in 1965 by this author in two siblings aged 22 and 19 years. It is an autosomal recessive disorder and to date no genetic mutation has been found, unlike congenital dominant autosomal aniridia, which is the result of a mutation in the PAX6 gene.

From the clinical point of view, from birth the infant has bilateral pupillary dilatation which is areflexic to light. During its first few months of life, the infant keeps its eyes closed because of intense photophobia. Slit lamp examination shows partial aniridia with some specific features of this syndrome. Visual acuity is limited. It may present with palpebral ptosis and very often glaucoma develops. Computer tomography and MRI imaging studies show

cerebellar hypoplasia or atrophy. It is necessary to establish the differential diagnosis to distinguish it from WAGR syndrome[11].

Table 4.2. WAGR syndrome management

Newborn infants:	Genetic analyses Eye exam by a pediatric ophthalmologist E.N.T. examination Detailed information to parents
1 month to 1 year:	E.N.T. examination Ultrasound every 3 months Evaluate psychomotor development
1 to 5 years:	Abdominal ultrasound every 3 months Neurological symptoms evaluation Endocrine evaluation (obesity) Nephrology evaluation Orthopedic treatment E.N.T. exams
5 to 13 years:	Pediatric neurologist Ophthalmology exam Endocrine and orthopedic exams Ultrasound every 3 months until the age of 6, afterwards every 6 months Nephrology and dietary follow-up Dental examination
13 to 21 years:	Developmental follow-up Neuropsychiatric follow-up Nephrology and endocrinology Dietary follow-up Dentist and orthodontist

4.4. Potocki-Shaffer syndrome

This is a disorder in which aniridia is associated with multiple exostoses. These are cartilaginous growths that develop in the diaphysis of long bones. This anomaly is linked to deletion of the EXT2 gene, located at chromosome 11p11.2.

4.5. Marinesco-Sjogren syndrome

This is an extremely rare association of aniridia with congenital cataracts, cerebellar ataxia and mental retardation. Only a few cases have been reported and the genetic alterations that cause it are not yet well known

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CHAPTER 5

EXAMINING THE CHILD WITH ANIRIDIA

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5.1. Introduction

When examining a newborn with aniridia, we should remember that the disease is uncommon (it is estimated to affect one in 80,000-100,000 people in Spain) and that it is difficult in this first exam to assess the extent of damage within the severity of the disease itself. In most cases, aniridia is characterized by the partial or complete lack of iris tissue. It is a congenital bilateral ocular defect, sometimes asymmetric, with a strong hereditary component. Some authors dispute the use of the term aniridia since, as the name suggests, the whole iris is not lacking and gonioscopy generally shows the presence of the iris root. In addition, ocular structures other than the iris are affected. Thus, it is not unusual to find cataract, lens luxation, glaucoma, corneal opacities, foveal and optical nerve hypoplasia and other primary or secondary conditions such as ptosis, ocular motor alterations or nystagmus.

The cardinal sign of aniridia is iris hypoplasia. The residual root stump varies in size to give rise to a lesser or greater amount of iris, such that it may be confused with a coloboma.

Another characteristic manifestation of aniridia, which determines greater visual impairment, is foveal hypoplasia. Its typical sign is a reduced or lacking foveal reflex. Fluorescein angiography shows a diminished or absent avascular foveal zone. In some patients, retinal vessels crossing this zone cause macular pigmentation alterations. These foveal anomalies appear from birth. Optic nerve hypoplasia is less frequent, but is also an important limiting factor for patient visual acuity. Both abnormalities, which usually lead to the appearance of a sensorial nystagmus, determine a low visual acuity, generally under 20/200 (0.1).

The development of glaucoma in patients with aniridia is fairly common, affecting more than fifty percent. Glaucoma secondary to aniridia is mainly caused by congenital anomalies of the angle, including absence of Schlemm's canal and gradual closure of the camerular angle due to anterior rotation of the iris root.

Keratopathy occurs later. It usually commences in the periphery, appearing as a haze in the Bowman's membrane and anterior stromal neovascularization. Over time, it may progress to affect the central area, diminishing visual acuity to an extent that warrants a penetrating keratoplasty.

Photophobia is the initial symptom of aniridia in most cases.

5.2. Examination

Aniridia is usually diagnosed at its early stages, except abortive or rudimentary forms, in which the presence of a greater amount of iris tissue decreases the clinical manifestations and typical signs of this disease. In dark eyes, diagnosis may be delayed for the same reason. In general, the newborn with aniridia will closes the eyes in response to light and feel more comfortable in the dark. Diagnosis is hindered by a child's lack of cooperation and the imprecision of available tests.

The examination of a child with suspected aniridia should seek to confirm or rule out its presence, detect other ocular manifestations of this syndrome, screen for the systemic abnormalities that may accompany sporadic forms, and guide genetic tests. We should bear in mind the patient's age and level of cooperation to select the most appropriate tests and interpret their results.

5.2.1. Anamnesis

Before the initial examination, we should obtain the following data in a complete anamnesis:

5.2.1.1. ***Patient clinical history.*** It is important to collect information about any possible *accompanying systemic disorder,* possible genital-urinary alterations along with information on the child's mental development, especially if the disease is sporadic. The results of *genetic studies* should also be obtained. Parents should also be asked about possible incidences during pregnancy, labor, etc.

5.2.1.2. ***Family history*****.** Almost two thirds of patients with aniridia have the familial, or autosomal dominant, form of the disease. However, affected family members are uncommon, though these could have abortive or minimally expressed forms. This determines a need to examine the family members of children with aniridia, since they may have a less obvious form of the disease without being aware of this. Further, we should obtain information on any other family ophthalmologic condition.

5.2.1.3. ***Disease history and progression.*** Parents should be asked when symptoms commenced in the child, how the child is doing and whether there are any complications. Patients with aniridia have intense photophobia, this being the initial symptom in most cases. We should also ask about previous treatments and the use of protective glasses for outdoors or visual aids. Finally, parents should be encouraged to contact the Spanish Society of Aniridia or, if applicable, the Spanish organization for the blind ONCE.

5.2.2. Examination procedure

The examination assesses both the child's ocular sensory (including visual acuity) and binocular, or motor, function.

5.2.2.1. Visual Acuity (VA)

Visual acuity is generally low (under 20/100, 20/200). As mentioned earlier, impaired visual acuity is related more to foveal or optic nerve hypoplasia than to the aniridia itself. However,

VA cannot be accurately predicted in the fundus exam conducted in the first months of life. In addition, further manifestations of the syndrome such as keratopathy, congenital cataract or glaucoma, may reduce vision. To determine VA, we first need to select the most appropriate test for the child's age. In babies up until the age of two years, fixation assessment, the preferential looking test or visual evoked potentials test can be used; the OKN drum can be used in some. Table 5.1 lists the different tests suitable for children of different ages along with normal test results.

In a healthy infant under two months of age, we can only assess the photomotor reflex (absent in patients with aniridia) and fixation (e.g., detecting whether the infant follows a moving face (usually this is possible around week 4-8 of life). Responses, nevertheless, vary because of considerable variations in maturity (without repercussions throughout life). When examining a premature baby, the response should be interpreted after correcting for age. Examining a young child is not easy and sometimes several tests are needed, or a single test with contradictory results will have to be repeated.

Table 5.1. Acuity testing and normal test results in children

Age (years)	Test	Normal result
2 or younger	Fixation	CSM
2 or younger	Preferential looking	0.23 to 9.8 cy/cm
2 or younger	Visual evoked potential (VEP)	
2-3	Pigassou test	0.4-0.8 (projector at 3 m) 0.5-0.6-1.25 (screen at 3 m)
3-4	Lea-Hyvärinen	0.32-1.0 (at 3 m)
4-6	Snellen	0.5-1.0
> 6	Letters	0.5-1.0

From 2-3 months of age, responses are more reliable, and the fixation test, preferential looking test and in some cases visual evoked potentials test can be performed.

Ocular fixation. Monocular and binocular fixation are assessed. For monocular fixation, the infant is shown a small object and we observe if the child fixes centrally with the

fovea. If he/she does not fix with the fovea this means that VA is 20/200 or lower. After separately covering each eye, the smallest object the infant can follow is determined. Fixation is recorded as good, intermediate/bad, central or eccentric and maintained or sporadic using the acronyms CSM (*Central, Steady, Maintained*) and UC, US and UM as follows:

The first letter, C, indicates "central fixation", detected as a central light reflex in the center of the cornea that is symmetric in both eyes. If the reflex is eccentric, fixation is recorded as UC (UC=*uncentral*). The second letter, S, describes how stable the fixation is (S= *steady*, US= *unsteady*) and the third letter, M, describes the extent of monocular fixation maintenance and binocular alignment. When fixation is maintained in one eye but not the other, there may be considerable difference in VA between both eyes (M=*maintained*, UM=*unmaintained*). This fixation can be assessed using the Gracis biprism as described below. In some children, inducing a small squint using a vertical prism (of 10 D) is useful to assess fixation maintenance capacity. Finally, the child's capacity to realign is recorded as rapid, after blinking or after a more prolonged blink.

Some clinicians prefer to use the acronym FF (=*fix and follow*) or Spanish terminology for the CSM system.

Preferential looking test. This test is based on a young child's natural preference to look at a bold pattern rather than a blank area. This was initially a fairly sophisticated test but has been simplified over time and is currently performed using Teller acuity cards (see Fig. 5.1). The test consists of a set of cards (9 in the basic test, 16 in the extensive) each with a central peephole, through which the infant's response is observed. Each card has on one side a patch of vertical black-and-white stripes with constant contrast (90%). From one successive card to the next, gratings decrease in thickness such that their spatial frequencies measured in cycles per centimeter (Cy/cm), each time go up by half an octave. The cards are presented through a window, alternating cards with the gratings on the right with those with the gratings on the left. Without knowing which side the gratings are, the observer determines which side the child looks at and after checking that the response is correct, moves on to the next card until the card with the thinnest stripes distinguishable is detected. The preferential looking test provides an illustrative measure of visual perception. Tables exist of measures of normality of perception obtained using Teller cards in children of different ages and possible Snellen equivalences [1,2,3]. Although this test requires no identification skills, it is affected by psycho-affective factors and the child's attention at the time of the test. When macular lesions exist, VA is overestimated. The test, nevertheless, is non invasive and gives a rapid measure of visual perception in patients unable to cooperate avoiding the need to wait for the results of electrophysiological tests. It is usually available (or should be) at most pediatric centers. It is more effective in children under 12 years. Beyond this age, it is difficult to keep their attention.

Figure 5.1. Preferential looking test

In Table 5.2, we provide a list of Teller card visual acuity measures obtained in children with aniridia examined by us and in a control group of 40 healthy children. All children were 1 to 12 months old. Up until the age of two months, many of the healthy children did not cooperate. In all the infants with aniridia, lower VA values were obtained than in the children without the disease, although VA varied among the affected infants. Also, newborns of only a few months of age were unable to distinguish even the clearest test, though with age, some perception was acquired.

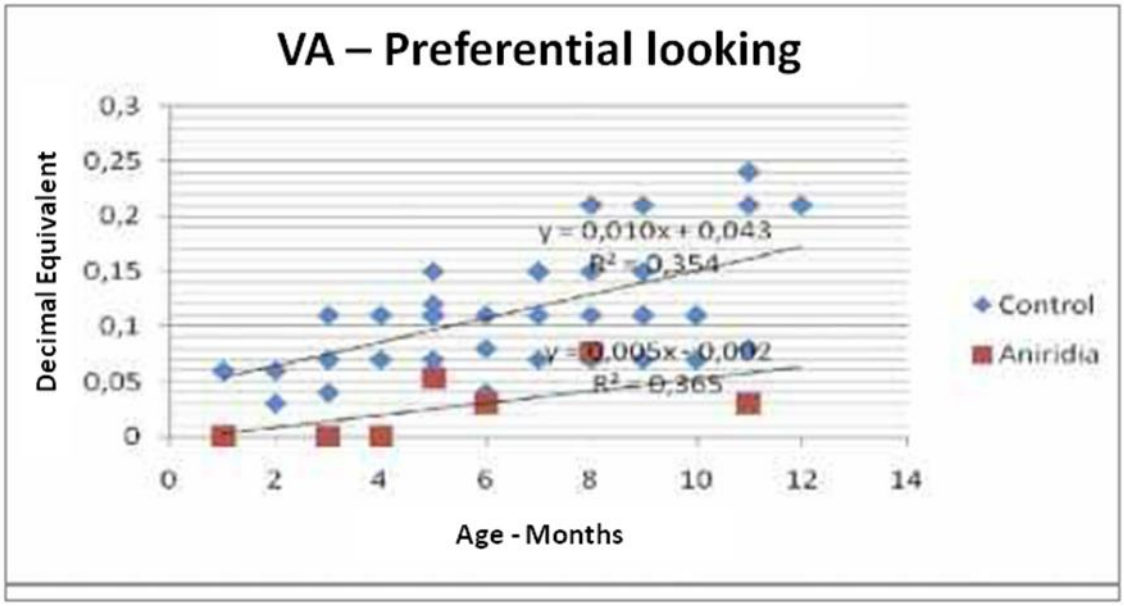

Table 5.2. Teller visual acuity measures recorded in infants with aniridia and in 40 healthy age-matched controls

Visual evoked potentials (VEP) test. The results of this test are usually abnormal in patients with aniridia although a normal test result may be obtained[4]. In the flash VEP test, latency and amplitude are modified while the response to the pattern VEP test indicates improved central retinal activity[5]. Electrophysiological experiments have generated Snellen equivalences. The drawbacks of this test in children are the need to sedate the child and a need for a team of pediatric electrophysiologists. In contrast, the electroretinogram is normal in patients with aniridia[6].

In children older than two (and nearly up until the age of three, depending on the child's cooperation), we can use verbal tests with pictures, such as Pigassou optotypes (Fig. 5.2A) or Lea-Hyvärinen symbols, also known as the LH (light-house) test (Fig. 5.2B). In most children, the first test with which we can measure visual acuity is the Pigassou. This test provides an improved measure over the preferential looking test but it is still imprecise: images are not equivalent in identification difficulty and may be comparable to larger images. In addition, memory contributes to the score obtained. A Pigassou visual acuity of 1.25-1.50 may vary by two lines from the result of the Snellen test[2]. In our patients, we recorded a VA of 0.1-0.6 using Pigassou symbols compared to 0.05-0.3 using the LH test. When some of these children were examined as adults, their Snellen VA was lower than their Pigassou value (e.g., Pigassou 0.6 versus Snellen 0.1), while correlation was much higher for the LH test. Most children had a different VA in each eye attributable to their aniridia per se and to amblyopia. Most had an associated tropia/microtropia/anisometropia and responded well to treatment.

In children with aniridia and very low vision, tests with larger pictures are required. However, fewer symbols on the chart will determine that memory may affect the results. It is

therefore best to use individual optotypes although this will prevent overlapping. For a VA worse than 0.05, we use the Feinbloom chart.

VA is usually determined first in the right eye and then the left. The eye is covered using a patch to make sure the child is unable to use the covered eye. If the whole line can be distinguished, then the corresponding VA is recorded (e.g., 0.3). If the line is difficult to identify, the figure recorded should be 0.3 (-1 or -2) for one or two erroneous symbols respectively, or 0.3 (+1 or +2) if one or two symbols in the next line are distinguished. When using isolated optotypes, the size of the test and the number of symbols seen by the child should be recorded; for example 0.1 (4/4) or 0.1 (3/4). Children with aniridia often show considerable variation in VA between visits (e.g., 0.1 to 0.2). Cooperation is poor in young infants so it is difficult to identify differences. Often tests need to be conducted at half distance so that more optotypes may be used. If the patient has a latent nystagmus, rather than occluding the non-examined eye it is better to penalize it with a translucent filter or a +8 D lens.

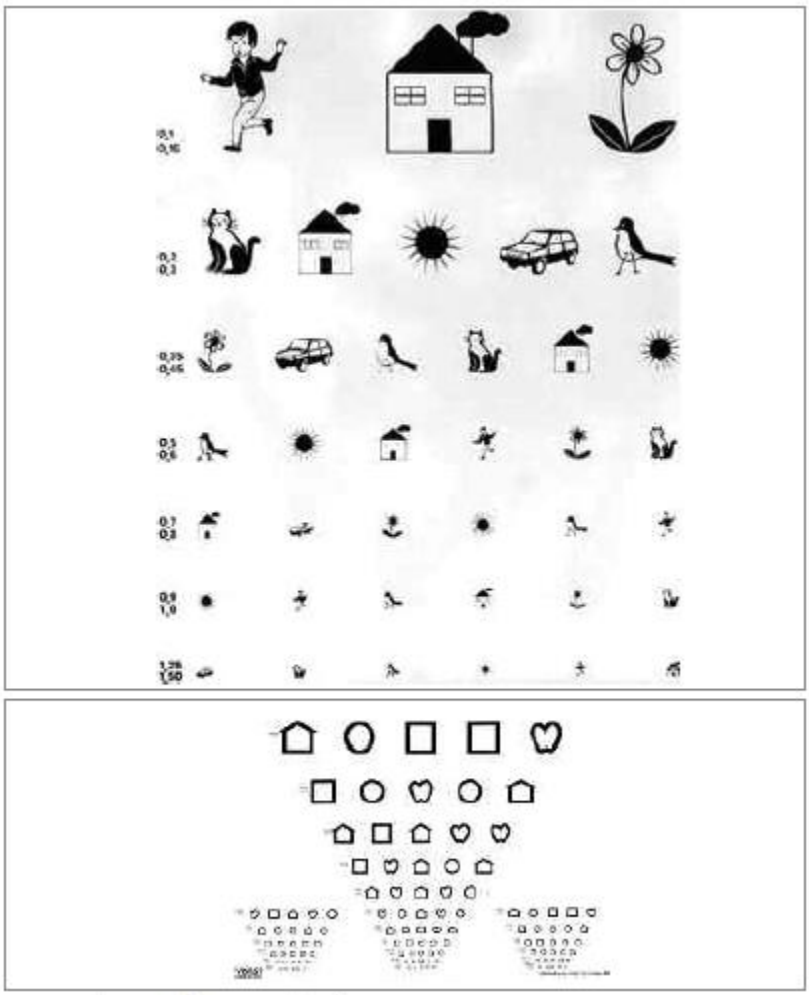

Figure 5.2. A) Pigassou optotypes B) LH test

The Snellen test can be used from the age of 4 years and letter optotypes beyond 6 years. Some aniridia patients will have a VA<0.05 and others a high VA, but typically VA is 0.1-0.2.

Near VA should also be recorded. Pediatric scales exist based on the same optotype formats as for distance visual acuity. Near VA is usually higher than distance VA. It is important that the test distance is 33 cm. Accommodation may be preserved in patients with aniridia despite being lower than normal. Near VA should be measured using plus lenses.

5.2.2.2. Refraction

In a general ophthalmologic examination, the pinhole is used to determine whether vision will be improved by correction or if the patient's normal correction can be improved. This test is inconsistent in children both with and without aniridia, and is even less useful if the disease is accompanied by a nystagmus.

Child aniridia patients usually have refractive defects. In a series of 14 children younger than 3 years, mean refraction was -1.37 ± 4.07 D. Eight children had myopia (spherical equivalent -1 to -7.75 D), 3/14 had a refraction of +1 to -1 and 3/14 had moderate hyperopia (+3.5 to +6). Most had astigmatism (2-4 D), and 10/14 had 1 to 3 D of anisometropia. In addition, in successive exams we noted a higher than usual variation in the refractive state of these children, that we attribute to an abnormal lens position during growth, corneal modifications and to the inherent difficulty in testing low-vision eyes, in which foveal fixation cannot be assured.

Refraction testing in children with aniridia should be conducted with *cycloplegia*. The use of *retinoscopy* is more precise though in our experience both methods should be used. Pupil dilation might be considered unnecessary because of iris hypoplasia though some accommodative power persists, albeit lower than in healthy children. For cycloplegia, we prefer the use of two drops of cyclopentolate given 5 minutes apart 45 minutes before testing. Atropine is not recommended since accommodation is normally reduced, an additional visit is needed and near vision is impaired for longer. In our series of 13 children (aged 2-36 months) with aniridia, refractive state was determined by pediatric autorefractometry and band retinoscopy with and without cycloplegia (Table 5.3). A myopic shift of -2.64 ±2.1 D spherical equivalent was detected between refraction with and without cycloplegia and of 1.13 ±4.65 D between retinoscopy with and without cycloplegia. The pediatric autorefractometer is used for refractometry without cycloplegia in the young child (Fig. 5.3). This method has its drawbacks since we cannot control fixation when VA is low. We have also detected higher variation due to accommodation. Finally, some child patients have cataract, which even if mild can modify the autorefractometry response.

Children older than two are able to cooperate more and, depending on the patient, we can gradually introduce more adult tests. The use of lens frames is recommended. In every check up visit, at least one retinoscopy without cycloplegia should be performed and pupil dilation indicated if a marked change is detected. A large shift to myopia may be a sign of ocular hypertension.

Table 5.3. Refractive state determined in 13 children with aniridia (aged 2-36 months) by pediatric autorefractometry and retinoscopy with and without cycloplegia

SPHERICAL EQUIVALENT	(Mean ± standard deviation)
Autorefractometry without cycloplegia	-3.57 ± 3.42 D
Autorefractometry with cycloplegia	-1.59 ± 4.22 D
Retinoscopy without cycloplegia	-2.19 ± 3.74 D
Retinoscopy with cycloplegia	-1.37 ± 4.07 D

Figure 5.3. Pediatric autorefractor

5.2.2.3. **Ocular motility and binocular vision tests**

Most children with aniridia have strabismus, amblyopia or fusion defects. Even if mild, strabismus needs to be assessed to detect a possible amblyopia requiring treatment. In our patient series, most children had low-angle microtropia or endotropia, which in some cases progressed to exophoria/tropia. Some children (those most affected) showed considerable exotropia (Fig. 5.4).

Most of the children in our series presented with amblyopia during check up visits and were treated. When possible to measure, stereopsis was 400 seconds of arch. In most of the patients, nystagmus, usually pendular/jerky was observed. Nystagmus was more intense in the children with more severe disease and sometimes improved between the age of 1 and 2 years. Two children had severe chin-up torticollis and "setting sun" eyes which improved over time.

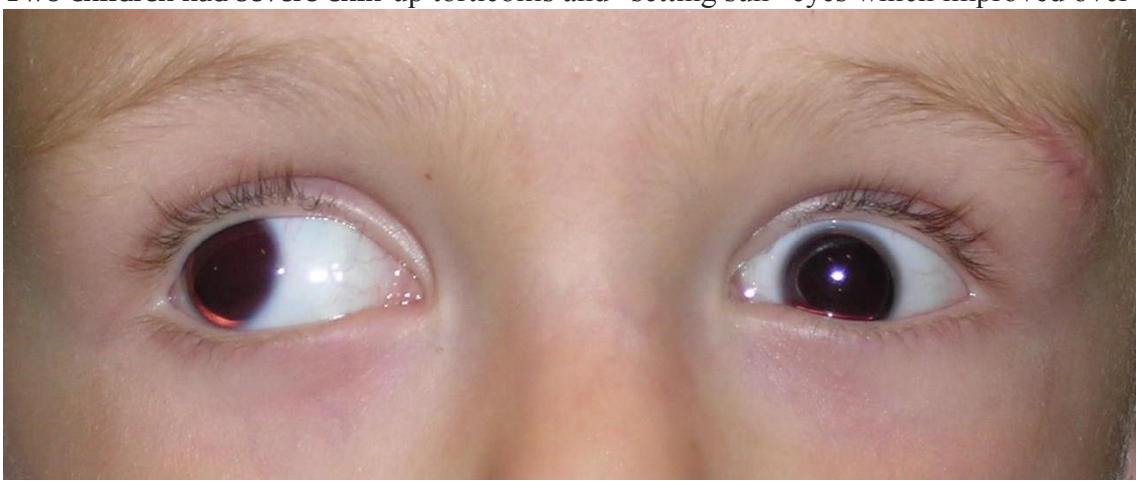

Figure 5.4. Exotropia in a child with aniridia

The motor and sensory examination of strabismus is extensive, and there are numerous published reports on this topic. The description below is a general overview of the basic examination normally performed in pediatric aniridia patients.

Motor Evaluation

1. We should first determine if there is manifest tropia upon visual inspection and describe the nystagmus in terms of its: direction (horizontal, vertical or rotatory), frequency (constant or variable – the latter is common in severe cases), pendular or jerky, right- or left beating, and amplitude. Later on a more precise assessment is conducted using measuring devices, but this initial examination gives an overall picture of the child's condition without much cooperation needed. During this exam, the presence of orbito-facial asymmetries or torticollis should be recorded.

2. Ocular rotations in the 9 positions of gaze. In children it is better to start with ocular versions. If the patient is unable to follow the object, the head may be moved to examine its movements. We should record any vertical deviations in lateral versions or oblique positions and also make note of the behavior and characteristics of the nystagmus. To draw the child's attention, rather than using numerous toys we should replace the object presented. It is common in a patient with aniridia and low vision that he/she cannot always follow small objects. If this occurs, we recommend the use of colorful stimuli such as the top of the cyclopentolate bottle.

3. Near and distance deviation. Usually the examination starts with the child in primary (distance) viewing position (PP), but before the age of one year, it is difficult to get the child to fix his/her sight at distance. We recommend the cover test and rarely use the Hirschberg test since it is very vague, especially in patients with aniridia. A morphoscopic object is used to stimulate accommodation. We then observe whether there is a tropia or phoria. If we cannot observe the strabismus, a Gracis biprism may be used but this test is not always conclusive because of the reduced VA. Besides finding out the magnitude of the deviation, we should also establish which eye is dominant. This will provide information about a possible amblyopia. The overall extent of strabismus is provided by the cover-uncover test.

4. The horizontal component of the strabismus is assessed when looking up or down to determine the A-V pattern.
5. Vertical strabismus is assessed during lateral movements, in oblique positions and with the head tilted towards one shoulder and the other.
6. The size of the deviation is measured using prisms (horizontal and vertical if necessary).
7. To examine torsional components, the bilateral Maddox rod, torsiometer, synoptophore or fundus pictures/retinographies can be used.
8. The near point of convergence (reduced in most of our patients), fusion vergences and the modified AC/A are measured. For these measurements, the child needs to be 5-7 years-old.
9. Special tests: passive ductions, generated forces, speed of saccadic movements.

Sensory tests

1. In child patients with aniridia, we usually use the synoptophore to assess retinal correspondence (from the age of 3.5-4 years). Vision is generally too low for foveolar and we use larger tests (macular tests). The Bagolini striated glass test is also useful, but to detect small abnormal angles/angular defects, this test can only be used in children of 6 years or older. The maculo-macular test is very accurate for a retinal correspondence study but in patients with low vision results are imprecise (Fig. 5.5).

2. To assess fusion at distance, we use the Worth 4-dot test and polarized vectograph test. The Worth test serves to identify gross or peripheral fusion (Fig. 5.6), while the variable vectograph system requires finer fusion (though charts of different levels of difficulty exist) (Fig. 5.7).

Figure 5.5. A) Synoptophore B) Bagolini striated glass test

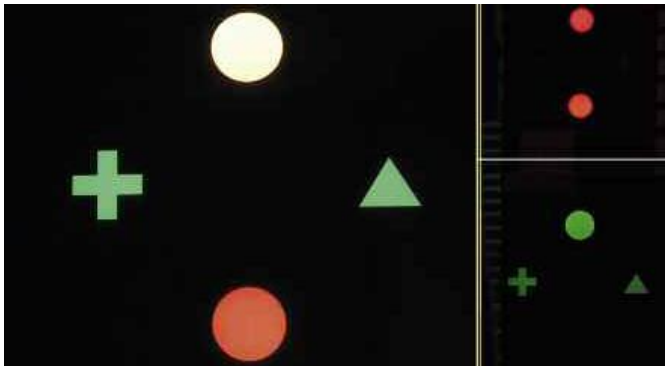

Figure 5.6. Worth 4-dot test

Figure 5.7. Polarized vectograph test

3. Amplitude of fusion is measured with the synoptophore using prisms at distance and near. Normal values for distance fusional amplitudes measured using prisms are 4-6 PD for divergence and 18 PD for convergence. Amplitudes of divergence should be determined first. In most of our patients with aniridia, fusional amplitudes are reduced especially convergence amplitudes.

4. Stereoscopic vision is assessed using the Lang test (Fig. 5.8) in the youngest patients. The TNO (Fig. 5.9) or Titmus (Fig. 5.10) stereotests are used for children older than 3-4 years.

Figure 5.8. Lang stereotest

Figure 5.9. TNO stereotest

5.2.2.4. Examining the anterior segment and ocular adnexa

A systematic search for any abnormality that could be associated with aniridia should be performed. The pediatric slit lamp (Fig. 5.11) is of help in younger children who are uncomfortable with the conventional slit lamp. This instrument can also be easily transferred to the operating room. The ocular structures listed below are those most frequently affected in patients with aniridia. These anomalies are described in more detail in other chapters.

1. Eyelids. Ptosis is relatively common. It may be congenital or appear during the first years of life. Sometimes a young child will retract the eyelids when he/she tries to look at a presented object (Figs. 5.12 and 5.13).

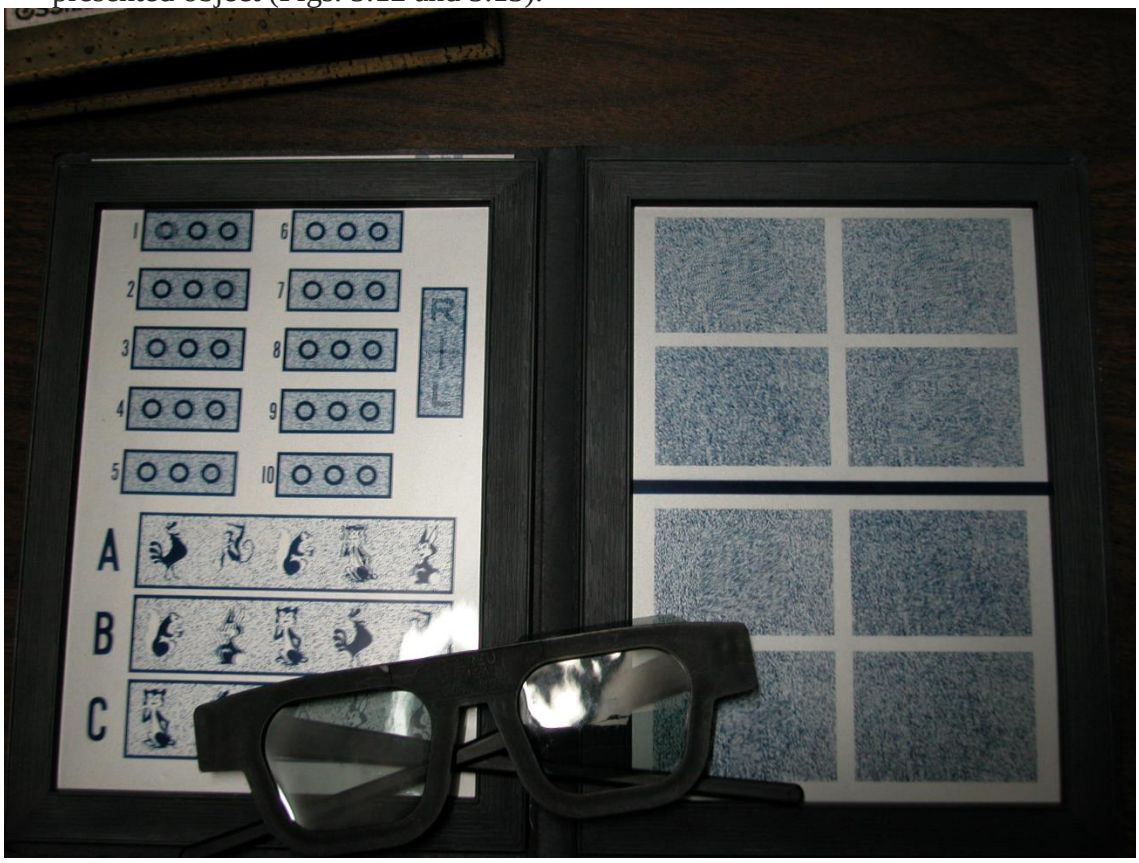

Figure 5.10. Titmus test

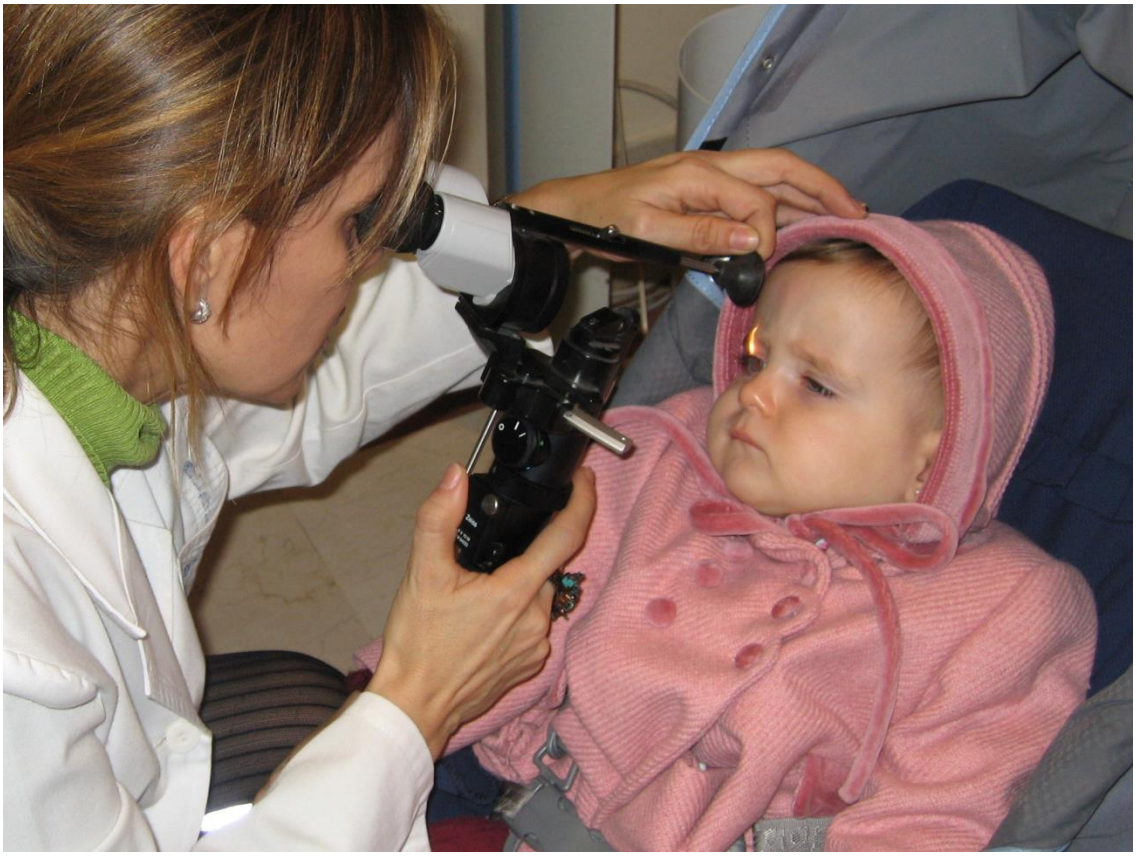

Figure 5.11. Pediatric slit lamp

Figure 5.12. Patient with ptosis associated with aniridia

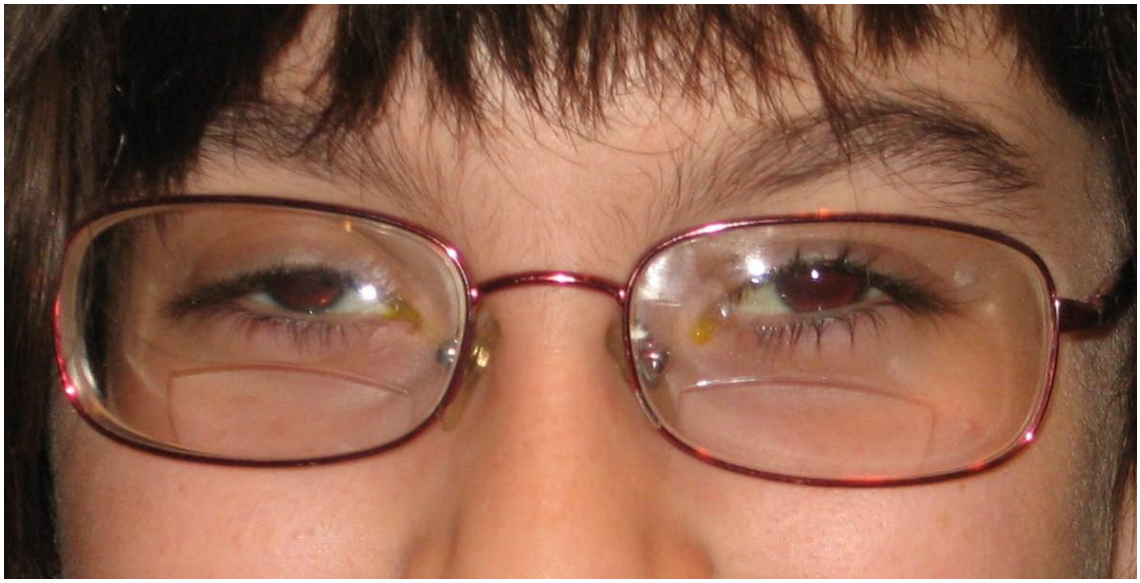

Figure 5.13. Patient with ptosis associated with aniridia

1. 2. Cornea. Dry eye and meibomian gland disease[7], microcornea, corneal clouding, neovascularization, keratoconus in adults, Peters' anomaly and corneal pannus. The extent of limbal insufficiency should be evaluated.
2. Iris. Hypoplasia varies from the presence only of an iris stump to milder forms involving an irregular pupil margin. An exhaustive exam of the eye under general anesthesia should be performed at least once to observe the iris root and the remains of iris by gonioscopy. If this is insufficient, ultrasound biomicroscopy (UBM) is a good option. This procedure is able to detect a reduced ciliary body[8].
3. Lens. Aniridia patients frequently have cataract. This may be polar or sutural, but also nuclear. Some patients have ectopia lentis. In early examinations of a child with aniridia, the need for cataract surgery should be assessed. Given that cataract is usually partial, surgery is not necessary at a very early age. This exam requires transillumination biomicroscopy (Fig. 5.14).
4. Intraocular pressure (IOP). Glaucoma occurs in 30-50% of patients with aniridia and usually appears between the ages of 8 and 15 years. IOP should be checked yearly or even every six months. Sedation is required in children too young to cooperate. Given that corneal thickness can modify IOP, pachymetry (central corneal thickness measurement) may be useful. This variable is often high in these patients[9].

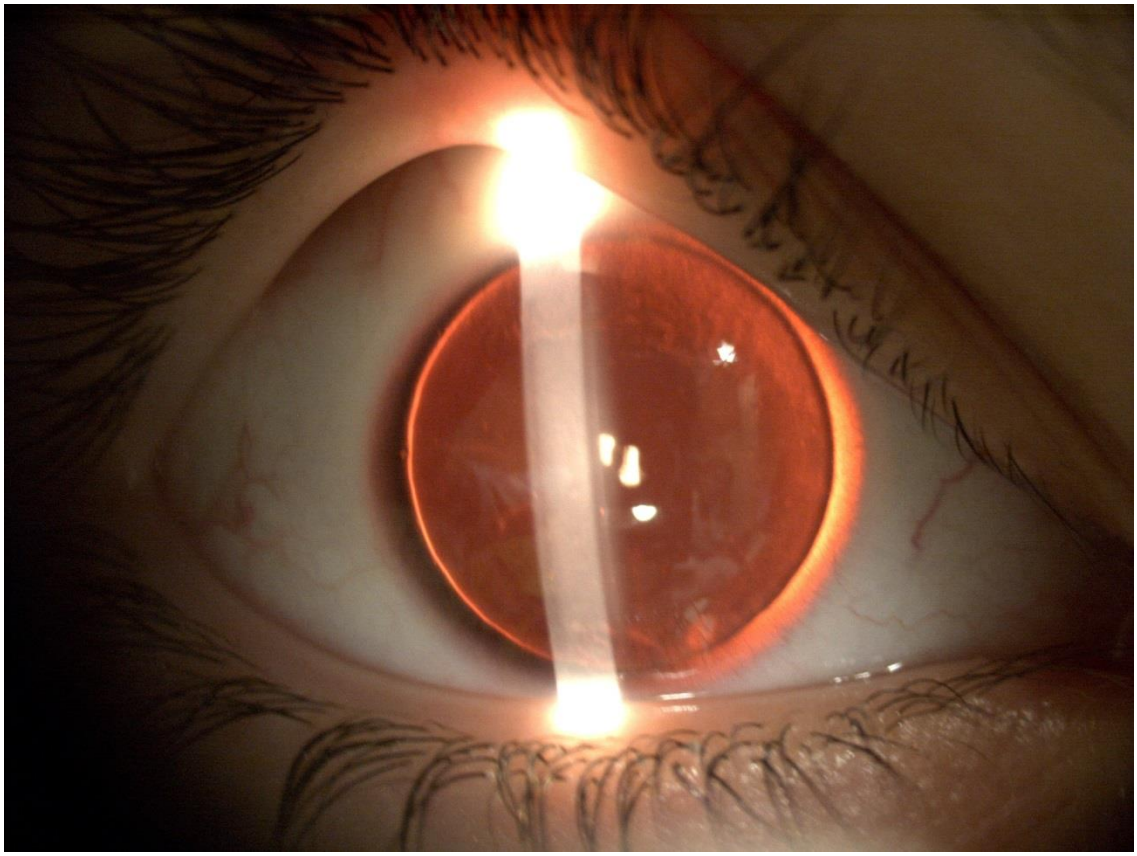

Figure 5.14. Transillumination biomicroscopy detection of cataract in a patient with aniridia

5.2.2.5. Fundus examination

In this exam we should look for the two signs that can be associated with aniridia and compromise the visual prognosis: foveal hypoplasia and optic nerve hypoplasia. Signs of glaucoma should also be checked for (Fig. 5.15). This exam may be difficult because of ocular immaturity, the absence of a foveal reflex in the first few weeks of life and poor patient collaboration, which is worsened by the intense photophobia patients suffer.

5.2.2.6. Other tests

In select patients, the tests normally included in a general ophthalmology examination may be indicated.

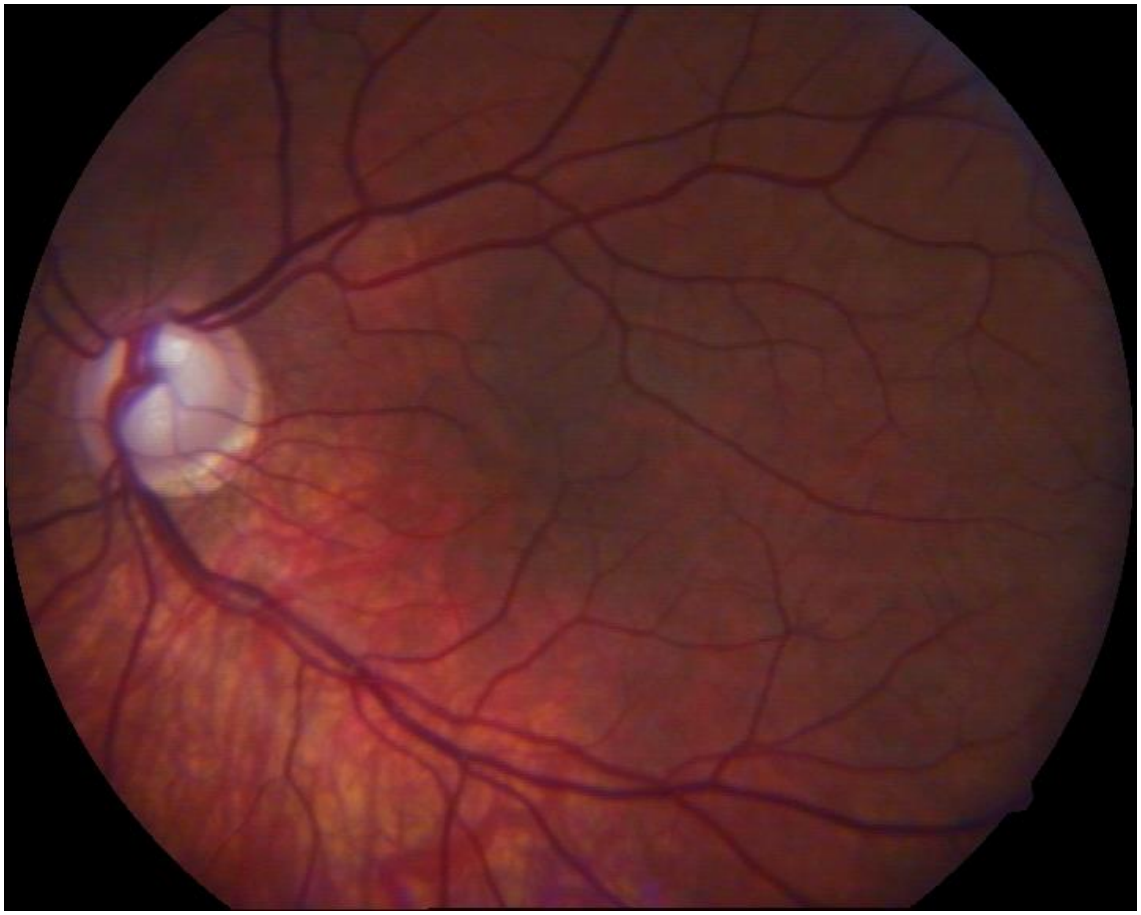

Figure 5.15. Glaucomatous appearance of the optic nerve in a patient with aniridia

C***olor vision*** assessment using the Farnsworth test can be conducted in children after the age of 6-8 years. There are color tests available for younger children (Fig. 5.16) but most of these tests screen for red-green color blindness. Even if a child is unable to identify letters and numbers, Ishihara's test can be used from an early age because the child can trace the images with his/her index finger.

Figure 5.16. Pediatric color test

C***ontrast sensitivity*** may also be affected in the patient with aniridia though it is not usually routinely assessed because of its wide variation in children. Scales adapted for children exist, among which the one based on the LH test is most recommended (Fig. 5.17).

Computerized ***visual field*** testing can easily be performed in 8-10 year-old children. Under this age, cooperation is not good. From the age of 3 to 4 years, visual field defects may be identified by the confrontation method but this is only useful for gross hemianopia or visual field defects.

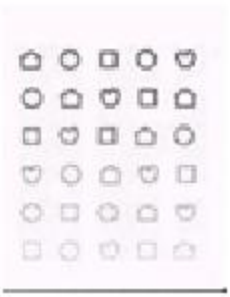

Figure 5.17. Pediatric contrast sensitivity test based on the LH test

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SECTION II

CHAPTER 6
Dry Eye Syndrome and Aniridia

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6.1. Introduction

Aniridia is a rare congenital disorder (1:65.000-100.000) which a well- known genetic fault[1,2], representing an overall eye disorder in which the iris hypoplasia, that names the disease, is only the most obvious clinical sign. It can also be associated with extraocular manifestations, among which it should be noted Wilms tumor. The disease affects therefore to all ocular structures including cornea, iris, chamber angle, ciliary body, lens, retina and optic nerve[3,4].

The most common ocular signs, in addition to the iris hypoplasia, are keratopathy, glaucoma, cataract, foveal hypoplasia and nystagmus. In recent years has been shown a growing interest in the limbal insufficiency accompanying the disease and characterized by the loss of Vogt palisades and progressive corneal pannus, which eventually leads to a central stromal scarring and neovascularization[5-12], while other surface alterations as dry eye disease have been less studied.

In this chapter some etiological, clinical and laboratory aspects are reviewed, as well as therapeutic measures to face the dry eye syndrome associated with congenital aniridia. The correct diagnosis and treatment of dry eye will favorably influence the evolution and improve the symptoms of other aniridia associated ocular surface disorders[13,14].

6.2. Pathogenesis of dry eye associated with congenital aniridia

Currently, the dry eye or tear dysfunction syndrome is considered an alteration of the tear film caused by a decrease in production or an inadequate composition that tends to instability and causes chronic ocular surface abnormalities[15]. Thus, the tear film becomes insufficient in quantity and / or quality to maintain important functions, failing in its role of preserving the integrity of the corneal epithelium and, therefore, reducing the visual quality, inducing symptoms of chronic ocular discomfort.

The tear film consists of three layers: mucous, aqueous and lipid, although we know that the first two are mixed to form a single layer mucous-aqueous. Each of these layers is produced by different specialized cells: mucous layer by goblet cells, the aqueous layer by the lacrimal glands and the lipid layer by the Meibomian glands. Disturbances in any of them will cause

instability and / or deficiency in the tear film, producing eventually a hyposecretor dry eye. However, in some instances, even with normal tear production, a proper use of it by the ocular surface is not shown. In these cases the evaporative dry eye, which might be due to a defective eyelid (lagophthalmos, ectropion, exophthalmos, etc.) or an alteration in the lipid layer secondary to epithelium failure (limbal insufficiency, bullous keratopathy...) which is unable to capture the tear.

When dealing with a patient with dry eye, there is not usually an exclusive involvement of one of these factors, usually several of them are more or less altered either by the etiologic agent primarily or secondarily because one influences the others. In this section, we will try to see which are these factors and how produce dry eye associated aniridia in order to assess a better understanding.

First, we know that the existing limbal insufficiency in aniridia might produce an abnormal epithelium which retain tear poorly and that determine an epithelium failure dry eye, as it occurs in any other case of limbal insufficiency. Some authors consider that the limbal alterations are the main cause of dry eye in patients with congenital aniridia and the rest of the ocular surface would be affected secondarily, altering both non-secreting epithelial cells of the conjunctiva as goblet cells, and extrapolating the results, most or possibly all patients with aniridia presents dry eye disease[13].

There are few references in the scientific literature about the pathophysiological mechanisms that explain the origin of dry eye in congenital aniridia, including two articles that may clarify some aspects[13,14]. To determine dry eye associated to aniridia different measurements were performed as tear meniscus level, non-invasive Break-up time tear film (NIBUT), the presence of mucous secretions, Schirmer test with and without anesthesia and staining with fluorescein and rose bengal plus impression cytology, which will be studied in another section of this chapter.

The most used parameter in the literature has been the Schirmer test, but while some authors describe abnormal test results in aniridia patients others found no such association[13,14,16,17] .

Al-Rajhi and Jastaneiah studied 36 eyes with aniridia and found a normal Schirmer test without anesthesia in 86.1% and 100% with anesthesia[14].

Similarly, Rivas and Murube also analyzed 36 eyes, and found only altered Schirmer test in 28% of the eyes[13].

Given these results we can conclude that the watery secretion produced by the lacrimal glands, seems considerably affected in aniridia eyes[14].

The Schirmer test, therefore, should be considered as a nonspecific diagnostic test to assess ocular dryness in aniridia patients.

The study of the mucous layer also provides mixed results in the literature. Rivas and Murube consider aniridia associated dry eye caused by a mucous deficiency, finding a decrease in the number of conjunctival goblet cells[13,18].

However, other authors note that in the aniridia exists an increased number of goblet cells in both conjunctiva and limbus[4,14], observing as well an increased mucous secretion and corneal filaments in 95% of the examined eyes that relate goblet cell hyperplasia found in impression cytology and symptoms of dry eye[14].

Regarding to lipid layer, we find mixed results again. While Murube and Rivas remark a mild blepharitis, which do not attach importance, in 10 of the patients studied[13], Al-Rajhi and Jastaneiah note an important stenosis and atrophy of the orifices in Meibomian glands observed in 77.8% of the eyes in their study, showing a positive correlation with deterioration in the ocular surface[14].

Based on this finding, these authors describe the dry eye associated with aniridia secondary to meibomian glands dysfunction, describing it as a problem of increased evaporation rather than a deficit of aqueous secretion[14].

The remaining tests rated show more consistent results between the two studies: tear meniscus of 0.5 mm or lower in 88.6%[14] , BUT altered in 72%[13] and 80.6%[14] respectively, staining with fluorescein and rose bengal positive over 80%[13,14] and impression cytology showed squamous metaplasia in 100% of both studies[13,14].

Furthermore in the two studies was observed a positive correlation between the degree of dry eye and corneal disease severity[13,14].

6.3. Impression cytology

Impression cytology is a useful complementary test for the diagnosis of dry eye syndrome in patients with congenital aniridia. This test has many advantages over other ophthalmologic measurements, since it detects suffering of the ocular epithelial surface and the existence of a dry eye, showing the squamous metaplasia process before keratinization might be observed clinically. Due to its nature of surface biopsy is a technique with a sensitivity and specificity close to 100 %, much higher than other ophthalmologic tests[19].

Moreover, impression cytology is an easy technique, quick and non-invasive allowing the study of the different cell types of the ocular surface. It is an anatomo-pathological technique that involves to collect the outermost epithelial layer of the ocular surface through HAWP304 Millipore filter paper strips. Impression cytology is performed after instilling one drop of double anesthetic (Alcon Cusi, El Masnou , Barcelona, Spain) in the conjunctival sac. The filter paper is placed on the ocular surface, causing slight pressure with the tip of a clamp during 3-4 seconds. For a better understanding of the ocular surface status in aniridia patients, samples should be collected from different conjunctival areas (upper bulbar, interpalpebral bulbar, inferior bulbar and lower lid) and peripheral cornea. These samples are fixed in ethanol 96 ° during 20 minutes, and stained first with periodic acid Schiff (PAS) and subsequently with hematoxylin, dehydrated in increasing concentrations of ethanol and xylene, and are permanently assembled in Entellan resin[20].

Graduation of the squamous metaplasia process brings a convenient approach for a better understanding in both clinical and morphological aspects of the disease[21], consisting of one normal degree and 5 increasing degrees of squamous metaplasia. The description of the density of goblet cells is only applicable to the conjunctiva, while the features of squamous metaplasia in no secretory cells serve both the conjunctival epithelium as the peripheral corneal epithelium. The level 0 corresponds to a normal conjunctival epithelium with numerous goblet cells (> 500 cells/mm^2) and with small no secretory epithelial cells joined together. The level 1 is present in

people with incipient dry eye subjected to adverse environmental conditions. The ocular surface shows a slight decrease in goblet cells (500-350 cells/mm^2) and a mild increase in size of the non -secreting epithelial cells. Grade 2 presents usually symptoms (itching, foreign body feeling, ocular fatigue, photophobia, etc…) but not clinical signs.
The ocular surface shows fewer goblet cells (350-150 cells / mm^2) and epithelial cells are slightly disturbed, with larger cell size and cell separation. Grade 3 is characterized by showing almost permanently reversible clinical signs and symptoms (vasodilation, keratopathies, short BUT, etc...). In ocular surface exists a small goblet cell density (150-50 cells/mm^2) and a marked increase in cell size and cell separation. Grade 4 shows irreversible symptoms and clinical signs (corneal vascularization, corneal scarring, symblepharon, etc…), epithelium has few goblet cells (<50 cells/mm^2), and usually empty of mucin. Secretory epithelial cells are wide, they are far apart and with large nuclear alterations. Finally, the grade 5 shows irreversible clinical signs and symptoms that decrease vision. Cells ocular surface are isolated, with keratinized cytoplasm and usually without nuclei. No goblet cells are found.

From the second decade of life aniridia patients which have not been subjected
to any surgical treatment, present a normal conjunctival or corneal epithelium (Grade 0) or very slightly altered (Grade 1) (Fig. 6.1); about 10% of patients with aniridia and non surgical treatment have a conjunctival epithelium with mild to moderate impairment (Grade 2 squamous metaplasia) (Fig. 6.2); and approximately 40% of patients also without surgical

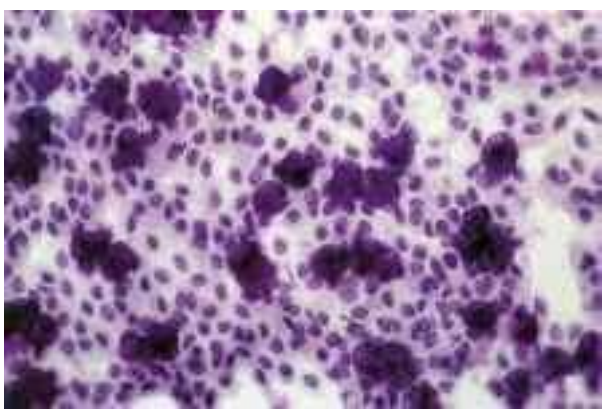

Figure 6.1: Impression cytology of the conjunctiva in a healthy subject. Small and together non-secreting epithelial cells are observed, while the number of goblet cells is high. PAS-hematoxylin staining. Original magnification x40.

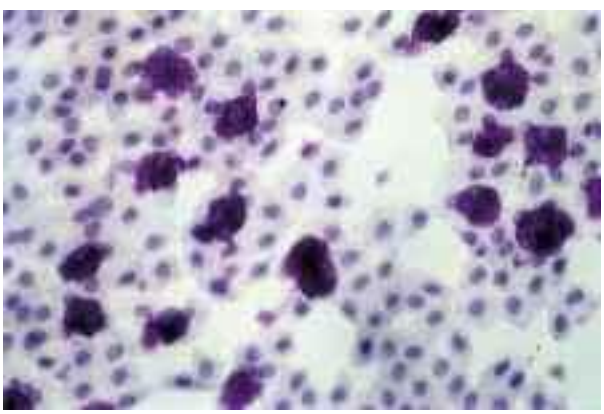

Figure 6.2: Impression cytology of the conjunctiva in a patient with congenital aniridia, with a mild degree of squamous metaplasia (Grade 2). The non-secretory epithelial cells are slightly larger and more separated than in healthy subjects (Figure 6.1). Goblet cells are present with a markedly lower density than in normal subjects (Figure 6.1). PAS-hematoxylin staining. Original magnification x40.

treatment have moderate alterations in the conjunctival epithelium (Grade 3), another 40 % have severe conjunctival alterations (Grade 4), (Fig. 6.3 and 6.4), while 10% usually have very serious changes in the epithelium of the conjunctiva (Grade 5 squamous metaplasia), (Fig. 6.5.). In these patients, impression cytology shows much more severe disturbances. Something logical due to the limbal deficiency observed in these patients, and moreover, all aniridia subjects without surgical treatment usually have a moderate to severe degree of squamous metaplasia. About 10% of patients have a Grade 3 squamous metaplasia in the corneal epithelium, about 50% have a Grade 4 and the remaining 40 % show usually very serious corneal changes (Grade 5). Besides the corneal squamous metaplasia, approximately 10% of

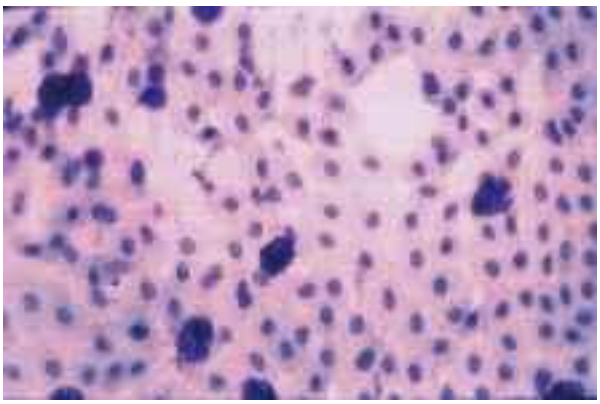

Figure 6.3: Impression cytology of the conjunctiva in a patient with aniridia, which has a moderate squamous metaplasia (Grade 3). The non-secreting epithelial cells are larger as in the previous grade (Figure 6.2), with some nuclear alterations and are further apart (Figure 6.2). Goblet cell density is small. PAS-hematoxylin staining. Original magnification x40.

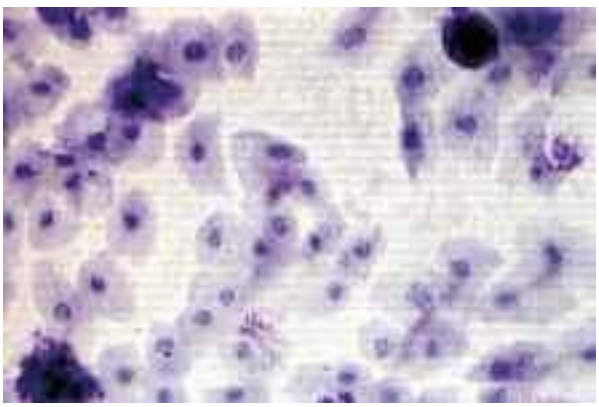

Figure 6.4: Impression cytology of the conjunctiva in a patient with aniridia, showing severe squamous metaplasia (Grade 4). The non-secretory epithelial cells are large, separate and have numerous nuclear alterations. Small number of goblet cells are observed. PAS-hematoxylin staining. Original magnification x40.

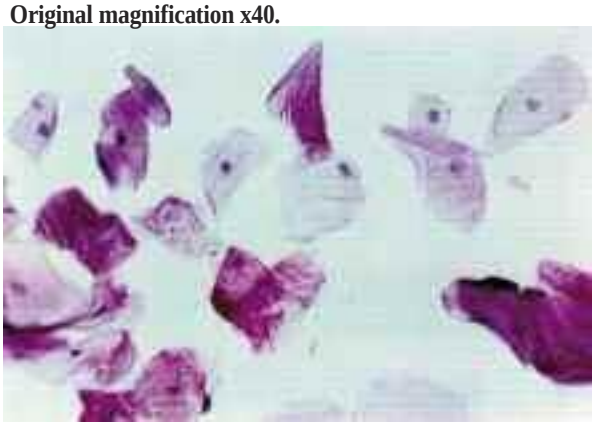

Figure 6.5: Impression cytology of the conjunctiva in a patient with aniridia, which has a very severe squamous metaplasia (Grade 5). The non-secretory epithelial cells are very large and isolated. They have a keratinized cytoplasm and altered or absent nucleus. Goblet cells are rarely observed. PAS-hematoxylin staining. Original magnification x40.

these patients display severe conjuntivalization. But when the histochemical determination of CK 3+ cytokeratin (corneal staining cell phenotype) and of CK 19+ cytokeratin (characteristic of conjunctival goblet cells) over the corneal impression of these patients is performed, the percentage increases to conjunctivalization in 40 % (observing a decrease in CK3 + and the increase of CK19 +), (Figure 6.6 and 6.7).

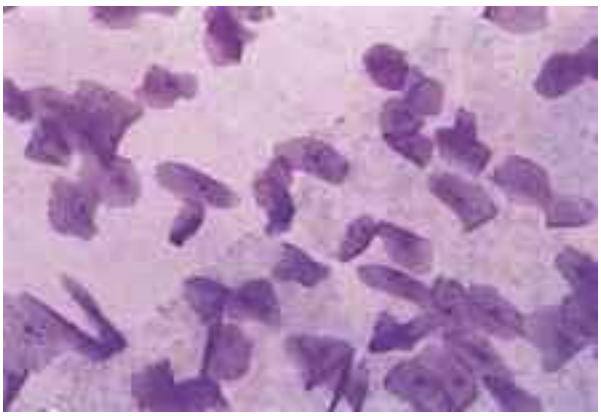

Figure 6.6: Impression cytology of the conjunctiva in a patient with congenital aniridia, previous to limbal transplant. Very altered limbal epithelial cells were observed. PAS-hematoxylin staining. Original magnification x40.

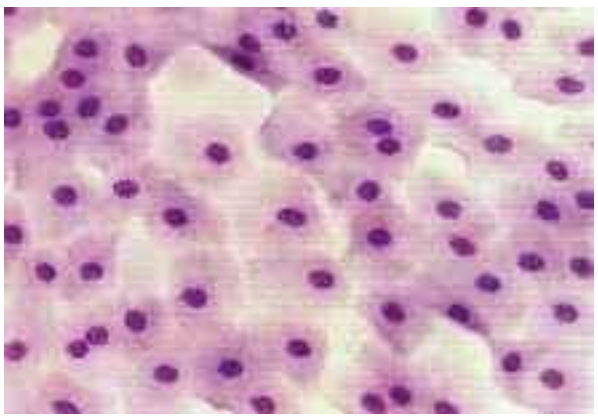

Figure 6.7: Impression cytology of the conjunctiva in a patient with congenital aniridia, twelve months after limbal transplant. Metaplastic corneal epithelial cells together with normal cell are observed. PAS-hematoxylin staining. Original magnification x40.

Although the degree of dry eye defined by clinical manifestations is not closely linked to clinical tests, there is a large relationship with cytology results. Thus, in
cases with mild ocular manifestations of aniridia, ocular surface usually shows a
mild-moderate dry eye (Grade 2), in cases of moderate ocular manifestations in aniridia patients, dry eye usually has a moderate degree of squamous metaplasia (Grade 3);
and aniridia patients with serious ocular abnormalities have severe level of squamous metaplasia (grade 4 or 5).

The results of clinical tests and impression cytology in the eyes of patients with
congenital aniridia indicate a predominance of certain deficiencies in the mucous layer tear film over the others, although there are also failures in the other two layers of the tear film. Only 10-15 % have mild blepharitis and 5% show a decrease in the
tear secretion, while 70 % of patients generally exhibit a significant goblet cells decrease, although some authors report an increased number[14]. These results show that, in general, aniridia is associated with the presence of dry eye disease, particularly aniridia is primarily considered a mucous-deficient disorder with low lipid component. But above any alteration in the tear film, dry eye syndrome in patients with aniridia has its origin in an epitheliopathy due to the absence or scarce limbal stem cells
and 100% of these patients display severe corneal epithelial disorders. In the ocular surface exists a vicious circle between the three tear film layers and the corneal epithelium, since alterations in one of these represents a serious impact in the entire ocular surface. Therefore, although the origin of dry eye syndrome in aniridia patients
is caused primarily by limbal abnormalities, alterations in the different layers of the tear film emphasize the degree of ocular dryness.
Most patients with aniridia (over 90%) come to our office with medical treatment based on artificial tears without preservatives, showing significantly better ocular surface state than patients who do not use. Although this is a palliative treatment, results in an o improvement in the ocular surface symptoms, a decrease in cellular disturbances, allowing corneal epithelium recovery, and including increases in the density of goblet cells. This treatment stops the squamous metaplasia process, enhancing subjective discomfort in these patients. For these reasons, aniridia patients require daily treatment with artificial tears preservatives-free.

6.4. Clinical impact of dry eye in patients with aniridia

The cause of dry eye aniridia is due to a poor quality and not quantity of the tearfilm produced, therefore, Schirmer test is not substantially altered in aniridia as other tear function tests[14].
To assess the tear function in these patients we will use impression cytology, quantification of tear meniscus, BUT, fluorescein and rose bengal staining, search of secretions and mucous filaments and exploration of the meibomian glands and not the Schirmer test.
Different studies have shown that techniques such limbal cell transplantation and amniotic membrane implant are effective methods in the reconstruction of the ocular surface in aniridia[5-9], besides it has been observed that these patients achieve a great benefit in ocular surface from the dryness improvement[13].

Finally, we emphasize here the extense relationship between dry eye and keratopathy associated with congenital aniridia:

1. When greater keratopathy, greater severity of dry eye disease and vice versa. Therefore it is originally a multifactorial dry eye disorder but with a prominent epithelium failure component.
2. Treating dry eye properly, we can slow the disease, stop and even in some cases improve keratopathy.
3. Treating keratopathy properly (limbal transplantation, amniotic membrane...), dry eye might improve significantly. Surgical treatments aimed at restoring limbal stem cells indirectly cause a rapid and significant improvement in dry eye signs that accompanies these patients (Fig. 6.8 and 6.9). After recovery of the corneal epithelium, a major anchor of the tear film is produced, and thereby also a better recovery of the conjunctival epithelium.
4. Another medical treatment often used in the clinical practice is autologous serum. Its main purpose is not only to care dry eye syndrome, but also to improve corneal epithelium state. Autologous serum is not used as a replacement of artificial tears but provides significant growth factors to the corneal epithelium.

Patients with mild or moderate degree of clinical aniridia have a marked improvement in symptoms and signs of dry eye, although in severe cases of the disease, the improvement is much more slight and transient (Figs. 6.10 and 6.11) [13].

Treatment with autologous serum, after obtaining informed consent in cases of central and confluent superficial punctate keratitis, refractory to treatment with preservative-free gel tears is suggested.

In conclusion, dry eye disease seems to be present in almost all patients with congenital aniridia, agravating severity as corneal disease increases. The recognition of this fact and the early initiation of treatment with lubricants can break the vicious circle and delay corneal changes. Artificial tears without preservatives produce an improvement in symptoms with decreased cellular alterations of the ocular surface, which allows to recover the epithelium, slowing or even improving the process of squamous metaplasia. Other measures related to the treatment of dry eye are creating wet environments, sunscreen, and in more advanced cases, the use of autologous serum.

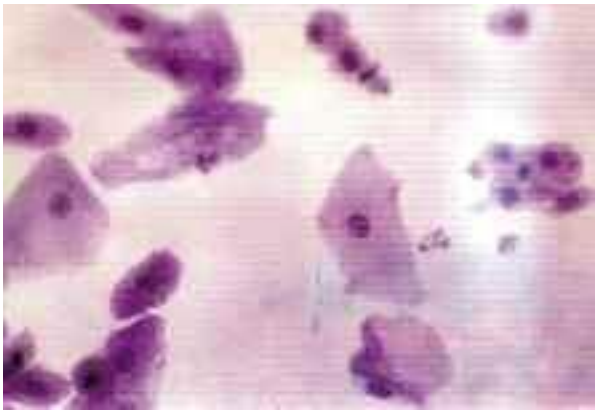

Figure 6.8: Impression cytology of the cornea of in a patient with congenital aniridia, two months after the amniotic membrane transplant. Epithelial amniotic cells are together with dramatically altered corneal cells are observed. PAS-hematoxylin staining. Original magnification x40.

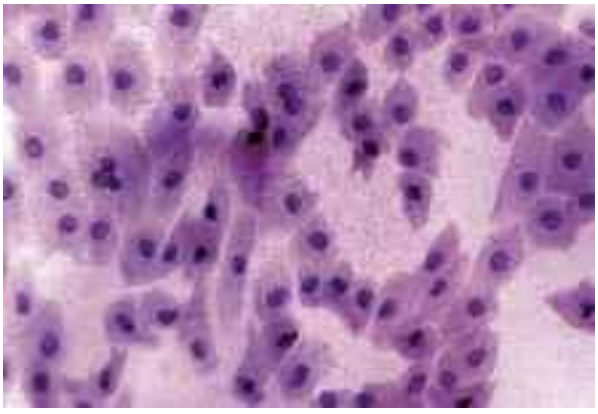

Figure 6.9: Impression cytology of the cornea in a patient with congenital aniridia, six months after the amniotic membrane transplant. Very altered epithelial cells together with normal cells are observed. PAS-hematoxylin staining. Original magnification x40.

Figure 6.10: Congenital aniridia. Note the severe limbal failure with full conjunctivalization of the ocular surface and central stromal scarring as well as a persistent paracentral epithelial defect.

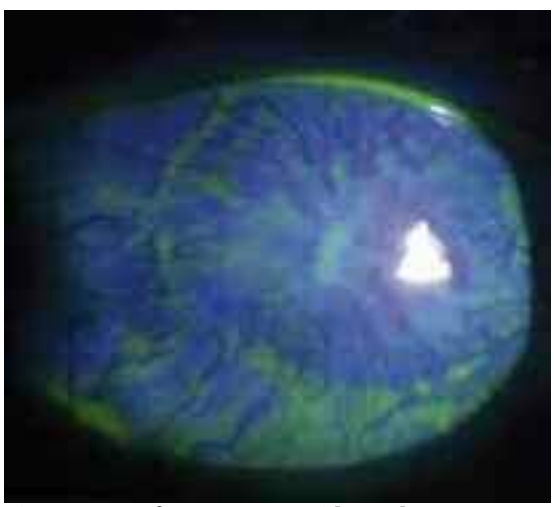

Figure 6.11: After treatment with autologous serum a full re-epithelialization of the cornea and a reduction of the inflammatory component is observed in the ocular surface.

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CHAPTER 7
DISORDERS OF THE CORNEAL LIMBUS AND ANIRIDIA

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7.1.- Anatomy and physiology of the corneal limbus

When we speak of the ocular surface we are referring to the anatomical and functional unit that is formed by various eye structures and its appendages, which makes it possible to maintain corneal transparency and protect the eye from external damage. The conjunctiva, corneal limbus, corneal epithelium and tear film are included in this definition. The eyelids, main lacrimal gland and lacrimal drainage system are vital appendages for the preservation of proper homeostasis on the ocular surface[1].

The corneal limbus, a key component of the ocular surface, consists of an approximately 2 mm wide area located between the transparent cornea and white sclera, coinciding macroscopically with the palisades of Vogt (Fig. 7.1). At the limbus the conjunctiva forms radially oriented ridges through which blood vessels and nerve fibers run and, as already noted, are known as the palisades of Vogt; they are more prominent in the superior and inferior limbus. The limbus is the transitional zone between the columnar conjunctival epithelium and the stratified squamous corneal epithelium. Although the border between conjunctiva and cornea is higly defined macroscopically, histologically speaking, it is not easy to distinguish.

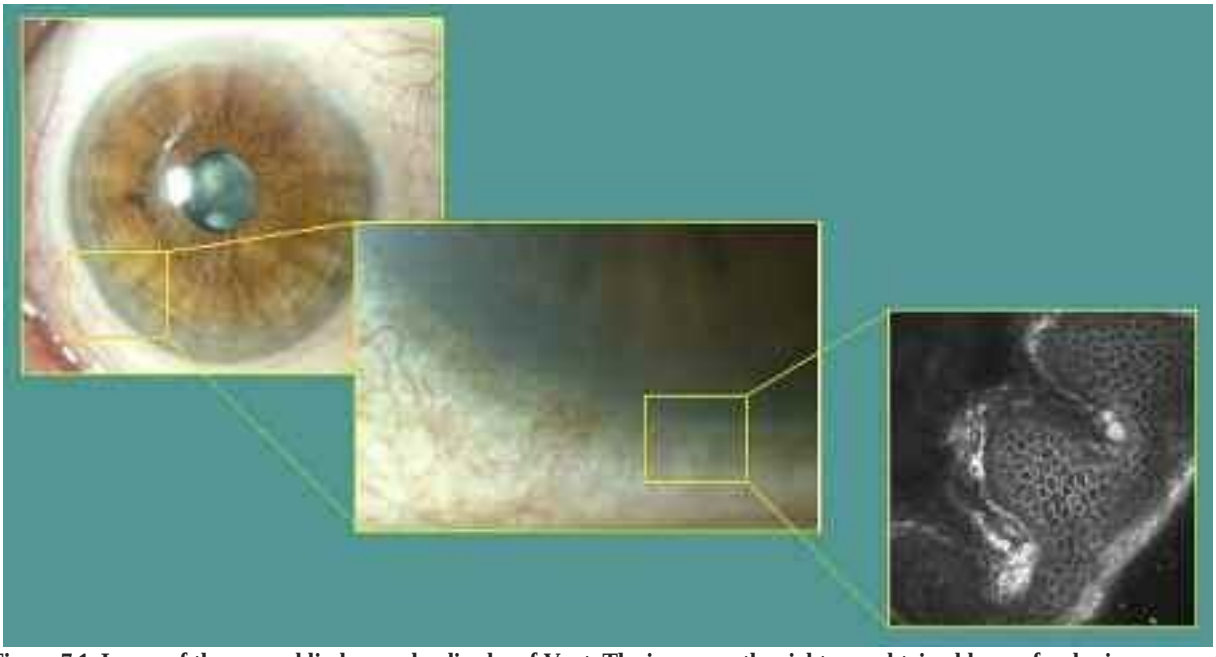

Figure 7.1. Image of the corneal limbus and palisades of Vogt. The image on the right was obtained by confocal microscopy of the palisades of Vogt.

Histologically, the corneal limbus comprises 2 layers: epithelium and stroma. The epithelium consists of 8-12 layers of stratified cells, with a few melanocytes and Langerhans' cells[2]. The basal layer is made up of cuboidal basal cells that include germ cells, progenitor cells or stem cells between the basal cells (Fig. 7.2). Asymmetric cell division is the key feature of these stem cells, which produces a new stem cell and a transient amplifying cell (TACs). The TACs subsequently undergo differentiation to generate basal corneal epithelial cells, which in turn give rise to all the other cell layers in the cornea. In addition to collagen fibers, proteoglycans and glycoproteins, the stromal layer of the limbus is composed of various types of cells (melanocytes, macrophages, mast cells, lymphocytes) as well as blood vessels, lymphatic vessels and unmyelinated nerve fibers.

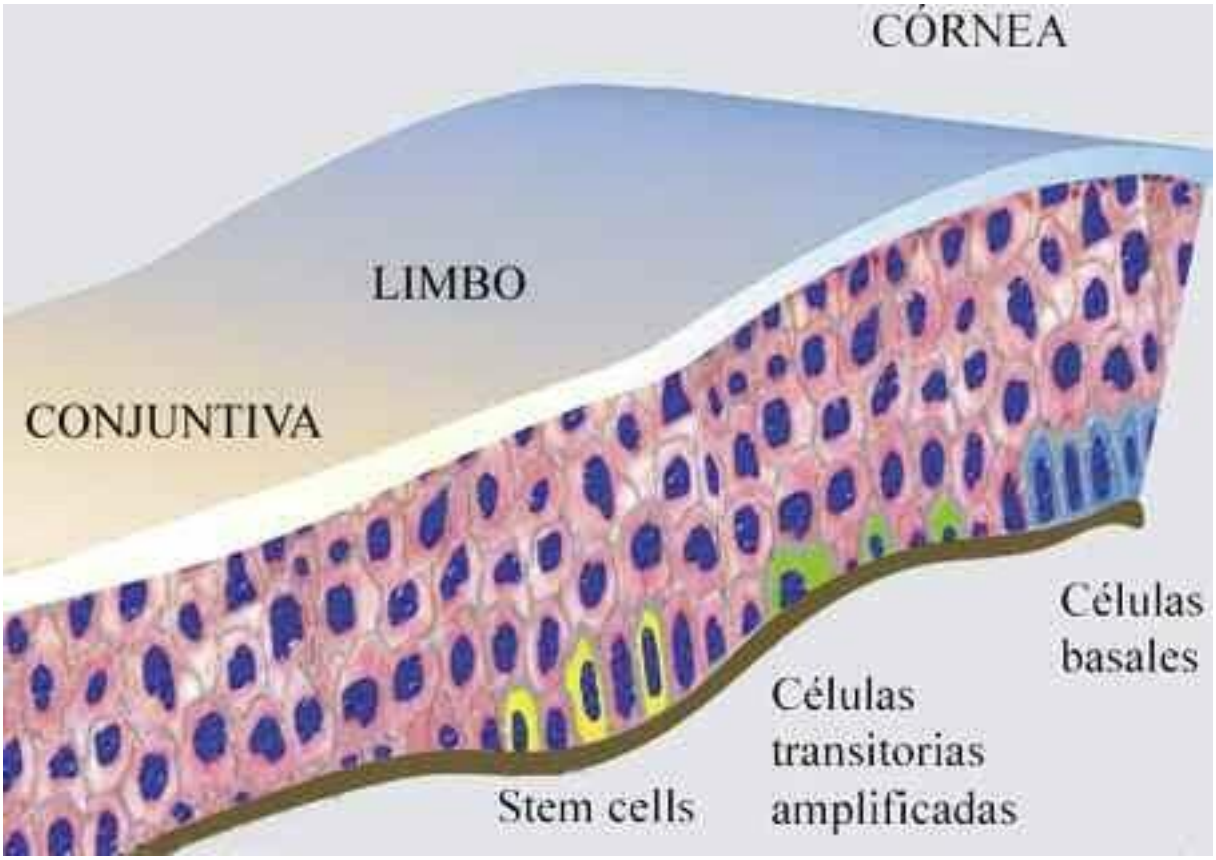

Figure 7.2. Schematic drawing of the anatomy of the limbus. The basal layer is made up of cuboidal basal cells that include germ cells, progenitor cells or stem cells.

7.1.1.- Limbal stem cells

Current evidence strongly suggests that stem cells (SCs) or corneal epithelial stem cells are located in the limbus. These cells would be responsible for the centripetal repopulation of the corneal surface and in turn would prevent the conjunctival epithelium from extending onto the central cornea. Several lines of evidence provide support for the limbal stem cell theory[3,4,5]: a) studies with tritiated thymidine have provided evidence of cells with long cell cycles (2 weeks) in the limbus; b) cell cultures have shown that these cells have a high proliferative potential; c) they have markers of undifferentiated cells (vimentin, alpha-enolase); d) experimental studies have shown the usefulness of limbal transplantation for corneal surface reconstruction; and e) limbal transplantation studies in humans have yelded favorable results.

As previously discussed, the fundamental characteristic of limbal stem cells is their capacity for asymmetric division, giving rise to a new stem cell and a transient amplifying cell (TAC)[6]. The TACs will then migrate centripetally and differentiate, producing the basal layer of the corneal epithelium, which generates the post-mitotic suprabasal cells of the corneal epithelium that are known as the wing cells. These cells will continue to migrate superficially in the squamous epithelium until they reach the surface as terminally differentiated cells and are eventually shed[7]. In a normal cornea this process takes about seven days. The XYZ theory by Thoft proposes that migration of epithelial cells takes place in three stages from the stem cells in the corneal limbus, whereby X represents centripetal movement, Y is the vertical movement and Z equals desquamation of cells on the surface (Fig. 7.3).

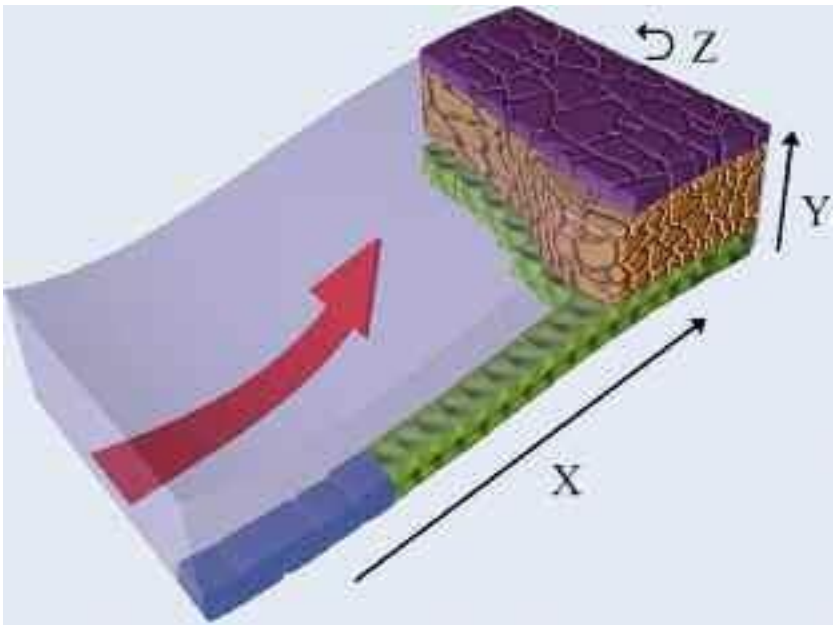

Figure 7.3. Migration of the epithelial cells in three stages, starting out as stem cells in the corneal limbus.

On the other hand, in a process that is not well understood, some limbal stem cells migrate superficially within the limbal region. It is likely that these stem cells act as a barrier to prevent conjunctival tissue from encroaching upon the corneal epithelium[8].

In the corneal limbus it is not just SCs that are important, but also the niche where they reside, which has special characteristics. Its location within the palisades of Vogt provides mechanical protection from external damage. Similarly, the presence of melanocytes creates an environment rich in pigment that protects the SCs from the harmful effects of UV radiation. The limbal stroma consists of loose connective tissue containing a dense vascular network, lymphatic channels and some cell groups.

7.2.- Pathophysiology of the corneal limbus

The corneal limbus has 2 main functions: a) the regeneration of the corneal epithelium, and b) to act as a barrier between the corneal and conjunctival epithelia. Therefore, when there is a failure in the corneal limbus that results in a loss of its functions, the epithelial cells of the conjunctiva invade the cornea, accompanied by fibrovascular tissue, in a process known as conjunctivalization[8] (Figs. 7.4 - 7.6). The vascularized conjunctival epithelium that grows on the corneal surface affects corneal transparency and induces a chronic inflammatory response. Thus, the main clinical manifestations of limbal deficiency are persistent corneal epithelial defects, conjunctivalization of the corneal surface, corneal scarring and loss of vision[9,10]. This process can be detected early by using impression cytology of the corneal surface to demonstrate the presence of goblet cells[11].

Figure 7.4. In stage 1 ARK, the conjunctival epithelium migrates onto the the peripheral cornea although it may go unnoticed unless stained with fluorescein.

Figure 7.5. Cornea seen in figure 7.4 a few seconds after a drop of fluorescein was instilled. The areas where there is conjunctival epithelium are permeable to the stain and turn into a greenish color, standing out clearly from the corneal epithelium.

Figure 7.6. The image shows the peripheral superficial vascularization and irregularities in the conjunctival epithelium in a patient with stage 1 ARK. There are no corneal opacities.

Limbal stem cell deficiency diseases can be classified into two major categories[8,12], according to the mechanism by which the damage to the stem cells occurs: a) those entities in which there is a loss of stem cells due to direct destruction (secondary limbal deficiency) (56% of cases), and b) those entities in which there are no external factors to which the loss or functional impairment of the stem cells can be attributed, or those in which an alteration of the limbal stem cell microenvironment occurs with gradual loss of the stem cells (primary limbal deficiency) (representing approximately 43% of cases) (Table 7.1). The first group includes diseases of the ocular surface in which stem cells are destroyed, such as chemical or thermal burns, scarring conjunctivitis, multiple surgeries through a limbal approach, prolonged use of contact lenses and serious microbial infections. Primary limbal stem cell (deficiencies due to an alteration of the limbal microenvironment with subsequent loss of SCs) occurs in congenital aniridia, keratitis associated with multiple endocrine deficiency and idiopathic limbal stem cell deficiency (Table 7.1).

7.3.- Limbal stem cell deficiency and aniridia

Aniridia is a congenital disease with an autosomal dominant inheritance pattern that can occur sporadically. Its incidence is estimated at 1 case per 65,000 to 95,000 births. Clinically it is characterized by the development of various ocular abnormalities: glaucoma, cataracts, absence of the iris, nystagmus, foveal hypoplasia, optic nerve hypoplasia and changes in the cornea. Aniridia is the most common cause of congenital limbal stem cell deficiency[13,14].

Although the corneal disorder may not be present at birth, it appears in the first few years of life in the form of neovascularization and grayish nodular opacities in the anterior stroma, initially located at the periphery. Over time they progress toward the center of the cornea causing irregular astigmatism and scarring that contribute to increase the already existing vision impairment caused by other eye conditions.

Table 7.1. Limbal stem cell deficiency diseases and their relative frequency.

1. Loss of stem cells due to direct destruction (56%)
- Chemical or therman injuries (36%). - Scarring conjunctivitis: Cicatricial pemphigoid (¿%?) and Stevens-Johnson syndrome (7,5%). - Multiple surgeries/cryotherapies of the limbal region. Radiation. (Iatrogenic) (4%). - Drug toxicity. (Iatrogenic). - Contact lens induced keratopathy (5,3%). - Severe microbial infection (3%). - Peripheral inflammatory keratitis.
2. Alteration of the limbal stem cell microenvironment (gradual loss of stem cells) (43%)
- Aniridia (hereditary) (14%). - Ectodermal dysplasias (hereditary). - Keratitis associated with multiple endocrine deficiencies (hereditary) (2%). - Neurotrophic keratopathy (neuronal o ischemic) (14%). - Idiopathic (8,5%).

Histopathology studies[15] have demonstrated the presence of goblet cells, epithelial cells with a conjunctival phenotype and absence of the palisades of Vogt in the cornea of patients with aniridia. All of these findings indicate a progressive loss or dysfunction of the limbal stem cells, which may be related to a deficient stromal microenvironment. Patients with aniridia are probably born with a reduced number of limbal stem cells that do not function properly. The keratopathy seen in these patients worsens over time. This deterioration may be secondary to the progressive loss of stem cells and the inability of the poorly-functioning remaining cells to ensure the stability of the ocular surface[14].

Aniridia is now considered to be a disease with a well known genetic cause, which occurs in both sporadic and familial forms and shows an autosomal dominant inheritance pattern with variable expression in different members of a family. The gene responsible for the disease (PAX6) is located on the short arm of chromosome 11[16]. This gene is widely expressed during the development of different eye structures such as the cornea, lens, the chamber angle, ciliary body and all layers of the retina. It is therefore a panocular disorder of the eye.

The PAX6 gene is considered to be an important regulator during eye morphogenesis as it controls the processes of cell proliferation, differentiation and apoptosis in normal eye development. Normal expression of PAX6 is considered to be essential for the development of ocular tissues such as the corneal epithelium, iris, ciliary body, lens and retina[17,18]. The regulatory role played by the PAX6 gene continues into adulthood and occurs at various levels.

The majority of the recent work about the genetic causes of congenital aniridia is based on animal studies, especially mice. Expression of the CK-12 and CK-3 cytokeratins by corneal epithelial cells is regulated by PAX6. These are cytoskeletal proteins that form the intermediate filaments in epithelial cells, whose main function is to keep the corneal surface stable. A

reduction in the expression of these cytokeratins has been observed in experimental models of aniridia, which translates into vacuolation and fragility of the corneal epithelium[19]. Studies in experimental models have also shown a reduction in the expression levels of desmoglein as well as of proteins alpha- and beta-catenin, whose synthesis seems to be regulated by PAX6 gene and that are involved in the adhesion mechanisms of cells in the corneal epithelium; their reduction causes gaps to appear between the epithelial cells[20]. These findings suggest also a considerable fragility of the corneal surface caused by this impairment.

Matrix metalloproteinase-9 (MMP-9) is also regulated by PAX6. In PAX6 mutation animal models with MMP-9 deficiency, it has been observed an accumulation of fibrin and an infiltration of inflammatory cells, which is related to increased levels of IL-1. The accumulation of fibrin alters the orderly structure of collagen fibers, reducing corneal transparency. Additionally, cellular infiltration results in a significant stimulus for neovascular formation.

To summarize this section, it should be remembered that in aniridia-related keratopathy there is intrinsic limbal involvement that alters the functions of the limbal SCs. Alterations of the ocular surface that occur in aniridia are caused, at least initially, by SC dysfunction[14]. As in other cases of partial limbal stem cell deficiency, patients with aniridia may remain asymptomatic until some external factor causes a disruption in the limbus and upsets the unstable equilibrium that maintained the integrity of the corneal epithelium. Accordingly, aggravation of keratitis symptoms following surgery with excessive limbus manipulation has been described.

7.4.- Aniridia-related keratopathy. Clinical signs and sympthoms and stages.

Corneal injuries invariably occur in patients with congenital aniridia. Corneal changes can be observed in the early years of life in 100% of patients, both clinically or histologically[21,22], and in 90% of cases the typical lesions of aniridia-related keratopathy (ARK) can be seen when a patient is examined with a slit lamp[23].

ARK is caused by limbal stem cell deficiency in these patients, which has been discussed above. In the early stages, the only sign that can be seen is conjunctival epithelium at the periphery of the cornea as a result of the limbal deficiency (Fig. 7.4). Unless fluorescein staining is used, this epithelium, which has a conjunctival phenotype, may go unnoticed during the examination. However, if we instil a drop of fluorescein and wait a few seconds, the conjunctival epithelium, which is permeable to the stain, will present a typical speckled pattern that clearly contrasts with the areas of corneal epithelium, much more compact and showing no staining (Fig. 7.5). As with any type of limbal stem cell deficiency from any etiology, in cases of diagnostic uncertainty the use of impression cytology will confirm the conjunctival epithelial phenotype by detecting the presence of goblet cells on the cornea. In this early stage, the rest of the cornea is normal, with a stroma that is totally clear and no lesions in the endothelium. As time passes, the conjunctival epithelium extends progressively towards the center of the cornea, followed by fine vascularization of the surface, although the underlying corneal stroma still remains clear (Fig.7.6). The conjunctival epithelium on the cornea causes the mild and sustained inflammation. This often translates into chronic discomfort for the patient (foreign body sensation, itching, photophobia, etc.), which, in some cases, may be increased by associated dry eye syndrome (see Chapter 6). The photophobia experienced by patients with aniridia is multifactorial and may be due not only to corneal injuries, but also to the aniridia, the

presence of a cataract or a neuroretinal disorder. With time, the cornea becomes totally covered by the conjunctival epithelium, which leads to reduced vision when it affects the visual axis. Since this epithelium is less adhesive to the corneal surface it may cause recurrent corneal erosions and persistent epithelial defects, thereby increasing the patient's symptoms, and inflammation of the corneal surface. As a result of the continuous inflammation, grayish-white, nodular, subepithelial lesions, similar in appearance to those in Salzmann's nodular degeneration, occur (Fig. 7.7), always located beneath the conjunctival epithelial phenotype (Fig. 7.8). In congenital aniridia, these lesions may be located in the peripheral cornea as in Salzmann's degeneration, or they may be closer to the central cornea and cause a greater impact on vision (Fig. 7.9). Finally, in the most advanced stages of aniridia-related keratopathy, the stroma is involved and opacification appears (which can be total) and even deep neovascularization (Fig. 7.10). Classical aniridia-related keratopathy rarely involves the endothelium. The patients with corneal edema whom we saw presented with endothelial damage secondary to cataract surgery, which was normally associated with the presence of mobile intraocular lenses in the anterior chamber.

Figure 7.7. Stage 2 ARK. In addition to stage 1 signs, nodular lesions occur.

Figure 7.8. After instilling fluorescein, the areas covered by the conjunctival epithelium as well as the nodular lesion under the abnormal epithelium can be clearly observed.

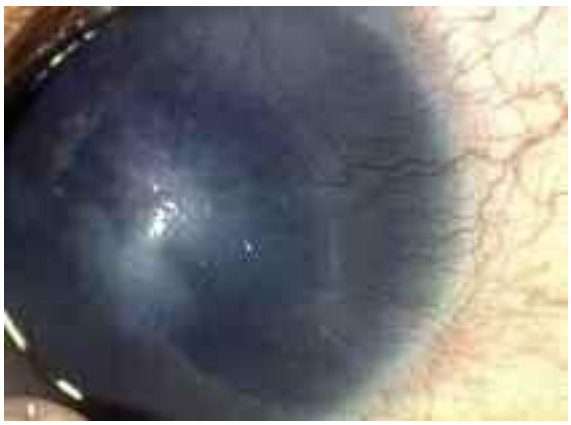

Figure 7.9. Advanced stage 2 ARK. The nodular lesion involves the central cornea, limiting vision and begins to involve the underlying stroma.

Figure 7.10. Stage 3 ARK. As a result of long-term limbal deficiency, the cornea presents with significant, diffuse stromal opacification.

Patients with congenital aniridia develop dry eye due to both of a lack of tears[24] as well as tear film instability and Meibomian gland dysfunction[21]. Not only does limbal deficiency progress with age, but dry eye also worsens and both factors are related to the progression of keratopathy.

Although the corneal lesions that are typical in patients with aniridia were described in 1979[25], there is still no globally accepted and standardized grading system. Probably, the system that is most commonly used internationally is the one originally described by Mackman that includes 4 grades[25]:

- Grade 0: examination finds no clinically observable signs
- Grade 1-A: presence of one or more areas where there is centripetal migration of the conjunctival epithelium from the limbus, although it does not extend to the center of the cornea (the original description does not mention the conjunctival epithelium but refers instead to "superficial dystrophy")
- Grade 1-B: conjunctival epithelium is growing in a 360º ring around the cornea periphery, although it does not reach the central cornea
- Grade 2: lesions such as those found in grades 1-A and 1-B that extend to the center of the cornea and/or stromal scarring

As we can see, this classification is based on the location and extent of the lesions rather than on the type of lesions. We use and propose a different classification that comprises three stages depending on the type of lesions:

- Stage 1: presence of conjunctival epithelium on the peripheral cornea, together with superficial vascularization in the periphery
- Stage 2: occurence of areas of subepithelial fibrosis (nodular degeneration) beneath the conjunctival epithelium
- Stage 3: stromal opacity

We believe that this classification, which describes the various lesions that the patient's cornea progressively develops over the course of the disease, is more practical for the

ophthalmologist (Table 7.2). Each stage involves a different type of lesion that requires different management and therefore, according to this classification, there is a different therapeutic indication for each stage.

Table 7.2. Clinical stages of aniridia-related keratopathy

Stage	Slit-lamp examination findings
Stage 1	Conjunctival epithelialization of the peripheral cornea. Superficial vascularization in the periphery.
Stage 2	Stage 1 lesions + appearance of fibrocellular nodular lesions beneath the conjunctival epithelium.
Stage 3	Stage 2 lesions + presence of stromal opacities.

Aside from ARK, that is due to limbal stem cell deficiency, it is interesting to know that, in general, the thickness of the corneal stroma in patients with congenital aniridia is greater than in the normal population. Different studies[26,27] published in recent years describe a mean central corneal thickness between 630 and 690 microns for patients with congenital aniridia, as compared to a mean of 550 microns in a normal population control group. This finding should be taken into account when measuring intraocular pressure in these patients, by adjusting the intraocular pressure reading obtained with the tonometer according to the patient's central corneal thickness. Otherwise, some patients may end up receiving unnecessary antihypertensive treatment, which a resulting drug-induced toxicity to the cornea and ocular surface. Microcornea is another finding that may also be associated with aniridia[28,29].

7.5.- Corneal surgery procedures

There are four surgical procedures that can be useful for the treatment of the different types of lesions related to ARK: corneal epithelial stem cell transplantation as treatment for limbal stem cell deficiency, superficial keratectomy for the removal of nodular subepithelial lesions, keratoplasty to improve vision in cases of significant stromal opacification and amniotic membrane transplantation to accelerate and enhance epithelialization following any of the surgical procedures mentioned above. Epithelial stem cell transplantation is the only procedure for treating the causes of keratopathy. The other procedures may be useful as adjuvant treatment (or, sometimes, as palliative therapy) but they do not, in and of themselves, slow disease progression.

7.5.1.- Corneal epithelial stem cell transplantation

There are two ways to perform a corneal epithelial stem cell transplantation: either by performing a **limbal transplant** (which includes the stem cells in the donor's keratolimbal tissue) or by using **stem cells that have been expanded in the laboratory** (in this case only epithelial stem cells obtained from a small fragment of donor limbus that have been cultivated-expanded in the laboratory on a substrate which usually consists of amniotic membrane are transplanted). In any case, as aniridia patients have bilateral disease, they always require allogeneic transplants

7.5.1.1.- **Allogeneic limbal transplantation**

Today this surgical technique is the one most widely used in patients with aniridia. Allogeneic keratolimbal allografts use fragments of the donor's tissue that contain stem cells in their natural environment (Fig. 7.11 and 7.12). The donor may be a close relative of the patient who is unaffected by aniridia, but we prefer to use tissue (preferably fresh) from a cadaveric donor for two reasons. In the first place, complete immunological compatibility is exceptional, so even if the tissue comes from a close relative there is always a chance for rejection and systemic immunosuppression is still required. Secondly, with immunosuppression the risk of rejection is low and using a graft taken from a cadaveric donor allows us to transplant the limbus onto 360º of the recipient cornea (as opposed to using a graft taken from a living donor, which significantly limits the amount of tissue and thus the number of stem cells that can be transplanted).

The surgical technique and postoperative management of the patients undergoing this procedure were recently described by the authors[30]. Under ideal conditions, the tissue used for the graft should preferably come from a young donor (less than 40-50 years), with a preserved corneal epithelium and it should be obtained as soon as possible after death. If the surgeon prefers to use a whole eye it should be stored at 4°C in a moist chamber until ready for use and must be transplanted within 24 hours of having been obtained. The limbal graft can also be obtained from conventionally processed sclerocorneal buttons including a 3-4 mm peripheral rim of conjunctiva. The usual methods of corneal storage will maintain the epithelium and sclerocorneal button stem cells in good condition for up to five days[31].

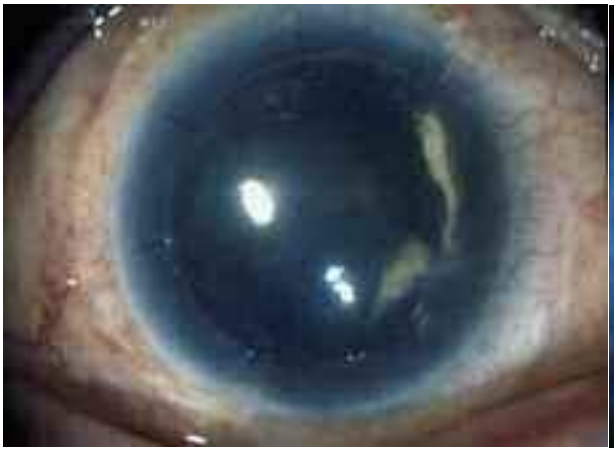

Figure 7.11. Partial limbal stem cell transplantation (180º) performed in the region of greatest involvement of the limbus in a patient with aniridia. Care was taken to avoid damage to the recipient limbus on the nasal side.

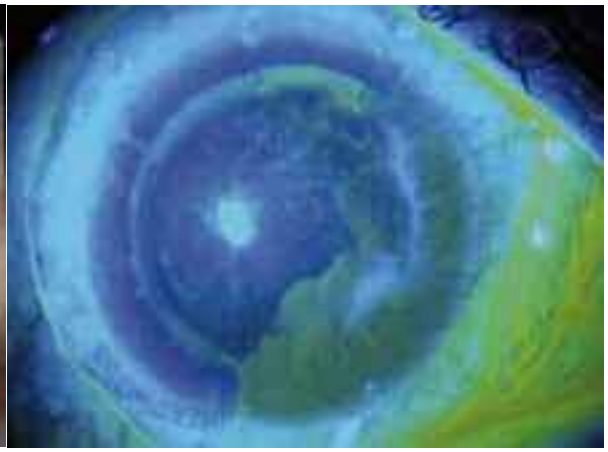

Figure 7.12. With the use of fluorescein staining, the borders between a corneal epithelial phenotype (no stain) and a conjunctival epithelial phenotype (positive for staining) that extends from the recipient's abnormal limbus can be seen

7.5.1.1.1.- Obtaining donor tissue

To obtain donor tissue, a 360º lamellar dissection of the corneal limbus including with about 2 mm of the peripheral cornea and about 4-5 mm of the conjunctiva must be carried out. If a whole eye is used, it must be firm in order for the dissection to be performed; this is achieved by injecting air or fluid intraocularly through the optic nerve. To dissect the eyeball, it can either be secured to a suitable stand or held in the hand using gauze. If the latter option is used, it is important not to exert excessive pressure when holding as high intraocular pressure carries a greater risk of perforation during dissection. A 9-10 mm diameter manual or suction trephine is used to mark the cornea, which is then trephined to a shallow lamellar depth. A 360º lamellar dissection of the ring around the corneal limbus (from the center toward the periphery) is performed using an angled blade and the scleral edge is cut with curved scissors. The entire ring from a donor globe and a quadrant from the ring of a second donor eye are commonly used. As mentioned previously, a lamellar ring-shaped limbal graft for transplantation may also be obtained from a sclerocorneal button that has been preserved in a conventional corneal storage medium. In this case, an artificial anterior chamber may be used or the lamellar dissection may be performed manually using scissors (removing approximately 2/3 of the posterior corneal thickness).

7.5.1.1.2.- **Surgical technique**

The procedure can be performed under local or general anesthesia. Although we use local anesthesia, some surgeons prefer general anesthesia because of the length of the procedure, which, in cases of combined surgery, may extend to up to three hours. When there is total limbal stem cell deficiency with conjunctivalization of the entire cornea, a 360º peritomy is performed and the conjunctiva is excised. The major bleeding points are cauterized using

bipolar diathermy at the lowest power setting needed. A plane of dissection is created just beneath the conjunctivalized fibrovascular tissue on the cornea and dissection is carefully begun in this plane, from the limbus to the central cornea, using an angled blade. In areas where it is possible, blunt dissection with a dissector or corneal scissors is preferable so as not to lose the plane of dissection and to avoid deepening it. After the fibrovascular tissue has been removed completely, the bleeding points are cauterized again so that the recipient bed is ready for the graft.

First an incision is made anywhere on the donor limbal ring to open it and then place it along the circumference of the recipient's limbal area, slightly posterior to the anatomical limbus. This leaves a gap in the circumference, which is then covered with a graft of adequate size taken from the quadrant of limbal tissue that was obtained from the second donor eye. The inner circumference of the graft is sutured with 10-0 monofilament nylon simple interrupted stitches (12 to 14 stitches) taking care to bury the knots in the recipient cornea, away from the graft. Next, a similar number of stitches are placed around the outer circumference of the graft, directly opposite the inner ones, using nylon 10-0 or 9-0 absorbable sutures according to the surgeon's preference. The peripheral stitches include the episclera and the conjunctival edge of the peritomy; the tension should be carefully adjusted as it also directly determines the final tension on the inner stitches. In the outer perimeter the knots of the stitches are placed facing the conjunctiva and may remain unburied. Lastly, a large diameter therapeutic contact lens that covers the graft completely is placed to promote epithelialization during the postoperative period. As an alternative to the contact lens, a suture can be placed at the outer margins of the upper and lower eyelids so that the trauma to the cornea caused by blinking is lessened during the first few days.

7.5.1.1.3.- **Concurrent amniotic membrane implantation**

Amniotic membrane grafts used to cover the cornea and sclera provide a basement membrane and a number of biological factors that promote tissue re-epithelialization[32-34]. In cases of limbal stem cell deficiency in which the recipient bed is found to be very rough and irregular, limbal stem cell transplantation may not be sufficient to achieve corneal re-epithelialization, as it is difficult for epithelial cells to grow on stroma with these properties. In these cases, an amniotic membrane graft (with the basement membrane-epithelium side facing up) covering the entire denuded area of the cornea and sclera promotes epithelialization. After suturing the amniotic membrane, the limbus is transplanted so that the inner edge of the limbal ring is placed on the amniotic membrane. In this way epithelial cells from the transplanted corneal limbus can grow more easily on the amniotic membrane substrate.

7.5.1.1.4.- **Postoperative management**

Standard postoperative treatment consists of topical prophylactic antibiotics and corticosteroids together with systemic immunosuppression. Topical treatment can be supplemented with preservative-free artificial tears and/or a 20% solution of autologous serum eye drops. Corneal epithelialization as well as intraocular pressure should be monitored on a regular basis. A graft rejection episode should be suspected when the following are observed: increased eye hyperemia, vascular thickening in the transplant area, edema of the graft, loss of the epithelium and persistent corneal epithelial defects in the area where the rejection has

developed. Early diagnosis and aggressive treatment when managing a rejection episode are two factors that are essential for graft survival. Treatment consists of the hourly application of topical steroids and high doses of systemic steroids (either orally or in the form of methylprednisolone intravenous pulses). If necessary, dosages of systemic immunosuppressive agents should be revised and increased.

Since the corneal limbus is a vascularized tissue with a high antigenic load, whenever an allogeneic limbal transplantation is performed (whether from a cadaveric donor or a close relative) there is always need for systemic immunosuppression in the recipient. Limbal transplantation is considered to require a level of immunosuppression similar to that used in renal transplantation. To achieve the necessary immunosuppression different drugs have been used successfully, including cyclosporin A, tacrolimus (FK506), azathioprine and mofetil mycophenolate, in addition to the oral corticosteroids which can be used in the first few weeks after transplantation. These immunosuppressants can be used alone or in combination, thus allowing for lower doses of each drug. Different immunosuppression protocols have proven effective[23,35-38] and, in our opinion, best practice requires the collaboration of a specialist in internal medicine or a rheumatologist whith an interest on post-transplantation immunosuppression. How long to maintain immunosuppressive treatment after transplantation remains a matter of debate but from a theoretical point of view, it should last indefinitely or until side effects become intolerable.

7.5.1.1.5.- **Results**

Most patients have a good postoperative outcome following allogeneic limbal transplantation when the keratolimbal graft covers the entire 360º degrees of the cornea. In our experience, partial grafts have a worse outcome and, at present, we only perform them in other types of limbal stem cell deficiency when an autograft can be obtained from the healthy eye. Few studies on limbal transplantation in patients with aniridia have been published in the international literature. In the reported results[23], which are consistent with ours, ocular surface stability has been achieved in approximately 75% of cases in which a keratolimbal allograft covering the entire 360º degrees of the cornea has been performed, after a mean follow-up of 2-3 years. Outcomes are better in the first year, while extended follow-up for more than 3 or 4 years shows that a significant proportion of limbal transplants fail and retransplantation is required. It is believed that this new failure of the transplanted stem cells may be related to a chronic inflammatory process or subclinical rejection episodes, or a combination of both.

Another important factor to consider when analyzing the results is the need for systemic immunosuppression. Immunosuppressive therapy is well tolerated by young and middle-aged patients and increases graft survival significantly. Success after transplantation is achieved in 90% of cases when systemic immunosuppression is used but this proportion drops to 40% when systemic immunosuppression is not used[23]. So, provided there are no medical contraindications for the use of systemic immunosuppression, it should be used, although there must be close monitoring during the course of the treatment by an internist or rheumatologist who has experience in managing patients who undergo this type of therapy.

7.5.1.1.6.- **Thoughts on limbal transplantation**

As we have already mentioned, stem cell transplantation is the only treatment that can stop the progression of the ARK and the outcome of limbus transplantation is good when systemic immunosuppression is used. However, we must not forget that systemic immunosuppression has potential adverse effects and surgery may cause iatrogenic injuries to the patient. Therefore, we believe that the ophthalmologist should always consider three key points before recommending limbal stem cell transplantation for a patient with aniridia:

a) **Confirm the diagnosis of total limbal stem cell deficiency**. To transplant a donor limbus the recipient's limbus must be completely removed. If we perform a limbal transplantation in a patient whose limbus was working (even if only partially) and our transplant fails, the result will be worse than the preoperative condition.
b) **Assess how much improvement** the patient can obtain from surgery. The goal of limbal transplantation is to stabilize the corneal surface as that translates into an improvement in vision and reduces discomfort for the patient. And yet, reduced vision and chronic discomfort in patients with aniridia are multifactorial in origin, so sometimes that makes it very difficult to predict to what degree a limbal transplant will help to achieve better vision or improve clinical symptoms. If this factor is not carefully evaluated together with the patient before surgery, the postoperative outcome may be disappointing for both the patient and the ophthalmologist.
c) **Risk-benefit should be assessed** on a case-by-case basis. Immunosuppression may pose too high a risk for patients who are elderly or those with systemic disease. In these cases one should consider the possibility of performing the limbal transplantation without systemic immunosuppression or the use of other surgical procedures to help the patient (even if only partially) or, as the case may be, even decide not to perform any surgery at all.

However, once limbal stem cell transplantation has been decided upon, surgery should not be delayed for too long. In stages 1 and 2 of our classification, in which stromal transparency is preserved, limbal transplantation is enough to stabilize the corneal surface and improve vision. If surgery is delayed and stromal opacities occur, keratoplasty will have to be performed simultaneously with limbal transplantation, which not only complicates treatment but also worsens the outcome.

7.5.1.2.- **Cultivated limbal stem cell transplantation**

This procedure uses epithelial stem cells that have been obtained from a small fragment of donor limbus and are cultivated and expanded in the laboratory, being subsequently transplanted on a substrate that usually consists of amniotic membrane (the non-functioning limbus and abnormal epithelium are first removed from the recipient's cornea). This is a relatively new technique, still in the development stage. The main advantage it has is that it allows autotransplantation to be performed for unilateral injuries (e.g., chemical burns) by transplanting cells from the contralateral healthy eye to the injured eye. In these cases the risk of rejection is eliminated and not having to use immunosuppression is a major advantage for patients. However, the financial cost of the procedure is much higher than that of a limbal transplantation and the number of stem cells that can be transplanted is lower than those provided with 360º limbal graft.

We used this technique to perform transplantations in three patients with aniridia a few years ago, using cells from direct family members in two cases and in one from a cadaveric donor. Although the initial results were satisfactory in all three cases, a few months after surgery we had to perform limbal transplants in two cases because of a depletion of stem cells. In the third case we have not had to re-transplant the limbus although at present there is also some degree of limbal stem cell deficiency. We believe that in patients with congenital aniridia, long-term outcomes are better when 360º allogeneic limbal transplantation is performed. The main advantage of the cultivated stem cell technique (i. e., the ability to perform an autotransplantation) is lost in patients with aniridia as they present with bilateral disease and the disadvantages we have spoken of remain. Moreover, it is believed that the causes of limbal stem cell deficiency in aniridia are not to be found in the stem cells themselves but rather in the surrounding microenvironment. In a situation like this it seems to make more sense to think that a limbal graft (in which the stem cells that are implanted within their own niche) will be more likely to survive than cultivated stem cell transplants (whereby the cells are placed directly on the recipient cornea that presents with an altered microenvironment).

7.5.2.- Superficial keratectomy

Superficial keratectomy removes the nodular subepithelial lesions that form on the cornea beneath the conjunctival phenotype epithelium. Histologically, these are avascular fibrocellular lesions (Fig. 7.13), which occur after a slight but maintained inflammatory process and whose slit-lamp biomicroscopic appearance is similar to Salzmann's nodular degeneration.

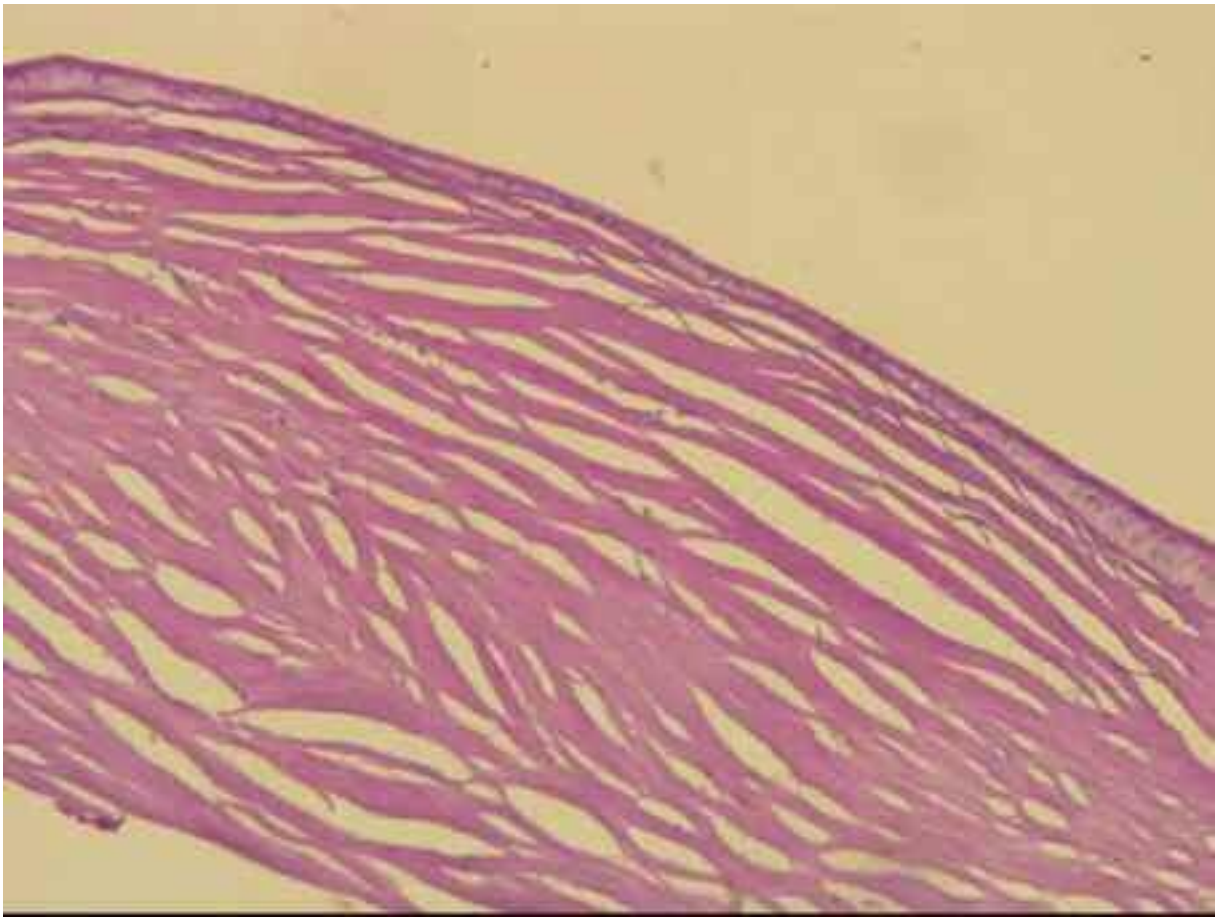

Figure 7.13. Nodular degeneration in a patient with congenital aniridia. Avascular fibrocellular lesions, similar in appearance to those in Salzmann's nodular degeneration, can be observed histologically.

The procedure can be performed using peribulbar or topical anesthesia. First the epithelium that covers the lesion is mechanically removed using a blunt spatula or similar instrument. Next the lesion is grasped with corneal forceps and pulled upwards while at the same time trying to dissect it by using a spatula or flat, blunt tool inserted under it. In the early stages the degenerative nodule is not strongly attached to the stroma and it is relatively easy to remove it using this technique while preserving the underlying stroma. As the lesion progresses it penetrates within the stroma making it more difficult and even impossible to separate the two. At this stage it is necessary to use an angled blade to remove the lesion. This should be done along a single dissection plane, parallel to the corneal surface. However, in these advanced stages the underlying stroma is rarely transparent and the resulting stromal opacity may limit the patient's vision so a keratoplasty may subsequently become necessary.

Ideally, superficial keratectomy should be performed at the same time as limbal transplantation because this procedure eliminates nodular lesions but does not have any effect on the limbal stem cell deficiency, which is the underlying cause for the occurrence of these areas of fibrosis. In the event that no limbal transplantation is going to be performed, it may be combined with amniotic membrane grafting to speed up and improve epithelialization after

surgery, although with this option there is generally a recurrence of the lesions within a few weeks or months after the surgery.

7.5.3.- Keratoplasty

Keratoplasty, whether penetrating or deep lamellar, is useful to remove stromal opacities that develop in the more advanced stages of keratopathy. However, it has no effect on limbal stem cell deficiency so therefore, if it is not performed prior to or simultaneously with limbal transplantation, there tend to be chronic problems with epithelialization during the postoperative period, which ends up causing recurrent opacities[23,39] (Fig. 7.14).

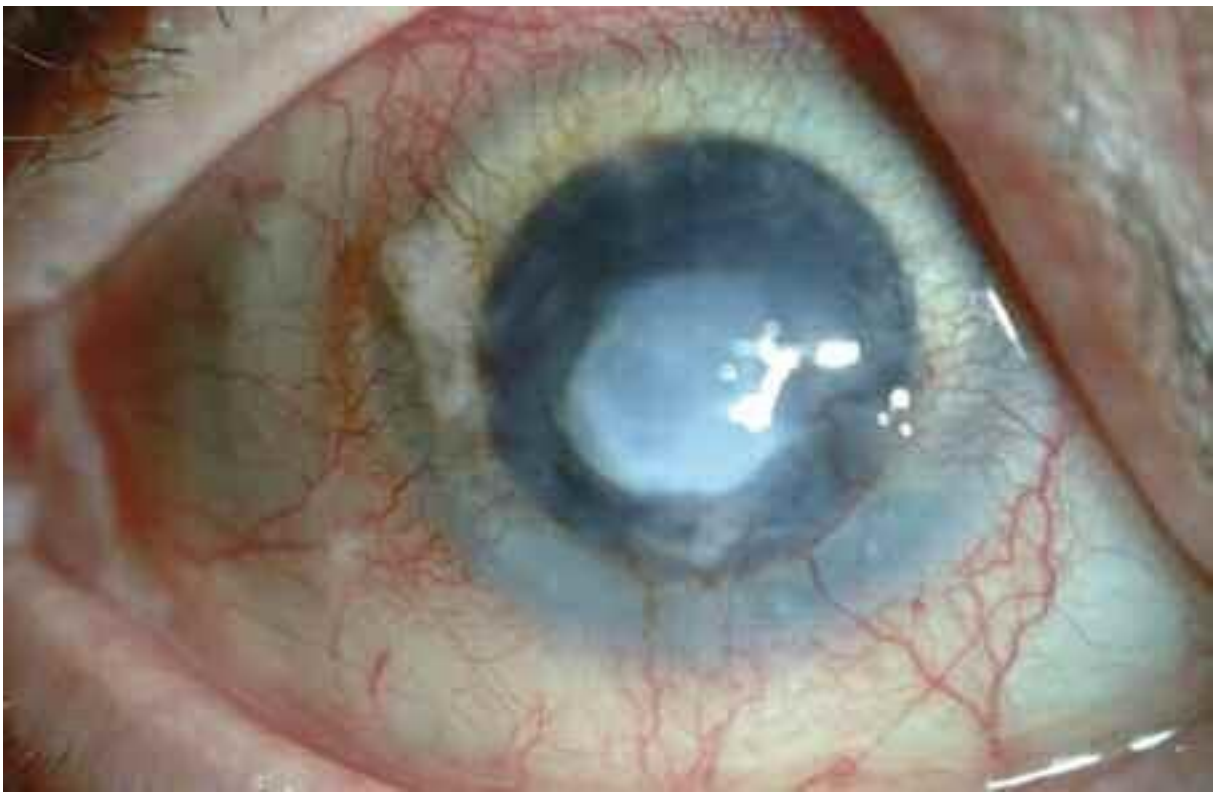

Figure 7.14. Patient with congenital aniridia and corneal opacities after undergoing keratoplasty. Months after performance of surgery, opacification and stromal neovascularization can be observed in the corneal graft as a result of untreated limbal stem cell deficiency.

Reports on the results obtained after using keratoplasty alone in the treatment of aniridia (without treating the limbal stem cell deficiency) make it clear that very few patients can benefit from this procedure (probably only those with partial limbal stem cell deficiency). In some reports[40], survival of the graft when a corneal transplantation alone was performed in patients with aniridia is 0%, while in others[41] it reaches 9%. Use of therapeutic contact lenses can protect the corneal surface and help stabilize the epithelium postoperatively in patients for whom limbal transplantation must be ruled out because of immunosuppression problems and in whom keratoplasty alone may be performed, improving outcomes[42].

As we have previously discussed, keratoplasty in and of itself does not usually have a good long-term outcome in the majority of patients with opacities secondary to limbal stem cell deficiency. Nevertheless it is useful and effective in treating corneal opacities that are not related to ARK or postoperative corneal edema in patients who do not have total limbal stem cell deficiency. In any case, one thing to keep in mind when considering keratoplasty or limbal transplantation for any patient is the risk for postoperative ocular hypertension. In some series[23], the percentage of patients who required shunts for postoperative intraocular pressure control was 32%.

7.5.4.- Amniotic membrane transplantation

In recent years amniotic membrane transplantation in ophthalmology has become popular since this tissue has been found to have a number of biological properties that make it unique[43]. These biological properties can be defined as a range of clinical effects, which can be expected after transplantation of amniotic membrane for ocular surface reconstruction:

a).- It **promotes the epithelialization of tissue** (corneal and/or conjunctival) from the adjacent healthy epithelium, while maintaining the epithelial phenotype present in the area.

b).- It **reduces inflammation** in the tissues beneath the graft and in the surrounding area.

c).- It **reduces neovascularization** of the corneal stroma.

d).- It **minimizes residual scarring** associated with tissue regeneration in both the conjunctiva and the cornea.

The amniotic membrane can serve as a graft or as a patch and may produce different clinical effects based on how it is placed. When implanted as a **graft** it fills in a tissue defect, replacing lost stromal matrix (in the cornea or conjunctiva) and providing a basement membrane upon which epithelialization can occur. In these cases, the amniotic membrane is placed with the basement membrane side facing upward. When used as a **patch**, it acts by protecting the ocular surface against external insults and providing biological factors that reduce inflammation and promote epithelial regeneration under the graft. In these cases, it is preferable to place the amniotic membrane with the stromal side facing up.

In patients with aniridia, amniotic membrane can be used as a graft in combination with limbal stem cell transplantation. As described above, the membrane is sutured to the cornea to serve as a substrate for epithelialization. Next the limbus is sutured, taking care that its inner edge is placed on the amniotic membrane to ensure that the epithelium will grow over it. In most cases, the corneal surface is smooth enough after superficial keratectomy so that only limbal transplantation need be performed and it is not necessary to combine it with an amniotic membrane graft. However, in cases in which the recipient bed is found to be very rough and irregular, the amniotic membrane graft provides a smooth and homogeneous basement membrane that facilitates epithelialization.

The amniotic membrane can also be used as a patch to promote epithelialization during the postoperative period following superficial keratectomy or keratoplasty. In these cases, the amniotic membrane must be placed with the basement membrane facing down (stromal side up) and must be larger than the underlying recipient limbus to ensure that the epithelium grows under it.

It should be remembered that the amniotic membrane uses the stem cells present in the area to stimulate growth of the epithelium and that, therefore, in cases of limbal stem cell deficiency, the amniotic membrane alone cannot provide proper long term epithelialization.

When the amniotic membrane is used as a patch it often falls off within a few days after grafting because of the continuous friction with the eyelid produced by spontaneous blinking. To prevent the graft from rubbing off too early, we place either a therapeutic contact lens over the amniotic membrane[44] or suture together the upper and lower eyelids at the outer margins in the first few days after surgery.

7.6.- Treatment for aniridia-related keratopathy

Up until a few years ago, management of ARK consisted in waiting for the corneal stroma to develop enough opacity to reduce vision significantly and to then perform keratoplasty to restore transparency. However, limbal stem cell deficiency (which was unknown at the time) always ended up by compromising corneal transparency in most patients again just a few months after surgery. In the last few years there have been numerous advances that have led to a better understanding of ocular surface disease and management of the lesions presented by patients with congenital aniridia.

Our treatment protocol is based on the classification system defined above, in which each stage involves different types of lesions and treatments (Table 7.3).

Table 7.3. Treatment of ARK according to stage.

Stage	Tratamiento
Stage 1	- if epithelium is stable preservative-free artificial tears. - if recurrent erosions or persistent epithelial defects --> 360º allogeneic limbal transplantation. - Alternatives to limbal trasplantation: therapeutic contact lens, autologous serum, lateral tarsorrhaphy.
Stage 2	- superficial keratectomy + 360º allogeneic limbal transplantation. - alternative (if limbal trasplantation is not feasible): superficial keratectomy + amniotic membrane patching.
Stage 3	- 360º allogeneic limbal transplantation + keratoplasty. - alternative (if limbal trasplantation is not feasible): keratoplasty + amniotic membrane patching therapeutic contact lens. - if opacity is not associated with ARK keratoplasty + amniotic membrane patching.

7.6.1.- Stage 1:

Stage 1 is characterized by the presence of conjunctival epithelium on the peripheral cornea, together with superficial vascularization in the periphery. These lesions are purely epithelial and there is usually no involvement of the central cornea. At this stage there is no vision loss due to corneal lesions although the patients may experience discomfort secondary to dry eye or the presence of abnormal epithelium on the peripheral cornea. In this phase surgery is never indicated and we only treat medically using preservative-free artificial tears, with dosing frequency varying according to the symptoms. Treatment with preservative-free artificial tears not only relieves the symptoms but also helps to prevent cell damage, so that patients thus treated have a better epithelium[24]. It is also very important to do the utmost to prevent drug toxicity in these patients. To that end, it is important to avoid the overmedicalization of patients with aniridia by not using topical treatments unless they are absolutely necessary and, wherever possible, preservative-free preparations should be used. These indications are valid, not only for patients with stage 1 ARK, but also for all patients with congenital aniridia, regardless of the grade of keratopathy.

In cases where the conjunctival epithelium on the cornea causes recurrent corneal erosions, 360º allogeneic limbal transplantation should be considered, as it is the only curative treatment for these patients. If limbal transplantation cannot be performed (e.g., due to systemic immunosuppression problems), other alternative treatments can be used, such as autologous serum, therapeutic contact lenses, tarsorrhaphy or amniotic membrane patching[23,42,45,46]. Nonetheless, it must be remembered that these procedures only provide temporary relief for symptoms and do not prevent keratopathy progression because they do not address the underlying limbal deficiency.

7.6.2.- Stage 2:

In stage 2, nodular fibrous lesions occur beneath the conjunctival epithelium. Superficial keratectomy can remove these lesions and they are easiest to treat when it is performed early. Nevertheless, once again, we must not forget that they result from an underlying limbal stem cell deficiency and as such the keratectomy should be combined with 360º allogeneic limbal transplantation in order to avoid recurrence. In cases where it is not possible to perform limbal transplantation, an amniotic membrane patch can be placed after the superficial keratectomy has been performed. That said, although initial outcomes are usually good when this technique is used, recurrence within weeks or months after the procedure is likely.

7.6.3.- Stage 3:

Stage 3 is defined by the presence of significant stromal opacity and therefore, keratoplasty (whether penetrating or deep lamellar) is required for the recovery of corneal transparency. Deep lamellar keratoplasty may be a good option for patients with stromal opacity without edema, especially in cases of advanced keratopathy with stromal neovascularization. However, stromal dissection must reach down to Descemet's membrane and this represents a major surgical challenge. Otherwise, the presence of an interface in the stroma can reduce postoperative vision and this can be particularly bad in patients with congenital aniridia whose vision is already limited.

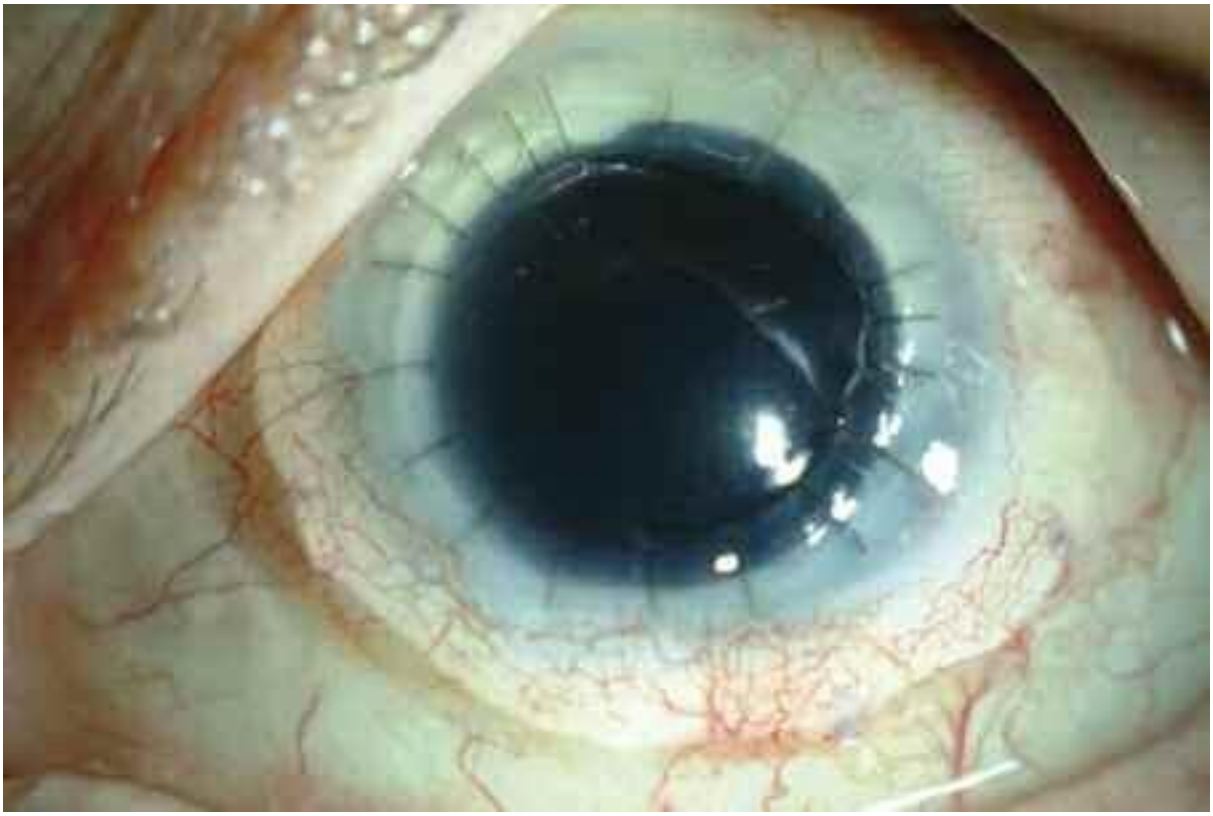

Figure 7.15. The same patient as in figure 7.14 after performance of 360º limbal transplantation and keratoplasty. The properly working limbal stem cells provide an adequate epithelium to the transplanted cornea allowing it to remain transparent over the long term.

As in previous stages, it should be remembered that limbal deficiency is the cause of ARK and that, therefore, keratoplasty alone will provide only temporary improvement before lesions recur in the short to medium term[39-41]. In our opinion, stromal opacities that occur in ARK should be treated with 360º allogeneic limbal transplantation and keratoplasty whenever possible (Fig. 7.14 and 7.15). One issue that continues to be debated is whether both procedures can be performed simultaneously or whether limbal transplantation should be performed first. Some authors recommend limbal transplantation (regardless of the type of limbal stem cell deficiency) should always be performed first and then keratoplasty can be proceeded with a few months later. In our opinion, this is the best option in cases of limbal stem cell deficiency accompanied by inflammation and major tissue destruction on the ocular surface (such as acute chemical burns, ocular pemphigoid and Stevens-Johnson syndrome). In these cases, the risk of limbal graft failure is high and keratoplasty should not be performed until the viability of the limbal transplant has been ascertained. However, this is not the case in patients with congenital aniridia and for them, with the use of systemic immunosuppression, the rate of survival of 360º allogeneic limbal transplants is really high for the first few years. In our experience in patients with congenital aniridia, surgery that combines 360º allogeneic limbal transplantation with keratectomy obtains the same results as surgery in two stages and enables the patient to have faster, more comfortable visual rehabilitation. Nevertheless, we recommend surgery be

performed in two stages whenever limbal stem cell transplantation is performed without the use of systemic immunosuppression or when the surgeon does not have enough experience with this type of surgery. If limbal stem cell transplantation has to be ruled out because systemic immunosuppression cannot be used, an alternative treatment for stromal opacity is to combine keratoplasty with an amniotic membrane graft to promote epithelialization in the postoperative period. However, this option is often accompanied by postoperative persistent epithelial defects that can lead to opacification of the transplanted cornea in the short to medium term. In such cases, some authors[42] recommend using a long-term therapeutic contact lens postoperatively to try to improve results.

Patients with corneal opacity not secondary to ARK fall into a different category. This includes corneal edema (with endothelial damage, for example, due to cataract surgery) and corneal leukoma (e.g., secondary to trauma or infectious keratitis). In our experience, the main cause for stromal opacity not secondary to limbal stem cell deficiency in patients with aniridia is corneal edema due to cataract surgery. We must not forget that visualization during surgery in these patients is poor because of the keratopathy and the absence of an iris, so the intraocular lens must always be implanted within the capsular bag. Trying to implant an intraocular lens when the capsulorhexis is not intact usually ends with a dislocated intraocular lens in the anterior chamber and secondary corneal edema.

These types of opacities, which is not secondary to limbal deficiency, is seen much less frequently but we mention them because they can be successfully treated with keratoplasty alone (after first ruling out that it is not associated with a major limbal stem cell deficiency).

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SECTION III

CHAPTER 8
PATHOGENESIS AND CLINICAL FEATURES OF GLAUCOMA IN PATIENTS WITH ANIRIDIA

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Aniridia consists in the congenital absence of the iris arising from a complex embryonic malformation of the iris, trabecular meshwork and cornea associated with limbal deficiency caused by haploinsufficiency of the PAX6 gene located on the short arm of chromosome 11. Glaucoma is an important disease in aniridia patients, as it is estimated that over 50% of patients with aniridia will develop it in the course of their lifetime.

In aniridia, the iris stroma, which originates in the neuroectoderm of the neural crest, does not form properly between the 12th and the 14th weeks of gestation because mesenchymal cells fail to migrate. In addition, apoptotic mechanisms that are normally involved in the development of the iris and anterior chamber are excessively active in these patients. However, the iris is never entirely absent since a rudimentary iris stump is visible on gonioscopy. Additionally, the angle is incompletely developed and abnormal retinal processes appear that cover both the pars plana and the pars plicata of the ciliary body.[i]

The incidence of childhood glaucoma in patients with aniridia varies between 6% and 75%, which means that the risk of developing glaucoma increases with age.[ii,iii] It develops because of the progressive migration of the iris stump towards the iridocorneal angle, resulting in the formation of synechiae that obstruct the trabecular meshwork. The severity of glaucoma is related to the degree of malformation of the angle: not all patients with aniridia develop glaucoma, and when it does develop, it is rare in the first decade of life, the onset of the disease typically being most frequent after the 2nd decade.2

There are important differences in the gonioscopic findings of aniridic patients with and without glaucoma. In the former we find angles that are closed or blocked by a web of tissue in front of the trabecular meshwork, whereas in the latter the angles are wide open.iv The angles of patients with aniridia but no glaucoma are completely free of adhesions between the iris stroma and the trabecular meshwork along all or almost all of the angle and the rudimentary iris is in a normal plane, perpendicular to the axis of the eye and without any portion of it extending forward. However, the angles of patients with aniridia and glaucoma are not in the same condition. There are variations in the degree of severity and the shape of the malformation from one patient to another, which determine the severity of disease, but in all cases the rudimentary iris stump extends anteriorly to cover the filtrating portion of the trabecular meshwork in part or wholly, leading to increased resistance to the flow of aqueous humor that is proportional to the increase in intraocular pressure and degree of obstruction that can be observed.

This anterior migration of the iris is progressive, so that at birth the angles of babies with aniridia are open, but fine strands and adhesions between the iris stroma and the trabecular meshwork (that are synechiae-like) can already be seen, giving the space between the periphery of the rudimentary iris stroma and the scleral spur and ciliary body band of the trabecular meshwork a jagged or "sawtooth" look. With time, the consistency, thickness and pigmentation of these "adherences" increases, leading to the anterior migration of the peripheral area of the rudimentary iris ("as if it was being pulled"), blocking off the formerly visible angle.2 In addition, the anterior migration of the peripheral area of the rudimentary iris causes the iris plane to tilt, altering its normal position in relation to the angle and causing it to close, so that on gonioscopic examination an optically closed angle is seen. Progression of the disease occurs at different rates in different patients and progresses asymmetrically in the individual patient.

In a minority of aniridic patients with glaucoma, the angle appears to be optically open, but it is covered with a thin layer of an amorphous, homogeneous and avascular material, which has a different degree of pigmentation and seems to increase as the patient gets older.2

There are also some cases, although rare, of patients with aniridia and open angles who develop glaucoma in adulthood.v In these patients the increase in intraocular pressure (IOP) is not caused by progressive closure of the angle and the clinical manifestations are barely different from those of chronic open-angle glaucoma (COAG). It should be noted, however, that these patients have many other ocular abnormalities (cataract, nystagmus, corneal dystrophies, limbal deficiencies, strabismus, optic nerve hypoplasia, disorders of the fovea and retina, etc.) that are also causes for impaired visual acuity and visual field defects. Even so, the gonioscopic anatomy of the angle in aniridia patients with COAG is not normal, with abnormal pigmentation, abnormal ciliary processes and areas of angle recession, so although the angle is optically open, the trabecular meshwork probably does not function correctly.5

As has already been mentioned and although it is a congenital disease, the onset of glaucoma in aniridia patients does not usually begin in the first few years of life, but in late childhood or adolescence instead, so they do not typically present with buphthalmos, megalocornea or the typical Haab striae that are characteristic of congenital glaucoma. There are, however, cases in which glaucoma is diagnosed in the first months of life. Thus, in a recent review of cases of congenital glaucoma treated in our department (more than 400 children with congenital glaucoma), only eight patients (male to female ratio: 1:7, 14 eyes) were identified as having aniridia and early-onset glaucoma (mean age at diagnosis of 6.57 months). One patient was diagnosed at 3 years of age and the other seven were diagnosed before the first six months of life.The clinical features of these eyes at the time of diagnosis were as follows:

IOP:	29.9 SD 6.3 mmHg
Horizontal Diameter:	11.6 SD 1.14 mm
Vertical Diameter:	10.9 SD 0.74 mm
AL:	21.1 SD 3.14 mm
C/D ratio:	0.28 SD 0.2

During follow-up (mean 81 months) a total of 20 surgeries (1.43 surgeries/eye) were performed. In four eyes goniotomy was the first surgical procedure and in 10 eyes

trabeculectomies were performed in the first place, since corneal transparency did not allow the performance of ab interno surgery.

Three eyes were controlled with a single goniotomy (follow-up of 84, 12 and 12 months), one eye was controlled with a goniotomy (failed at 1 month) and a trabeculectomy, and five were controlled with two trabeculectomies.

In spite of adequate intraocular pressure control in all of the cases, the final visual acuity was less than 0.1.

In cases of late-onset glaucoma (usually in the 2nd decade of life), the clinical features are similar to chronic angle closure glaucoma (CACG) in adults, but with the difference that neither the crystalline lens nor iridotomies play any role in these patients. Several other associated ocular abnormalities are involved in the loss of vision and visual field defects that may also occur.

As in any type of glaucoma, the diagnosis is based on measurement of IOP, angle examination, visual field testing and examination of the optic nerve. However, in these patients diagnosis is complicated by ocular abnormalities that accompany the disease (cataracts, corneal dystrophies, nystagmus, strabismus, etc.), which complicate the examination of both the anterior pole and the optic nerve and interfere with the results of the perimetry test. A characteristic of this disease is that patients with aniridia are able to withstand relatively elevated IOPs with barely any changes in optic disc cupping5 and so, while taking into account the absolute value of the IOP, delaying treatment of these patients could also be considered until they begin to show an increase in cupping in addition to increased IOP. In consequence, it is important that these patients are closely followed with routine comprehensive exams, including taking photographs of the optic nerve (and where possible, by using other structural tests) in order to monitor for any changes. Patients with elevated IOP alone should not be treated, unless it is higher than 30 mmHg.5

In the early stages of the disease the angle may remain partially open, so patients may respond to topical hypotensives and therefore it may be possible to control the disease with medical treatment. Nevertheless, it is not uncommon that the rate of disease progression to gonioscopic angle closure is such that it inevitably fails to respond to topical management and surgery becomes mandatory. Goniotomy in patients with angle abnormalities may help to improve the disease prognosis as well as the degree of response to medical treatment and seems to be the most appropriate surgical technique for the treatment of such patients.2

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CHAPTER 9
STRATEGIES IN THE MEDICAL TREATMENT OF GLAUCOMA IN ANIRIDIA

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(translation by a resident doctor)

9.1. Introduction

Aniridia includes a spectrum of diseases derived from an eye morphogenesis alteration. These diseases share the presence of a variable degree of bilateral iris hypoplasia and they may be associated with systemic anomalies. This alteration of the eye development derives from a defect in the PAX6 gene located on chromosome 11, with an autosomal dominanEt or acquired inheritance.

Among the different eye malformations affecting the anterior segment of aniridia patients, we can find trabeculodysgenesis, which can lead to glaucoma in up to a 75% of aniridia cases. 1 Glaucoma can develop at any age in these patients.

(Trabeculodysgenesis is one of the different eye malformations affecting the anterior segment of aniridia patients, leading to glaucoma in up to a 75% of cases. Glaucoma can develop at any age in these patients.)

In addition to this misdevelopment of angle structures responsible for aqueous humor drainage, a chronic synechial angle closure can be found, secondary to the anterior rotation of iris traces present in the angle region.

In the same way, structural alterations derived from anterior segment surgeries or steroid induced intraocular pressure (IOP) elevation are factors that can worsen a pre-existing glaucoma in these patients. 2

Apart from the high prevalence of glaucoma, we must take into account that population affected by aniridia has several added difficulties in glaucoma diagnosis and treatment (diagnosis and treatment of glaucoma). 3

Regarding the diagnosis, IOP measurement with applanation tonometry becomes more difficult to obtain due to the frequently associated nystagmus, also making difficult other explorations as gonioscopy. IOP measurement can also be less accurate as a result of corneal alterations that often accompany aniridia, i.e. corneal leucomas or keratoplasty. On the other hand, it has been reported that aniridia patients have an increased corneal thickness compared to normal population, what could lead to an incorrect measurement by applanation tonometry. This increase in central corneal thickness (CCT) is not the result of an altered endothelium, but could be determined by the effect on the cornea of the PAX6 gen mutation responsible for aniridia. 4

Structural and functional examination of the optic disc also results more difficult due to poor cooperation because of photophobia and nystagmus, ocular media opacities and low vision.

Although secondary glaucoma in aniridia patients usually requires surgery for its control, as it is often refractory to medical therapy, 5 sometimes it is necessary to prescribe medical treatment and then keep peculiarities of these eyes in mind.

9.2. Antiglaucomatous drugs and aniridia

Among the hypotensive agents available today we can find prostanoids or hypotensive lipids. They have been shown to have a strong hypotensive effect and few side effects in adults, and that is why they have become very often used antiglaucomatous drugs as first-line treatment or adjuvant treatment. Nevertheless, glaucoma in aniridia usually appears in the first two decades of life, 6 and we must also take into account that experience with this group of drugs at these ages is limited and they seem to be less effective the younger children are. 7 In the same way, although there are very few reports about the effectiveness of prostanoids for glaucoma in aniridia, apparently their hypotensive effect is not as strong as (that described) for glaucoma in adults. Eye malformation typically affecting these patients is supposed to include also uveoscleral outflow pathway, where these drugs act (work, which one is more formal?). 8,9

As in adults, prostanoids show few ocular side effects, although literature reports about their use in pediatric population (childhood) are limited; it has been reported a conjunctival hyperemia incidence similar to that in adults (5,3%), but less incidence of hypertrichosis and iris darkening (1,8%). 10 Systemically, there is only one case report of a possible sleep disorder 11 and another one of hyperhidrosis 12 related to latanoprost treatment.

The effect of prostanoids on the pathologic cornea that usually accompanies aniridia is another important aspect of the medical management of glaucoma in these patients, as these drugs could increase the risk of ocular surface failure (fig. 1). It is worth remembering that aniridic keratopathy affects up to 90% of cases, 13 and even though it can remain asymptomatic, external aggressions, such as topical hypotensive treatments, can break the weak balance of corneal epithelium. This ocular surface alteration generates low vision, corneal ulcers and leucomas; it can appear in the first decade of life as a thickening of the peripheral corneal epithelium, followed by superficial neovascularization, that can extend over the whole cornea, evolving to a subepithelial fibrosis and stromal scarring.

The ocular surface disorder in aniridia could have its origins in a primary limbal stem cell deficiency, 14 which lead to a poor corneal epithelium regeneration, with ocular surface instability, secondary dry eye, and the loss of the corneal epithelium function as a barrier. The chronic aggression over the ocular surface epithelia creates a response of metaplastic transformation in them. This unstable situation could be destabilized by the use of hypotensive lipids since they may produce a superficial punctate keratopathy, 15 (fig2) the same as other antiglaucomatous eyedrops. This superficial punctate keratopathy is mainly associated with the eyedrops preservative, benzalkonium chloride. 16 In addition, this kind of drugs could inhibit, at least experimentally, epithelial cell migration. 17

On the other hand, the alteration in functions regulated by PAX6 gen may cause the different signs of this keratopathy. Defects include a reduction in adhesion molecules levels of epithelial cells that, along with a cytokeratins deficiency, leads to an ocular surface fragility. 18 Furthermore, there is a metalloproteinase-9 (MMP9) or gelatinase B 19 deficiency which causes a collagen instability in the corneal extracellular matrix, with fibrin accumulation and cellular infiltration, a collagen fibers organization disorder with subsequent corneal transparency loss.

Indeed, it seems that central corneal thickness in aniridia patients is higher than that in the average population for the same age. 20 Prostanoids also act over the same metalloproteinases, however, the effect is the opposite, they trigger increase of these enzymes synthesis, leading to collagen degradation in the extracellular matrix, which (what) would be related to a corneal stromal thinning. 21

Another important drug class is the topical beta-blockers group. Their mechanism of action is based on a large (big) reduction, up to 50%, of aqueous humour production (, up to the 50%). 22 They have been used for over (more than) 20 years, but their mechanism of action has not been proved, accepting the beta-adrenergic receptor blockage in the ciliary body.

Beta-blockers hypotensive efficacy is good in the short-term. Even though it has been reported the possibility of an effectiveness loss in long-term treatments (drift). They are also excellent agents for the combination therapy, as they show an appropriate additive effect when they are administered in combination with other therapeutic drug group.

Systemic side effects are well known, we could highlight cardiovascular effects (bradycardia) 23 or respiratory effects (dyspnea, respiratory insufficiency). 24 These side effects are more common (frequent) in old (elderly) patients and disappear once the treatment is stopped.

In the context of aniridia, local side effects of these drugs are more important.

Besides the presence of benzalkonium chloride as a preservative in most (most of the) beta-blockers commercial preparations, the drug itself causes a mild anesthetic effect on corneal surface. It has also been reported that long-term treatment with these drugs induces a decrease in tear production 25 and goblet cells density. 26 These latter facts together with the superficial anaesthesia may cause dry eye symptoms and the subsequent corneal epithelium damage.

Another drug class is the selective alpha-2 adrenergic agonists group, whose prime example for long-term treatments is brimonidine.

Brimonidine is the most selective alpha-2 adrenergic agonist available nowadays, 27 although the human eye shows some vasoconstriction after its instillation, 28 that (what) suggests an alpha-1 agonist effect. The accepted mechanism of action is a decrease in aqueous humour production by about 20%. 29 Its use is contraindicated in children due to the possible central nervous system depressant effect.

Locally, the most important problem related to long-term use of this substance is the development of an allergic conjunctivitis in 10% of cases during the first year of treatment. 30

The last therapeutic group we are mentioning here is the topical carbonic anhydrase inhibitors group. There are two commercially available compounds, dorzolamide and brinzolamide, with very little difference between them, except for local tolerance (stinging sensation upon instillation), which seems to be better with brinzolamide. 31 Their mechanism of action is a decrease in aqueous humour production at (in) the ciliary body, by the inhibition of carbonic anhydrase enzyme. Their hypotensive efficacy is moderate and they must be administered between twice and three times a day. Local tolerance is only acceptable, as a 10-15% incidence of punctate keratitits has been reported in chronic treatments. 32 Moreover, it has been proved that treatment with these agents induces a slight increase in corneal thickness, suggesting an effect on these drugs pumping ability.

Clinical management

As we have mentioned before, all drugs have a potentially toxic effect on ocular surface. This toxicity derives from the presence of preservatives as well as from the drug effect itself. Preservative-free preparations are ideal for medical treatment in aniridic patients, where (in whom) the epithelium will probably get significantly altered throughout their life. Nevertheless, we would like to point out that even preservative-free eyedrops contain active ingredient, which is also potentially toxic.

As a basis in medical treatment of glaucoma in aniridic patients, we must highlight two different situations. A) Aniridic patients with no previous surgery, usually young, in whom (where) elevated IOP is the most relevant find in exploration. B) Aniridic patients who have already undergone surgery (cataracts, keratoplasty, etc. Fig 3) usually with an important alteration in corneal transparency (superficial corneal leucoma related to limbal stem cell failure) and/or intraocular structures alteration (retained lens fragments, intraocular implants…). Below, we will describe the most important aspects, in our opinion, for the management of these two situations.

A) In virgin eyes, it is indispensable an ultrasonic pachymetry, because, as we have already said, aniridic patients have an increased corneal thickness. In this situation, cornea may be extremely thick and we have to modify applanation tonometry IOP readings, in practice, subtracting 3 to 4 mmHg in most patients. This reduces drastically the number of virgin eyes needing medical treatment.

In the same way, target IOP for a young aniridic patient with an elevated IOP and an optic nerve with a well preserved neuroretinal rim may rise (go) up to 24 mmHg, in the likely case of (a very thick cornea) the cornea being very thick.

Visual field in these eyes is (of little help) not very helpful, as it will show visual field defects caused by glaucoma, but also those caused by amblyopia, cataract, etc. The ideal assessment method is the ophthalmoscopy fundus examination, to perform a classic optic disc evaluation.

In these cases we will medically treat only if the optic disc shows signs of glaucomatous damage, and IOP is clearly elevated (>24 mmHg if thick pachymetry). We will start with preservative-free drugs (like beta-blockers), avoiding combination therapy as much as possible (high target IOP). In case of other drugs use (if we must use other drugs), the physician should keep an eye on the possible appearance of limbal insufficiency signs, in order to stop responsible drug or strengthen lubricant treatment.

B) In complicated eyes, usually with previous surgeries, the approach will be similar (regarding the thick pachymetry and high target IOP), but polytherapy will be needed more often. We just want to point out that prostaglandin analogues can induce cystoid macular edema and some degree of intraocular inflammation in predisposed eyes, so they must not be considered as first-line treatment in these complicated cases. Carbonic anhydrase inhibitors have also been related to corneal edema in predisposed eyes. On the other hand, alpha-2 adrenergic agonists (if well tolerated, regarding allergic conjunctivitis) add a mild vasoconstrictor effect and therefore an anti-inflammatory effect, which is beneficial for these cases, mainly in the immediate postoperative period.

Also in this case, first-line treatment should be preservative-free drugs (only beta-blockers are available with (on) this formulation).

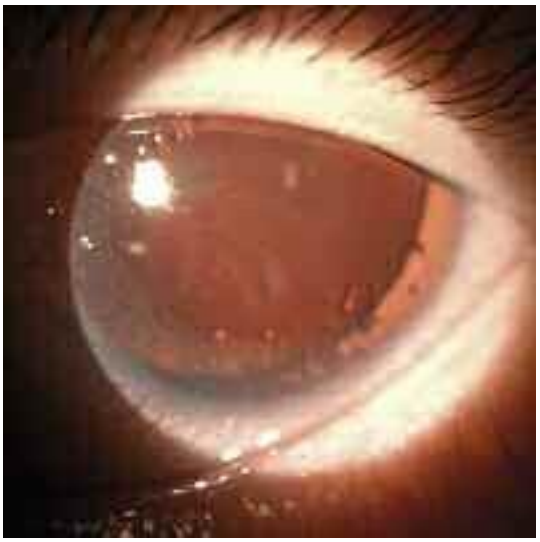

Figura 9.1. Aspecto típico de la epiteliopatía cor- neal en un paciente afecto de aniridia.

Figura 9.2. Queratopatía punctata (con tinción fluoresceínica) como respuesta tóxica corneal al tratamiento con un análogo de las prostaglandinas.

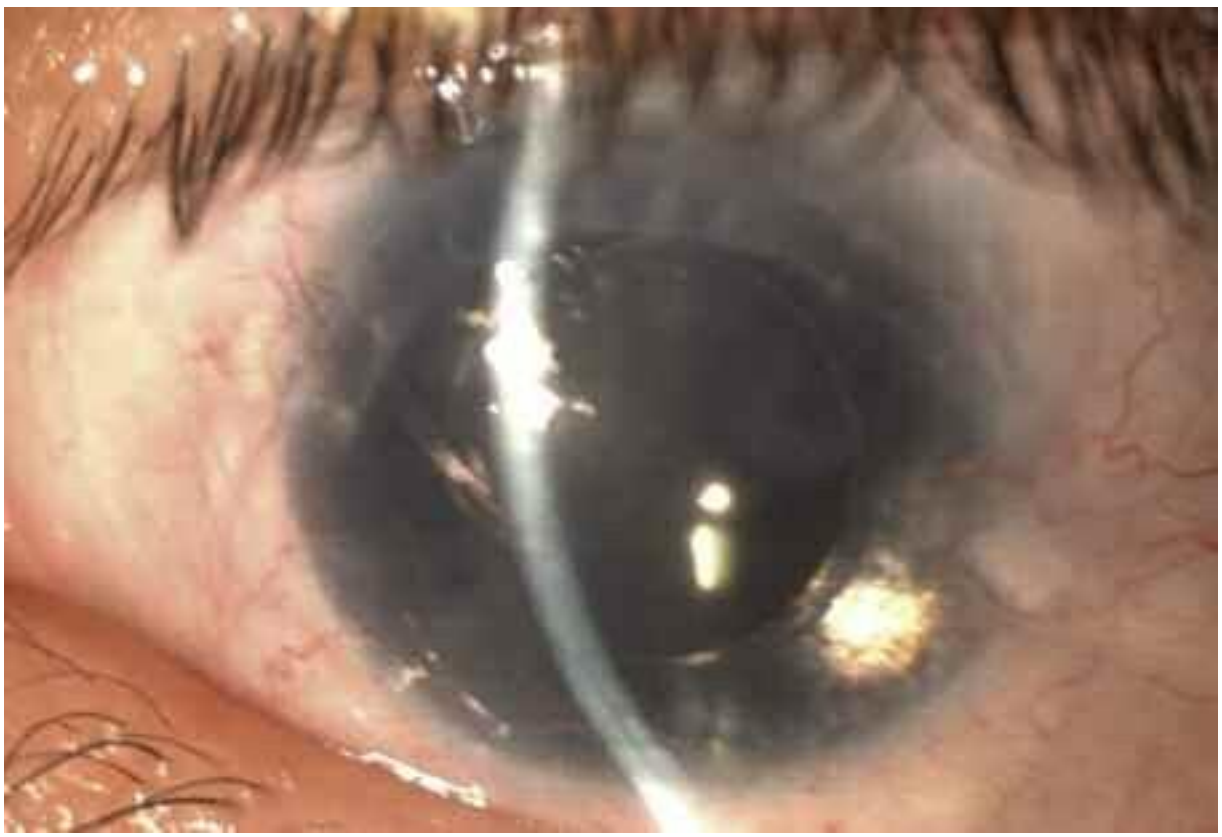

Figura 9.3. Aspecto del segmento anterior de un ojo aniridíco con cirugía de catarata e implante intraocular y epi- teliopatía corneal avanzada.

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CHAPTER 10
SURGICAL TREATMENT OF GLAUCOMA SECONDARY TO ANIRIDIA

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10.1. Introduction

Glaucoma is one of the most common causes for loss of vision in patients with aniridia and occurs in 50% to 75% of cases1. The mechanism that causes glaucoma in patients with aniridia is related to changes in the angle that occur in the first decade of life. Although glaucoma develops in childhood or adolescence, it does not exhibit typical signs such as enlarged corneas or myopia. Aniridic glaucoma is rare in childhood and is associated with abnormalities of Schlemm's canal or angle function2.

The development of glaucoma is correlated with changes in the angle, which is normal in childhood and undergoes progressive changes between the ages of 5 and 15 years, resulting in blockage of the angle owing to the anterior positioning of the rudimentary iris stump. The gradual obstruction of the angle may be due to a confluence of tissue elements between the peripheral iris and the angle wall. These synechiae migrate forward causing occlusion from the scleral spur to the posterior trabecular meshwork. This alteration may be accompanied by a change in the angle of the iris from its normal plane to a plane parallel to the axis of the eye. Other mechanisms that cause glaucoma in these patients include absence of Schlemm's canal or angle closure secondary to the use of pilocarpine3. In eyes with moderate IOP elevation the angle obstruction is limited to the upper quadrant, whereas in eyes with advanced glaucoma the iris stroma covers most of the trabecular meshwork.

10.2 Surgical management

After all medical options for the treatment of glaucoma associated with aniridia have been exhausted (which, in most cases, prove to be ineffective) surgical treatment should be considered. However, surgical management is complex and requires unconventional techniques. A review of the experience of 41 ophthalmologists concluded that no single technique had been shown to be effective4. Of the techniques described in the literature, the following should be highlighted:

10.2.1 Argon laser trabeculoplasty

Although use of the argon laser may sound interesting in theory, the results of cases reported in the literature have been unsatisfactory, so it is not recommended for the treatment of this disease1,2.

10.2.2 Trabeculectomy

Traditionally, classic trabeculectomy has been the first line treatment but it tends to fail in most cases, especially when performed in children (success rate of 9% to 17%)2,5. In another study by Nelson et al., 5 of 14 patients required reoperation or failed with primary trabeculectomy. Other authors have reported success rates ranging from 0% to 9% for filtering procedures (partial or full-thickness2, 6).

Okada and colleagues reported good results in a study in which they performed 17 trabeculectomies without antimitotics and 3 trabeculectomies with mitomycin C in 10 eyes of 6 patients under the age of 40 years who had glaucoma and aniridia. Patients had IOPs less than 20 mmHg for at least 14.6 months after surgery7.

However, another study in which all of the patients underwent trabeculectomies with mitomycin C reported a failure rate higher than 60% in patients with aniridia8.

10.2.3 Trabeculotomy

Other procedures that have been used are trabeculotomy9 and internal sclerectomy with an automated trephine10.

A study by Wiggins and Tomey2 found that trabeculotomies were ineffective. Nonetheless this procedure has been recommended for the initial treatment of aniridic patients with uncontrolled glaucoma. In another study in which trabeculotomies were performed on 12 eyes, glaucoma was controlled on 10 eyes (83%) (6 after only one procedure and 4 following two trabeculotomies); mean follow-up was 9.5 years. Only 3 eyes (25%) controlled IOP without medications. Younger patients had better outcomes, which was attributed to their not yet showing progressive changes in the angle9.

10.2.4 Drainage tube implants

Drainage tube implants (success rate of up to 83%) are a good alternative in most cases although it may sometimes be necessary to use techniques affecting the ciliary body such as cyclocryotherapy or diode laser cyclophotocoagulation11.

Molteno et al.12 reported on a series of 3 aniridic eyes with glaucoma that achieved IOPs of less than 20 mmHg without medications following Molteno valve implantation. Another case has been reported of a patient with aniridia and glaucoma in whom a double-plate Molteno valve implant was used, achieving an IOP of less than 20 mmHg in both eyes13.

Wiggins and Tomey2 reported IOP control being achieved in 5 out of a series of 6 eyes (83%). Although the success rate of Molteno implant surgery is favorable, most authors do not recommend it as an initial procedure because of the high risk for complications. In a more recent study by Arroyave and colleagues14 that included 8 eyes of 5 patients and a mean follow-up of 92 months, Baerveldt drainage implants were used. A decrease in mean IOP from 35 mmHg to 14.9 mmHg (without medication) was observed; final visual acuity improved in 5 of 8 eyes (63%) and remained unchanged in 2 eyes (25%). One patient lost light perception due

to retinal detachment. Success rates based on survival analysis were 100% at 6 months and 88% at 1 year. Another more recent study in which Baerveldt implants were used had a success rate of only 50%15.

Another device that has been used is the Ahmed valve (Figures 1 and 2), which is associated with a success rate in controlling IOP that ranges from 50% to 75%16. The advantage of this valve is that there is a smaller pediatric-sized model, as well as a double-plate version that allows for drainage of a larger surface area. As is the case with other procedures, the most common problem is medium- or long-term failure. One cause that has been described for this failure is fibrovascular ingrowth within the Ahmed valve17. Other complications that have been reported with valves implanted in eyes with aniridia include athalamia (33%) and tube migration (17%)2.

10.2.5 Ciliary body destructive procedures

Cyclocryotherapy has not shown good results in patients with aniridia. Despite reports of higher rates of successful IOP control (from 17% to 66%)2, 6;18-20 compared to other methods, the high incidence of phthisis bulbi especially in patients with aniridia (up to 50%) and loss of vision (up to 57%) do not favor cyclocryotherapy as a primary intervention for patients with aniridia and glaucoma18-20.

The results of argon laser cyclophotocoagulation have not been very successful either, and it has been reported that it may even accelerate the preexisting tendency of tissue proliferation within the angle19. There is little experience in the use of diode laser cyclophotocoagulation as primary treatment of glaucoma associated with aniridia, although this procedure has been used successfully in association with a valve implant11.

10.2.5 Goniotomy

Goniotomy has also been used for the treatment of glaucoma in aniridia patients. There are several studies that report low success rates with this technique (20% or less)6, 9, 21. Although goniotomy is not useful in advanced cases, there are studies in the literature suggesting that a prophylactic gonioplasty technique to separate the extensions of tissue between the iris and the trabecular meshwork may prevent the development of glaucoma22-23. The surgical technique is the following: the patient's eye is treated preoperatively with 1 drop of 0.5% atropine to prevent the iris stroma from rotating anteriorly and with 1 drop of 0.5% apraclonidine to reduce post-surgical bleeding. The anterior chamber is entered either from the nasal or the temporal side, using a smaller-than-usual gonioscopy lens to allow easy entry through peripheral clear cornea. The rotation of the eyeball by an assistant enables treatment of a larger area. Using the gonioscopy knife, light downward pressure is applied to the abnormal tissue to begin separating it from the trabecular meshwork. This tissue is usually vascularized, but does not bleed readily. This maneuver is repeated all around the angle that can be visualized, using the knife to eliminate any iridotrabecular synechiae. The anatomical result can produce a permanent separation between the iris and the filtration tissue. With the use of this technique, IOP was controlled without medication in 89% of patients and with medication in 11% of patients, after a mean follow-up of 16 years.

Since prophylactic goniosurgery frees the trabecular meshwork from iris synechiae, damage to the trabecular meshwork is delayed or stopped23.

10.3 Conclusion

Surgical treatment of patients with glaucoma and aniridia is a challenge for ophthalmologists who specialize in glaucoma, since there are no clear criteria defining the ideal technique for managing these patients. A multidisciplinary approach combined with early surgical treatment may prevent glaucoma from becoming an intractable problem leading to total loss of vision in the patient.

With respect to surgical treatments that provide the best results, at present these would be prophylactic gonioplasty and valve implants.

Figure 10.1. Patient with aniridia and glaucoma in whom an Ahmed valve was implanted in the anterior chamber, with a reduction in IOP from 32 mmHg to 18 mmHg (using one IOP-lowering drug).

Figura 10.2. Detail of the anterior chamber tube of the above patient.

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SECTION IV

CHAPTER 11
ALTERATIONS OF THE CRYSTALLINE LENS IN CONGENITAL ANIRIDA

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11.1. Introduction

Congenital aniridia (or simply "aniridia", as we will use from now on) is a panocular development anomaly associated to mutations of the Pax6 gene. [i,ii] This is a gene that regulates other genes, which explains the multiple ocular structures affected, without respecting the classic division into embryonic layers. Aside from the defect in the iris that the condition gets its name from, aniridia produces alterations from the cornea to the retina and the optic nerve, including the crystalline lens and the ciliary zonule.

The impact on visual function of alterations to the lens in aniridia is variable and often relatively minor, among the multiple problems that these patients usually present. However, they currently represent the aspect of this condition that can most readily be corrected by surgical treatment. This also allows the iris defect to be corrected (at least in part) thanks to diaphragm implants, whether or not integrated with intraocular lenses. This chapter focuses on describing the changes observed clinically in the crystalline lens of the aniridia patient. Later chapters will deal with treatment and possible complications.

11.2 Clinical and biomicroscopic considerations

Alterations to the lens in aniridia can essentially be included in three clinical categories: those relating to transparency (opacities or cataracts), those relating to position (subluxation) which in general show zonular weakness, and those relating to size and shape (microspherophakia, etc.).

11.2.1 Transparency alterations

Crystalline lens opacities are very frequent in eyes with aniridia. In young patients, their prevalence has been estimated at between 50 and 85%.[iii] In an extended family in which 38 of the 76 members suffered from aniridia but with relatively conserved visual function - 61% of the affected members had visual acuity of 20/30 or better and only 5% of 20/200 or worse -, cataracts were observed in 18%. No patient in this group had nystagmus or corneal pannus.[iv]

Cataracts associated to aniridia have a variable morphology and are described as polar, cortical, subcapsular, lamellar and, more rarely, nuclear. Small opacities, either anterior or posterior polar or both, typically exist from birth, which do not usually seriously compromise vision, especially compared to other defects such as macular hypoplasia and hypoplasia of the

optic nerve or nystagmus. This may explain the scant attention paid to the presence of cataracts in some studies, including recent ones. [v] In the cases at our centre we have found the following clinical types according to the degree of opacity of the crystalline lens:

1. Lenses that are especially transparent, or have only small anterior or posterior polar opacities, or other opacities outside the visual axis. These are usually paediatric patients (Fig. 1). A transparent or almost transparent lens frequently occurs in cases of incomplete aniridia (Fig. 2), but it can co-exist with glaucoma, microspherophakia or lens subluxation (Fig. 3), or with corneal opacities.
2. Small to medium-sized predominantly anterior opacities, either polar, subcapsular or cortical. There are several variants, almost always with bilateral symmetry, from the typical flat polar plaque measuring about 1 mm, either isolated (Fig. 4, top and centre left) or associated to coronary peripheral opacity (Fig. 4 top right), or with annular reinforcement around the edge (Fig. 4 bottom). Sometimes they are prominent, pyramidal (Fig. 5) or can have lamellar extensions towards the foetal nucleus or even multiple plaques (Fig. 6).

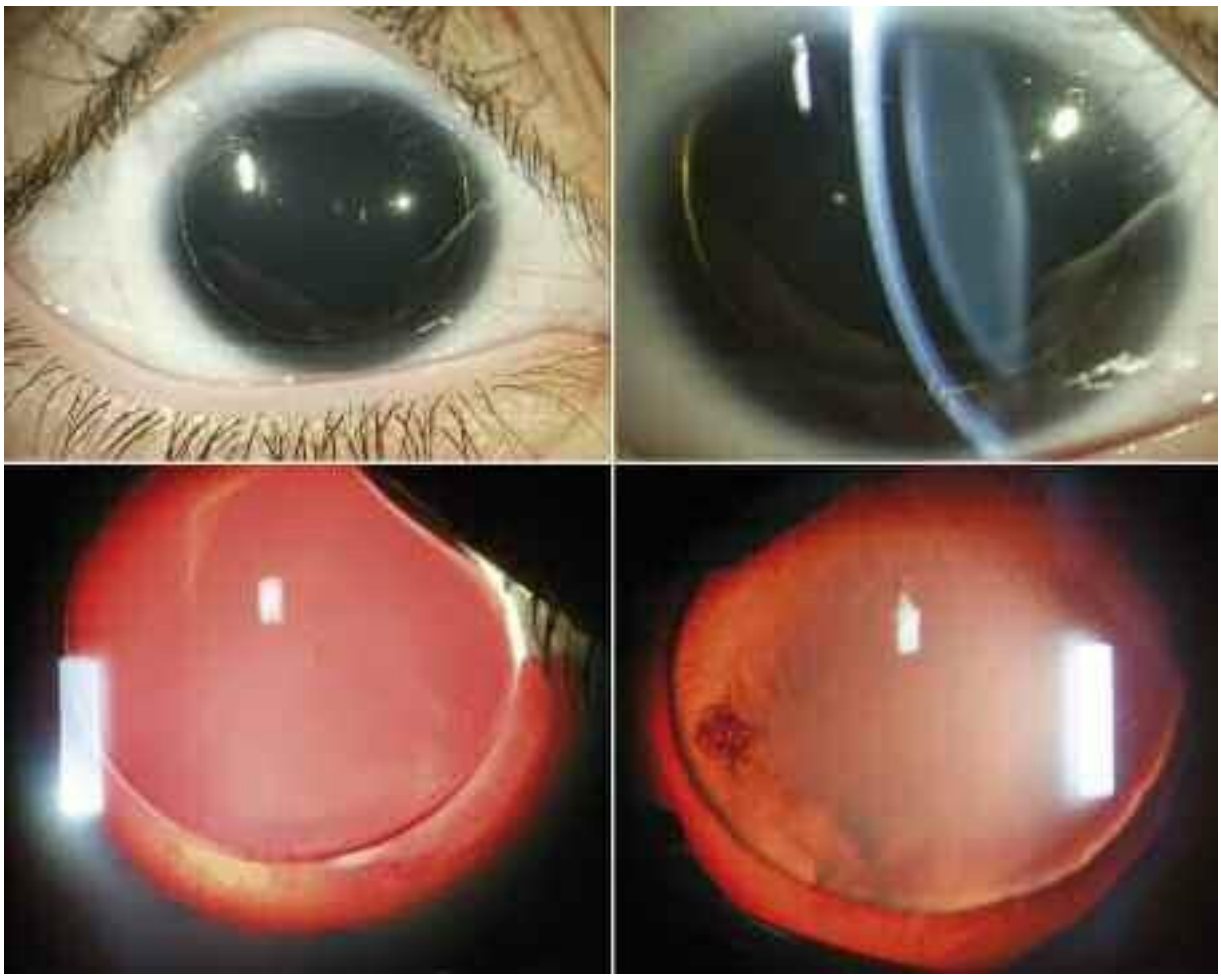

Figure 11.1. 10-year-old girl with complete aniridia and lenses described as "transparent", although there is a punctiform posterior polar opacity in OD and small peripheral anterior cortical plaque in OS. Note the incipient bilateral inferior zonular distension. She presents glaucoma which required valve implant surgery in OS.

Figure 11.2. OS of a 4-year-old boy with bilateral incomplete aniridia, transparent lens and VA < 0.1 due to possible foveal hypoplasia. IOP was normal (the white mark in the centre is a reflection of the flash).

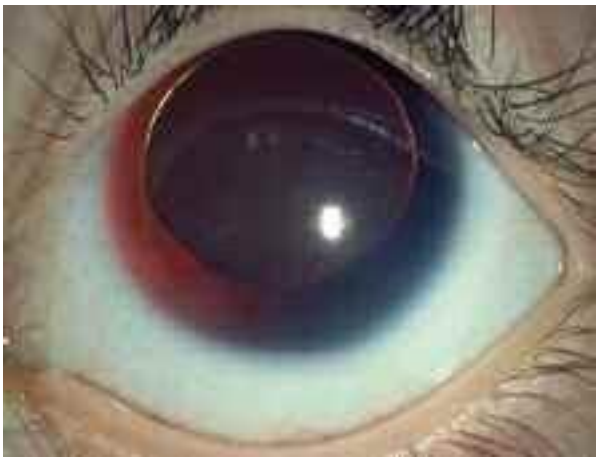

Figure 11.3. OS of a 6-year-old boy with bilateral complete aniridia and appearance of microspherophakia. The zonule has a weak and distended appearance. The lenses were transparent except for a punctiform anterior polar opacity, although he already presented glaucoma (see evolution in fig.14).

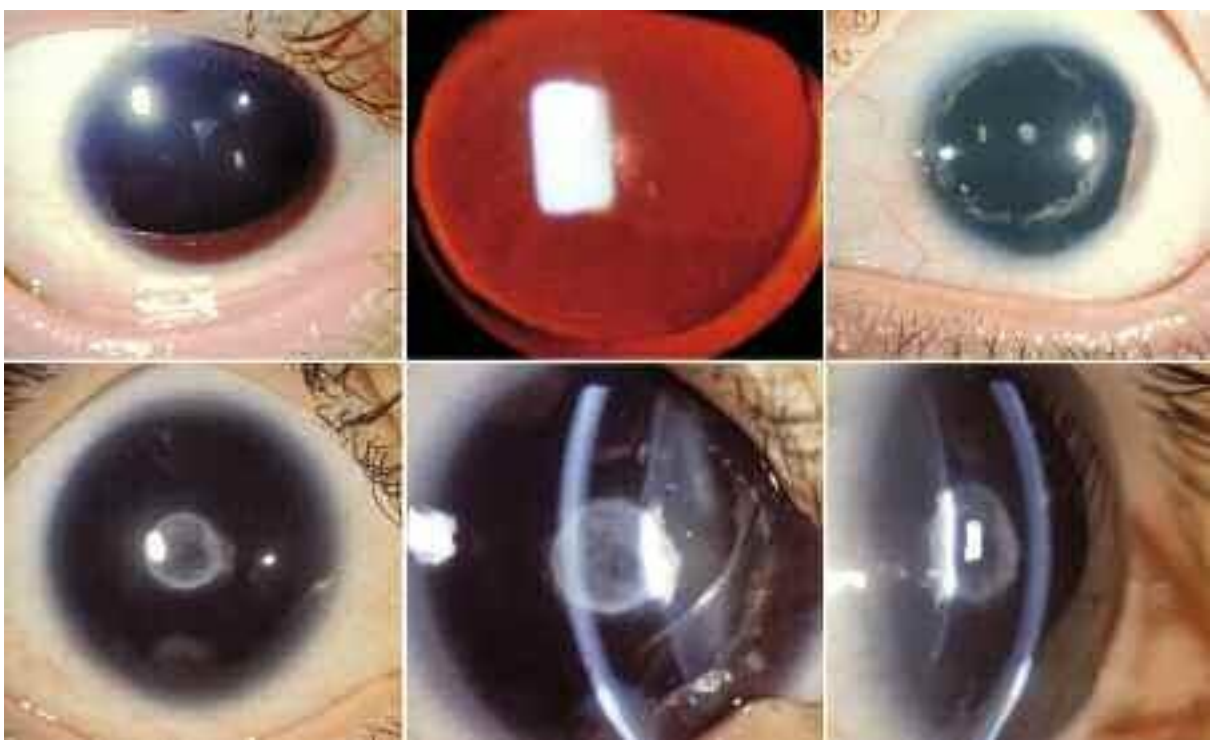

Figure 11.4. Shapes of anterior subcapsular cataracts in aniridia. Top and centre left: flat anterior polar plaque of around 1 mm, isolated, in a 22-year-old male. Top right: id. Associated to coronary peripheral opacity in a 26-year-old woman. Bottom: central plaque of 2.5 mm, with peripheral circular reinforcement, in a 4-year-old girl who also presented choroidal coloboma.

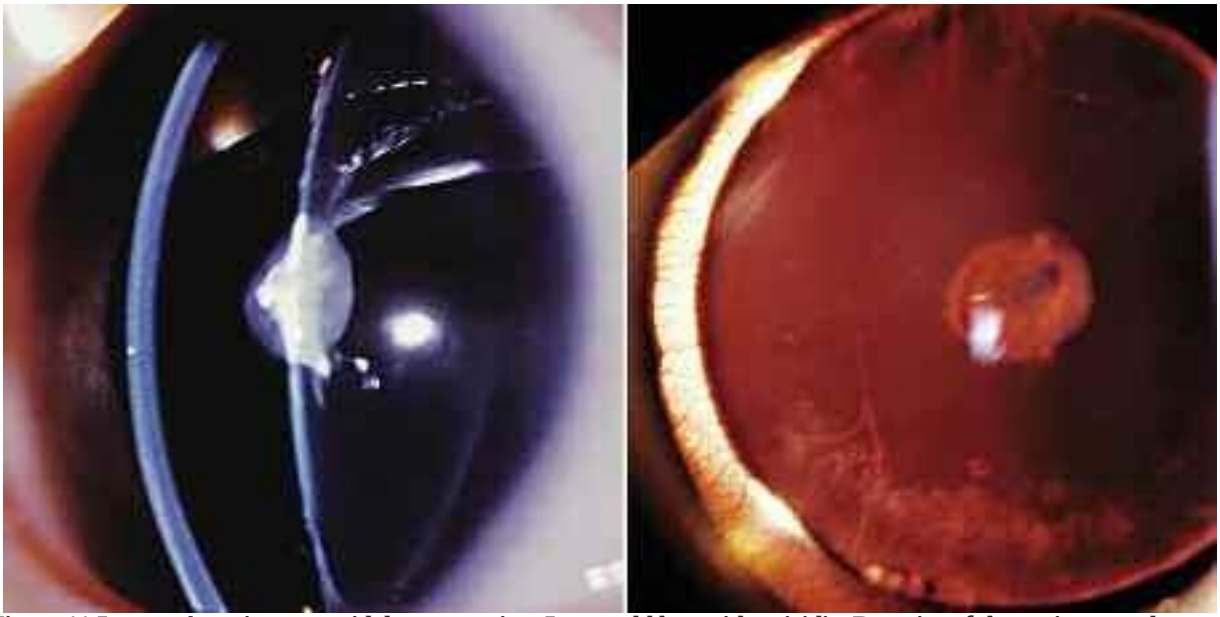

Figure 11.5. Anterior pyramidal cataracts in a 5-year-old boy with aniridia. Remains of the tunica vasculosa can be observed.

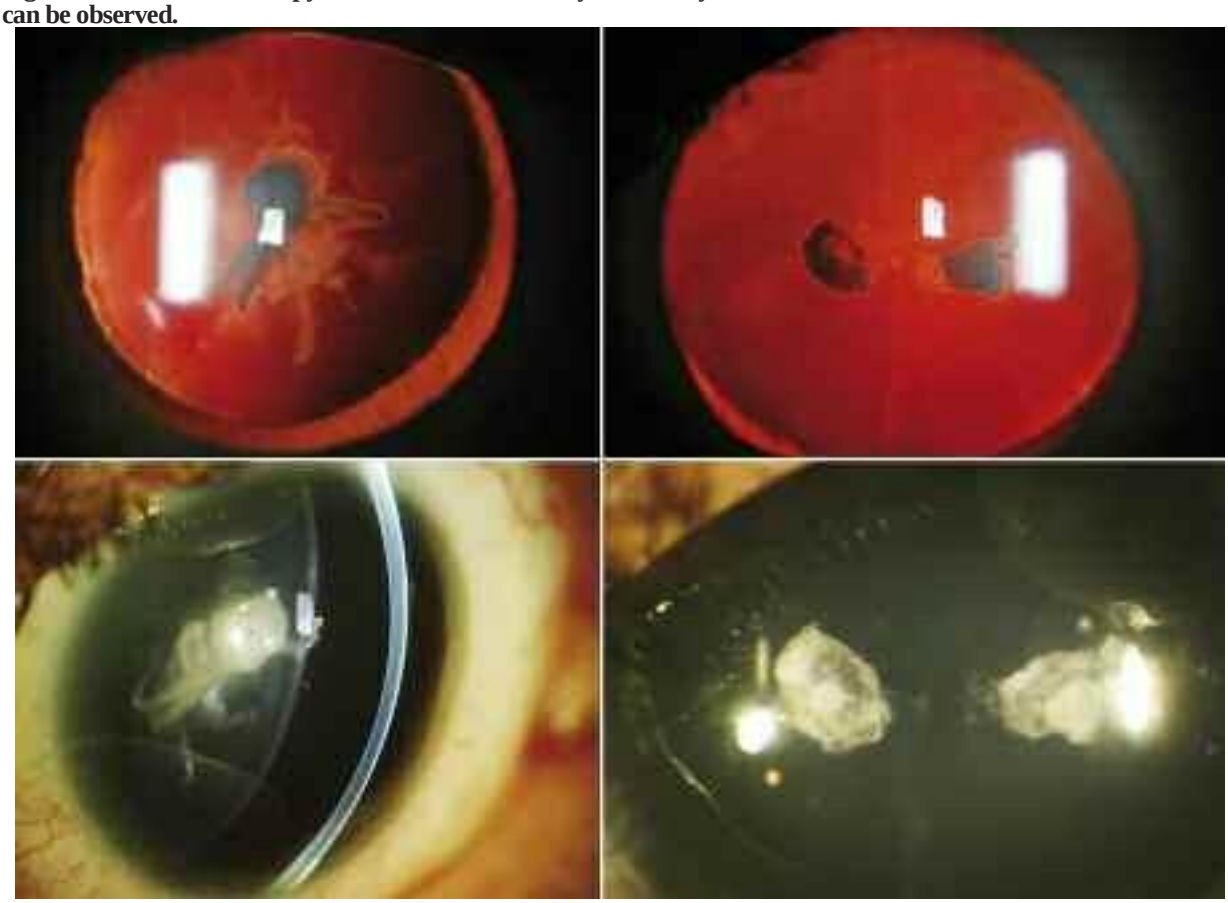

Figure 11.6. 17-year-old male with aniridia and IOP controlled with hypotensor eye drops. The OD presents an "anterior polar" cataract, actually a nodule just in front of the foetal nucleus with lamellar

extensions arching backwards and others starting at the posterior pole of the foetal nucleus. The OS presents two plaques in the anterior cortex, on a plane just in front of the nuclear interface.

3. Polar posterior subcapsular or predominantly axial cataract, sometimes associated to anterior polar or subcapsular, and which tends to progress peripherally, either on the cortical plane (Fig. 7) or another slightly deeper plane (subcortical or lamellar). This may also occur in cases of partial aniridia (Fig. 8).
4. More extensive posterior cataracts than those in the preceding group, sometimes described as subcapsular, although they usually occupy a subcortical plane. Typically with a coral-shaped or petaloid appearance: a series of radial opacities converge towards a posterior axial nodule (Fig. 9)
5. Lamellar (zonular) or annular cortical cataracts. Perhaps a variant of the above, but predominantly peripheral and with certain respect for the visual axis (Fig. 10), at least until the cataract progresses with age.
6. Nuclear cataract. Can occur rarely in isolation in congenital or infantile aniridia (Fig. 11). The nuclear component is seen as an evolutional form associated to any of the above patterns in an older age group. (Fig. 12).
7. Very evolved or ripe cataracts. The progressive affectation of the cortex causes a profuse opacity, either predominantly cortical, corticonuclear or irregular. In some cases a rapid evolution takes place with the formation of an intumescent cataract (Fig.13).
8. Finally, there is a group of patients where the degree of cataract cannot be established with precision (sometimes, not at all), due to the presence of corneal opacities or prior surgery.

In most cases of aniridia, the lens opacities progress during the first two decades of life until they become subcapsular, lamellar or cortical cataracts. Although it is normally a slow process, the gradual extension with age or the development of a nuclear component justifies in many cases the surgical approach between the third and fifth decades, apart from cases that experience a rapid maturation and subluxated cataracts.

When congenital cataracts severely affect the transparency of the optical media, their removal before 3 months has been recommended to prevent nystagmus due to sensory deprivation. However, it is not easy to calibrate the possible benefit of early removal, as nystagmus is also frequent in cases of aniridia without cataracts, especially taking into account the possible complications. In the presence of opacity of the optical media, the assessment of the state of the macula and of the optic nerve (of the potential visual acuity) is difficult, and even the information that can be obtained from electrophysiological tests is often of limited usefulness. In any case it is advisable to exercise extreme prudence in recommending surgery, due to the great inflammatory and scar-forming reactivity of eye tissue in aniridia in general and especially in infants.

11.2.2 Alterations of position

The presence of subluxation of the lens (*ectopia lentis*) is also frequent in aniridia. It has been estimated to occur in at least half of eyes and always consists of an upward displacement. As long ago as 1947, Beattie described superior subluxation in 12 members of a family of 28 with aniridia, and a much higher risk of glaucoma in cases with subluxation. [vi] As glaucoma in

aniridia is associated, at least in some eyes, to a progressive angle closure due to anterior synechiae [vii], it is possible that the presence of subluxation favours the formation of such adhesions on account of the lens movements. Alternatively, the pathogenesis of the zonular weakness could be related to more severe anomalies of the angle. Degeneration of the zonule in aniridia is possible due to the alteration (hypoplasia) of the ciliary body; an aspect has been evaluated clinically by ultrasound biomicroscopy[viii]. We have observed that cortical opacities in subluxated lenses tend to develop more in the sector that has the greatest zonular defect (Fig. 14).

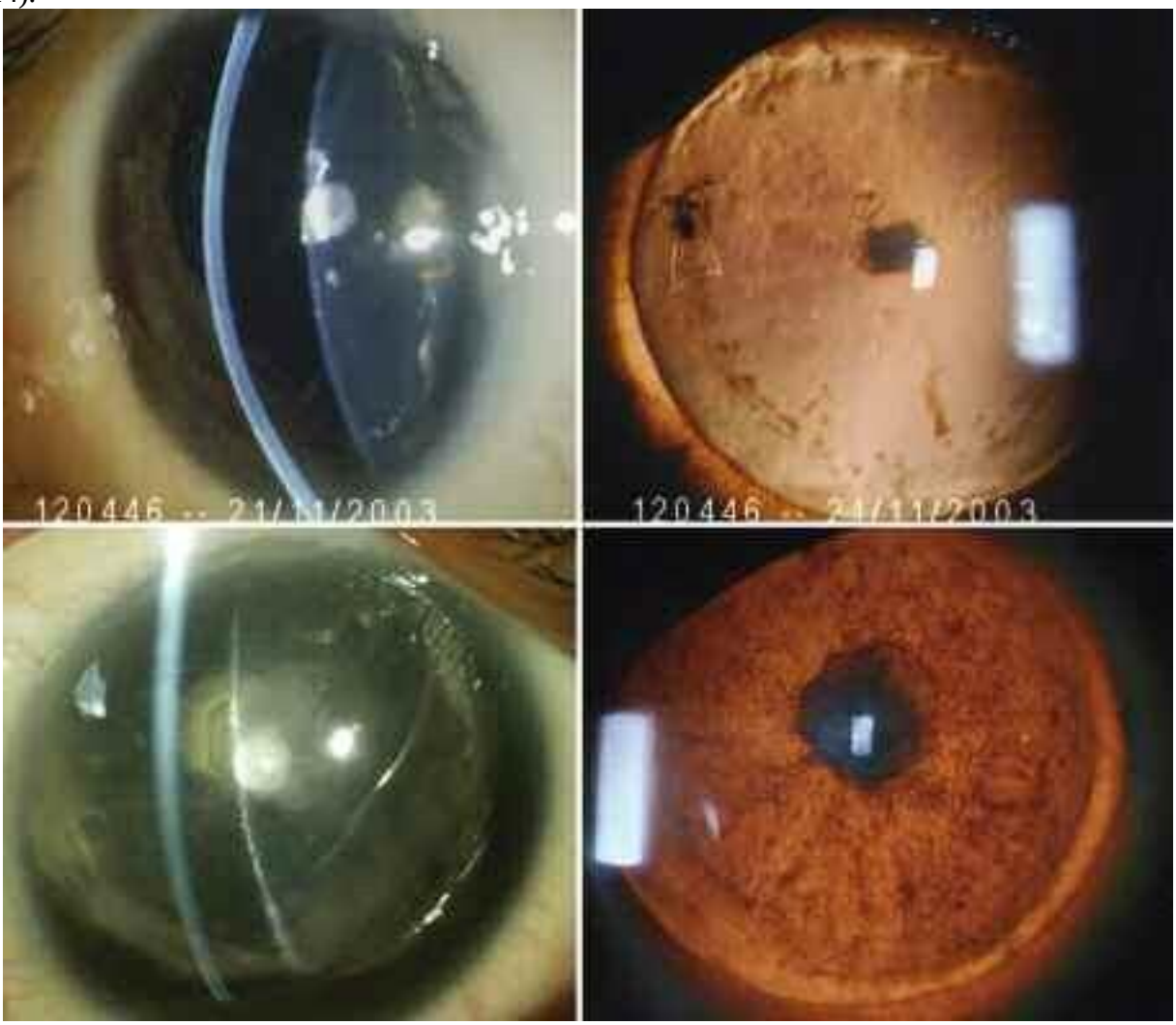

Figure 11.7. Female with WAGR syndrome, who presented polar posterior and anterior subcapsular opacities from the age of at least two years, en OU. Required glaucoma surgery in OU. At the age of 25, the opacities in OS had progressed very little (top), with discreet posterior peripheral extension, while OD (bottom) developed a diffuse cortical cataract as well as denser axial opacities.

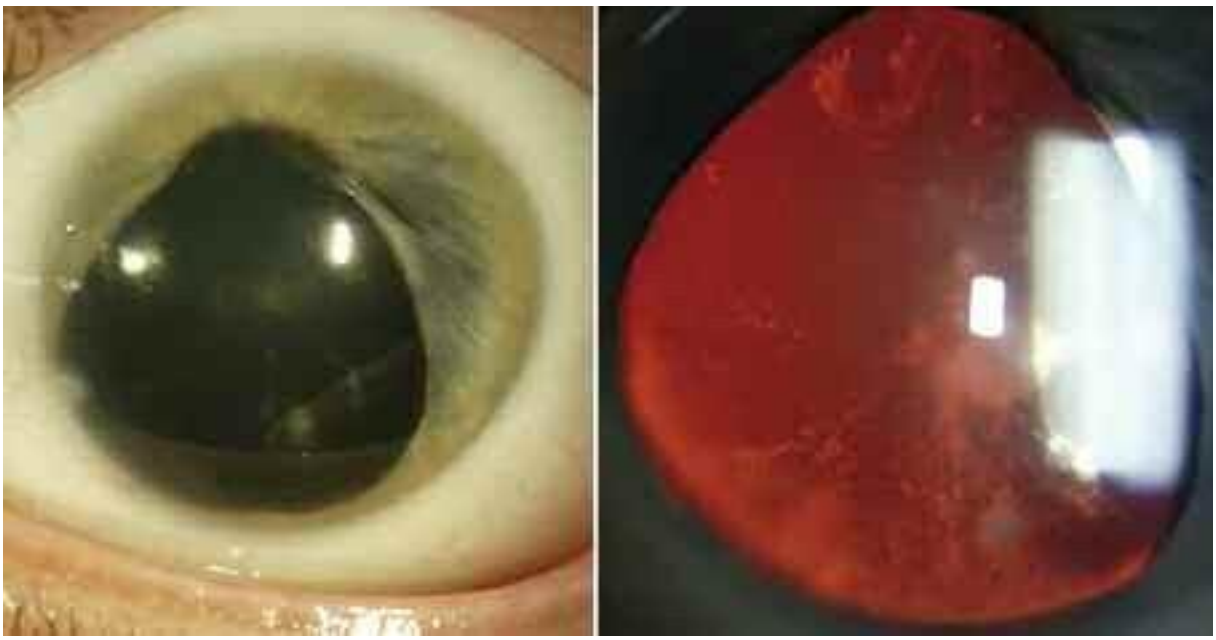

Figure 11.8. Partial aniridia associated to central posterior subcapsular opacity with peripheral satellites or branches, in a 32-year-old female patient with a family history of aniridia in four generations. There was a slight pulverulent opacity in the embryonic nucleus.

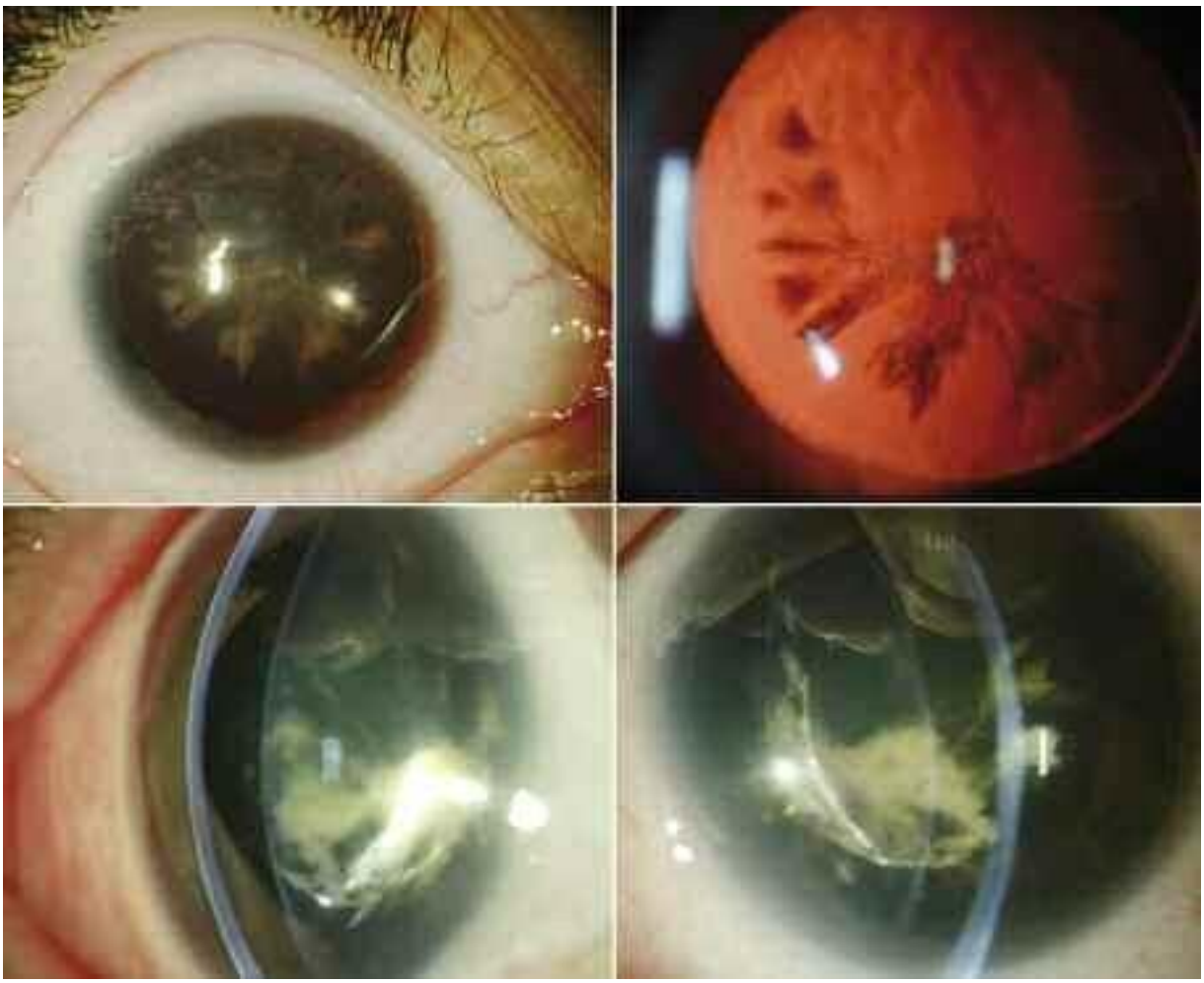

Figure 11.9. Extensive cortical or subcapsular posterior cataract in two cases of complete aniridia. Top, 13-year-old male patient where coral-shaped or petaloid aspect can be observed with branches that converge on an axial nodule, both in direct light and with back lighting. Bottom, in a 34-year-old female patient, the plane of the opacities appears to be subcortical, with a posterior lamellar cataract. In both cases there is a certain inferior predominance.

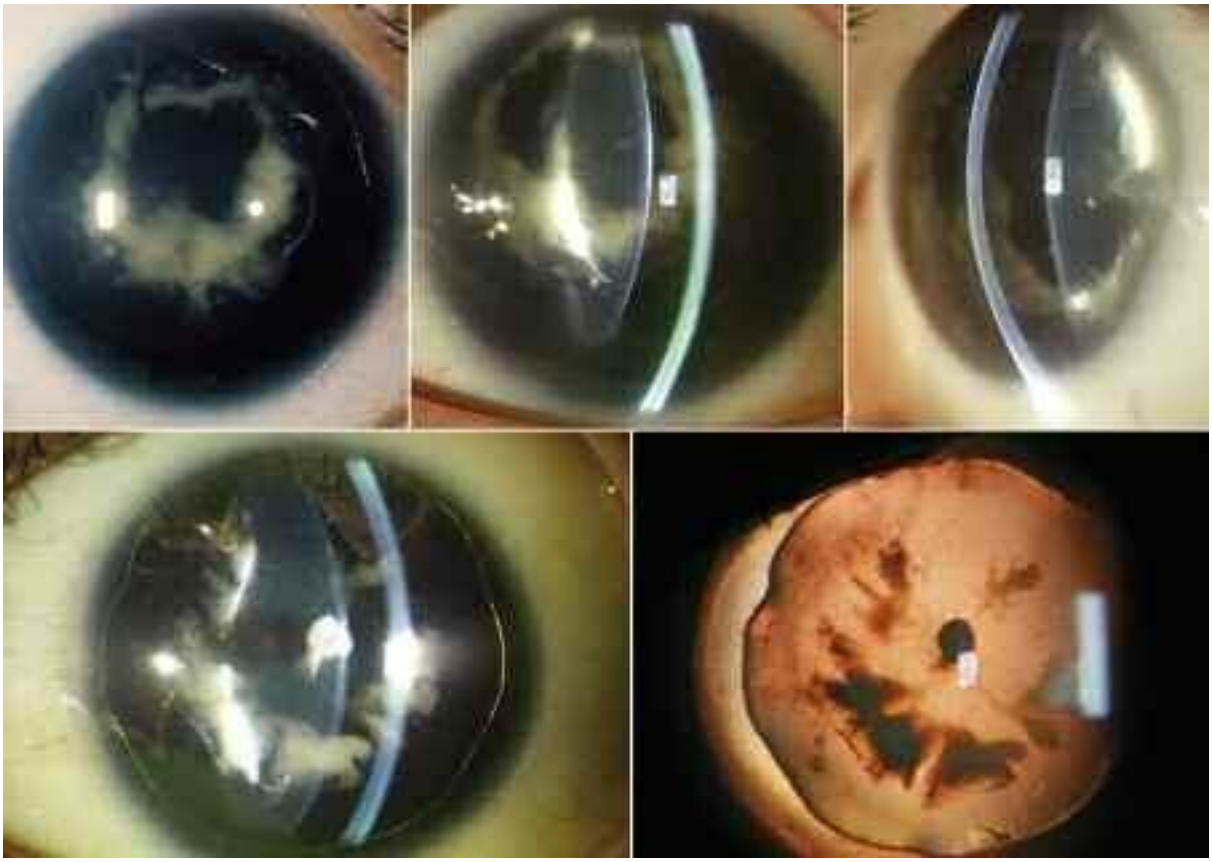

Figure 11.10. Four patients with aniridia and approximately ring-shaped cataract. The opacities are located on the subcortical or zonular plane (top left, 17-year-old male; centre, 27-year-old woman), or in the deep portion of the equatorial cortex (top right, 33-year-old female patient, sister of the patient in fig. 8). The axial region may be respected or have small opacities, either posterior or anterior (bottom, 34-year-old woman).

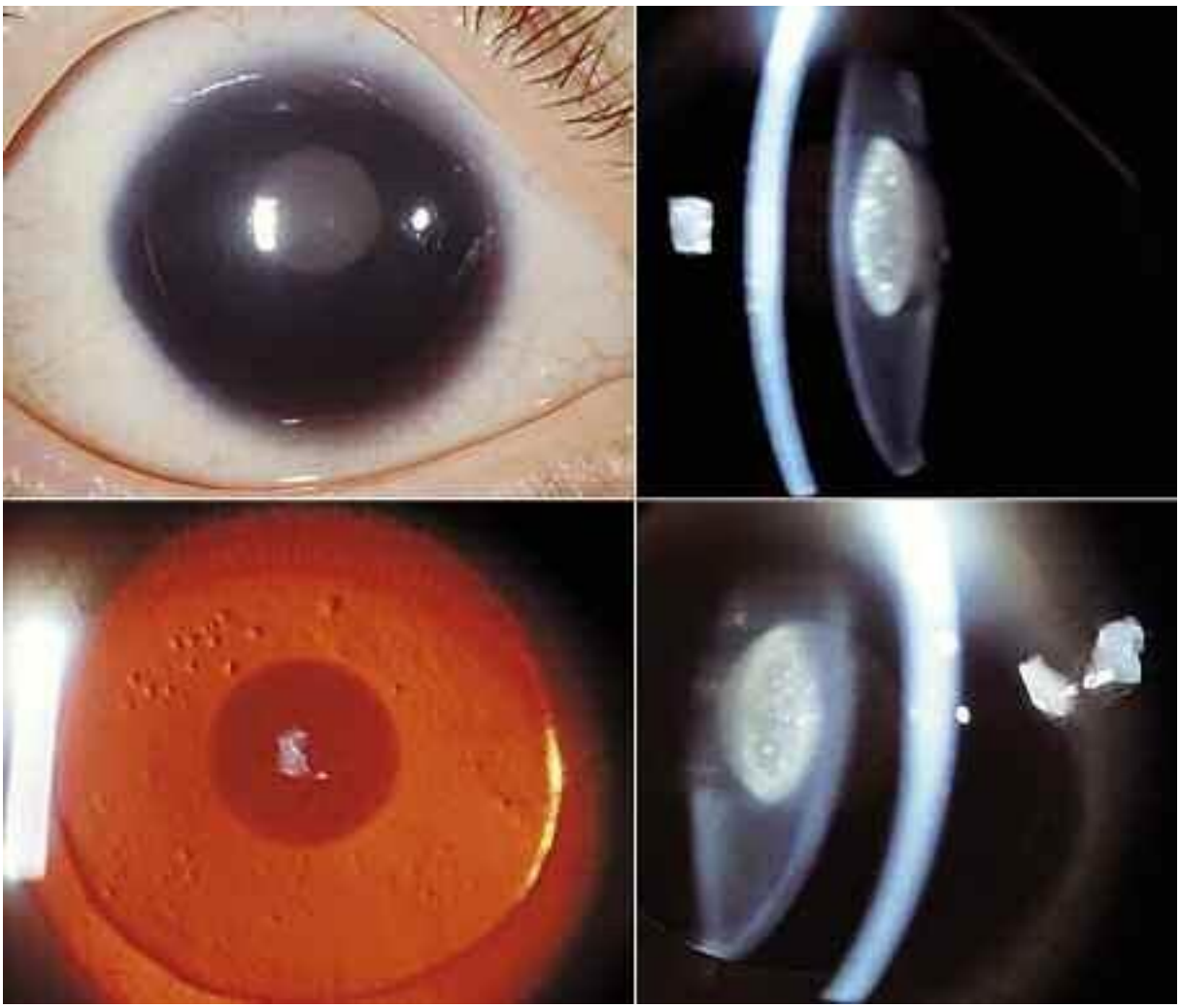

Figure 11.11. 12-year-old boy with aniridia, cataracts observed since birth, glaucoma and psychomotor retardation. Renal alterations were excluded. There is no family history but there is however parental consanguinity. The opacity appears to be limited to the foetal nucleus of the lens.

Figure 11.12. Evolution to nuclear cataract in two women with aniridia, aged 41 (left) and 28 (right). The former, with a moderately brunescent nucleus, previously presented only subcortical or lamellar opacities. The latter, with an opalescent nucleus, previously had a dense central posterior subcapsular plaque and carries an annular limbus homograft. Note the incipient subluxation in both.

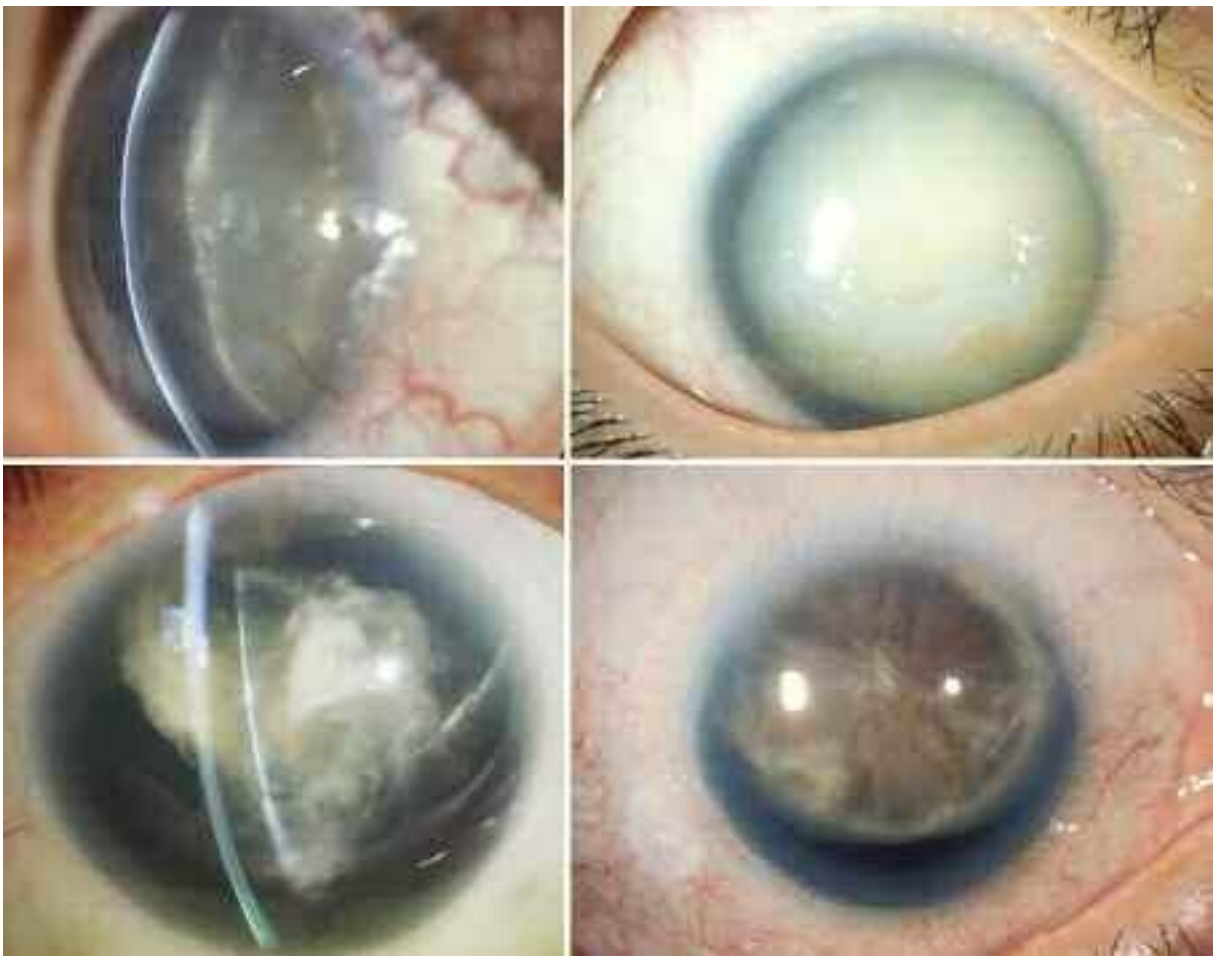

Figure 11.13. Four advanced evolution shapes of cataract in aniridia. Top left, 40-year-old woman with diffuse cortical opacity and discreetly opalescent nucleus. Top right, 19-year-old woman who experienced rapid vision loss, presents an intumescent cataract. Her other eye, which at that time only had small subcortical opacities, developed the same aspect six months later. Bottom left, 45-year-old woman with significant irregular opacity and signs of partial reabsorption. Bottom right, 44-year-old man with mature corticonuclear cataract, very brunescent nucleus and subluxation.

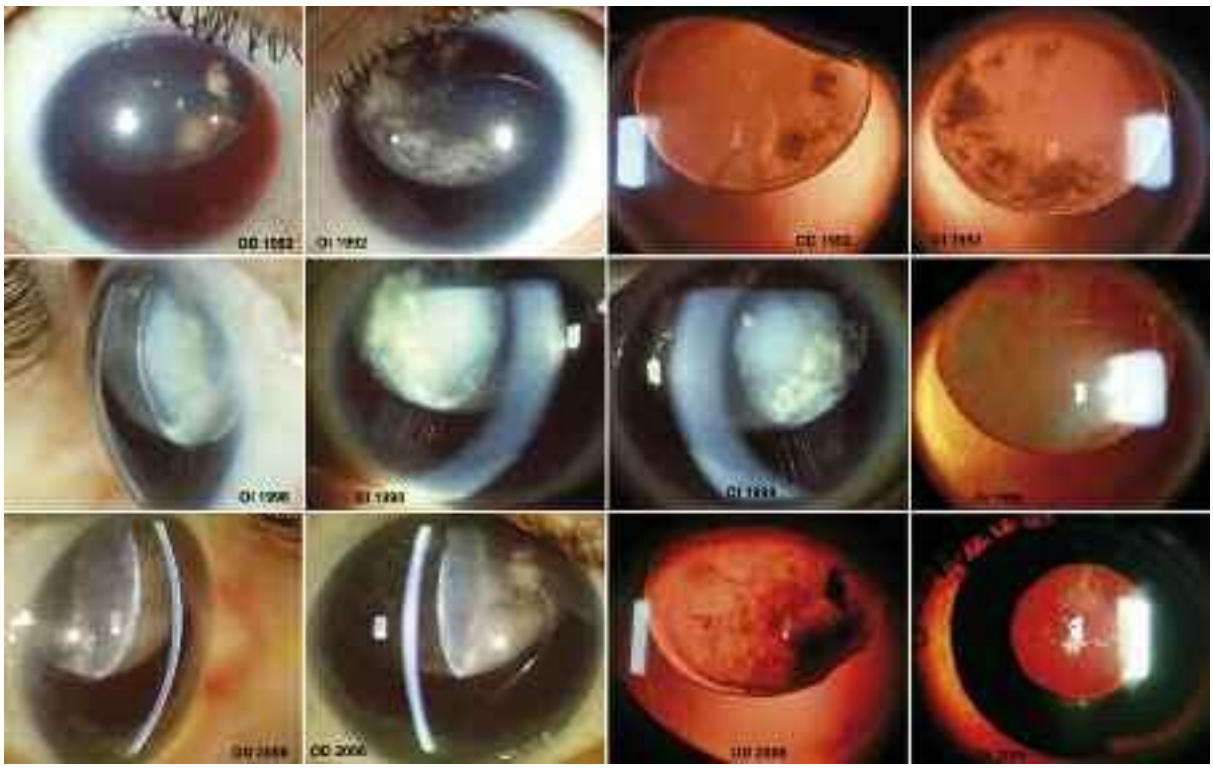

Figure 11.14. Evolution of the patient in fig. 3 with development of opacities, while the subluxation does not really appear to have increased much. Top: aspect of OU at the age of 21. Cortical opacities have appeared in the inferior sectors. Middle row: OS at the age of 27, with progression of the cortical opacities and development of opalescent nuclear cataract. Bottom: OD at the age of 35, with more diffuse cortical but even more transparent nucleus. The densest opacity coincides with the area of greatest subluxation, as shown with back lighting. Bottom right, OS with diaphragm IOL implant.

Another possible alteration of the lens position would be the presence of a keratolenticular adhesion. This has been described associated to aniridia and causing cataracts. [ix] We have observed this configuration in an infant with incomplete aniridia, opacity of the entire corneal quadrant and keratolenticular adhesion, but still with very little lens opacity, all of these bilateral and symmetrical (Fig. 16). This is probably a manifestation of anterior segment dysgenesis similar to Peters anomaly –which has also been linked in some cases to the Pax6 gene.[x]

Even in cases with no evident subluxation, biomicroscopic examination of the zonular area permitted by the absence of iris often shows a greater amplitude of the space outside the lens midpoint, typically in the inferior sector (see Figs. 1, 3, 9, 12, etc.). This is a sign of incipient zonular distension due to weakness or loss of fibres. The possible spontaneous evolution towards subluxation or complete luxation should be considered in the surgical consideration of all cases of aniridia with cataracts, as they often concern young patients, with many decades of life in front of them. For this reason some authors advise against using in-the-bag fixation for implants,[xi] or even advocate simple lensectomy without implant when there is a clear subluxation.[xii] However, the various fixation techniques in the absence of capsular support

allow a lens to be implanted in any case in which it is considered useful. It could be argued, on the other hand, that the great variability in the forms of presentation of lens alterations in aniridia could correspond to different forms of evolution, not necessarily towards zonular autolysis. A study of diaphragm lenses implanted in front of the capsular bag, supported on the ciliary sulcus, indicates good stability after 46 months of average follow-up, although complications are not avoided in certain cases, such as deterioration of the glaucoma (4 out of 19 eyes) or chronic endothelial loss (3 out of 11 eyes). [xiii]

11.2.3 Alterations in size and shape

Changes in lens shape have been described, which could be a result of zonular weakness or the manifestation of a malformation of the lens itself. Using computerized tomography, Mehta el al. observed an apparent inversion of lens curvatures in a case of aniridia, with greater convexity on the anterior than the posterior, which does not occur in traumatic subluxations. However, the B-scan showed that this change occurred "in the cataract and not in the capsule". [xiv] On the other hand, the conservation of the shape of a membranous cataract after luxation into vitreous has been noted in a female aniridia patient who had undergone valve implant surgery for glaucoma.[xv] In several cases of aniridia we have observed an increase in the anterior curvature of the lens, sometimes described as "anterior lenticonus", which appears more convex than the posterior in the absence of cataracts (Fig. 16).

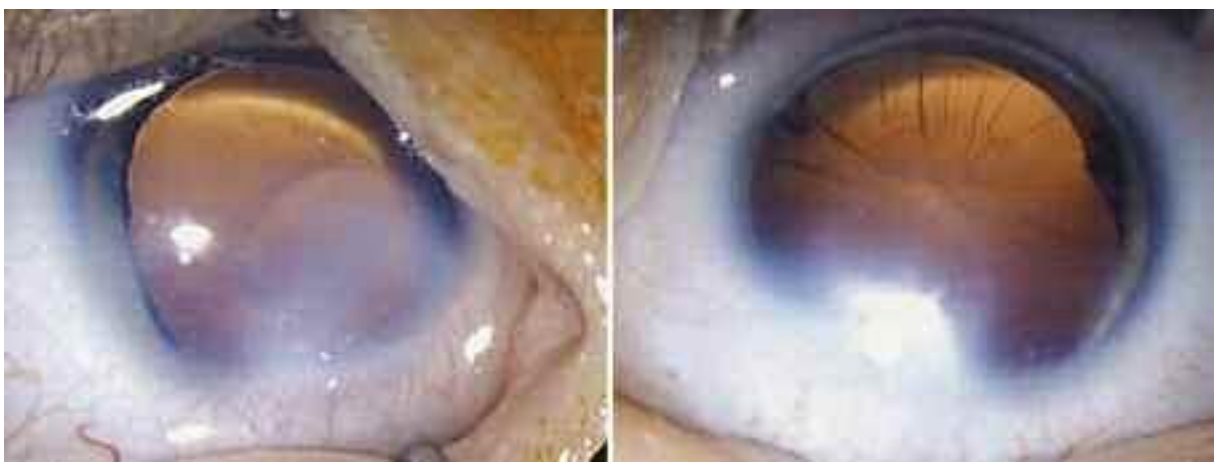

Figure 11.15. Both eyes in this 2-month-old boy presented subtotal aniridia subtotal, finely vascularized corneal opacity in the entire inferior nasal quadrant and adhesion of the cornea to the lens in that sector, although the lens was still transparent. Note the persistence of remains of the tunica vasculosa lentis.

Figure 11.16. Two different patients with aniridia and lenses–with only small opacities, in which the anterior curvature appears increased, larger than the posterior. Subluxation is barely observed.

In cases with microphakia or microspherophakia (Fig. 3), the small lens size could be due to lens hypoplasia, secondary to the weakness of the zonule, or even to a reabsorption process. When the subluxation appears in childhood, the sector with the greatest zonular defect is observed to be reduced as well as rounded. This is also seen in other forms of *ectopia lentis* and appears to be a consequence of the great elasticity of the lens in children. If the zonular weakness is general, a soft lens will tend to adopt a spherical shape and a smaller diameter.

Spontaneous reabsorption of the lens has been described in a Japanese family with aniridia and microcornea, over three generations and possibly a fourth. [xvi] We have occasionally observed partial reabsorption with the formation of a membranous cataract, almost always associated to a significant subluxation. In at least one case we found – whilst performing a keratoplasty – a total absence of the lens with no history of surgery or trauma, and no ultrasound evidence of posterior luxation, while the other eye did have a cataract. This could be a case of spontaneous reabsorption, with or without prior luxation, or agenesis of the lens, which we have also observed in eyes with Peters anomaly.

11.3 Pathological anatomy

Histopathological studies of the lens in aniridia are not in abundance. However, the presence of cataracts is the norm among eyes with aniridia which are enucleated, with several degrees of severity being described in posterior nuclear, cortical, subcapsular and anterior subcapsular cataracts.[xvii],[xviii] In one case described by Zimmerman and Font, small excrescences were found on the posterior capsule, and foci of calcific degeneration and globoid cells in the surrounding area, similar to those observed in Lowe syndrome. [iii],[xviii]

In some eyes with aniridia, particular fragility of the lens capsule has been found during surgery. Schneider et al. measured thicknesses of between 4.72 and 7.36 μm in the anterior capsules of two aniridia patients aged 23 and 35 years, thinner than the mean of 17.56 μm found both in four control patients aged 59 to 77 years and in another two cases of aniridia patients aged 71 years. [xix] Zonular alterations in aniridia are probably related to hypoplasia of the ciliary epithelium, which would give rise to deficiencies in the components of the zonular fibres such as collagen IV or glycosaminoglycans, although the specific defect is unknown. Similarly, it has been speculated that the possible capsular thinness or fragility would correspond to low levels of one or several of the habitual components of the lens capsule: type IV collagen, laminin, entactin, heparan sulfate proteoglycan and fibronectin, as a result of a lesser synthetic activity of the lens epithelium[19]. However, it is well known that the thickness of the anterior capsule, unlike the posterior, increases with age. [xx]

11.4 Ethiopathogenesis

It is currently accepted that aniridia is due to mutations or to chromosome deletions that affect the activity of the Pax6 gene, located in humans in chromosome 11p13. The former association to chromosome 2p [xxi] markers - called "type I" aniridia, while "type II" was associated to Pax6 – has been disproved by later genetic studies of the same families.[xxii] Sporadic cases due to major chromosome deletions involve a greater risk of developing a Wilms' tumour and other anomalies (WAGR syndrome), due to the loss of the gene that inhibits this tumour (WT1) and other genes located in the vicinity of Pax6. The genetic origin of the association of aniridia, congenital cataracts, mental retardation and cerebellar ataxia (Gillespie syndrome) is unknown, although the presence of mutations in the Pax6 gene has been ruled out.[xxiii] Other mutations in the Pax6 gene have been linked with some cases of Peters anomaly [x], and, more recently, with bilateral microphthalmia, cataracts, glaucoma and nystagmus in a family, and with diverse ocular (nystagmus, cataracts, foveal hypoplasia, colobomas…) and neurological malformations in another, but with no iris defect.[xxiv] The genetic aspects of aniridia are covered extensively in another chapter.

The mechanisms whereby genetic defects cause ocular alterations in aniridia are not known with accuracy. Although the cells of the optic vesicle and the lens originate in different embryonic layers –the neuroectoderm and the superficial ectoderm respectively -, their development involves a series of mutual relationships of induction. This leads to the question of whether alterations in aniridia are due to the primary dysfunction of the Pax6 activity in one, the other or in both cell types. Until recently, there was a certain consensus concerning certain aspects that pointed to a predominant role of the lens[xxv],[xxvi] (however, see below):

- The primary defect that gives rise to the "small eye" phenotype – due to mutation of Pax6 in mice used as animal model of aniridia – is the failure of the superficial ectoderm to form the lens placode.
- The development of the lenticular vesicle requires Pax6 activity in the prospective lens ectoderm but not in the optic vesicle.
- Pax6 would not be essential for the formation of the optic vesicle, although it does participate in subsequent phases of retinogenesis.

However, it has been proven that the expression of Pax6 in the mouse optic vesicle is necessary to maintain its contact with the lens epithelium, which in turn is necessary in order

for the subsequent inductive interactions to take place.[xxvii] More recent studies in the chicken embryo show that the expression of Pax6 in the optic vesicle does play a role in regulating cell survival in it and in lens development (beyond the state of lenticular vesicle), at least during a narrow time window. [xxviii,xxix] Normal eye development would therefore require the correct expression of Pax6 in *both* structures.

Furthermore, Collinson et al. observed that in small eye mouse chimeras with up to 80% of mutant Pax6 cells, these cells are replaced in the lenticular vesicle by normal cells (which are reproduced more actively) as of the sixteenth day of development, but this does not occur in other ocular tissue. In spite of this, the eye size, iris and cornea defects returned to normal in the foetal and adult periods. Therefore, these aspects of the phenotype could be *secondary* to the primary alterations in the lens and not to an effect of the mutation in Pax6 in these structures.[xxx] Extrapolating to humans, these authors suggest that the partial therapeutic correction of the lens through gene therapy (even if it were only efficacious in a percentage of cells) in embryos with aniridia genotype identified by prenatal diagnosis,[xxxi] could significantly improve several of the clinical defects responsible for subsequent poor vision, ocular degeneration and blindness.[xxx]

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CHAPTER 12
CATARACT SURGERY IN CONGENITAL ANIRIDIA

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12.1. Introduction

There are several factors that define low visual acuity associated with congenital aniridia: ocular surface disorders, macular and optic nerve hypoplasia, nystagmus, amblyopia, cataract and glaucoma.

Patients suffering from aniridia often have congenital cataracts, usually anterior and posterior polar opacities, which most frequently do not cause significant visual impairment. However, in patients with aniridia, it is estimated between 50% and 85% of lens opacities, progress for first two decades of life (1) to become cortical, subcapsular and lamellar opacities, and many need surgical treatment even before puberty.

It has also been reported an uncommon case of total cataract reabsorption in a family with congenital aniridia (2).

Therefore, since the knowledge that lens opacities is one of anterior segment defect in congenital aniridia that can significantly affect visual acuity, we think it is important general ophthalmologist be familiar with this entity and know proper therapeutic management.

12.2. Surgical indications

First of all, we want to emphasize anterior segment surgeon should hold a moderate attitude when denoting cataract surgery due to poor visual prognosis in these patients and the risk of both, intra-and postoperative complications that cataract surgery can bring.

Hence, many authors agree the decision to perform cataract surgery in patients with aniridia must be justify by presence of a significant impaired visual function due to cataract.

Other authors, even in cases of cataract associated with ectopia lentis, claim surgery should be avoided if visual acuity improves with an appropriate refraction through aphakic section of the visual axis (1).

12.3. Surgical skill

Then, we will detail specific aspects of the surgical cataract steps in eyes affected with congenital aniridia.

First, we find no evidence data in literature about which is the best incision to perform in aniridic eyes. In theory, given these eyes show limbal and corneal anomalies, ideally we must perform a scleral incision, avoiding making corneal or limbal incision in order to not damage ocular surface, congenitally impaired.

However, Reinhard published a series of 19 aniridics cataract operated eyes (10 extracapsulars and 9 phacoemulsifications) performed a 170º corneal incision and a single-piece IOL-artificial iris was placed in the sulcus. (3). Despite the incision size and location, corneal surface worsen in only four cases, with progression of a preexisting pannus in two eyes.

Therefore, we do not recommend the widespread practice of a special incision and the choice of incise is given by surgeon's experience.

We can have surgical difficulties in the first step of the capsulorhexis. Schneider et al, warned in some patients with aniridia the anterior capsule is extremely breakable (4), which is technically difficult to perform a continuous circular capsulorhexis (CCC) and is partial to come tears along the edge. After histological examination of anterior capsules from several aniridic patients, it has defined two groups: young patients in whom the anterior capsule were abnormally thin (less than 8 microns) and weakness, and elderly patients with normal capsular thickness average (mean of 17.56 microns) and normal behavior during rhexis step.

This fact highlights the abnormal capsular thinness, and, although the etiology is unknown, it is postulated it could be on account of the decrease or absence of any of the components of the capsule.

For this reason, we must be especially careful when performing CCC in patients with aniridia, mainly on two assumptions: young patients and when puncture the anterior capsule we can notice the appearance of folds in, since we may have difficulties to control the rhexis with the appearance of loosers or tears or in the posterior capsule.

The recommendations when making CCC are:

- Use highly cohesive viscoelastic agents.
- Make a little capsulorhexis, smaller than normal.
- Staining the anterior capsule with trypan blue or indocyanine green for better visualization of the rhexis
- Avoid contact of any instrument with anterior capsule, to prevent damage

Once successfully completed capsulorhexis and hydrodissection is achive, continue with phacoemulsification. At this point, we have bear in mind other lens pathology associated with congenital aniridia: the ectopia lentis.

This entity has been described up to 56 % of aniridic eyes (1). A histological study showed the hypoplasia of ciliary processes but have not been published changes in zonula or pars plana. However, although morphologically the zonule is normal, the high incidence of ectopia lentis may be due to variations in their molecular structure.

In this way, we advice you should make a careful preoperative ophthalmic examination in order to detect this defect.

Even so, although anatomic position lens variations are not detected, we recommend using careful maneuvering during phacoemulsification and aspiration of the masses. It is important not to push or pull excessively the nucleus and also recommend setting flow values in order to reduce turbulences. So it should use a low flow and vacuum parameters but not

necessarily less ultrasound power. These steps prevent the intraoperative zonular dialysis and if it appears, it will be necessary to handle like any other patient.

Finally, proceed with implantation of an intraocular lens. Some surgeons have strongly advised against intraocular lens implantation in the capsular bag or ciliary sulcus during cataract surgery, given the high incidence of decentration and subluxation/luxation of them, making the patient to wear aphakic contact lenses. Currently, it is well known that these eyes have limbal stem cell deficiency, which discourages the use of contact lenses. This scenario, joined with new designs of intraocular lenses and artificial iris leads us to conclude that there is no contraindications for implanting an intraocular lens in patients with congenital aniridia.

12.4. Artificial iris devices

Artificial iris devices were popularized in Europe in 1994 by Reinhard *et al.* who used an intraocular lens with a black iris diaphragm for treatment of congenital aniridia (5). Rosenthal, subsequently introduced the first artificial iris device through a small incision for treatment of iris dysgenesis.

It is known that many factors have influence in low vision in these patients, photophobia is a common symptom in aniridia, due to the lack of complete iris diaphragm. The main advantage of inserting an intraocular lens with a device which acts as artificial iris is to reduce the amount of light entering the eye, resulting in a reduction of photophobia, an improvement in depth of focus and a reduction of spherical and chromatic aberrations associated with the edge of the lens (6).

The first lens with artificial pupil was a monoblock intraocular lens with full iris diaphragm developed by Sundmacher. Nowadays, there are available 2 Morcher intraocular lens models: 67F and 67G.

Both lenses have a full iris diaphragm diameter of 10mm, leaving a central optical zone of 5 mm, and differ only in the overall diameter of the lens (67F and 67G 13.5 mm 12.5 mm).

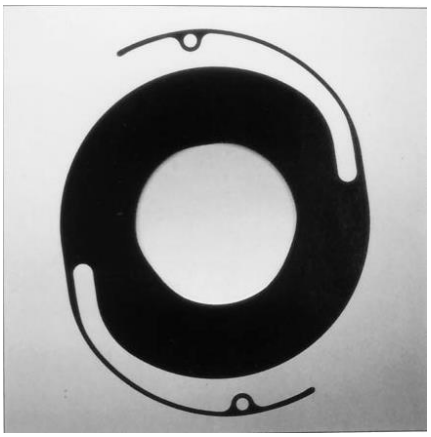

Figure 1. - 67G model monoblock intraocular lens with a full iris diaphragm. 67F model is similar, differs only in total diameter of the lens that is 13.5 mm.

These intraocular lenses are appropiate for placement in ciliary sulcus if capsular bag is damage or absence, but have two disadvantages: they require be inserted through a large incision of 10 mm and can be difficult to properly setting up, so it is advisable to perform an intraoperative gonioscopy to confirm the correct placement of haptics (6).

These devices are also used in cases of secondary implants in aphakic aniridic patients who complain of photophobia or if they can not tolerate contact lenses. In these cases, you can proceed with placement one of the two models in the ciliary sulcus trans-scleral suture fixation.

The disadvantage of practice large incisions for inserting these lenses boosted the development of new models which could be managed through small incisions (3.2 mm). Thus, intracapsular rings were developed. It is available 2 models: Morcher 50C and 96G types.

50C iris ring model consists of 2 rings (each one with many colour iris segments) that must be rotated into the eye to the ensure segments assembled, making a complete diaphragm. After achieving the correct assembly, proceed to insert the foldable intraocular lens. This model form an artificial pupil of 6 mm of diameter, which allows a good window to explore the ocular fundus but main disadvantages are the fragility of the device, and the difficulty of assembly.

Even in case of rupture of posterior capsule Osher found 50C design provides a good stability in the capsular bag (7), although in these cases, it is advisable to insert a one-piece iris-IOL in sulcus or scleral sutured when capsular support is insufficient.

Figure 2 - . 50C Morcher iris ring model.

The 96G model is an intracapsular ring of 11 mm diameter that only has a pigmented iris segment, so it is an archetype for treatment of acquired sectorial aniridia up to 3 hours.

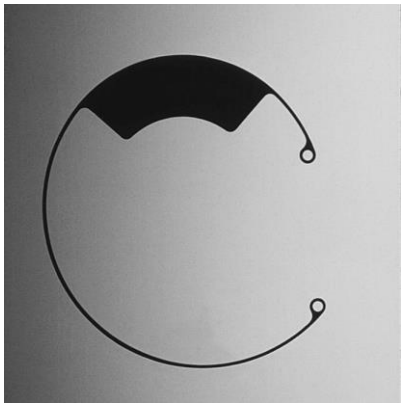

Figure 3 - . 96G Morcher iris ring model

A third design of intraocular lens, developed by Pozdeyeva, is called artificial diaphragm iris-lens (8).

It is suggested, this elastic lens with 5 equidistant haptics supports the lens making a uniform distribution of forces, good stability and a correct well-balanced. Blunt side of haptics does not allow damage the capsule during implantation.

Lens can be placed into capsular bag, but it is necessary perfunctorily remove support elements and in cases of capsular instability, support components may be mechanically join to iris remains or employ three scleral tunnels for suture fixation. Incision size varies between 5 and 6 mm depending on the presence or absence of support parts.

Experience with this lens is very limited since only it has been implanted in 19 eyes, but only 3 cases were in congenital aniridia.

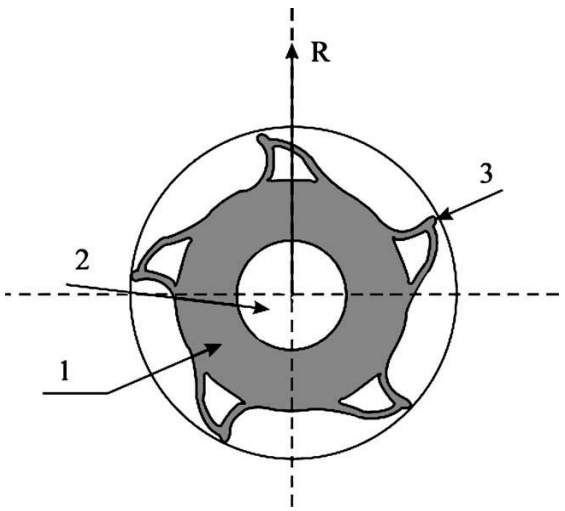

Figure 4 - . Lens - iris diaphragm . 1 - . Artificial pupil. 2 - . Lens optics . 3 - . Support elements

12.5. Results

It is difficult to make an assessment of yield results with this kind of lens in terms of visual recovery and improvement of glare in congenital aniridia since experience is limited but results seem promising.

Reinhard published long-term results with some different black diaphragm IOL models sulcus implanted in 19 eyes with congenital aniridia (3). 14 of them, improved visual acuity (half of them achieved more than 2 lines of VA), 1 kept preoperative VA of 0.1 and 4 slightly worsen VA. Furthermore, glare was reduced by 79 % (11 of 14 patients).

Burk *et al.* published their results with 67F, 67G, 50C and 96G models in 28 aniridic eyes with many etiologies (6). In congenital aniridia subgroup (10 eyes, 3 were implanted 67F model and 7, 50C model) the average VA increased at least 3.6 Snellen lines in 7 days; in 2 eyes VA correlated with preoperative, due to nystagmus and corneal opacities and 1 eye lost one Snellen line because of a significant corneal irregularity. Indeed, subjective glare before and after implantation of artificial iris was evaluated, and they observed glare sensitivity decreased from a mean of 2.8 preoperatively to 1.3 after surgery in these patients (0= no glare; 1= slight glare, 2 = moderate, 3= severe).

12.6. Complications

Frequently, surgery complications may take plase in aniridia, some has been discussed above. We have already commented capsular frailty assists ruptures of anterior and posterior capsule. Also, we have warned the risk of subluxation/luxation of the lens due to zonular weakness and worsening of corneal surface after anterior segment surgery.

However, it has been described specific complications associated with the use of intraocular lenses placed in ciliary sulcus. Thus, it has been observed a chronically impairment blood-aqueous barrier (BAB) in aniridia, and many hypotheses have been postulated to explain this variation:

- Contact of haptics and diaphragm with uveal remnants can cause chronic irritation.
- Arrangement of haptics is more difficult in an aniridic eye and might be inadvertently placed in angle or pars plana. Unsuitable support in iridocorneal angle is partial to a greater disorder of trabecular function and endogenously modified. Thus, intraoperative gonioscopy is recommended to confirm the correct setlement of haptics.
- Sulcus placement of lens in aniridic patients might have greater movement.
- BAB in aniridic eyes might be more susceptible to traumas compared to healthy eyes.

Chronic change of BAB mainly affect on intraocular pressure. So, although congenital aniridia involving glaucoma, disturbances in BAB may boost glaucoma or accelerate the progression (3, 6).

Series published by Reinhard about IOL- iris -piece (3) conclude the main complication associated was the variation of intraocular pressure control: 4 of the 14 patients who did not have glaucoma developed the disease (2 were controlled with medication and 2 needed

surgery). Furthermore, glaucoma worsened in 4 of 5 patients, needing more topical treatment and 2 eyes surgery.

Therefore, we must insist on the importance of close monitoring of postoperative intraocular pressure in eyes with sulcus-lens placement, even advise against to implant intraocular lens in eyes suffering from glaucoma.

Other complications associated with chronic impairment of BAB are: Chronic low-grade inflammation that might determine the need to use topical corticosteroids for months (3), the progressive loss of endothelial cells (3) and cystoid macular edema (3).

Finally, we highlight a specific complication of aniridic operated eye, the so-called progressive anterior fibrosis syndrome (9).

Tsai *et al.* described this disease in 7 cases of aniridic patients previously operated of cataract surgery associated or not with other anterior segment surgeries (drainage tube or keratoplasty). First findings include the appearance of thin membranes that cover the front and back IOL surface, with fibrosis and capsular contraction giving anterior displacement of the IOL, in absence of clinically observable inflammation. Late findings include, progressive development of fibrosis, with spreading above the membrane, causing further displacement and tilting the lens to cornea with subsequent endothelial damage and the extent of the membrane to ciliary body bringing about hypotonia and even tractional retinal detachment.

Contact or closeness of intraocular devices with immature blood vessels from rudimentary iris tissue remnant in eyes with congenital aniridia might be a mechanism for anterior fibrosis syndrome.

In this way, it is advisable a closely follow-up of patients who have intraocular devices or those who have some surgeries when this complication is diagnosed, must be performed a membranectomy early in order to prevent complications associated with anterior membrane spread and subsequent fibrosis.

12.7. Conclusions

Cataract surgery in congenital aniridia entails careful surgical handing and as we have seen, associate several complications. If we add this disease has a poor visual prognosis, cataract surgery in aniridic eyes should only be considered when cataract is responsible for a significant decrease in vision.

New intraocular lens implants seem to offer encouraging results, not only in terms of visual recovery but especially in reducing photophobia. However, multicenter studies are needed to understand the long-term results of these new designs.

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CHAPTER 13
COMPLICATIONS OF CATARACT SURGERY IN CONGENITAL ANIRIDIA

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13.1 Introduction

Cataract surgery presents specific peculiarities in congenital aniridia patients. The high incidence of opacity of the lens, the absence of iris and the current possibility of placing an artificial diaphragm, the zonular alterations, the associated limbal corneal alterations and the prevalence of glaucoma in these patients make it necessary to take into account the possible appearance of a series of complications which do not occur with the same frequency in cataract surgery in conventional patients. It is important to know these specific peculiarities and adapt our surgical technique to prevent them and, if they arise, to treat them appropriately.

The complications of cataract surgery in patients with aniridia can be divided into intra-operatory and post-operatory complications, depending on when they appear.

13.2 Intra-operatory complications

As discussed above and in the corresponding chapter, cataract surgery in aniridia patients presents particularities that should be known in order to avoid the appearance of intra-operatory complications which on occasions may be serious or make it necessary to carry out more aggressive manoeuvres that could worsen the visual prognosis in patients which, because of their condition, already have low visual expectations.

13.2.1. Anaesthetic technique

The type of anaesthesia used can help us in some particular cases to prevent the appearance of complications. In patients with very poor vision, with difficulty fixing on the microscope light, those on whom a large incision is going to be made in order to implant a diaphragm lens with a large diameter[1] or in whom the IOL is going to be placed using trans-scleral suturing, or in whom endocapsular ring segments are going to be implanted requiring an absence of motility, we would recommend peribulbar anaesthesia. Due to the high incidence of corneal opacity, even when incipient, visualization of the fine details of the structures and instruments in the anterior chamber under the microscope is generally not good, and therefore it may be difficult to employ a careful surgical technique under topical anaesthesia. In “simple” cases, topical anaesthesia can be used if we feel sure and comfortable.

13.2.2 Complications of the incision

The choice of incision for commencing surgery in each case has been discussed in previous chapters. The main complications we can find are bleeding of the incision, poor coaptation and residual astigmatism. Patients with aniridia habitually present limbal

insufficiency in an important percentage of cases, which leads to the appearance of a peripheral corneal pannus or vascularization (Figs. 1 A and 1 B) that facilitates the appearance of bleeding when we make the incision. If we are placing an implant with a large diameter and have to make a 10-mm incision in order to insert it, it would be advisable to use a classic extracapsular technique with conjunctival flap and cauterization of the limbal vessels, which would avoid this complication. In these cases surgery could also be performed with a small incision (2 mm.) or with microincision surgery (MICS) and after removing the cataract, extending the main incision to implant the artificial diaphragm IOL. In general, the recommendation is to avoid highly

Figure 13.1A: Pannus affecting the superior area in a patient with congenital aniridia and inferior lens coloboma.

Figure 13.1 B: Pannus in 360º in a patient with congenital aniridia and lens with hexagonal morphology.

vascularized areas in order to prevent this complication. The progression of epithelial alterations after cataract surgery by 21% and persistent epithelial defects have been described[2].

Furthermore, the peripheral corneal tissue in the presence of vascularization in the case of a large incision can cause problems with healing and coaptation if we do not use a careful suturing technique. In general, we prefer a corneoscleral incision with conjunctival flap if we implant a large-diameter IOL or when we associate the cataract surgery to trabeculectomy, although these IOLs can be implanted through large purely corneal incisions, 1 or 2 mm from the limbus (Fig. 2) in cases where the superior conjunctiva is to be respected in case filtering surgery is necessary in the future. On the other hand, in cases where a pair of ring stems are going to be implanted to form an artificial iris diaphragm, a corneal incision of 3 mm in an area of the cornea without vessels is recommended.

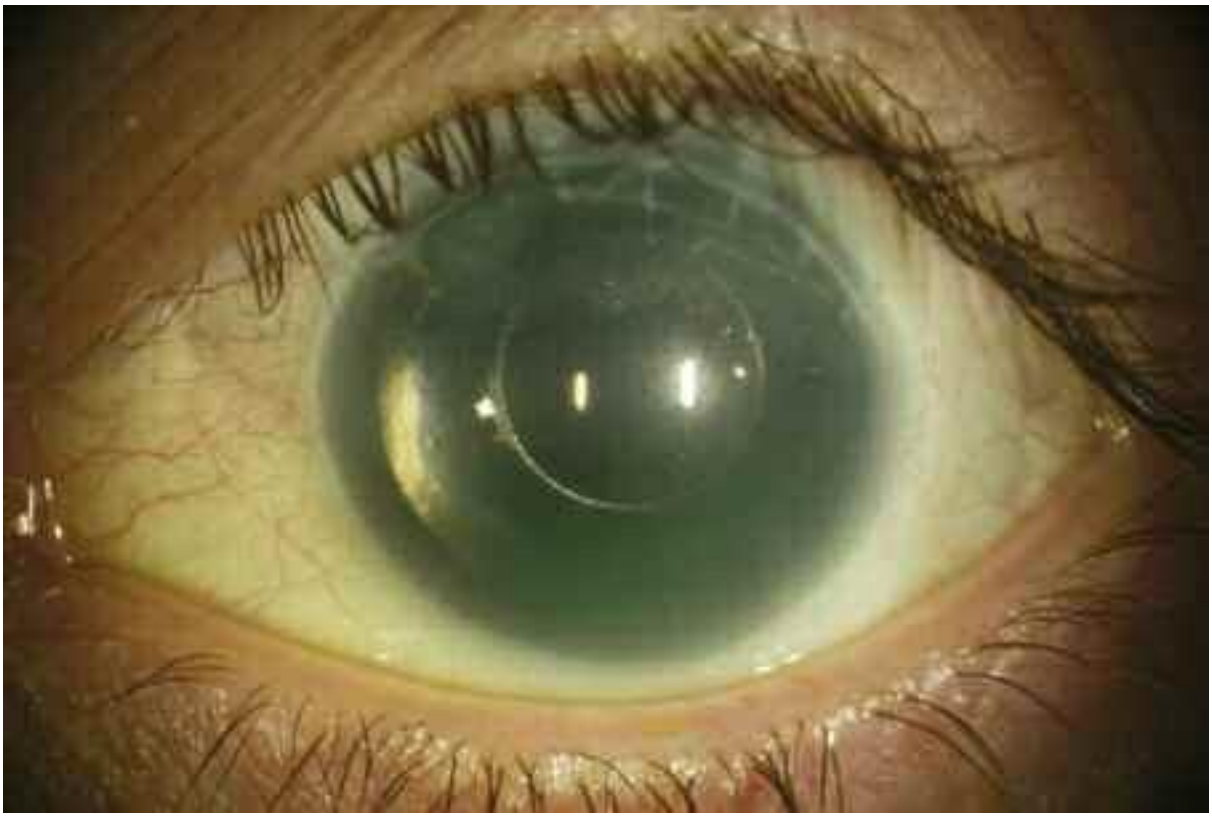

Figure 13.2: Lens with artificial diaphragm that was implanted through large corneal incision in superior part of the cornea.

13.2.3 Complications in capsulorhexis

The structural alterations of the anterior capsule in patients with aniridia[3,4], which make it difficult to perform the surgery, have been widely described. We have to take into account the type of implant we want to use and the location in the eye. If we have decided to implant a diaphragm lens, it is really difficult to place it in the capsular bag due to its large diameter, and performing a very wide capsulorhexis, still very difficult because of the circumstances

mentioned above, will not allow the implant to remain in the bag in a large majority of cases. Rupture of the capsulorhexis during implantation of a large implant tends to be the norm, and we therefore advise implantation in the sulcus.

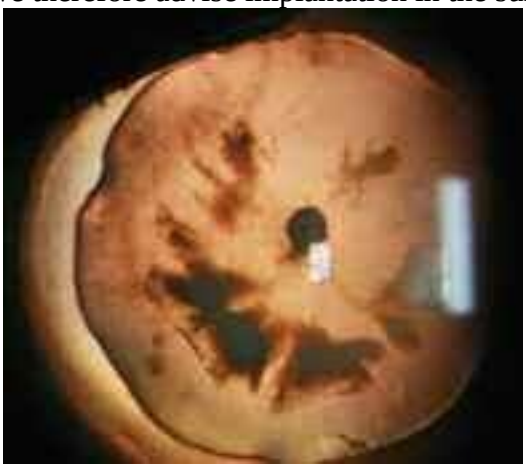

Figura 13.3. Small peripheral lens colobomas.

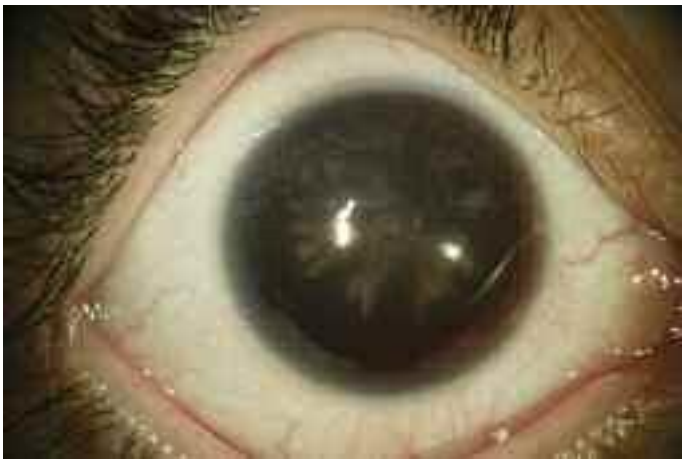

Figura 13.4. Lens with posterior cortical opacities and commencement of inferior conjunctivalization due to limbal insufficiency.

13.2.4 Complication in lens extraction

In general, there is no difference in the extraction of lens material between patients with aniridia and normal patients. Where phaco-expression of the nucleus is carried out, this must be done with the utmost care to avoid zonular rupture, as discussed above. In cases in which phaco-emulsification is used, using either coaxial or bimanual technique, (MICS), vacuum and/or aspiration values have to be adjusted to avoid chamber collapse, zonular detachments or ruptures of the posterior capsule, which in these cases of difficult visualization can be a challenge to resolve adequately. In general, these are not usually excessively hard cataracts as the posterior subcapsular or cortical opacity component generally predominates over the nuclear component.

In the event of rupture of the posterior capsule during phaco-emsulficiation technique and implantation of ring segments, we will proceed as usual, initially covering the perforation with viscoelastic, then proceeding to perform vitrectomy if there is vitreorrhagia and we will than perform posterior capsulorhexis. If the final conditions allow, we will continue with the in-the-bag implant, but if there are any doubts or visualization is difficult, it is advisable to change strategy and implant a diaphragm IOL in sulcus. This brings up the need to have it available in the operating theatre with the consequent cost this entails, as lenses are requested on order.

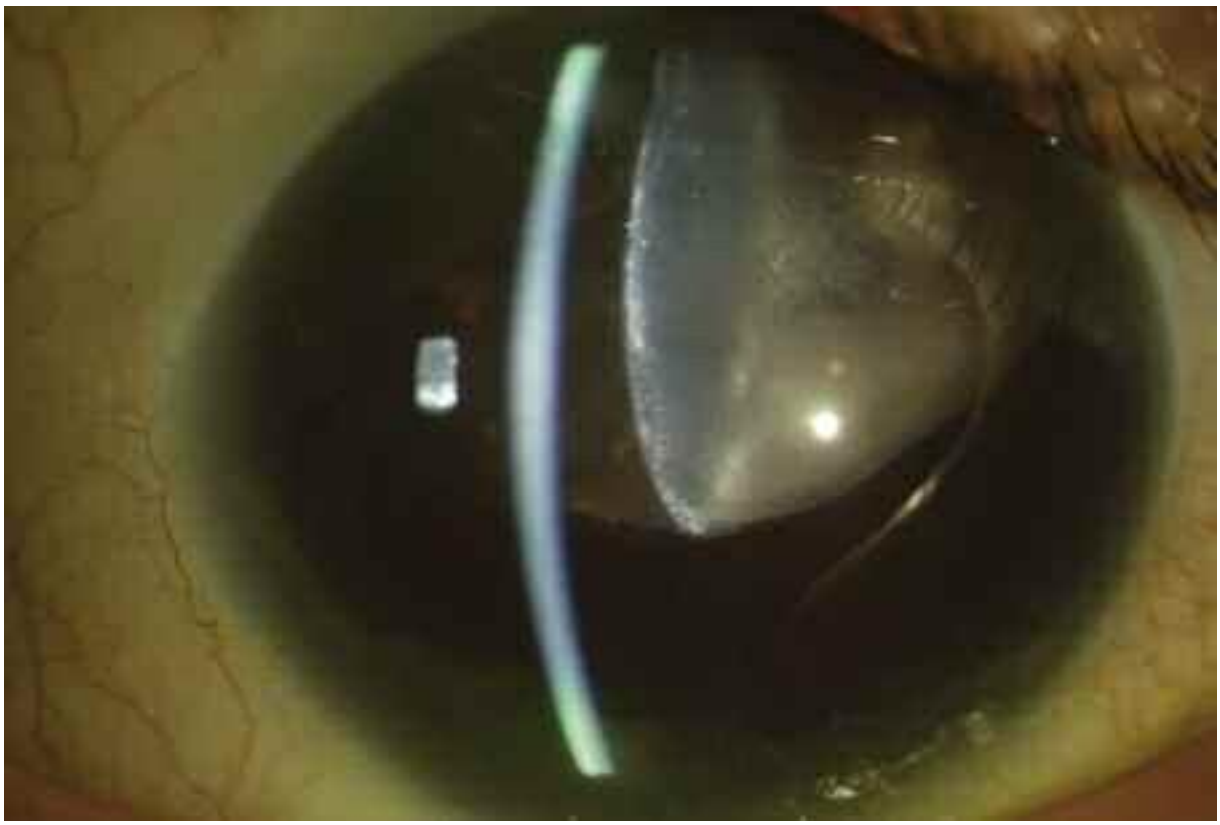

Figura 13.5 Ectopia lentis with dense cataract in a patient with aniridia.

13.2.5 Complications during IOL implantation

Implanting a rigid diaphragm IOL (Figure 6) does not present differences compared to the conventional technique except that the incision is 10 mm and there is no iris. Therefore, if there is vitreous pressure during implantation we may break the capsular bag or detach it on pushing the IOL inside the eyeball. Good hypotonia is therefore important, as well as making sure, by using not too much viscoelastic, that the capsular bag is not displaced by the haptic on implanting the IOL.

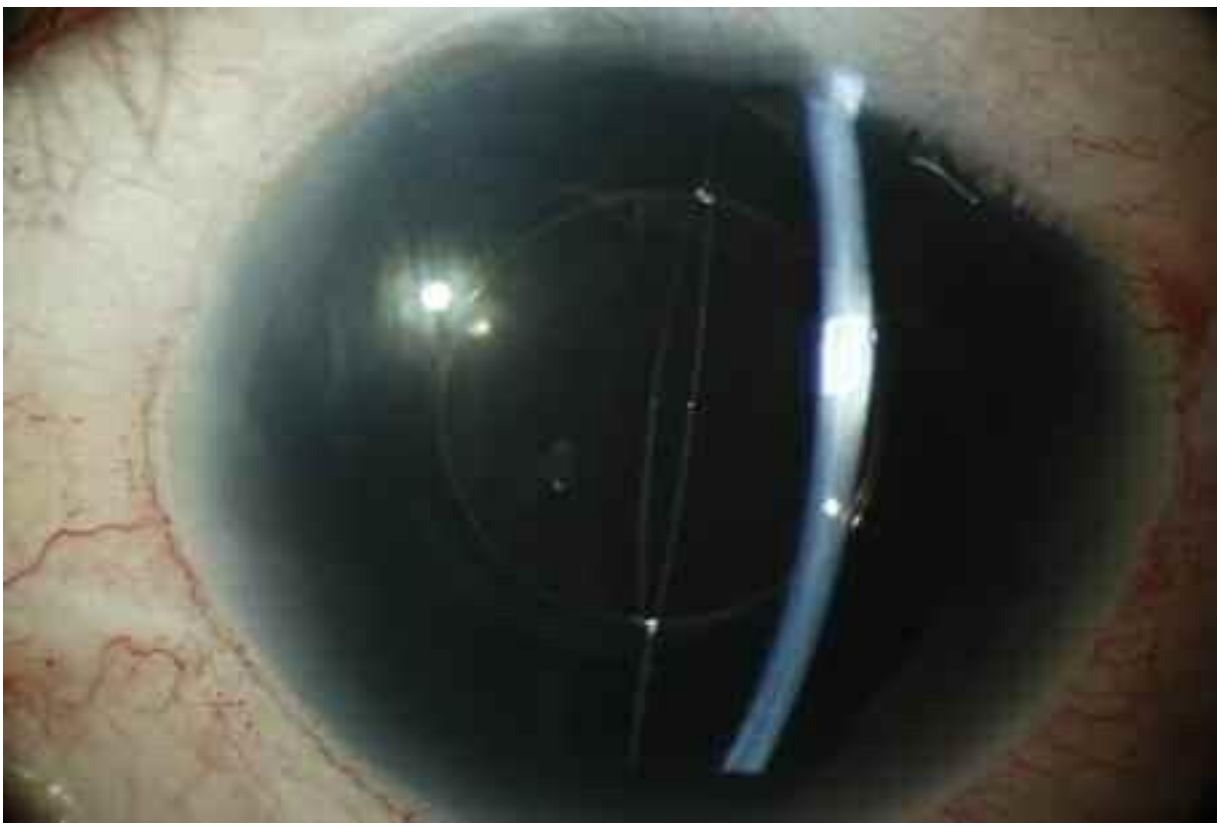

Figura 13.6. Typical image of diaphragm IOL implanted through corneoscleral incision.

It is advisable to remember the fragility of the haptic union with the diaphragm area in these lenses which makes breakage much more frequent than with conventional PMMA IOLs. As they are of the same material, ring segments also break frequently during implantation manoeuvres, and therefore it is necessary to remove the broken fragment and implant a new one. This problem arises particularly if we wish to implant them using smaller incisions than are recommended by the manufacturer. We must make sure that the ring segments are correctly positioned inside the capsular bag, because if they are placed outside they will not perform their function of 6-mm diaphragm adequately and will cause chronic inflammation phenomena of the anterior segment. As a rule, the recommendation is to place an in-the-bag ring first, followed by the next ring placed behind it while the capsular bag is kept dilated at all times with viscoelastic and finally place the foldable IOL also behind the second ring. Any rupture of the posterior capsule during these manoeuvres may force us to abort the procedure and place a rigid diaphragm IOL, so exceptional care should be taken during these manoeuvres.

In cases where the implant has to be sutured to the scleral wall, the technique the surgeon is most comfortable with will be used. The high incidence of post-operatory glaucoma and the possible need for filtering surgery should be taken into account, so it is recommended that anchor sutures be placed after 3-9 hours. When tensing the suture, it is also important to remember the greater fragility of the material that favours rupture of the optic-haptic union, which, if it happens when the IOL is already implanted, would complicate the surgery considerably.

13.3. Post-operatory complications

The main post-operatory complications that are usually observed after cataract surgery in patients with aniridia are post-operatory glaucoma, progression of corneal lesions, chronic inflammation of the anterior segment and progressive anterior fibrosis syndrome. There is obviously a possibility that other general complications found in any cataract surgery may also arise, which should be treated in the normal manner, but we will discuss those listed above on account of their greater frequency in this type of patients.

13.3.1 Post-operatory glaucoma

The higher incidence of glaucoma in aniridia is well known and has been widely discussed in the corresponding chapter. An increase in the incidence of glaucoma after cataract surgery has also been observed in these patients [2,5,6] and the involvement of diaphragm IOLs with a higher incidence was even postulated[7]. Some authors perform the surgery combined with trabeculectomy (Fig. 7) in cases of pre-existing glaucoma[8]. There are no conclusive studies on the implication that the use of diaphragm IOLs may have on the development or aggravation of glaucoma, although the combination of surgical trauma, the rupture of the blood-aqueous barrier with the resulting chronic inflammation and the possible inflammatory stimulus of the haptics placed in contact with the ciliary body in the sulcus probably constitutes the mechanisms that cause closure of an already pathological trabecular meshwork, resulting in ocular hypertension.

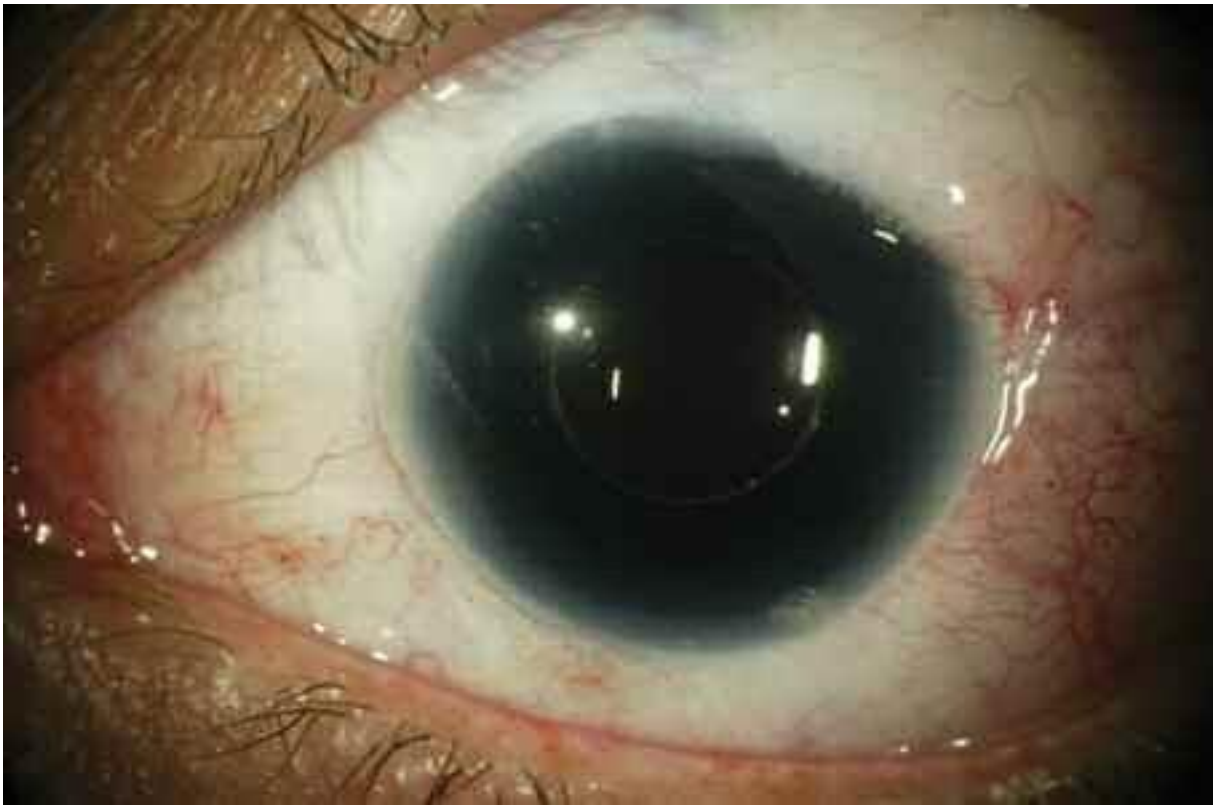

Figura 13.7. Filtration bleb after trabeculectomy performed with combined IOL implantation surgery on the patient of the preceding figure.

The treatment of post-operatory glaucoma includes medical treatment, which must be monitored frequently, noting and observing the possible effects on the eye surface altered by the concomitant limbal insufficiency that these patients suffer, and the different surgical alternatives such as trabeculectomy, valve implant (Fig. 8) and cyclodestructive procedures.

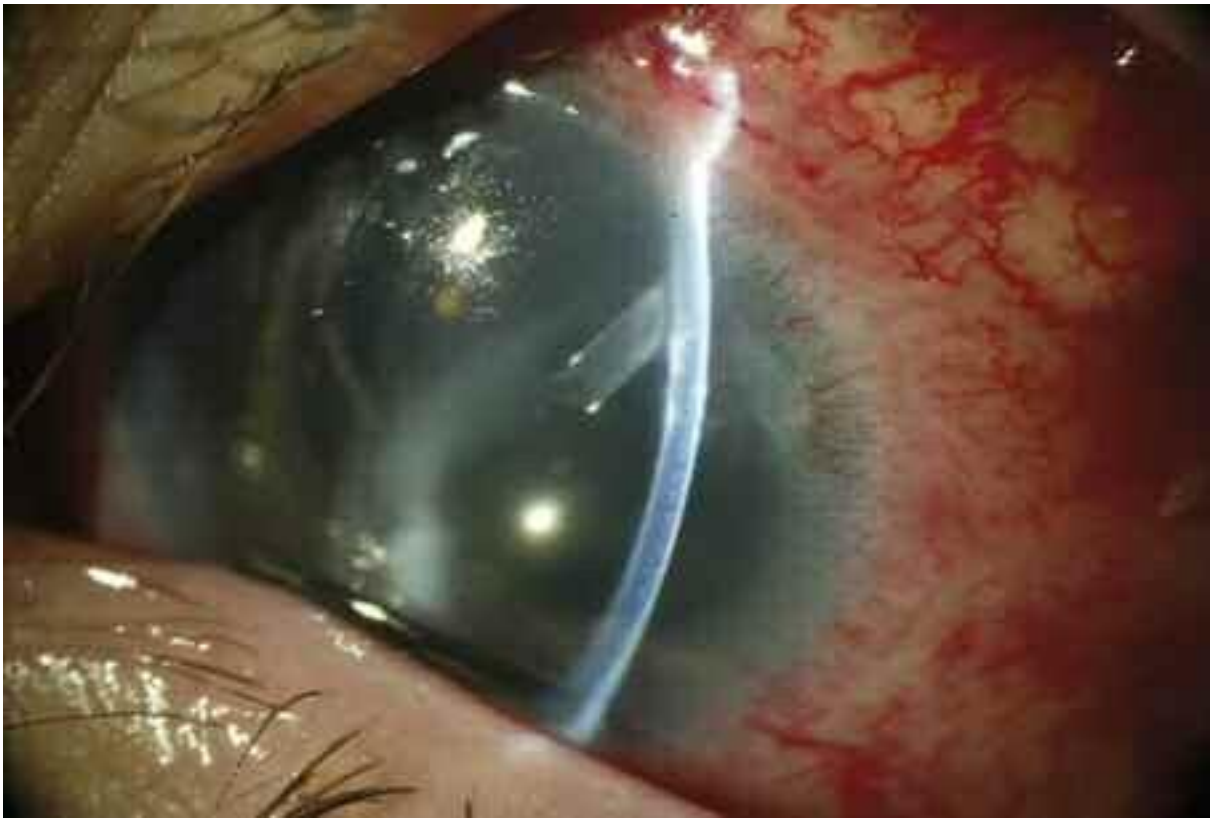

Figura 13.8. Valve drainage implant with draining tube in anterior chamber. Immediate post-operative image.

13.3.2. Progression of keratopathy

If there are corneal alterations secondary to the limbal insufficiency, large incision cataract surgery performed on some of these patients can aggravate them, with the resulting reduction in visual acuity and the appearance of symptoms that cause discomfort to the patient. These alterations may be incipient with pannus and appearance of conjunctival epithelium in the periphery of the cornea circumferentially, in which case we advise using a scleral incision technique if the IOL to be implanted has a large diameter (Fig. 9) or associated to a resection of the pannus and superficial keratectomy of the conjunctivalized area with the application of amniotic membrane (Fig. 10) which delays revascularization and the appearance of keratopathy by some years.

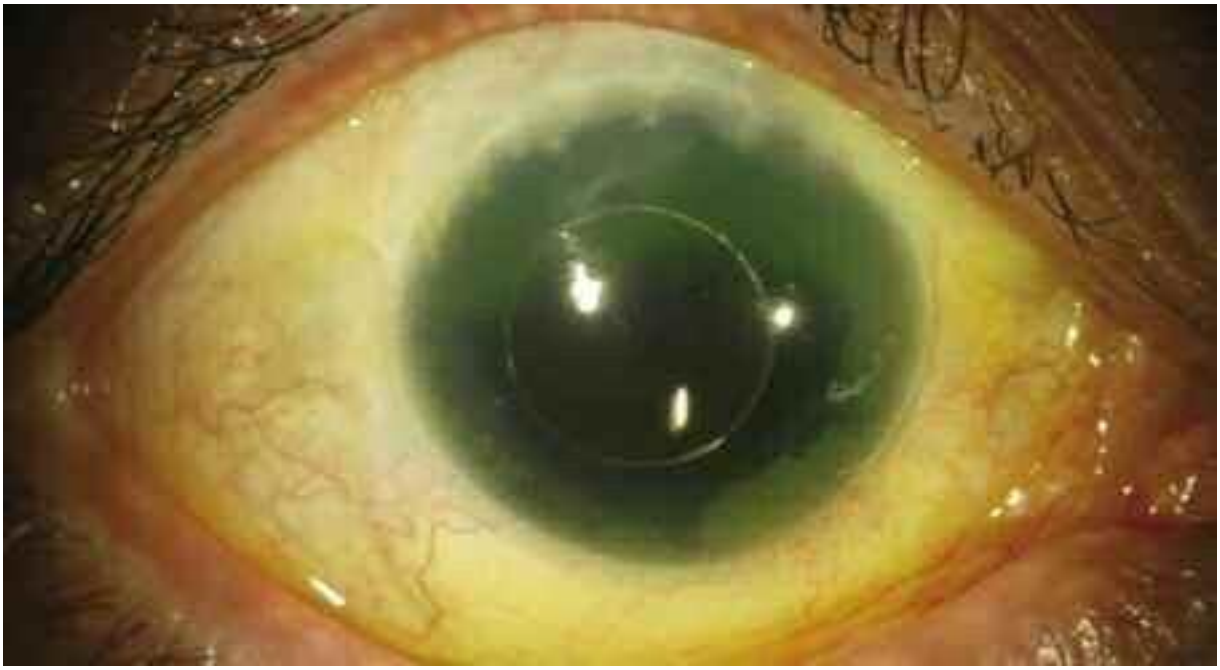

Figura 13.9. Image of incipient limbal insufficiency with fluorescein stain positive and thin vascular pannus in a patient previously operated for cataracts on superior limbal cornea.

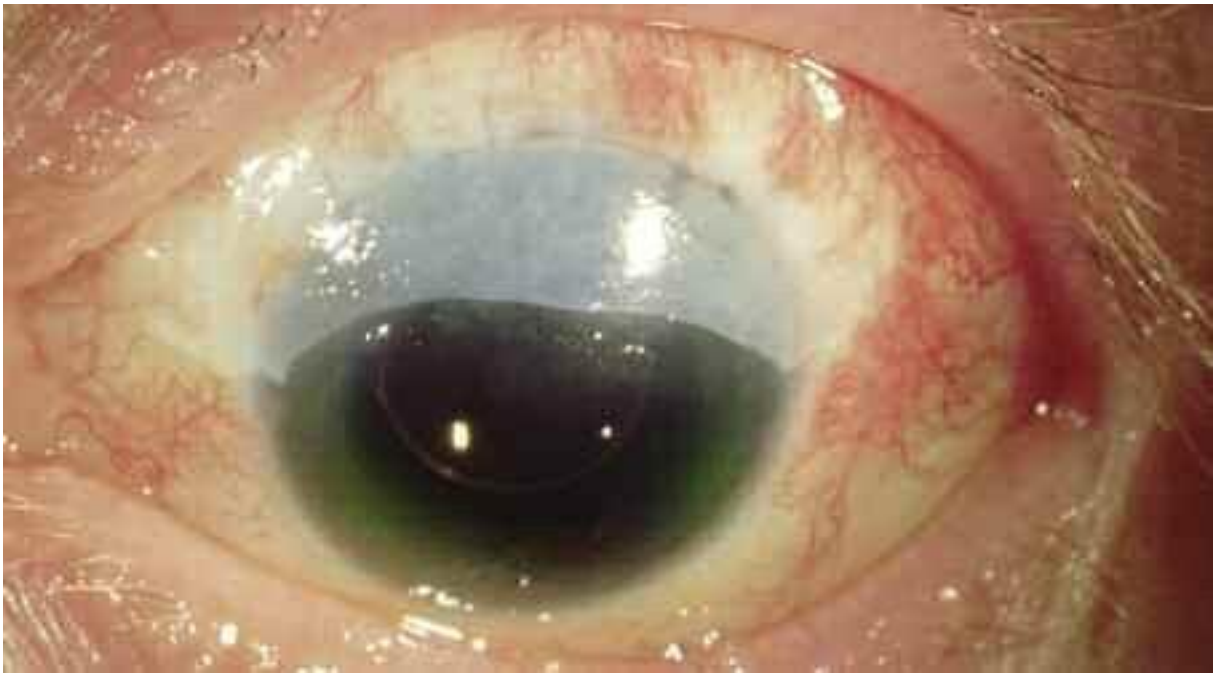

Figura 13.10. Covering with amniotic membrane as symptomatic treatment in a female patient with frequent epithelial micro-erosions in superior corneal area.

In more advanced cases of keratopathy we can carry out a total superficial keratectomy to improve visualization of the cataract surgery and upon completion carry out homotransplantation of the sclerocorneal limbus (Fig. 11). In cases where the limbal transplant

has been carried out prior to the cataract surgery, we advise performing the implantation using a small incision to respect, as far as possible, the transplanted ocular surface (Fig. 12).

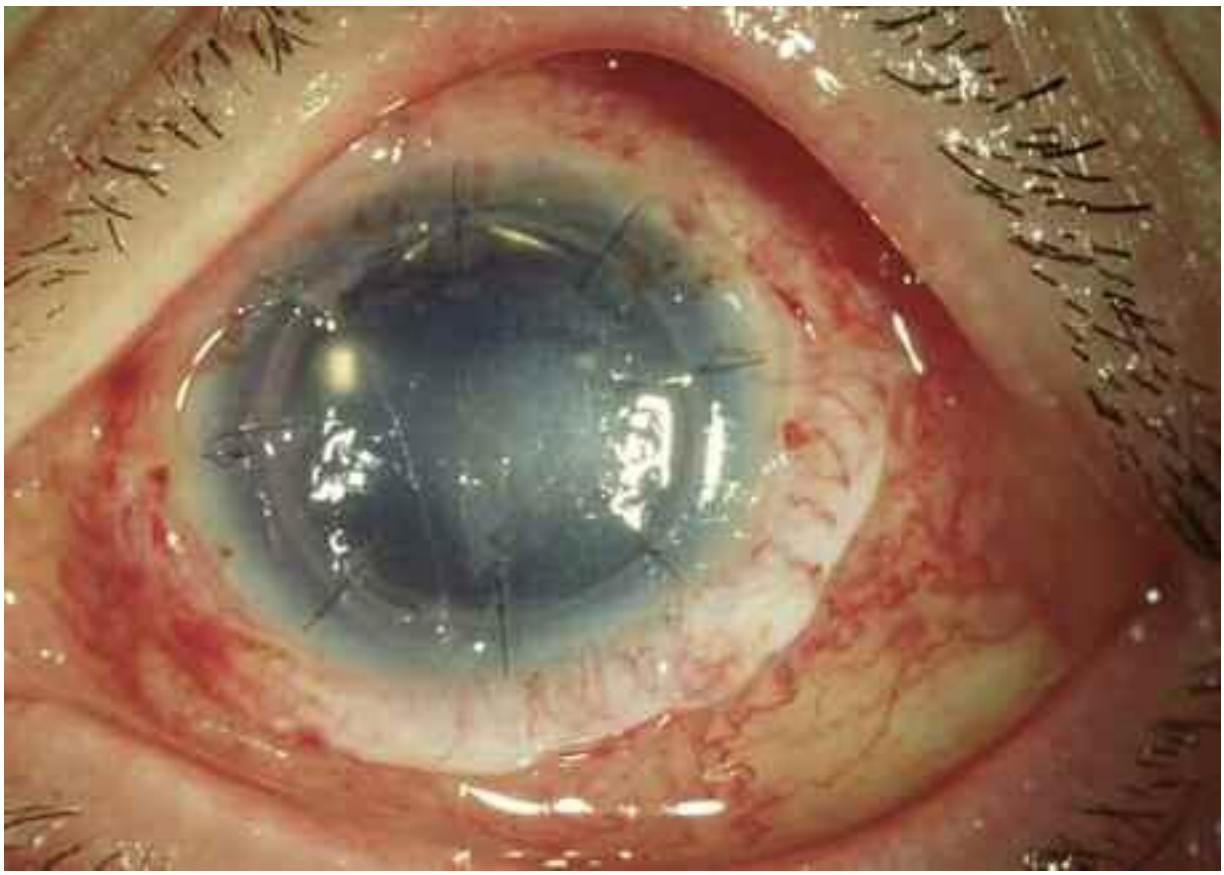

Figura 13.11. Limbal annular homograft in a male patient with congenital aniridia previously operated for cataracts. Result 15 days after surgery.

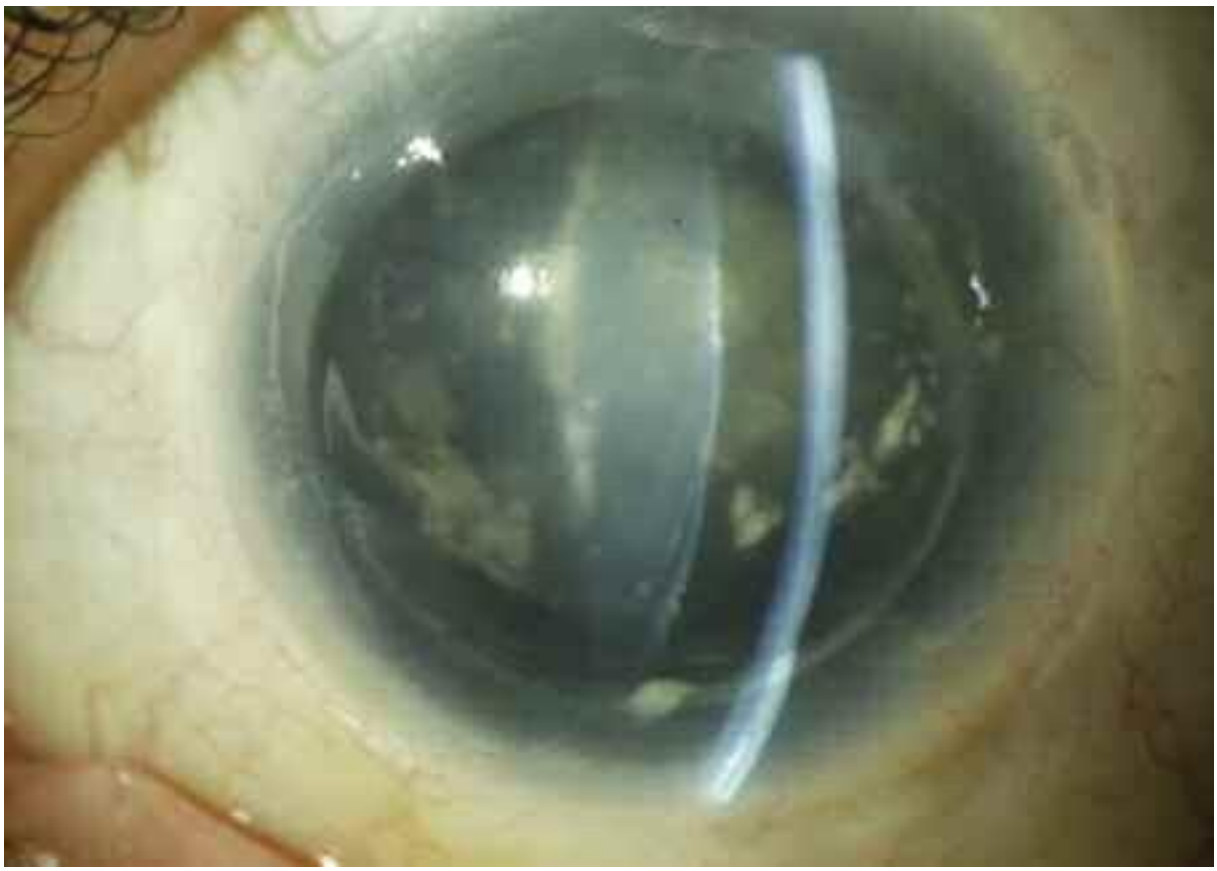

Figura 13.12. Patient with limbal homograft performed 2 years ago with excellent reconstructive result but with a cataract in evolution. Small incision surgery and placing of ring segments should be suggested to avoid the large incisions needed by diaphragm IOLs which could affect the limbal transplant.

13.3.3. Chronic inflammation of the anterior segment

One of the main problems we can find after cataract surgery in aniridia is post-operatory chronic inflammation[7]. The mechanisms are not well known, but the combination of surgical trauma and the implantation of IOLs with a large diameter or supported on the sulcus probably exert a chronic irritating stimulus on the ciliary body, increasing the inflammatory reaction in the anterior chamber. It is therefore important to monitor the degree of inflammation that these cases present and carry out more prolonged topical anti-inflammatory treatment and follow-up controls than normal.

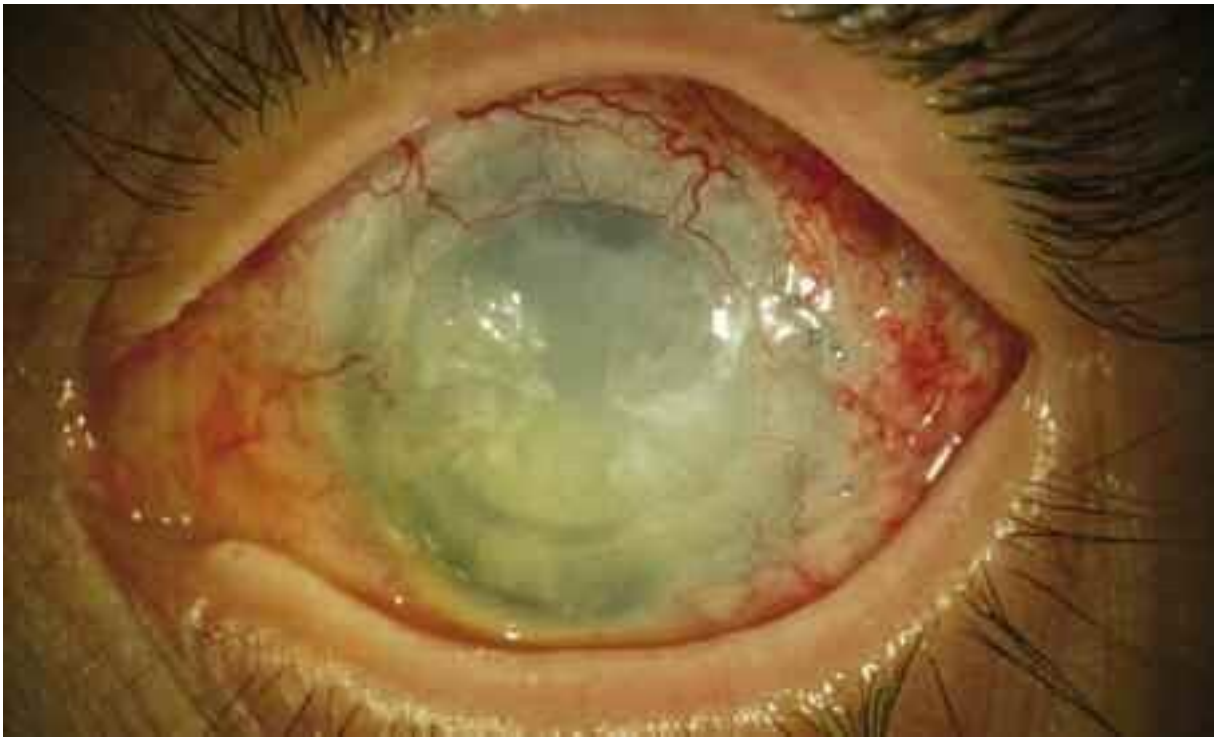

Figura 13.13. Progressive anterior fibrosis syndrome in a female patient operated for cataracts with segment ring implant. The rings are attached to the corneal endothelium and the previously performed limbal transplant is vascularized.

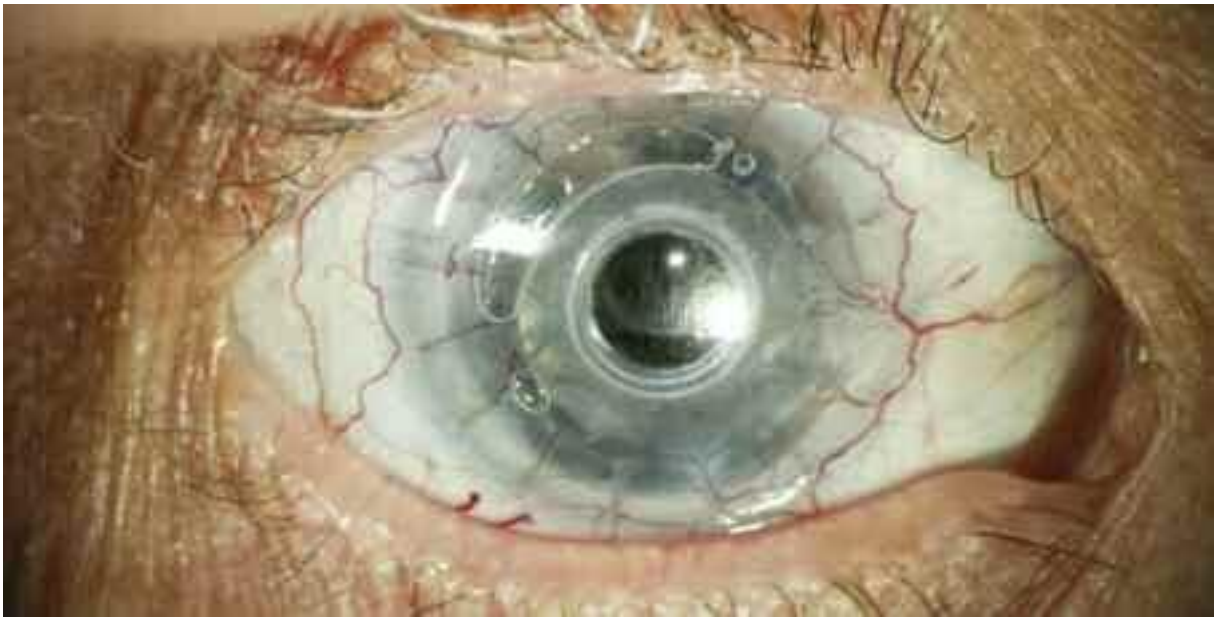

Figura 13.14. Boston type keratoprosthesis in a female patient with partial aniridia in whom keratoplasty had previously failed.

In the chapter dealing with anterior segment cataract surgery, a particular complication in these patients known as *progressive anterior fibrosis syndrome* was disccused[9]. In a study carried out by Tsai and collaborators in a series of 155 eyes affected by aniridia, they found 7 eyes that had undergone some type of anterior segment surgery, in general multiple procedures, in which a fibrosis reaction had been formed that originated from the rudimentary iris and enclosed the IOL displacing it towards the corneal endothelium, which was also covered in a fibrotic membrane or developed secondary endothelial decompensation. In our experience, we have seen 2 cases of this magnitude, one case after implantation of ring segments and IOL by small incision in a female patient who had a limbal transplant (Fig. 13), and another case after combined penetrating keratoplasty and IOL sutured to sulcus implantation surgery. In this case we had to perform a Boston type keratoprosthesis procedure (Fig. 14) to attempt to restore vision.

13. 4. Conclusions

The anatomical characteristics and affectation of practically all ocular structures in the eye with congenital aniridia facilitate the appearance of complications after any surgical act we perform. Due to their frequency, most of these patients will undergo cataract surgery at some time in their lives, so we must know the different surgical techniques and apply them adequately when we are dealing with a patient with aniridia. From trying not to damage or aggravate pre-existing corneal alterations, using ring segments or diaphragm IOLs according to the circumstances, to preventing or treating the post-operatory inflammatory component, avoiding serious complications such as endothelial decompensation, cystoid macular oedema or progressive fibrosis of the entire anterior segment, we must know the key points of cataract surgery in these patients in order to treat them adequately.

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SECTION V

CHAPTER 14
RETINAL DISORDERS IN PATIENTS WITH ANIRIDIA

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14.1. Introduction

Congenital aniridia is a rare bilateral condition that, as its name indicates, is characterized by its most obvious clinical manifestation: the total or partial absence of iris tissue. Yet it is a disease that can affect multiple ocular structures, both in the anterior segment (where it affects not only the iris, but also the cornea, the iridocorneal angle and the lens) as well as in the posterior segment (mainly affecting the retina and optic nerve, with these alterations being partially or fully responsible for the loss of visual acuity that affects patients with aniridia).

Congenital aniridia results from abnormal neuroectodermal development, so it is reasonable to expect aniridia to be associated with abnormal retinal development as both the pigmented epithelium and the iris musculature, as well as the retina, originate from the neuroectoderm. This abnormal development is secondary to a mutation in the PAX6 gene that is located on the short arm of chromosome 11 (11p13). This gene controls the development of many nervous system structures, which explains the wide range of ocular and systemic abnormalities associated with this disease. In the development of the eye it is involved in the interactions between the optic cup and the surface ectoderm; it is expressed early in the optic placode, which is where development of the anterior segment of the eye originates, and also in the optic vesicle and cup, which later differentiate into the retina, and that is why mutations in PAX6 can have a significant impact on retinal function[1,2].

The decrease in visual acuity in patients with congenital aniridia is due to several factors, among which are corneal and lens opacities, glaucoma, strabismus and retinal disorders (mainly foveal and optic nerve hypoplasia)[3,4]. In addition, the absence of an iris increases the optical aberration of light when it passes through the periphery of the lens and causes phototoxic degeneration of the retina due to the excessive exposure to light. Nevertheless, the presence of congenital sensory nystagmus in the majority of patients indicates that their vision is already impaired at birth, when many of these conditions have not yet developed, which underscores the importance of a congenital retinal disorder as a significant cause of visual impairment[1].

Electroretinographic disturbances are seen in most patients with congenital aniridia, so retinal dysfunction should be considered a cardinal feature of the aniridia phenotype. These alterations range from an almost normal retina to severe impairment, suggesting heterogeneity

in the retinal function of these patients. Electroretinogram (ERG) amplitude may be reduced for a number of causes: decreased photoresponse and hence reduced sensitivity, alterations in the synaptic integration and transmission in second-order neurons, or a reduction in the number of healthy retinal cells. Macular and optic nerve hypoplasia are often present in aniridic patients, which suggests that the primary cause for a reduction in ERG amplitude may be a reduction in the number of retinal cells[1].

Not all ERG components are equally affected: the amplitude of the oscillatory potentials, which are generated in the inner retina, is the parameter that is most affected, followed by the amplitudes of b-waves (which originate from Müller cells) and a-waves (which reflect photoreceptor activity). Thus, the electroretinographic parameters are affected with a gradient from the innermost to the outermost retina[1].

14.2. Retinal disorders

Disorders that can be seen in the posterior segment of the eye in aniridia patients are described below. It is important to highlight that it is often difficult to document them because of nystagmus and media opacitiy that is secondary to corneal and lens damage[5].

14.2.1. Optic nerve hypoplasia

14.2.1.1 **Definition**

Optic nerve hypoplasia is a non-progressive, congenital anomaly that can be segmental but is often diffuse[6] and tends to be bilateral. It may occur in isolation or be associated with other neurological or ocular abnormalities, including aniridia[7]. It is characterized by a lower than normal number of axons and retinal ganglion cells, although the mesodermal elements and glial supporting tissue of the optic nerve are normal[8]. Histopathological sections show that there is a reduced number of ganglion cells and optic nerve fibers but that the outer layers of the retina are normal[6].

14.2.1.2 **Ophthalmoscopic examination**

Ophthalmoscopically, a small optic disc (or papilla) (½ to ⅓ of normal size) that is pale grayish in color can be observed. The double ring sign is a landmark, although it is not always present[6,8]. It consists of a yellow-white halo of peripapillary hypopigmentation caused by concentric chorioretinal atrophy that is surrounded in turn by another ring of hyperpigmentation. Histologically speaking, the outer ring corresponds with the junction between the sclera and the lamina cribrosa and represents what would have been the margins of a normal optical disc; the inner ring represents an abnormal extension of retina and pigment epithelium over the outer portion of the lamina cribrosa. Together, the dimensions of the hypoplastic disc and peripapillary halo are about the size of a normal optic disc.

The double ring sign is an indication that the optic nerve is smaller than the scleral canal. Despite the small size of the disc, retinal blood vessels are usually of a normal size, although they can be tortuous.

14.2.1.3 **Clinical presentation**

Visual acuity is highly variable and depends, at least in part, on the number of intact

neurons[11] and the preservation of the papillomacular bundle[6]. Patients with severe hypoplasia may present with very altered visual acuity, sluggish photomotor reflexes and nystagmus, whereas in milder forms we may find minimal impairment of visual acuity and/or strabismus[9]. Strabismus is a common presenting sign in patients with unilateral or asymmetric disease, whereas in bilateral optic nerve hypoplasia diminished visual acuity and nystagmus are more frequent[6].

There are other conditions that are also associated with optic nerve hypoplasia, from a relative afferent pupillary defect (RAPD) to inability to maintain gaze. Numerous associated visual field defects have been described[8,9]: centrocecal defects with normal peripheral visual field, inferior altitudinal defects, bitemporal defects or homonymous hemianopsia, sectoral defects or generalized constriction. Bitemporal defects and generalized constriction occur more frequently in severe hypoplasia, while inferior altitudinal defects are more common in segmental optic hypoplasia. Bitemporal visual field defects may indicate the presence of midline central nervous system defects, which are frequently associated with optic nerve hypoplasia[6].

14.2.1.4 **Diagnosis**

Diagnosis is not always easy because of the wide clinical spectrum of this anomaly, which ranges from an easily detectable severe form (aplasia or nearly total aplasia of the optic disc) to a slight decrease in the size of the optic disc or a mild segmental hypoplasia[9]. It may be difficult to diagnose these milder or segmental forms of optic nerve hypoplasia on ophthalmoscopic examination and they can be easily overlooked unless particular attention is paid to the size of the disc in relation to the diameter of the vessels and the vessel patterns. If the diameter of the superior or inferior retinal arteriole at the spot prior its bifurcation is carefully compared to the diameter of the disc, even the mildest of cases can be documented. While the normal disc/arteriole ratio in an adult eye is 14.6 ± 2.4, in hypoplastic discs the ratio varies from 6.1 to less than 14.6[12].

Electroretinograms with a green filter can detect the loss of nerve fibers. A ratio of more than 3 between the distance from the center of the optic disc to the macula and the biggest diameter of the optic disc are considered to be a diagnosis of hypoplasia[8]. The peripapillary halos may lead to misdiagnosis because if these halos are mistakenly considered to be part of the substance of the disc, the true size of the hypoplastic disc can be overestimated[13].

14.2.1.5 **Optic nerve hypoplasia and aniridia**

Optic nerve hypoplasia is a common finding in patients with congenital aniridia. Layman et al.[13] found this anomaly in 9 of 12 patients with aniridia and postulated that most of these patients have some degree of optic nerve hypoplasia, even if it is mild. McCulley et al.[14] found clinically apparent hypoplasia in 6 of 56 aniridic patients (10.7%). Nonetheless, optic nerve hypoplasia, like other retinal abnormalities, is often difficult to document because of nystagmus and media opacities, as mentioned above[5].

Optic nerve hypoplasia may be a natural consequence of the poor retinal and macular development that is seen in many patients with aniridia[5,13]. Poor development of the retina results in a reduction of the retinal nerve fibers and, therefore, of the diameter of the optic

disc[13]. As in previous publications, McCulley et al.[14] observed the simultaneous occurrence of foveal hypoplasia (which will be discussed later) and optic nerve hypoplasia in several patients, which has led to suggest a causal relationship between the two. However, there should be another etiology in some or in all patients, given that 50% of cases of optic nerve hypoplasia occurred independently of foveal hypoplasia.

Mutations of the PAX6 gene have been implicated in multiple congenital ophthalmic anomalies, including foveal hypoplasia, and in a wide variety of optic nerve abnormalities including hypoplasia, so it appears that this mutation, which is responsible for the aniridia phenotype, may also be responsible for abnormal optic nerve development. It has therefore been suggested that hypoplasia of the optic nerve in patients with aniridia is, at least in part, a direct result of the PAX6 mutation[14].

True aplasia of the optic nerve is rare in the general population, but a case associated with aniridia has been described[15]. Most reported cases of optic nerve "aplasia" are in fact optic nerve hypoplasias or atrophies, as there is still a recognizable optic disc with some nerve fibers, a retinal vascular pattern that is normal or almost normal and, at least, light perception. In true aplasia, the optic nerve and disc, retinal ganglion cells and nerve fibers and central retinal vessels are either absent or are so rudimentary that they bear no resemblance to normal structure or function. In the zone normally occupied by the optic disc, the retina and choroid tend to extend without interruption.

14.2.2. Foveal hypoplasia

14.2.2.1 **Definition**

Foveal hypoplasia is a cardinal sign of aniridia and is characterized by a reduced or absent foveal reflex, abnormal persistence of vessels in the central avascular zone (retinal vessels pass through the center of the presumed foveal area) and a certain decrease in macular pigmentation[9,16]; also, in foveal hypoplasia the illumination with a blue light does not show the darkening at the center of the macula lutea[17]. These foveal abnormalities are present at birth and, in mild cases, can sometimes help establish the diagnosis of aniridia[3]. There does not seem to be any relationship between the degree of irideremia and foveal hypoplasia[18].

Although the existence of retinal dysfunction is accepted, its etiology is a matter of debate. Foveal aplasia or hypoplasia, directly due to mutation of the PAX6 gene, and phototoxicity, due to a poorly developed iris, contribute to retinal dysfunction in varying degrees[14].

14.2.2.2 **Clinical presentation**

Aniridia patients with foveal hypoplasia have a reduced visual acuity and pendular horizontal nystagmus that makes this abnormality hard to detect. It is thought that the degree of visual impairment in patients with aniridia depends largely on the degree of foveal hypoplasia and, in some cases, on the presence of optic nerve hypoplasia[3,5,16-18].

Although there are many causes of nystagmus and poor vision in infancy, the characteristic iris defect and the absence of foveal reflex in aniridia may be clinically useful when it comes to distinguishing this disorder from others in the differential diagnosis[5].

14.2.2.3 **Diagnosis and tests**

As previously mentioned here, the ophthalmoscopic appearance of foveal hypoplasia is characterized by a reduced or absent foveal reflex, abnormal persistence of vessels in the central avascular zone and some decrease in macular pigment.

Fluorescein angiography can reveal a foveal avascular zone that is reduced or absent with a regular vascular pattern, which may prove helpful in the diagnosis of patients in whom foveal hypoplasia is be subtle[9,17,18].

Using optical coherence tomography, McGuire et al.[17] observed an extension of all of the neurosensory retinal layers through the area in which the fovea would normally be located. They also found that the photoreceptor layer seemed to be more prominent in the central area and that no clivus, anticlivus or foveal pit could be seen.

Electrophysiological studies have shown mixed results in aniridia (*see 14.1. Introduction*). Tremblay et al.[1] and Wu et al.[19] have described abnormalities in the electroretinogram (ERG) tests but no findings have been consistent enough to be of diagnostic use[5]. The electrooculogram (EOG) is usually within normal parameters and the visual evoked potentials (VEP) tend to show amplitudes that are reduced when compared with the degree of loss of visual acuity[5,6].

14.2.3. Retinal lipid deposits

Jesberg[20] reported on a series of cases of aniridia patients with a circumferential area behind the ora serrata in which there were numerous white spots that were irregularly distributed along a band that did not reach the equator posteriorly. The diameter of each white spot was about half the diameter of a secondary retinal arteriole.

In the only case that was documented histologically, staining chemical reactions showed that these were lipid deposits (positive staining with Oil Red 0 and Sudan IV).

When added to its genetic features, the finding of these lipid deposits in the peripheral retina of aniridia patients suggests that the defect that causes the disturbance may be due to alterations in lipid metabolic pathways, resulting in the storage of an abnormal lipid compound in the tissues. This alteration in the metabolism would probably occur as a result of a change in DNA structure due to spontaneous mutations.

14.2.4. Giant tears and retinal detachment

Dowler et al.[4] were the first to describe the occurrence of retinal detachment due to giant tears in four aniridic eyes as a possible complication of congenital aniridia. Prior surgery, some type of vitreoretinal abnormality or buphthalmic ocular enlargement might have been be possible factors in the pathogenesis of retinal detachment. As has been noted above, Jesberg[20] described peripheral retinal abnormalities in congenital aniridia which Dowler et al.[4] subsequently found in the four eyes with retinal detachment, suggesting that these two disorders may be linked. However, lipid deposits in the peripheral retina are a common finding in aniridia, whereas retinal detachment is rare. In any case, regular monitoring of the retinal periphery should be conducted in these patients, especially in cases of aphakia.

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SECTION VI

CHAPTER 15

LOW VISION CARE FOR PATIENTS WITH ANIRIDIA

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15.1. Introducción

Prescriptions for low vision aids are not related to the type of disease, but rather to the needs and interests of those affected, whatever the reason for low vision, so therefore, there is no specific support for people with aniridia: prescriptions for special devices and techniques to make the most of remaining vision depend upon rehabilitation goal-setting, which must be agreed from the outset with the affected person.

The job of low vision specialists is to help people with vision loss to not give up the activities they enjoyed before losing vision. And to help them to prevent their handicap from turning into multiple disabilities.

Low vision centers will never be able to provide more vision to someone with low vision, as this is impossible, and in any event, the only one who can help a person achieve better vision is the patient's own ophthalmologist. The purpose of rehabilitation is to make the most of the vision the person affected still has. Thus, it cannot substitute for regular eye care, but is to be used as complementary care.

15.2. Low vision care

Care for low vision uses a standardized approach that is complex and multidisciplinary. Basic care requires six to eight hours per patient, with a team of qualified professionals: opticians, optometrists, occupational therapists, vision rehabilitation therapists and general rehabilitation therapists, appropriate equipment and time to care for the patients. Otherwise proper care will not be provided and the patient will drop out.

Rehabilitation for those afflicted with low vision involves four processes: evaluation of remaining vision, prescription of the technical aids needed to achieve the goals that have been defined beforehand, training in special techniques to make the most of remaining vision and proper use of the prescribed aids and follow up.

15.3. Low vision rehabilitation

15.3.1. Evaluation by an ophthalmologist

Low vision rehabilitation care is started on the basis of an assessment by an ophthalmologist, which must include: a diagnosis of the condition, whether treatment, either medical or surgical, is needed and a prognosis. Visual rehabilitation for low vision does not begin until the ophthalmologist has exhausted all other resources at his or her disposal to help the patient achieve the best possible eye health and the best possible vision.

15.3.2. Initial appointment

Before performing a comprehensive eye examination it is advisable to speak with the patient, either over the phone or during the visit, to have an idea of the prognosis.

Some of the key questions to ask are:

1. Can the patient walk alone without stumbling? That will gives us an idea about the visual field. The larger the visual field, the better the prognosis.
2. At what distance is the patient able to watch football or something similar on the TV? If he/she watches television at a distance greater than one meter, then the prognosis is more likely to be favorable.
3. Are any magnifying devices or other aids used for reading? How were they acquired? Interest and motivation predispose to positive rehabilitation outcomes.
4. Who knows about his/her visual impairment? Would he/she object to using visual aids in public? Acceptance of visual impairment is essential in rehabilitation.

15.3.3. Interview

15.3.3.1. **Characteristics**

This is the most important part.
It must be conducted after analyzing the previous assessment reports.
It must be thorough and comprehensive.
The optometrist must conduct it in person unless there is someone who specializes in interviews on hand who can help (social worker).
It is important to know how to listen and guide the course and direction of the interview. Identify the specific problem areas that concern the patient.

Find out what the patient wants to achieve: Goals. Compare level of demand (needs) with level of performance (abilities).
After the interview we must be able to establish a rehabilitation training program.

15.3.3.2. **Data to be included**

In addition to personal data, we should also include:

- History and symptoms.
- Lifestyle.
- Mobility.
- Patient's goals.
- Distance vision.
- Near vision.
- Lighting needs.

History and symptoms:

- Principal diagnosis.
- When did the problem start?
- Was it sudden or gradual?
- Has progression been steady, intermittent or none?
- How much decline in the last month, 3 months, 1 year, 3 years?
- Causes of the decline.
- General health and eye health history.
- Does he/she go for regular checkups? When was the last visit?
- Names of the health professionals who care for the patient.

Lifestyle:

- Current and former jobs.
- Social environment, does he/she live alone? with their spouse? etc.
- Highest level of education attained: grade school, high school, university.
- Driving: does he/she drive or want to drive?

Mobility:

- Is the patient able to walk: independently, with aid, outdoors, indoors?
- In known places, unfamiliar places, in daytime, at night?
- Is he/she able to cross streets alone, does he/she use a cane?
- When walking, is his/her gait normal, indecisive, unsteady, does the patient need help?
- Other mobility problems.

Patient's goals:

- Main reason for the visit.
- Other needs.
- Who encouraged him/her to come?

Distance vision:

- Can the patient: read street signs, house numbers, bus numbers?
- Recognizes faces, at what distance?
- Does he/she go to the cinema, sports events, theater, etc.?
- Does he/she watch TV, at what distance?
- Does he/she recognize the color of traffic lights, cars, clothing?
- Does he/she see better some days than others?

Near vision:

- Was the patient in the habit of reading?, when did he/she last read something?
- How much time does he/she spend reading, does the patient enjoy it?
- What size font is he/she able to read: headlines, subheads, magazines, newspapers, phone book?

15.3.3.3. **Setting rehabilitation goals**

We can not begin a Low Vision exam before knowing what we want to achieve.

It is very important to agree on the goals with the patient prior to starting rehabilitation.

The targets should be set following an analysis of the initial assessment reports and listening to the patient.

It is key to identify the specific problem areas that concern the patient. Why has the patient come to us? Why does he/she would like to see better? What does he/she need to see better? These are common questions.

The goals should be clear, specific and possible to achieve. For example: to be able to watch TV at 3 meters, read, write, etc.

The goals cannot be ill defined or vague. For example: he/she wants to see better when doing everything.

Rehabilitation will be focused on achieving the goals.

15.3.4. Evaluation of remaining vision

15.3.4.1. **Eye examination**

Similar to a standard optometric examination.

15.3.4.1.1. **Ophthalmoscopy**

Do not be influenced by abnormal fundus appearance. A large central lesion may have a good prognosis.

Use the ophthalmoscope to estimate the patient's sphero-cylinder refraction.

15.3.4.1.2. **Biomicroscopy (Slit Lamp Exam)**

Examine: Cornea, conjunctiva, eyelids, pupillary light reflexes.

Sometimes major diseases involve a loss of transparency that needs to be identified.

15.3.4.1.3. **Eye movement**

Examine: excursions, rotations, etc.

Proper eye movement leads to greater use of residual vision and a reduction of impairment.

Poor eye movement can help to detect functional problems associated with anatomy, which can be corrected with adequate training, especially in children.

15.3.4.2. **Visual field**

15.3.4.2.1. **Perimetry**

Provides us with very valuable information regarding the patient's mobility possibilities and whether or not he/she can move around without being at risk of stumbling and falling.

It is also very useful for knowing what areas of the retina have the best vision so that they can be used in rehabilitation, by teaching the patient to see through them or through an eccentric fixation, if necessary.

15.3.4.2.2. **Amsler grid**

Very useful for determining the direction of eccentric fixation that the patient uses and assessing whether or not it is adequate. Especially indicated for detecting near-distance visual field defects. It assesses visual acuity monocularly and binocularly and provides valuable information to determine whether a prescription should be for monocular or binocular use.

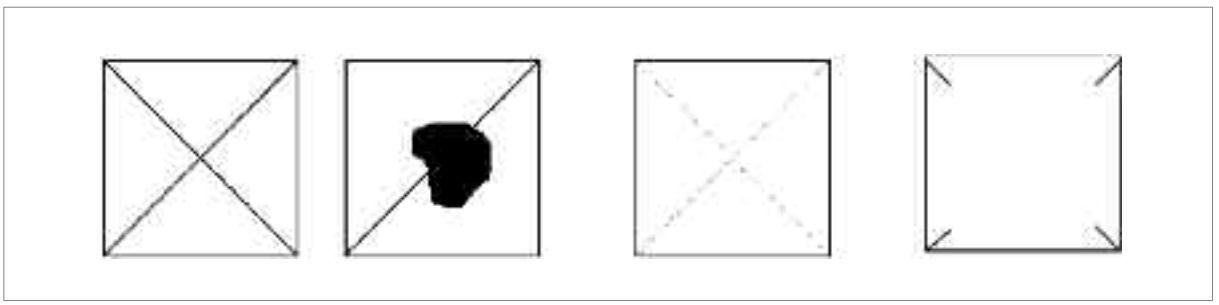
15.3.4.2.3. **Types of scotoma**

When it is **Positive,** the patient is able to perfectly see the margins of the scotoma. It appears as a spot in the visual field.

A **Negative** scotoma is when the patient is unaware of the missing area in his visual field, because the brain fills in the information.

A scotoma is **Relative** when the patient is still able to see something through it.

When the scotoma is **Absolute** there is no vision at all through it. (Figures 1, 2, 3 and 4)



Pictures 15.1, 15.2, 15.3 y 15.4

15.3.4.3. **Color vision**

Use the Farnsworth 28-hue test if patient has sufficient vision. If not, use samples of colors.

Degrees:
a) Able to distinguish between colors.
b) Able to match colors.
c) Unable to do either.

15.3.4.4. **Contrast sensitivity**

The tests vary by manufacturer.

They should all be held perpendicular to the patient's line of sight.

Test at one meter distance; if the patient's performance is less than normal, repeat at 50 cm with the appropriate refractive correction.

You should be able to set the lighting of the room in a range from 700 cd/m^2 to 10 cd/m^2.

Note the light level at which the best results have been achieved.

The background for the test should be dark and dull.

It is a more functional test than the one for visual acuity, which measures the ability to differentiate under ideal conditions.

Sensitivity to contrast provides us with more information about that person's functional behavior under real conditions. It is very useful to know how much ability the patient has to discern between, for example, a light gray curb and a dark gray pavement when out walking.

15.3.4.3. **Refraction**

Similar to a conventional refraction test; there can be changes in the technique, style and attitude.

15.3.4.3.1. **Keratometry**

It is important to measure the objective astigmatic error, as it is sometimes difficult to perform a retinoscopy.

It should be performed on the area through which the person usually looks, not the central part.

If nystagmus is present, keratometry should be performed in the position where it is blocked; if there is none, hold the eye still above the eyelid.

If the patient uses eccentric fixation, it should be performed on the area most often used for looking.

15.3.4.3.2. **Cover test**

Remember that when vision is poor and fixation is uncertain, the lack of movement does not

necessarily mean that there is no phoria or tropia.

15.3.4.3.3. **Retinoscopy**

This is the test that provides us with the greatest amount of information.

If the shadows cannot be seen at the usual distance, it is advisable to move closer to the patient, so the shadows become more clear, while bearing in mind that the margin of error is greater the closer we get. (Radical retinoscopy).

This test must also be performed on the area the patient most commonly uses to look.

15.3.4.3.4. **Measurement of distance visual acuity**

Use proportional optotypes, the Bailey-Lovie chart is ideal.

The ratio of the progression of size for each row of letters is obtained by multiplying the previous level of visual acuity by 1.25: 1, 1.25, 1.5, 2.0, 2.5, 3.1, 4.0, 5.0, 6.25, 8.0, 10, etc., thereby obtaining a scale of sizes that is proportional.

It uses letters that have the same blur effect (Sloan letters).
Proportional visual acuity progression.
Wide range of letter sizes.
Same number of letters per row.
Spacing between letters and rows equal to reference letter size.

Optotypes that use a decimal scale are not proportional and the progression of visual acuity between rows differs widely; the spaces between the upper rows are very small while the spaces between the lower rows are very large. They are not useful for the measurement of low visual acuity.

The optotype charts should be mounted on stands on wheels so they can be moved closer to and farther from the patient.

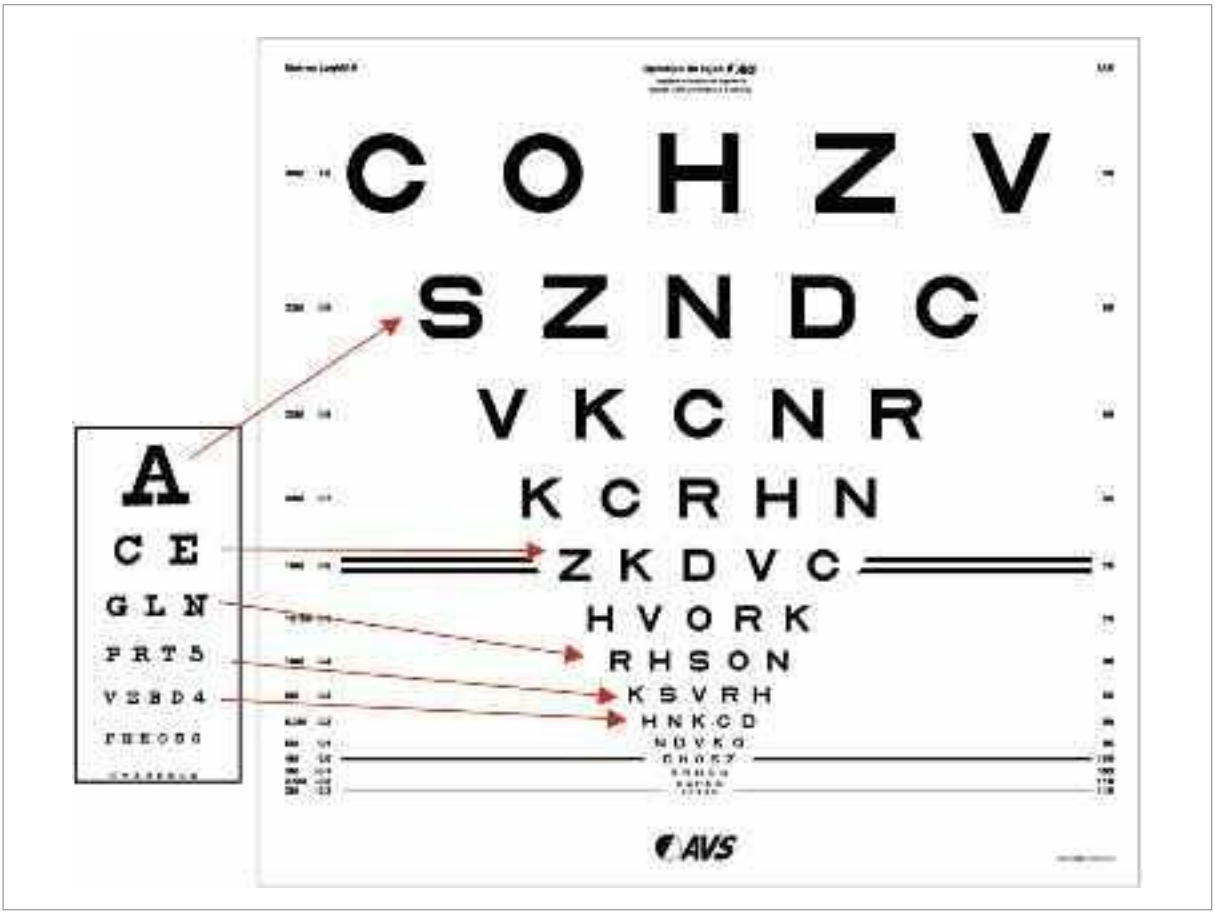
The optotypes should be transilluminated to achieve maximum contrast. Fig. 1 and Fig. 2.

Start at a distance of 3 meters. If the patient is unable to see any of the symbols, bring closer to 1.5 meters, visual acuity value is doubled. If the patient is still unable to see, move the chart nearer, to 1 meter, and if he/she can still not see it, to 50 centimeters. It is necessary to have optotypes that are large enough for the patient to recognize.

When measuring visual acuity, allow the patients to adopt the head position that they prefer in

order to obtain the best visual acuity.

Record visual acuity using Snellen's system. The numerator is the testing distance at which visual acuity is measured and the denominator is the distance at which a person with normal visual acuity (VA of 1 or 20/20) would be able to see, which is noted in each row. Example: 3/20.



15.3.4.3.5. **Subjective tests**

1) Two very important features:

- Do not rush.
- Emphasize the positive.

2) Always use a trial frame when testing patients whose visual acuity is equal to or less than 3/8.

- This way, the patients will be able to adopt the preferred position for their head and eyes.
- It is more natural and comfortable for patient.
- Eye movements and fixation are easier to observe.
- There will be less limitation of the visual field on account of the phoropter and the patients will be able to use eccentric fixation if they need to.
- There is practically no instrumental accomodation.

Figure 15.1. Bailey-Lovie Chart for testing low vision.

3) Determining the sphere

a) Concept of «Just noticeable difference» (JND).

- In people with normal VA it is ±0.25 D.
- In people with low vision it is calculated according to the following formula:

Formula for Just noticeable difference.

$$JND = \frac{\text{Snellen denominator at a distance of 6 meters}}{30}$$

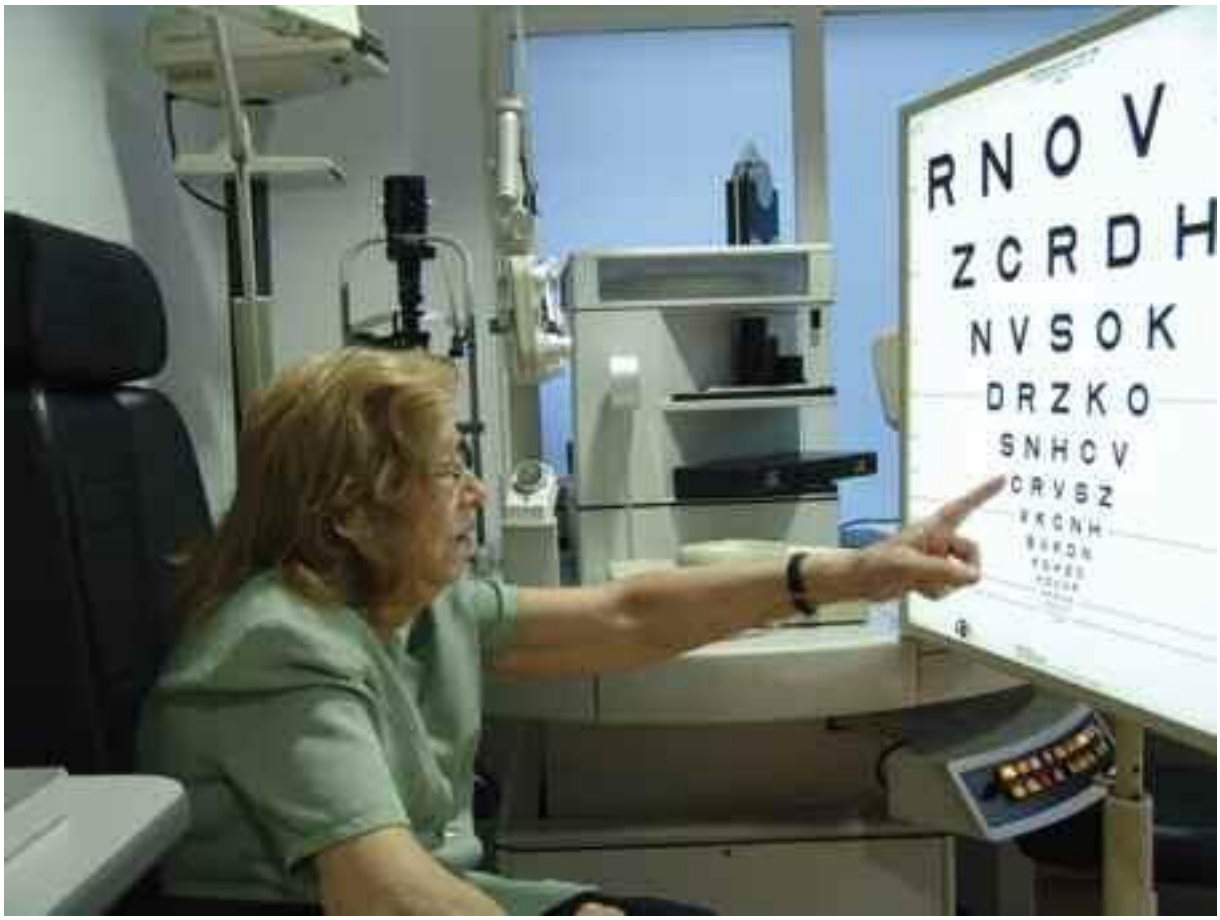


Figure 15.2. Measurement of distance visual acuity.

-Example: If vision is 6/60

$$JND = \frac{60}{30} = 2.00\ D$$

b) Begin with:

- Old prescription
- Retinoscopy value
- Ophthalmoscope power setting
- Neutral

- At which best vision is obtained

1) Calculate the (JND)

2) Add half of the JND value to the initial starting spherical value and compare; subtracting the other half, find out which direction is the preferred one.

3) Add half of the JND value again to the preferred power and compare the result to the spherical value that results from subtracting the other half of the JND value.

4) Apply the JND value over again as many times as necessary to find the upper and lower limits of the patient's definite dioptric power.

5) Apply the JND value to the mid-point in order to determine final refraction.

6) If there is no preferred power, return to the starting sphere. Add ± 1D to the JND and repeat.

7) If the patient is unable to discriminate between different powers, use the mid-point.

Example:

- Using JND = ± 2.00 D.
- Initial sphere = -2.00 D.

1) Apply the JND. Which power does the patient prefer, (-3.00) or (-1.00)?

-3.00
–2.00
–1.00

2) Patient prefers (-3.00). Tends more to negative values.

3) Apply the JND value again to the preferred power.

-4.00
–3.00
–2.00

4) Patient prefers (-2.00). The definite power will be more negative than (-2.00), and more positive than (-3.00). Therefore, the only powers that fulfill these requirements are: (-2.25), (-2.50) y (-2.75).

5) How to choose? Apply the JND value to the mid-point.

-3.50
–2.50
–1.50

– If the patient prefers (-3.50), definite power will be: **(-2.75).**

– If the patient prefers (-1.50), definite power will be: **(-2.25).**

– If the patient doesn't notice any difference between (-3.50) and (-1.50), definite power will be: **(-2.50).**

– The result we have obtained has a precision of ±0.25 D when the patient can only perceive –2.00 D differences.

– This precision is very useful for prescribing special aids for low vision.

4) Determining the cylinder power

a) Method for the amplification of the cylinder

1) Increase cylinder power using the initial JND without modification of the sphere.

2) Twirl the axis 45º while patient looks at the optotype where the best visual acuity is shown, three times in each direction.

3) Ask when it seems most blurry in each direction and make a mental note of the answer.

4) The mean of the six readings shall be taken as the axis.

5) To measure the power:

-Use the JND and proceed as for the sphere.

b) Conventional method.

1) Axis: use a cross-cylinder at the following powers:

a) If vision is equal to or greater than 0.33 or 6/18, use ± 0.37 D.

b) If vision is less than 0.33 or 6/18 or equal to 0.15 or 6/40, use ±0.75.

c) If vision is less than 0.1 or 6/60, use ±1.00 D.

2) Power:

-After the axis has been determined, superimpose the cross cylinder to determine the power of astigmatism.

5) Things to remember

– Always use a trial frame with acuities less than 6/16.

– Modify the sphere and cylinder to obtain the spherical equivalent. Check to see if spherical equivalent can be obtained by decreasing cylinder power if the change in graduation has been very sudden.

15.3.4.3.6. **Measurement of near visual acuity**

1) Use an optotype with proportional spacing, single letters and metric notation.
2) Add +4.00D to measure visual acuity at a distance of 25 cm
3) Use a typoscope instead of pointing with a finger.
4) Begin with normal room lighting, starting with the smallest line of letters the patient can see when light is increased or decreased. Use a gooseneck lamp, so light can be moved closer or farther away.
5) Record visual acuity of the right eye (OD), left eye (OS) and both eyes (US).

15.4. Optical treatment of low vision

15.4.1. Definition

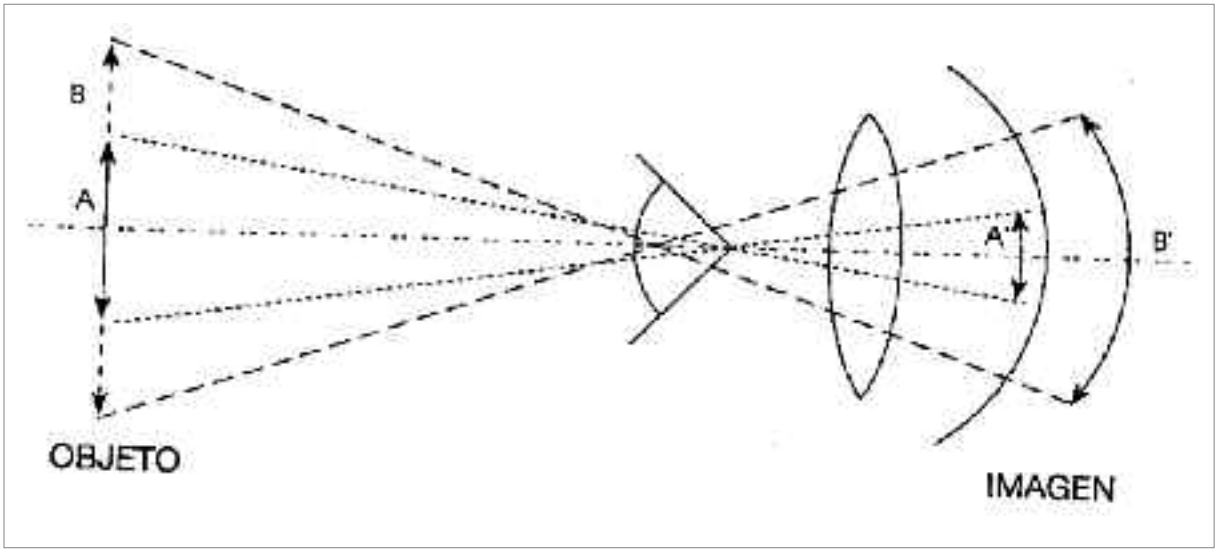
This consists in increasing the size of the image produced on the retina through the use of magnification systems, so more retinal cells are stimulated and are thus able to send more information to the brain for it to process the image.

15.4.2. Magnification systems:

- Size magnification
- Distance magnification
- Angular magnification
- Projection magnification

15.4.2.1**. Size magnification**

This approach consists in increasing the actual size of the object, so that by doubling the size of the object, the retinal image is also doubled and therefore visual acuity is doubled. (Figure 6)



Picture 15.6

Examples would be people who read large print or use felt markers instead of ballpoint pens.

Increasing the size and therefore the weight of objects tends to be inconvenient and costly but the advantage of this method is that it allows the patient to read at the usual distance.

15.4.2.2. **Distance magnification**

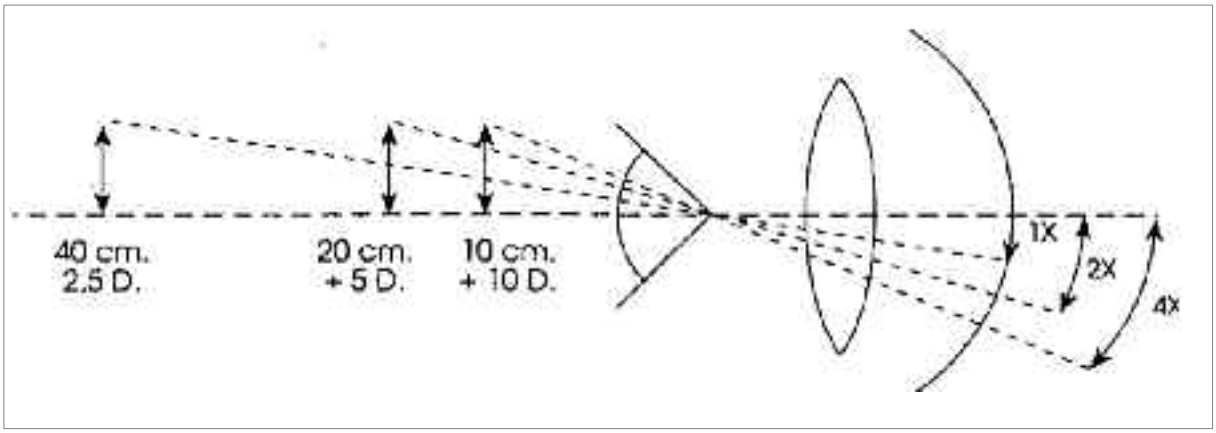
This approach is based in the fact that whenever an object is brought close to the eye, the retinal image becomes larger.

The relationship is such that when an object is brought to one half of the viewing distance, the size of the retinal image doubles; if the distance is reduced by one quarter, the size of the retinal image will increase four times and so forth (Figure 7).

By bringing an object closer to the eye, its light rays become more and more divergent and require compensation for the eye to see them clearly.

Diopters needed in order to be able to see an object clearly

The diopters needed in order to be able to see an object clearly are determined by the following formula:



Picture 15.7

$$D = \frac{100}{d}$$

D = Diopters needed
d = distance in cm

Therefore, if we want to see an object at a distance of 25 cm clearly we need:

$$D = \frac{100}{25} = +4D$$

The 4D that are needed to see an object at a distance of 25 cm clearly can be obtained in the following 3 ways:

1) By placing a converging lens (+) in front of the eye.
2) Making the eye accommodate. The eye is capable of increasing its dioptric power, enabling it to focus up close.
3) In the case the patient has myopia of –4D that is not compensated. When this patient takes off his –4D distance eyeglasses, his visual system is already focused at 25 cm without the need to make any effort.
4) It can also be obtained by using any combination of the preceding three methods.

The addition of a converging lens (+) to the eye will not increase the size of the object, but it will cause it to focus perfectly on the retina.

When the distance at which we want to see the object is very short and the accommodation of the lens is insufficient, we need converging lenses (+), unless the patient has very high myopia.

The main disadvantages of this type of magnification are that the working distance is short and the lens limitations with respect to the field of view.

The advantage of these aids is that they are inexpensive and easy to use, in addition to aesthetic considerations.

15.4.2.3. **Angular magnification**

This is the magnification that occurs when we look through a telescope constructed from two lenses.

The objective lens is the lens through which the rays enter the telescope (Figure 8).

The ocular lens is the lens that is closest to the eye (Figure 9).

The lenses are mounted so that secondary focal point of the objective coincides with the primary focal point of the ocular lens.

This results in the angular magnification of an afocal system (focused at infinity).

The telescope lenses deviate the rays of light so that, when they leave the telescope, they appear to come from an object that is closer to the eye, and therefore give the impression that the object is much larger.

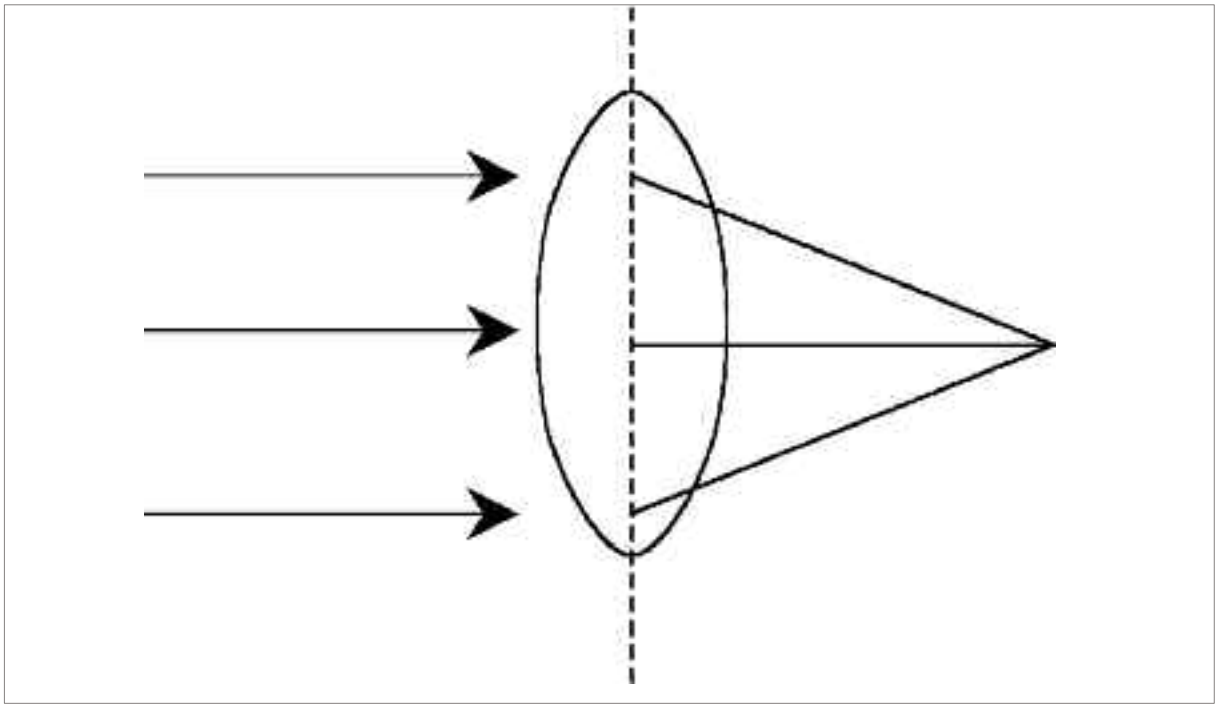
The angular magnification is the ratio between the angle formed by the optical axis and the ray that leave the telescope divided by the angle formed by the optical axis and the ray that enters the telescope (Figures 10 and 11).

The main advantage of angular magnification over other systems is that it is the only magnification system that enlarges distant objects that cannot be made larger or brought closer.

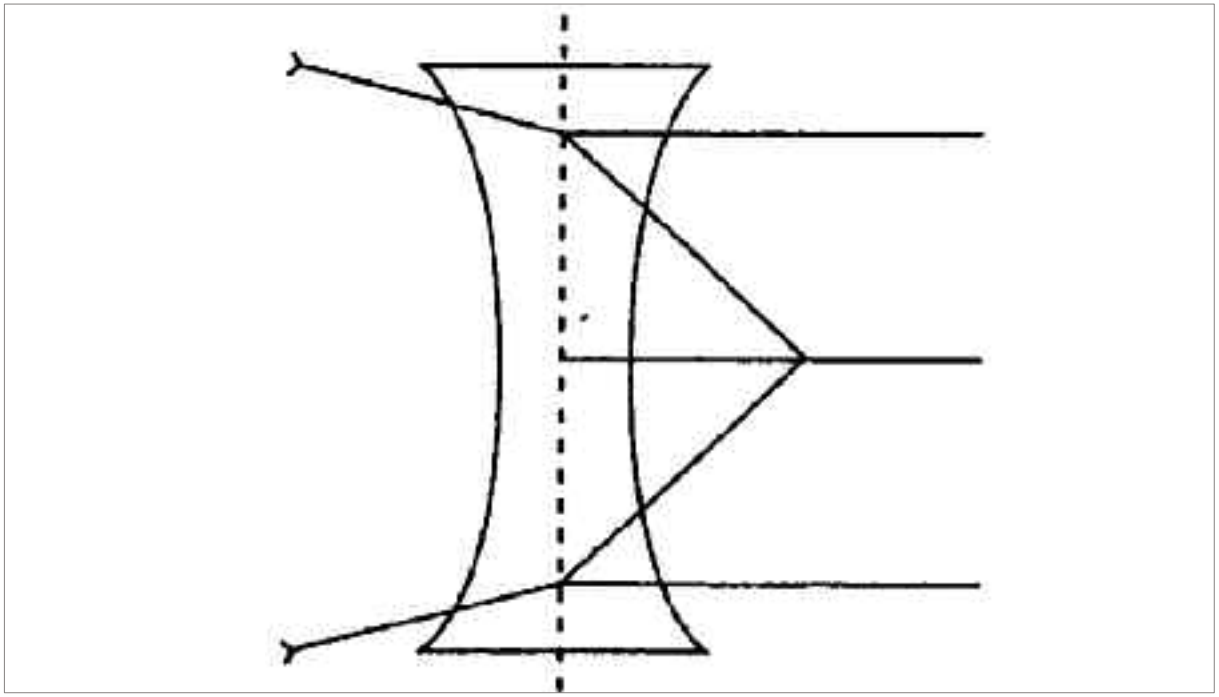
The disadvantages are: motion parallax, changes in the spatial perception of objects and a restricted field of view.

15.4.2.4. **Projection magnification**

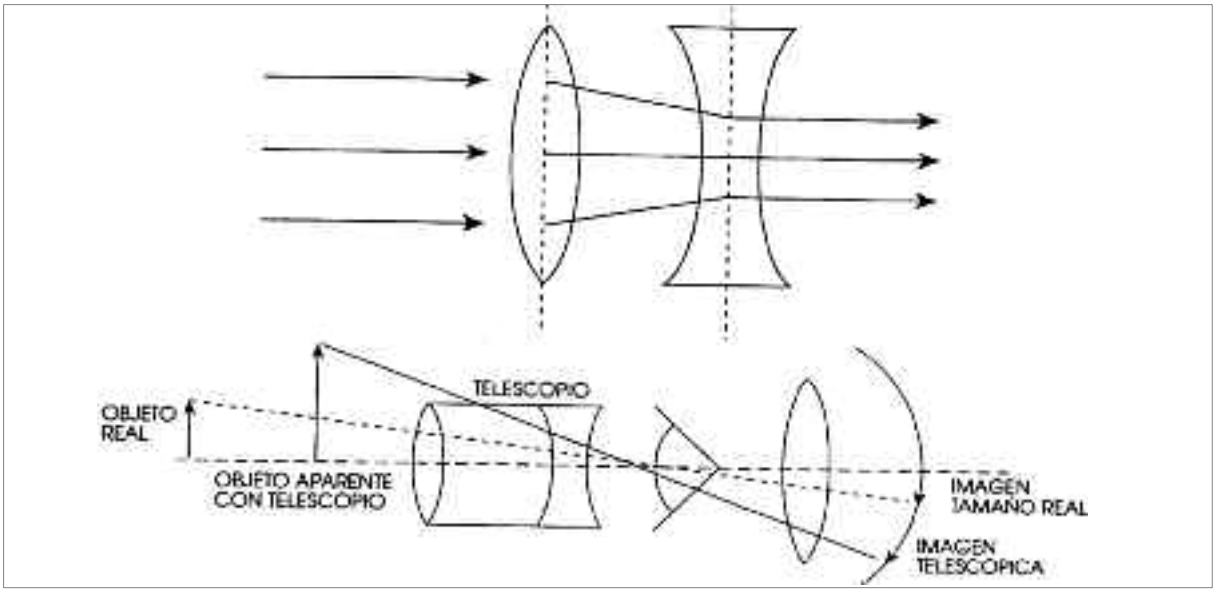
An object is enlarged by projection on a screen, as occurs with the slides or closed circuit television aids (Figure 12).



Picture **15.8**



Picture 15.9



Pictures **15.10 y 15.11**

Formula:

$$A = \frac{\text{Size of the image}}{\text{Size of the object}} = \frac{\text{Distance of the image}}{\text{Distance of the object}}$$

Advantages of this system of magnification

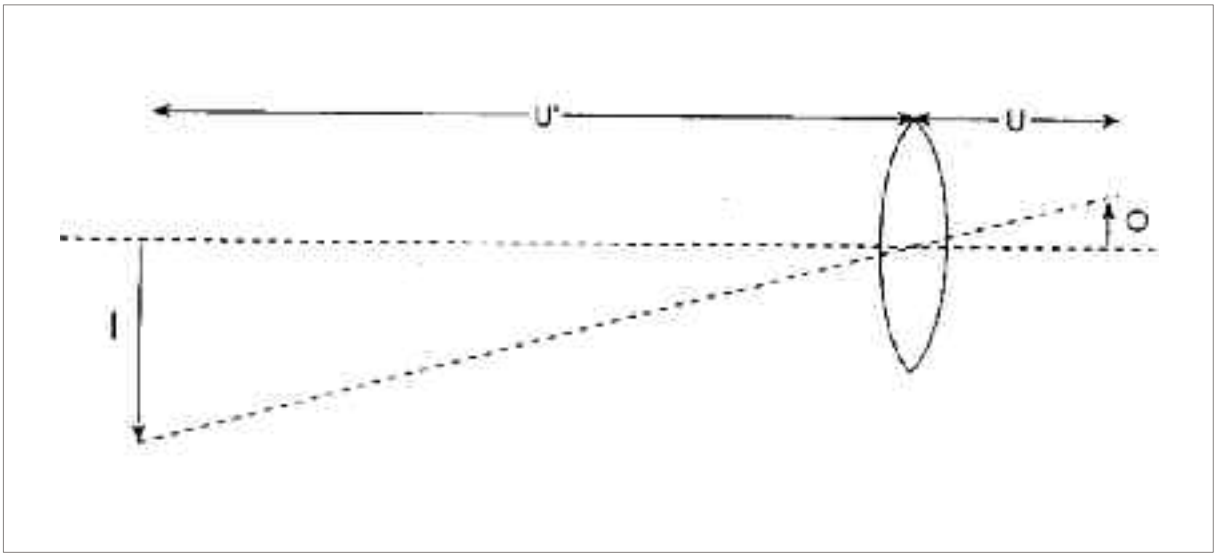
1) Normal working distance
2) Large field without aberrations

Main disadvantages

1) Not easy to move
2) The price is high

15.4.2.5. **Total magnification**

The magnification of the retinal image can only be obtained by the use of any one of the four magnification systems described above or a combination thereof.



Picture **15.12**

When several magnification systems are used, total magnification is the product of the magnifications produced by each one of them.

If the size of a text is doubled (2X) and the viewing distance is reduced from 40 cm to 20 cm (2X) the resulting total magnification will be: 2 x 2 = 4X.

If at the same time projective magnification of x4 is used, total magnification will be: 2 x 2 x 4 = 16X

The image will be 16 times larger than the original and therefore visual acuity will also be 16 times higher.

15.4.3. Selecting magnifying power and vision aids for near vision

Near vision is usually more important than distance vision, so therefore we will discuss it first.

If we know what the distance visual acuity is we can predict the near visual acuity, if we have used proportional optotypes, but near visual acuity tends to be higher than the visual acuity required for reading (Figure 3).

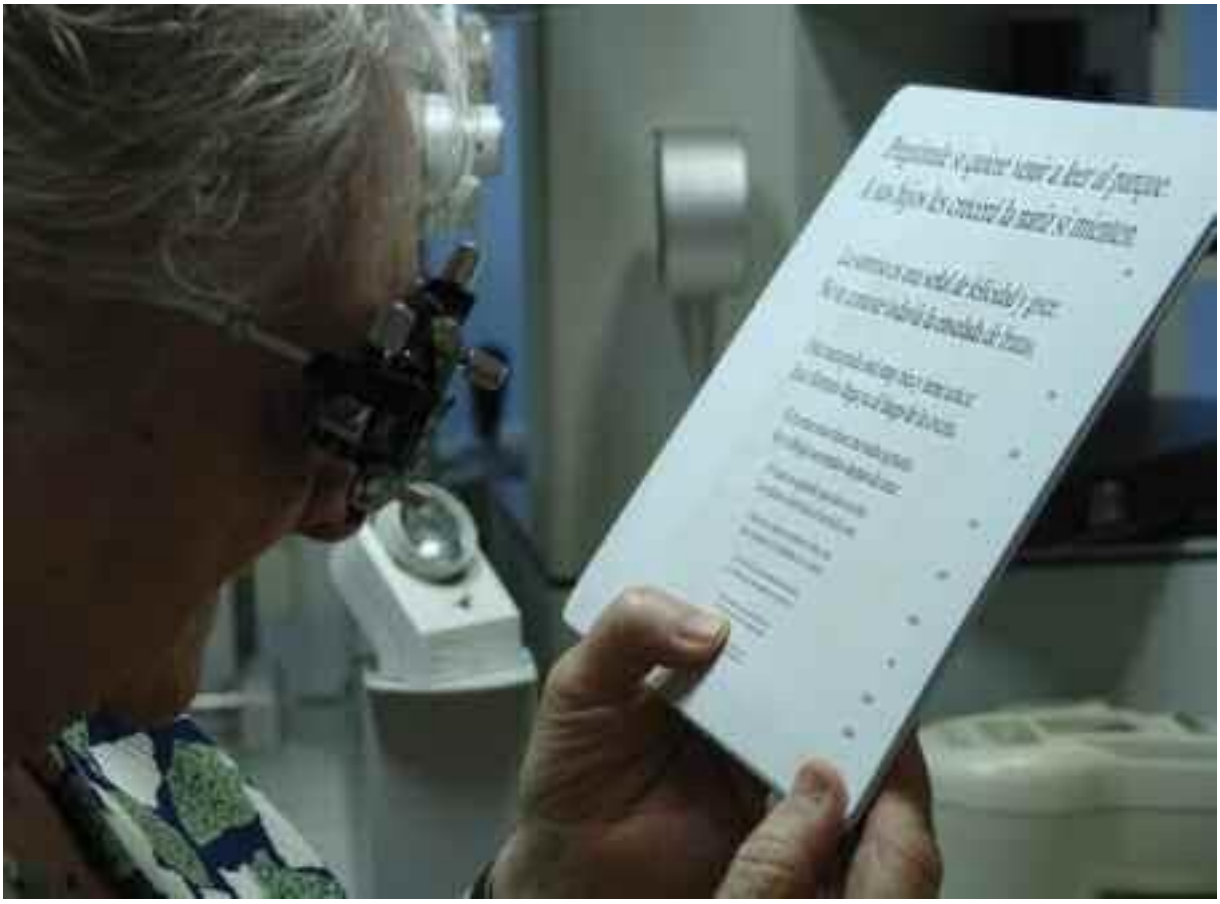


Figure 15.3. Measurement of near visual acuity.

15.4.3.1. **Calculating the magnifying power for near targets**

1) Use an optotype with a continuous text, proportional spacing between rows and metric notation.
2) Measure visual acuity at a distance of 25 cm with an addition of + 4.00 D.
3) Determine the visual acuity required for the patient to identify each of the near targets.
4) Calculate the magnifying power required to identify each of the near targets.

$$A = \frac{\text{Visual acuity of the patient}}{\text{Visual acuity of the target}} = X$$

5) Multiply the magnifying power times 4.00, the diopters required to see at the distance at which near visual acuity was measured. The result will be the plus diopters that need to be added to the spherical power in order to obtain target visual acuity.

If a patient is able to read 3 M print at 25 cm, there is a need of +4 D. In order to see 1 M print (newspaper size print), which is three times smaller, it would be necessary to multiply his visual acuity times three.

$$A = \frac{3M}{1M} = 3X$$

To find out how many plus diopters are needed, it would be necessary to multiply the magnifying power times 4.00.

3X (+4D) = +12D.

With a reading distance of three times less than 25 cm, the addition to the patient's distance prescription will be +12 D.

$$d = \frac{100}{12} = 8.3 \text{ cm}$$

We can decide later whether to prescribe a +12 D addition or less so that when he reads larger print he can do so at a greater distance, as long as he is able to accommodate for the plus diopters without any trouble in case he has to read print that is of target size.

6) Perform a reading test using the addition that has been calculated to identify the target.

7) Modify it if necessary. If the patient does not manage to read the target, increase the addition.

If there is any question between two powers, measure the speed of reading.

15.4.3.2. **Presentation of the different types of near vision aids**

1) Use the magnifying power that has already been found for each target and do not make any modifications to it when selecting the type of aid.

2) Decide what type of aid is most appropriate to identify each target.

– Microscopes
  Monofocal microscopes
  Bifocal microscopes
– Magnifiers
  Handheld
  On stands

– Telemicroscopes
– Electro-optical devices
– Non-optical aids
– Non-visual aids

3) Let the patient know what options are available, together with the advantages and disadvantages of each one in achieving the different targets.

4) Check that the patient is able to read at the right distance and achieves the target.

15.4.4. Selecting the magnifying power and vision aids for distance targets

15.4.4.1. **Calculating the magnifying power for distance targets**

1) Determine the visual acuity required for the patient to identify each of the distance targets. The specialist should be able to judge how much visual acuity is required to successfully perform the task the patient wishes to do.

For example: if the patient wants to be able to read street signs, visual acuity of 3/6 is sufficient. With a visual acuity of 3/6 we are able to do most of the tasks we are use to performing: read bus numbers, see the bus routes, metro station names, etc. Generally speaking, visual acuity using a telescope exceeds 0.5, which is the desired minimum target.

2) Calculate the magnifying power required to identify the target

$$A = \frac{\text{Snellen denominator according to the patient's visual acuity}}{\text{Snellen denominator according to the target visual acuity}} = X$$

If the patient has a visual acuity of 3/30, he will need 6X to achieve a visual acuity of 3/5.

3) Test the telescope power that has been calculated.

4) Modify it if necessary. If the patient does not reach the target, increase the telescope's power and compare.

15.4.4.2. **Presentation of the different types of distance vision aids**

1) Once the power has been selected we can decide on the type of telescope.

According to the patient's features:

-Monocular or binocular
-Handheld or spectacle-mounted
-Brightness, field of view, size, etc.

2) If the telescope has a fixed focus, calculate the power that needs to be added to the ocular lens.

3) Use an optotype at an appropriate distance to check visual acuity.

4) When prescribing the telescope, remember that the higher the magnifying power, the more the visual field will decrease, and that generally leads to an increase in difficulties. Prescribe the lowest possible magnifying power at which the target is achieved.

15.5. Low vision aids

15.5.1. Types of aids

-Microscopes
-Telescopes
-Telemicroscopes
-Handheld and stand magnifiers
-Electro-optical devices
-Visual field enhancement support systems
-Non-optical aids
-Non-visual aids

15.5.2. Microscopes

15.5.2.1. **Definition**

A microscope consists of a converging lens or system of lenses, specially designed to minimize aberrations and for use at a distance of less than 25 cm.

15.5.2.2. **Features**

Microscopes are based on the principle of magnification by means of decreasing relative distance.

It does not magnify anything in itself, but it allows us to see an object clearly when it is brought closer and it is this approximation which gives it its magnifying power.

It makes up for accommodative dysfunction at very short distances. Young people with high accommodative power use the same principle without any aids and without any of the constrictions on field of view that result from using microscopes, so by getting very close to the object they manage to see it enlarged. But if they become tired or need to read at a shorter distance, they need microscopes just as much as older people do.

When people with high myopia take off their eyeglasses, they function as though they have an inner microscope, so that when they take their glasses off they acquire additional positive power, which is inversely proportional to their degree of myopia, and by doing so their near vision performance improves when they take their eyeglasses off.

The higher the magnifying power, the lower the visual field and the shorter the working distance; therefore the number of tasks that can be performed easily is more limited.

15.5.2.3. **Types of microscopes**

-Classification based on:

- Use:

-Monofocal
-Bifocal (additions from +4.00 to +4000 D).

- Material from which they are made:

-Glass
-Plastic

- Lens geometry

-Spherical
-Aspheric

- Construction

-One lens
-Several lenses

15.5.2.4. **Working distance**

$$\text{distance in cm} = \frac{}{F\,(D)}$$

15.5.2.5**. Magnifying power effective at the reference distance of 25 cm**

$$A_{25} = \frac{F(D)}{4}$$

If the ametropia has not been corrected, the formula is:

$$A_{25} = \frac{F\,(D) - R}{4}$$

In which R is positive for hyperopia and negative for myopia.

15.5.2.6. **Advantages of microscopes**

They have a better aesthetic appearance and are less bulky than telemicroscopes.

They allow both hands to be kept free.

The field of view is larger compared to telemicroscopes and magnifying glasses with the same power.

They are comfortable when reading for long periods and for writing, if distance allows.

15.5.2.7. **Disadvantages of microscopes**

The working distance is very short and causes fatigue easily.

Posture can be very uncomfortable if suitable accessories are not used: stands, comfortable chairs, adequate lighting, etc.

Arm or head movements are needed, rather than eye movements, and learning to coordinate them is difficult.

Binocular vision is only possible up to a maximum magnification of 3X, using base-in prisms to

alleviate convergence.

High-power microscopes have a very shallow depth of field, although it is higher than telemicroscopes.

From a distance everything appears very blurry.

They need to be taken off when moving around.

15.5.3. Telescopes

15.5.3.1. **Definition**

Telescopes are optical aids that use angular magnification to create a larger retinal image size without the need to get closer or enlarge an object.

15.5.3.2. Features

They are the only instruments that help people do distance tasks.

Afocal telescopes are focused at infinity, which is anything beyond a minimum distance of 6 meters.

They must be used in combination with the patient's conventional distance correction or the distance correction be embedded in them.

The exit pupil is the optical window that is seen projected on the ocular lens and through which the patient must look. The higher the magnification of the telescope, the smaller it is.

When looking through a telescope there is a loss of brightness; the best results are obtained when it is used in good lighting conditions.

Telescopes are to the visually impaired as canes are to the blind.

15.5.3.3. **Main disadvantages**

The movement of objects is exaggerated when looking through them.

By giving the impression that objects are closer than they really are, they disrupt the spatial judgment of objects.

Visual field constriction.

The higher the power of the telescope, the more their disadvantages increase.

15.5.3.4. **Telescope parameters**

a) Powers available

b) Field of view

c) Range of focus

d) Weight

e) Possibility to have them mounted on glasses

f) Brightness

g) Possibility of embedding distance correction in them

h) Depth of field

15.5.3.5. **Categories of telescopes**

A) Galilean telescope

1.- Structure

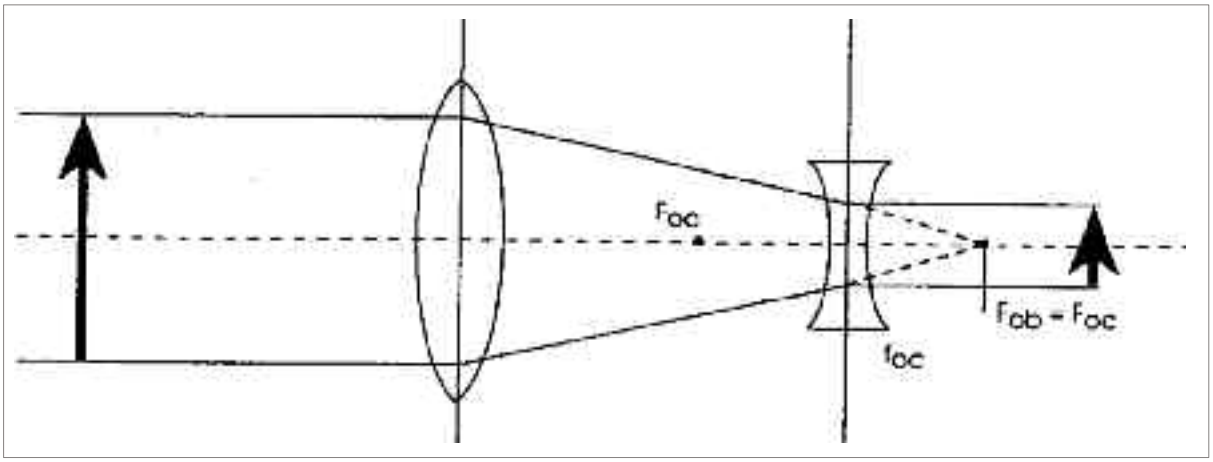
It consists of an objective, which is a converging lens, and an ocular lens, which is a diverging lens, mounted in such a way that the secondary focal point of the objective coincides with the primary focal point of the ocular lens (Drawing 13).

2.- Features

The image is upright.

Its usable magnification is limited to low values due to its image forming characteristics and the location of the diaphragms.

The field of view is not clearly defined around the edges and is vignetted.

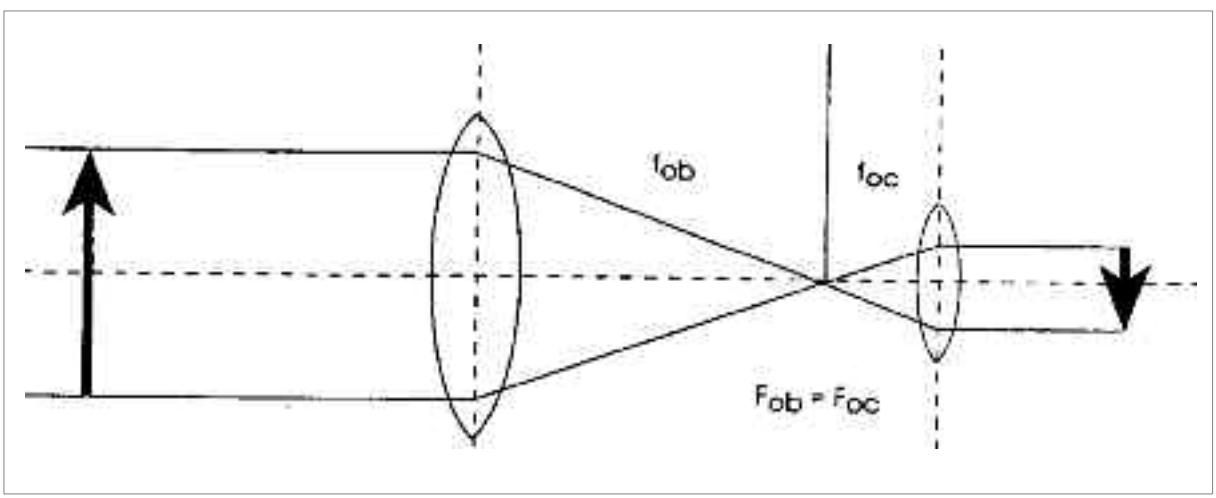


Picture 15.13

B) Keplerian telescope

1.- Structure

It consists of two converging lenses: one lens is the objective and the other is the ocular lens, positioned in such a way that the primary focal point of the objective coincides with the secondary focal point of the ocular lens (Drawing 14).



Picture 15.14

2.- Features

It yelds an inverted image that is corrected by inserting prisms.

It provides greater magnification than Galilean telescopes.

The field of view is clearly defined and wholly usable.

15.5.3.6. **Formulas for telescopes**

-Variables:

A= Magnifying power of the system
d= Separation between the objective and the ocular lens
Fob= Dioptric power of the objective lens
Foc= Dioptric power of the ocular lens

-Simple ratio

(1) $$A = \frac{-F_{oc}}{F_{ob}} = \frac{-f_{ob}}{f_{oc}}$$

(2) $$d = \frac{1}{F_{ob}} + \frac{1}{F_{oc}}$$

15.5.3.7. **Types of telescopes**

-Handheld

-Spectacle-mounted (positioned centrally or superiorly)

Figure 15.4. Using a manual telescope to cross a street.

Figure 15.5. Telescope placed on the upper part of eyewear to enable moving around.

- Some of these telescopes have clip-on systems that allow patients to slip them on over their regular glasses whenever they need them and which provide much the same results as spectacle-mounted telescopes.

The telescope needs to be in contact with the lens surface of the eyeglasses.

2) Positioned superiorly (Figure 7)

- These are small diameter telescopes that are placed on the upper part of the patient's eyeglasses, so that they can look through their normal distance lenses while moving around but when they need extra magnification to look at an object, they can tilt their head down and raise their eyes to look through the telescope.

- These provide greater visual acuity but a lower field of view.

- The main drawback is their small size and therefore restricted field of view.

15.5.4. Telemicroscopes

Figure 15.6. Training a girl in the use of a manual telescope.

15.5.4.1. **Definition**

These are telescopes that have a focus range equal to or less than 60 cm, a distance which allows objects to be handled.

15.5.4.2. **Features**

Telemicroscopes provide a greater working distance than microscopes, but have a reduced field of view.

Depth of focus is more restricted with regard to microscopes: they are only useful at a certain distance.

Some manufacturers have models with a built-in close-up lens on the objective, so it doesn't have to be superimposed, but the principle is the same: an objective with a close-up lens, which

in this case constitutes just one lens.

On occasion the patient may need distance vision, in which case a negative lens with a power equivalent to the inverse of the working distance at which the telemicroscope has been intended to be used can be placed.

The product of the magnification of the telescope times the dioptric power (diopters) of the microscope gives us the plus equivalent power in diopters that we have to add to the correction to obtain the same magnification as without the telemicroscope.

15.5.4.3. **Accommodation when looking through a telescope**

When an object at a finite distance is viewed with an afocal telescope, the accommodation of the eye when looking through the telescope is much greater than with the naked eye and is directly proportional to the square of the telescope's magnification.

$$U_t = \frac{A^2 U}{1-dAU}$$

Figure 15.7. Training in the use of a telescope on the upper part of eyewear.

Figure 15.8. Reading with a telemicroscope.

So that:

Ut= Accommodation required with the telescope
A= Magnification of the telescope
U= Accommodation without a telescope
d= Distance between the objective and ocular lenses

As d is very short in comparison with the other parameters, we can consider that:

$Ut = A^2 U$

If we use a 2.2X telescope to view an object at 1 m, the accommodation of 1 diopter multiplied by 2.2^2 is required. In this case, +4.84 D are necessary in order to see an object focused at one meter. If the same telescope is used to view an object at 40 cm, 4.84 x 2.50 = 12 D of accommodation would be required, which is next to impossible; some form of adjustment is needed so that the eye is not forced to accommodate to that power.

If we want to lessen the need of the eye to accommodate, we have three options:

1) Decrease the power of the ocular lens, making it more positive
2) Increase the distance between the objective and the ocular lenses
3) Increase the power of the objective lens

The first option is used to turn an afocal telescope into a telescope with a fixed focal length.

The second is used in focusable telescopes.

The third method is the one that is most widely used, by placing a positive powered lens cap over the objective and transforming the telescope into a telemicroscope.

15.5.4.4. **Magnifying power of telemicroscopes**

The resulting magnification is found by multiplying the telescope's magnifying power times the magnifying power of the microscope or approximation lens.

Atm = At Am

Remember that:

$$A_m = \frac{F\,(D)}{\text{---------}}$$ Reference distance of 25 cm

4

15.5.4.5. **Working distance**

Working distance is defined solely by the microscope.

$$d = \frac{100}{F\ (D)}$$

Whereby:
d= Working distance
F= Dioptric power of the microscope

Example:

A patient uses a 3X telescope for distance viewing, with a +4.00 D approximation lens to see close up.

Calculate:

a) The magnifying power of the telemicroscope, using the 25 cm reference distance

$A_{tm} = A_t\ A_m = 3x1 = 3X$

b) The magnifying power of the telemicroscope, using a 40 cm reference distance

Atm = At Am = 3 x 4/2.5 = 4.8X

c) Working distance:

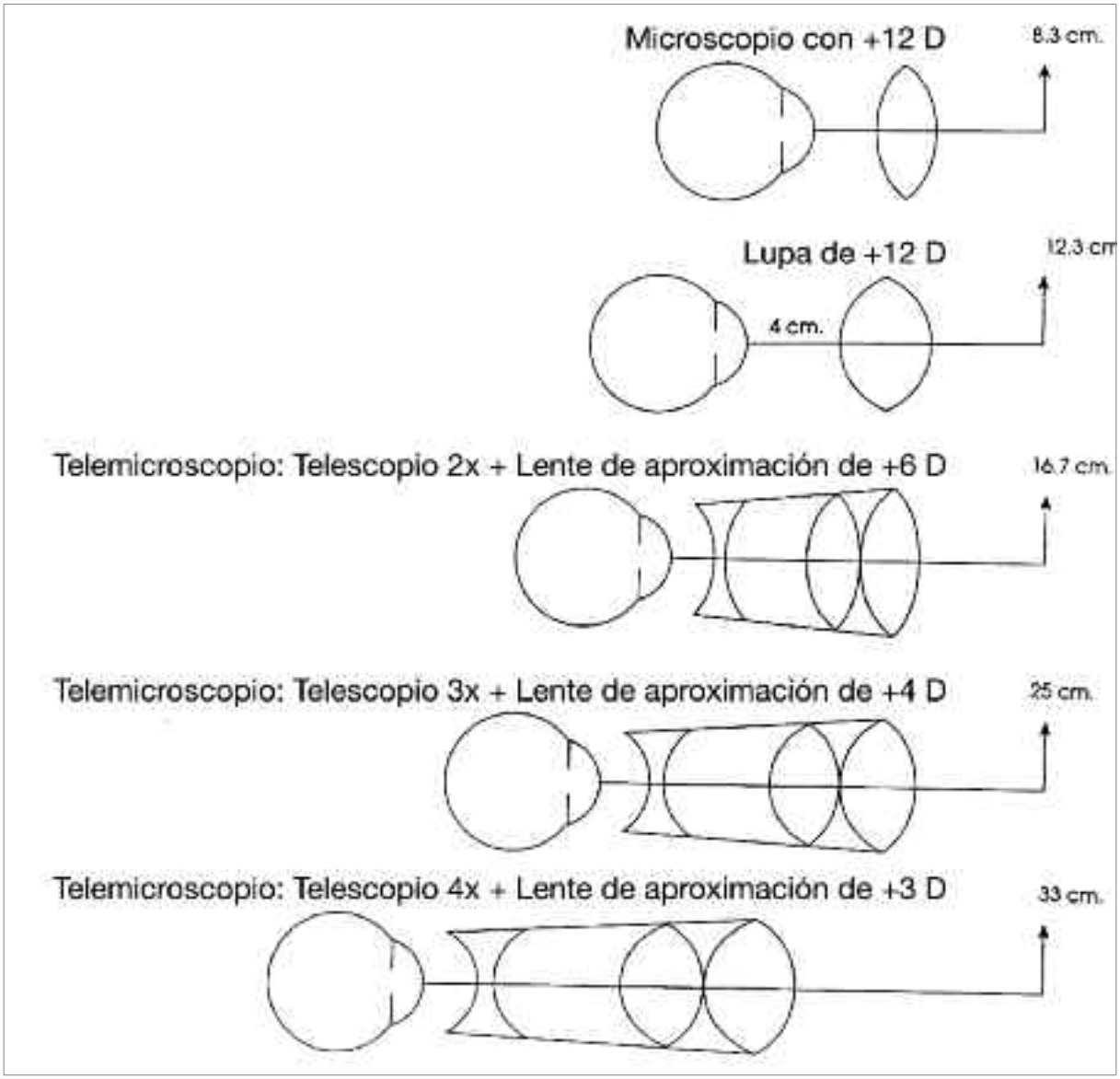
$$d = \frac{100}{F\ (D)} = \frac{100}{4} = 25\ cm$$

15.5.4.6. **The same magnifying power at different working distances**

+ 12D Microscope

+12D Magnifier

Telemicroscope: 2X Telescope + +6D approximation lens



Dibujos 15.15, 15.16, 15.17, 15.18, y 15.19

Telemicroscope: 3X Telescope + +4D approximation lens

Telemicroscope: 4X Telescope + +3D approximation lens

Figure 15.9. Using a manual magnifier.

Whenever we increase the power of the telescope and reduce that of the microscope or approximation lens, we obtain a greater working distance but the field of view is narrower. The most appropriate prescription will depend on what the patient's goals are.

15.5.5. Magnifiers (Handheld and stand) (Figure 9)

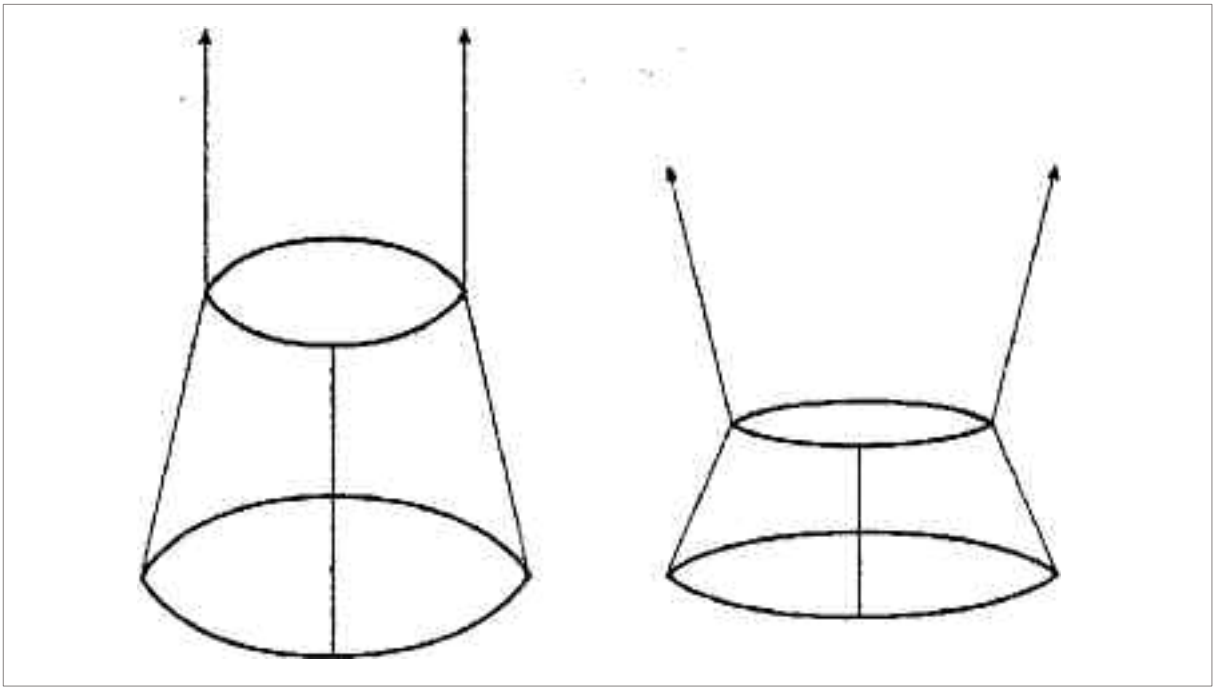
15.5.5.1. **Definition**

A convex lens or group of lenses (to correct aberrations) which allows the size of objects to be increased when viewing through it. It is handheld or mounted in a stand.

15.5.5.2. **Features and characteristics**

In the case of handheld magnifiers, the object must be placed at the focal length of the lens,

$$d_f = \frac{100}{D}$$



Picture **15.20 y 15.21**

for the virtual image that is produced by the handheld magnifier acts as if it came from the infinity.

In this way maximum magnifying power is achieved and no accommodation is required.

When the lens is positioned at exactly its focal length:

a) The user must wear his usual distance glasses while using it.

b) The magnifying power is independent of the separation between the eye and the lens.

The field of view does vary with respect to the distance between the eye and the lens. The closer the eye is to the lens, the wider the field of view for reading.

If the distance between the lens and the text is reduced, magnifying power decreases and the light rays emanating from the lens will diverge; in order to see clearly it will be necessary to use an addition to see close up or to make use of accommodation. (Drawings 20 and 21)

A virtual and erect image located at a finite distance will be formed.

The greater the magnifying power, the smaller the diameter of the magnifier and therefore, the smaller its field of view.

In the majority of cases, the magnifier's real magnifying power does not equal the manufacturer's stated magnification, so in order to define them it is best to use their power stated in diopters.

15.5.5.3. **The magnifying power of magnifiers**

If we take 25 cm (1/4 of a meter) as the distance of reference,

$$A_{25} = \frac{F\,(D)}{4}$$ Formula used to calculate effective magnification

Assuming that any refractive error is compensated and accommodation is not necessary.

Some authors believe that it is more real to compare the image in the magnifier with the accommodation required for the distance of reference. In this case, the formula to be used is:

$$A_{25} = 1 + \frac{F\,(D)}{4}$$ Conventional formula to calculate magnification

15.5.5.4. **Combination of magnifiers and reading additions**

If the patient puts the magnifier in contact with the glasses, the magnifying power of this combination is very close to the sum of the two magnifying powers.

Example:

If a patient with a +3D addition glasses puts a +8D magnifier in contact with his glasses, the effect will be equal to the one that would be achieved by using a +11D magnifier.

If the magnifier is not in contact with the addition lens, the sum of the two powers (i.e. the addition of the glasses and the magnifier) will be lower than their combined real power, according to the following formula:

Feq = F1 + F2 – CF1 F2

In which:
Feq = Equivalent power
F1 = Power of the magnifier
F2 = Power of the addition
C = Distance between the two in meters

15.5.5.5. **Types of magnifiers**

a) Handheld magnifiers

- These are the most common low vision aids.

- They range in power from +3D to +20D if they have only one lens, which must be aspheric at powers from +8D.

- Pocket magnifiers are also handheld; they have several lenses, are smaller in diameter and are available in up to +80D power.

- Some have a built-in light and are ideal as aids in dimly-lit environments (restaurants, theaters, etc.).

- Magnifiers ones with aspheric lenses can be used in two ways: held very close to the eye at a distance of 3 or 4 cm, in which case the flatter side must be the one closest to the eye in order to have a wider field of view, or separate from the eye, in which case the curvier side should be closest to the eye.

b) Stand magnifiers

- They are available with either fixed or adjustable focus.

- With or without light

- Fixed focus magnifiers

These magnifiers are the same as handheld ones but are mounted on a stand that is set at the correct focal distance for reading.

Most of the time the stand holds the lens at a shorter distance forming a virtual image at a finite

distance. In order to see this image clearly, either an addition for close up viewing or accommodation is required.

- Adjustable focus magnifiers

Adjustable focus magnifiers are able to compensate for the user's refractive error. There is no need for accommodation or for any additions in order to look through them.

15.5.5.6. **Advantages of magnifiers with respect to other devices**

Relatively normal reading distance. Greater useful distance, the eye-lens distance is greater than with glasses.

They are easy to manage in case of eccentric viewing training.

They are widely used and well-known optical aids (no hang-ups because of their use).

Magnifiers that are mounted in stands are very useful for children or older people with poor motor control.

They are practical for use for certain diseases in which the visual field is limited.

Some have their own light built in for use in places that are dimly lit.

15.5.5.7. **Drawbacks of magnifiers**

The field of view is smaller than that of microscopes of the same power.

Both hands are needed to use them.

Typically, reading speed is slower with regard to that with microscopes.

Handheld magnifiers need to be held at the correct focal distance in order to provide maximum magnification.

One has to look perpendicularly through the magnifier, otherwise aberrations will occur.

At magnifying powers over 20D the field of view is very small.

When using stand magnifiers with a fixed focus, it is also necessary to wear additional glasses in order to read easily.

Figure 15.10. Training in the use of a video magnifier.

15.5.5.8. **Main uses**

They are an excellent secondary device that are the perfect complement to other aids, e.g., glasses with microscopes can be used for prolonged reading, but is useful to have a pocket magnifier for quick, casual uses such as looking at prices of things when picking them, seeing a name on a business card, etc.

For patients with deficits in motor control, for whom maintaining proper focal distance is difficult, stand magnifiers are very convenient.

They are useful for patients who have a limited visual field, whose field of view is further reduced when they get closer to the image.

Stand magnifiers are first prescribed for children with low vision.

Stand magnifiers are also generally used as training devices for any type of magnifier.

Adjustable focus magnifiers help to compensate for slight refractive errors. If the lens is held closer than its focal distance to the page, the light rays emerge from the lens divergent and compensate for myopia. If the magnifier is held away from its exact focal distance, approachig it to the eye, the light rays that emerge from the lens are convergent and compensate for hyperopia.

15.5.6. Electro-optical devices (Figure 10)

15.5.6.1. **Definition and components**

Aids that allow the size of an image to be increased using electronic media, composed of a monitor, a camera and an optical system. (Drawing 22)

a) At present, most monitors display images in color but black and white can be used for reading, to improve contrast. One of their features is a controller to reverse polarity: white letters on a black ground (reverse polarity) or black letters on a white ground. They also have controllers to adjust light, brightness and contrast.

b) The camera also records in color and defines image quality.

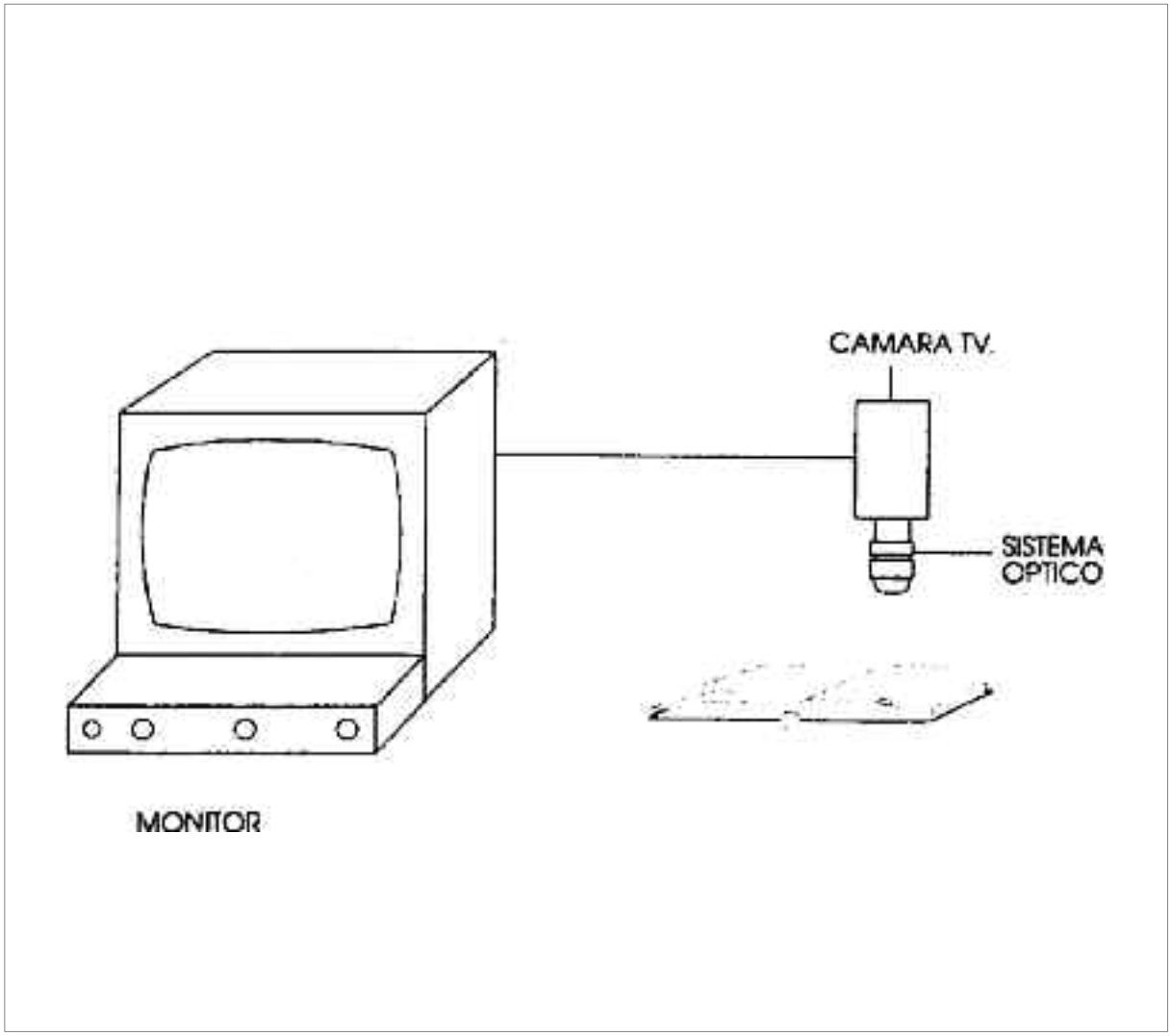
c) The optical system depends on the type of task that needs to be performed. There is a different one for each type of task.

For example:

- At a distance: to view a blackboard in class
- Up close: to read or write

There are two controllers:

- One to adjust the zoom that will provide magnification
- Another to focus on the image



Picture 15.22

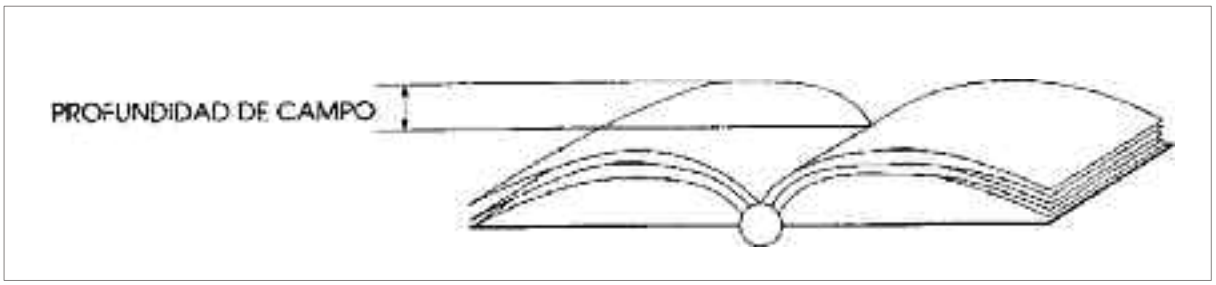
15.5.6.2. **Characteristics of electro-optical devices**

They make it possible to read at a normal distance.

The relative distance can be varied, away from or closer to the patient, depending on what we want to do (to enlarge the image).

When magnification of more than 8x is required, the field for reading is greater than with optical aids.

The polarity can be reversed; with reverse polarity the background is larger than the letters and there is less glare.



Picture 15.23

Contrast and brightness can be controlled.

Depth of field is greater than with optical aids. (Drawing 23)

There is a space between the camera and the text, so it is easy to turn pages, write, do crafts, etc.

Magnifying power is greater than for other optical instruments, ranging from 2x to 60x.

It is possible to read binocularly.

We can move forward or backward and the image remains sharp.

The screen can be split by using two cameras to perform different tasks at the same time.

15.5.6.3. **Negative features**

They are immobile.

Their size is considerable.

Their cost is high.

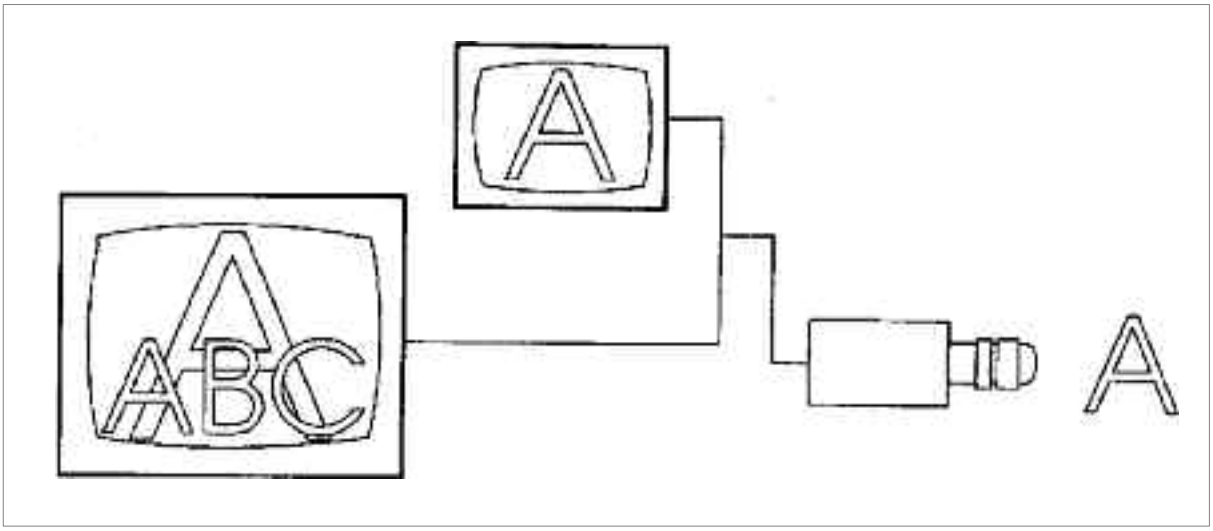
They are quite fragile.

They need maintenance.

Training is required to use them, which is a nuisance. It is hard to get used to reading on a screen while the hands move a text that is not viewed directly.

15.5.6.4. **How to increase the field of view in an electro-optical device**

a) By using a larger monitor, so that the size of the image is increased using the optical system's own magnification. Font size is then reduced using the zoom until it is the same size as before. (Drawing 24)



Picture 15.24

b) By getting closer to the screen. By getting closer, magnification through a reduction in relative distance is used, so the size of the font can be reduced and the same effect is achieved in terms of size, but the field of view is larger.

15.5.6.5. **Magnifying power of an electro-optical device**

a) This depends on:

- The focal distance of the optical system
- The screen size
- The zoom range that is used
- The distance at which the patient is positioned

b) How to measure?

1) Place a millimeter ruler beneath the optical system, in such a way that is visible in perfect focus on the screen.

2) Use another ruler to measure the image that is formed on the monitor.

3) Measure the distance from the patient's eyes to the screen. (Drawing 25)

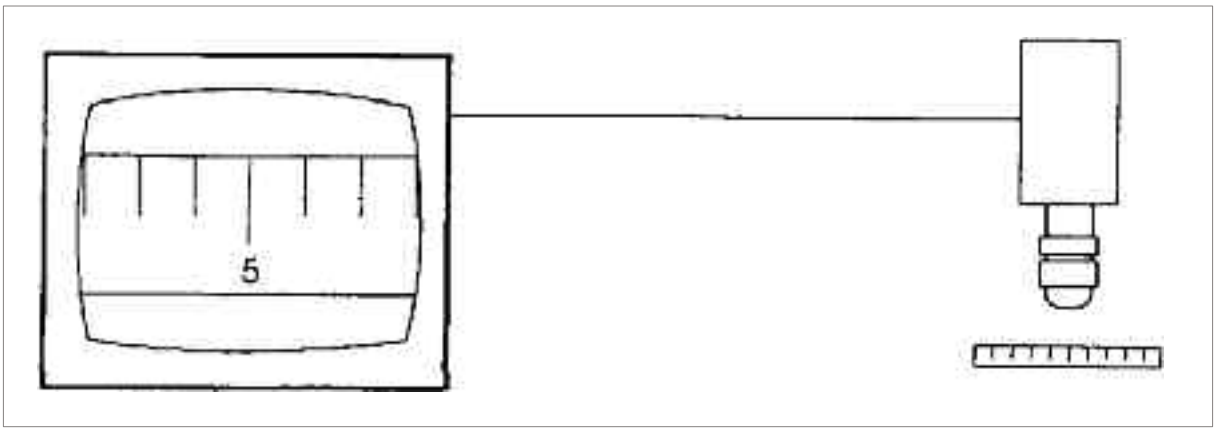
4) Multiply the result of "b" (the magnified image that is formed on the screen), times the 25 cm reference distance and divide by "c" (the distance from the patient's eyes to the screen).

5) The result is the magnifying power the patient is using.

6) To find out the diopters of a lens that will provide the same effect:

With respect to a 25 cm distance:

F= A25 x 4



Picture **15.25**

7) Example:

After placing a ruler in front of the optical system, we observe that 1 cm on the ruler equals 20 cm on the screen. The working distance is 40 cm

With what magnifying power are we working? What would the power be of a microscope that would obtain the same magnification?

a) 1 cm = 20 cm on the screen
With respect to a 25 cm distance

$$20\ X\ \frac{}{40} = 12.5x$$

b) Magnifying power of the equivalent microscope

$$F = 12.5 \times 4 = 50D.$$

This patient would obtain the same power with a +50D magnifying lens at 2 cm from the text.

15.5.6.6. **Cases where electro-optical devices use is essential**

a) Patients who have not been able to adjust to using optical instruments

b) Patients who need very high magnification, more than 12X, and for whom it is very difficult to function with optical aids

c) Patients who have no practice in using their vision. Video magnifiers are very efficient auxiliary devices for training in fixation and positioning techniques, even if other types of optical aids are used following the training.

d) Patients with small fields of view, 5º or under, who have been unable to adjust to using optical aids

e) Patients for whom prolonged reading is very bothersome when using optical aids (Figure 11)

15.5.7. Aids for using visual field (Figure 12)

People with a 30° visual field are considered to be able to function normally in day to day living. Problems appear as the field becomes reduced and are considered to be serious when the visual field is less than 10° or when there is hemianopsia (blindness in half the visual field).

These are patients for whom the use optical aids will be much more difficult; they therefore need more training, as well as training in orientation and mobility techniques, use of a cane, etc. These patients are functionally blind at night.

Sometimes magnification is not comfortable for them because it enlarges the image in a part of the retina that does not function and they only perceive something that has been enlarged in a small area, but they have no idea about the surrounding context.

Example: a patient using a telescope may see someone's mouth very clearly but does not know whom the mouth belongs to, as he/she cannot see the rest. Some patients take this to mean that there has been a reduction in their visual acuity.

They improve considerably after developing techniques for tracking and searching.

15.5.7.1. **Instruments that are used**

a) Reduction systems

- Reversed conventional telescopes

- Galilean telescopes with low magnification, 2X or 3X, are usually used to view through the objective.

Figure 15.11. Threading a needle using a video magnifier.

Figure 15.12. Using a lens to reduce the size of an image and expand the field of view.

- They are practical as long as visual acuity is not much reduced and the visual field is small. Visual acuity is decreased by as much as the field increases.

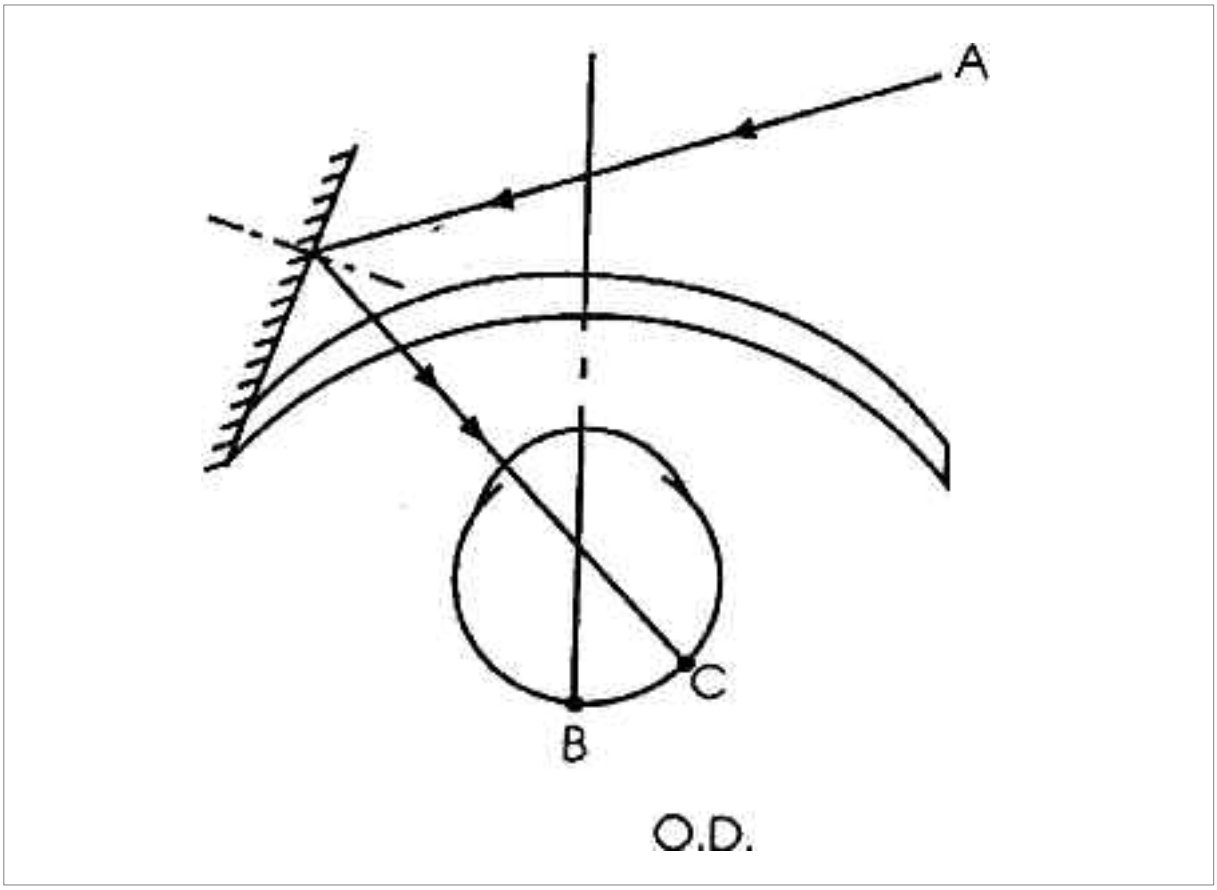
- Anamorphic systems

- These consist of a reversed Galilean afocal telescope with cylindrical lenses so that the size of the image is reduced in only one meridian, horizontally, and thus expanding the field

of view in that meridian.

- The horizontal visual field expands by as much as the image in that plane itself decreases.

- When the patient turns his head horizontally or vertically they function well, but if the head is inclined at an oblique angle, one has the impression that one is in a boat in the middle of the sea.



Picture 15.26

b) Eyeglasses with mirrors for hemianopsia

- They have different designs and have been described by several specialists. The most common is a little mirror fitted onto the bridge of the glasses that tilts slightly toward the temporal side.

- The objects in the patient's blind area are reflected in the mirror and the patient sees them without having to turn his/her head.

- The main disadvantages are:

    - The mirror reverses the image.

    - If the patient has binocular vision there can be a superimposition of the images. (Drawing 26)

- Training is cumbersome and the patient must be predisposed to going through with it. Comparable to the effect of a car's rearview mirror.

c) Fresnel prisms

- They are used to aid mobility skills of people whose visual field is greatly decreased. They are prisms with a 30D external base that are placed on the outer edge of the eyeglass lens, so that they do not interfere with vision in the primary position of gaze.

- When patients look through the prism peripheral objects are shifted toward the center, which allows them to move their necks less and to have an idea of what surrounds them with a small movement of the eyes.

- Vision through the prism is not too clear but it does help to avoid bumping into things, to find objects and, if they are of interest, to turn the heads and look at them straight ahead.

- Change in spatial awareness is the main drawback, because the patient has to look through the prism at objects that are placed 30º away from the point where he is looking. He will try to pick up objects that are not where he thinks they are.

- Training is complicated and the prisms are usually not useful when patients improve their techniques for tracking and searching.

15.5.8. Non-optical aids (Figure 13)

Figure 15.13. Non-optical aids: reading stand and lamp with a flexible arm.

15.5.8.1. **Definition**

Non-optical aids do not provide magnification but are used to enhance the use of vision, with or without optical aids. Generally non-optical aids improve lighting, contrast, posture or the working distance.

15.5.8.2. **Large-type**

These are texts that have been blown up, on the basis of the principle of relative size enlargement. To date it has been the method most commonly used by schoolteachers to work with children with visual deficits, since no other means were available to them.

It is achieved by making enlarged photocopies of the original text, by as many times as necessary. Each photocopy is approximately 1.8 times larger than the original. If several powers of magnification are wanted, it is necessary to make photocopies of photocopies and quality will decrease accordingly.

Advantages and drawbacks:

- If a text is enlarged sufficiently, it allows a better working distance in comparison with other optical aids.

- The size of the final text is too large to be managed with ease.

Primary use:

- When patients have very low visual acuity and a need for high magnifying power, large type can be used in combination with optical aids, thereby improving working distance.

- For people who have to read at a distance that is greater than normal and are unable to

adjust to the use of telemicroscopes.

- As a training for optical aids in general.

15.5.8.3. **Lighting controls**

The control of lighting is one of the determining factors for a successful rehabilitation.

Sometimes, by simply improving light conditions, we can help the patient achieve the same rate of performance with less magnification.

Most patients with low vision need more light on their work surface, but without glare or reflections on the text that is being read, so therefore, they need an appropriate level of light and proper orientation of the light source.

We need to be careful about reflections from reflective surfaces such as glossy paper magazines.

As they age, older people need about twice as much light as younger people. This is a very important factor since most low vision patients are over 60 years of age.

LIGHTING FORMULA

The amount of illumination is inversely proportional to the square of the distance and directly proportional to the angle of incidence.

$$I = \frac{I_o \cos a}{d^2}$$

In which:

I = Total illumination
Io = Light source strength
d = Distance of the surface from the source

The following aids that are related to lighting are recommended:

a) Lamps with flexible arms or goosenecks that can be adjusted to the best position for optimum light. (Figure 14)

If incandescent bulbs are used, 100W usually provides the most adequate power.

Figure 15.14. Adjusting light for reading.

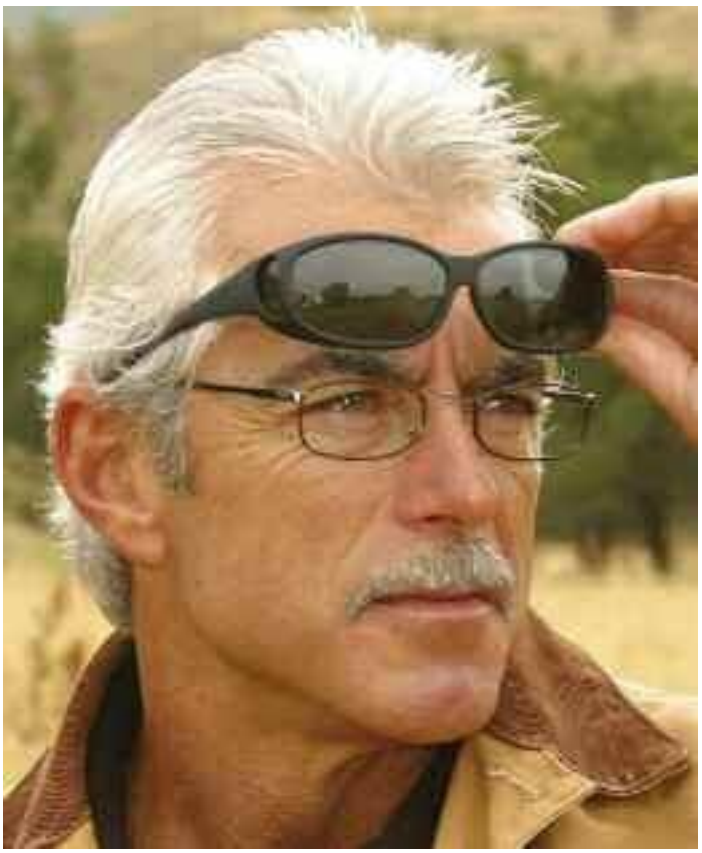


Figure 15.15. Glasses with an ultraviolet filter, with top and side protection, that are placed over the user's prescription eyeglasses.

Halogen bulbs are not recommended because they generate a lot of heat and their light tends to create glare.

Lamps with two 15W fluorescent tubes are the ones that give the best results. They provide more light than 100W incandescent bulbs.

Plus they can be placed closer to the user's face without the heat they give off being a bother. They consume less too.

b) Filters (Figure 15)

Patients with low vision are very sensitive to glare and need a longer than usual adaptation, under photopic or scotopic conditions, or both.

In order to make a definitive description, an assessment is needed both indoors and outdoors, evaluating the patient's response to glare, adaptation to light, to darkness, etc.

For this purpose the following are primarily used:

- Polarizing filters that only allow the passage of light traveling in one direction

- Filters that absorb UV radiation and shades of blue light, which filter short wavelengths (ultraviolet and blue light waves); this radiation wavelenghts are the most annoying as they create the most glare.

These filters are the most commonly used for retinal problems. Conventional filters do not help, as users complain about vision loss and they do not provide sufficient protection no matter how dark they are.

They are used:

- In tinted lenses, using the patient's refractive correction

- In shields, that fit over the patient's eyeglasses

- In glasses that fit over the user's own eyewear, with top and side protection; this is the best solution.

15.5.8.4. **Contrast**

Many patients with low vision have contrast sensitivity deficits. A higher contrast sensitivity is needed in order to better their performance.

This can be achieved by using the following techniques:

a) Using black markers instead of blue ballpoint pens

b) Placing colored filters the size of the page over the text. These are usually yellow, if the letters are black on a white background.

c) Using yellow supplements, over their glasses, so letters appear to be blacker.

d) Using typoscopes, which are pieces of black cardboard or plastic with a slot cut in it that frames a line of text, covering the rest. It helps the patients to stay on the line they are reading and increases its contrast.

(Drawing 27)

e) Using lined paper to help write in a straight line

15.5.9. Non-visual aids

15.5.9.1. **Definition**

These are aids that can help improve performance of any task without need of involving vision, by using other senses.

15.5.9.2. **Features**

They can be used by people who are visually impaired or blind.
They mainly use touch and hearing to gather information.

They are a very useful complement to optical aids under various circumstances.

Examples: Calculators, talking thermometers and clocks, signature guides, scented markers, cards for ordering taxis, etc.

15.6. Training

Figure 15.16. Training in the use of a telescope to watch television.

15.6.1. Things to take into consideration

Training is the key to successful rehabilitation.

Simply prescribing appropriate aids for patients is not enough. We also need to teach them how to use them.

Most of the time patients do not use their vision properly and we must teach them techniques that will help them make better use of their remaining vision.

It is important to explain the problem to them, what their disease entails, where it is located, and why they cannot see well. They are always grateful for that. Most patients have received next to no information about their problem.

Patients' needs are not always solved with the different aids, but rather with specific training for different tasks.

15.6.2. Types of training

a) Training in specific techniques to make the most of remnants of vision

b) Training in the proper use of the devices prescribed

c) Orientation and mobility training

d) Training in daily living skills

15.6.3. Training in specific techniques to make the most of remnants of vision

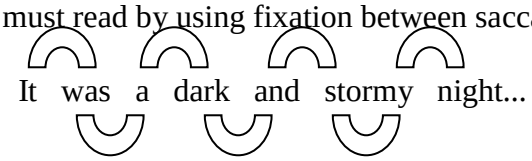
1) VISUAL FIELD RESTRICTIONS

a) When there is notable visual field restriction, as in the case of retinitis pigmentosa and glaucoma, head movements are required instead of eye movements.

b) For reading:

1. We have to measure how many letters fit into the patient's field of view.

2. The patient must read by using fixation between saccades, preserving the previous syllable.



3. Patients have a hard time moving from one line to the next. Begin with short lines as an exercise and as they improve with practice, use longer lines.

4. Use the least possible magnification so as not to reduce the field. Patients do not have an overview of the full page; sometimes they cannot even manage to see a whole letter in a headline.

5. Train their visual memory so that with one quick glance they will know what they have seen.

2) CENTRAL SCOTOMAS

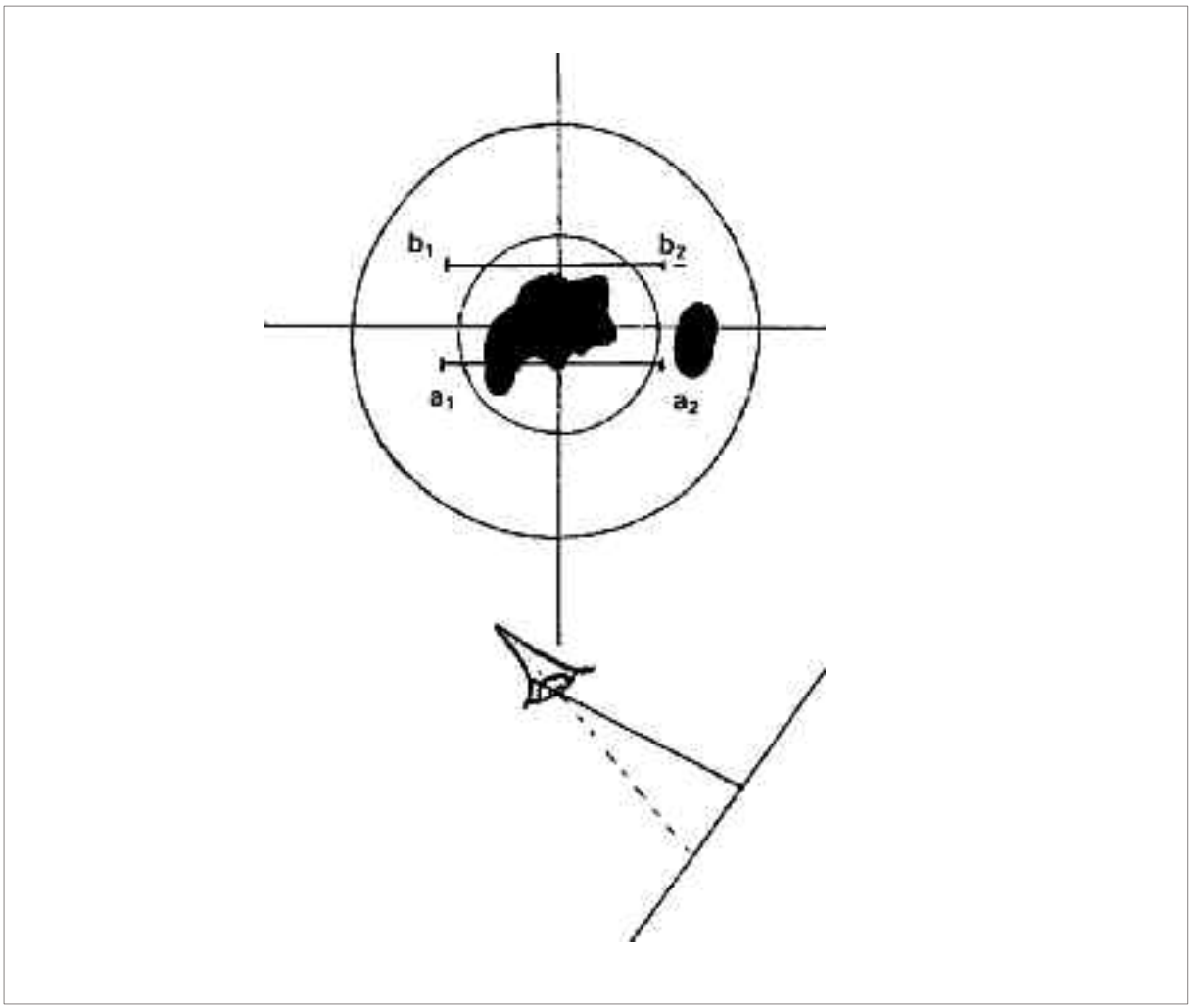
a) The loss of central vision because of macular degeneration or other conditions requires teaching the patient how to use eccentric fixation techniques.

b) Method for determining where to develop eccentric fixation

Identify the area that retains the most visual function closest to the macula, which is where visual acuity is greater. (Drawing 28)

a1-a2 is closer to the center, visual acuity is higher, but the field of view is smaller → less

useful for reading. b1-b2 is further from the center (macula) → worse visual acuity, greater need for magnification, but reading is more comfortable.



Picture 15.28

By turning the gaze downward, the scotoma moves in the same direction and the upper part of the retinal projection is used.

c) In practice

1. Draw lines above and below a typed line and ask the patient to fixate on these lines.

2. Observe by looking at what line the patient reads best; that is the line he/she will have to fixate when reading.

Mary had a little lamb

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3. When the patient has become used to it, delete the line and ask him to read by imagining it, now that he/she knows where to fixate.

4. It is sometimes useful, especially at the beginning, to move the text in front of the eyes, not the head. It is difficult to find a point of fixation and when we find one, we need to keep it.

15.6.4. Training in the proper use of the devices prescribed

Explain:

What the aid is called and how it is used. What problems may arise from its use and what are the solutions. How to properly hold the aid, how to keep proper working distance. How to find things and focus using a telescope. What kind of lighting is best for each type of task. What types of non-optical aids can be used. How to maintain, clean and care for the aid.

15.6.5. Orientation and mobility training (Figure 17)

Not all of a patient's needs are solved by prescribing an aid, it is sometimes necessary to teach different techniques for activities such as: managing with public transportation, crossing a street safely, learn to guide and to be guided, moving around using a cane, solving situations where light is poor, etc. These techniques are taught through modules that are designed specifically for each person on the basis of an evaluation of their skills.

15.6.6. Training in daily living skills

These are regular activities that are needed to perform in day-to-day life; they cannot be solved with technical aids, but instead require learning some skills and some tricks that will help people overcome situations that were simple before the loss of vision and now can be difficult to perform, such as: identifying money, table manners (eating without knocking food from the plate, pouring liquids, etc.), identifying clothing, personal care (shaving, applying makeup), cooking safely, safety in handling an iron, etc. These activities usually require working with a person in their own home.

15.6.7. Final recommendations

Always allow the patients to be the ones to decide what to wear.

Figura 15.17. Use of a cane in cases where the visual field is restricted.

It is best to not prescribe a device than to force patients to buy one and then not use it because they hate it; it can always be recommended again on a subsequent visit.

Advise patients about the type of care they will need in the future and where to get it.

Recommended period of time for using the devices.

Tell patients when they should return for their next visit.

Provide patients with a report detailing all that has been achieved. Specify clearly what the starting point was and how far they have come along, the aids they use, what they are able to perform with each one and the new activities they are able to do.

The success achieved and our report will be self-evident to others who are visually impaired.

15.7. Follow up

The program is not over until careful follow-up has been conducted.

Low vision rehabilitation is a young discipline so its evaluation and development over the course of many years is crucial.

Continued support for these patients is essential for a number of reasons:

1. Many of them suffer from progressive diseases, visual acuity varies and, in addition to close ophthalmologic monitoring, they need new solutions.

2. The majority of patients are elderly and sometimes their motivation falters; paying attention to them helps them to continue using their vision.

3. In some patients, use of their vision increases their motivation, so they need assistance to help them fulfill the new needs that arise, which they had not even thought about before.

Follow-up can be performed by telephone, with an appointment for a visit if needed.

Follow-up should be performed: at 1 week, at 1 month, at 3 months, at 6 months and at 1 year, unless the patient requires otherwise.

15.8. Conclusion

Low vision care uses a standardized approach that is complex and multidisciplinary. It is a complement to ophthalmologic care.

If done properly, it can be effective.

We should not confuse it with selling a technical aid.

We help patients have an independent and active visual life, so their quality of life is improved.

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SPANISH ANIRIDIA ASSOCIATION

The Spanish Aniridia Association would like to express its deep gratitude to the generous dedication of the medical expert group who authored this publication, for contributing to the promotion of knowledge about aniridia and to research into this rare disease as well as for developing a best practice protocol targeted to health professionals, in order to unify standards of medical care for these patients.

Given the low rate of prevalence of this disease and the geographical dispersion of those affected, the Spanish Aniridia Association fostered the publication of these guidelines because of a need to bring together ophthalmologists who have the most experience in the diagnosis and treatment of aniridia, to get them to share their knowledge with others who have not had any experience in treating patients with this disease while at the same time ensuring that these patients receive quality care from their doctors. If it is already difficult for a patient with aniridia to find a doctor who is familiar with this disease, it is even more so for children who, in addition to aniridia, suffer from kidney cancer, genitourinary anomalies (Wilms' tumor) and/or mental retardation - WAGR syndrome.

The Spanish Aniridia Association (known by its acronym in Spanish, A.E.A. - Tax Registration Number: G-81529729) is a nationwide non-profit organization that was founded on June 15, 1996. From the beginning itaimed at serving as a point of reference both for professionals and for those afflicted with the disease, acting as a bridge between the two communities and facilitating the exchange of information in both directions. The A.E.A. is legally registered in the National Registry of Associations at the Ministry of Health (No.161283) and the Autonomous Community of Madrid (E-1440.7).

The A.E.A.'s headquarters is located in Hospital Clínico San Carlos
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Asociación Española de Aniridia (A.E.A.)
Spanish Aniridia Association

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