CONGENITAL ANIRIDIA KERATOPATHY TREATMENT

MANEJO TERAPÉUTICO DE LA QUERATOPATÍA ASOCIADA A ANIRIDIA CONGÉNITA

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ABSTRACT

Objective: To attempt to review the aniridia keratopathy pathogenesis and establish a treatment protocol according to the severity of the symptoms.

Methods: Personal experience in aniridic keratopathy management and a bibliography review.

Results: The ocular manifestations of this anomaly include defects of the cornea, glaucoma, lens subluxation, cataracts, hypoplasia of the iris, fovea and optic nerve, amblyopia and nystagmus. The keratopathy occurs in a 20% of patients with aniridia. The correct PAX6 expression is necessary for normal corneal development, limbal stem cell activity and correct corneal epithelial cell migration and adhesion.

Conclusions: The management of ocular surface diseases due to limbal stem cell deficiency in aniridia is complex but has changed in recent years, as an understanding of the limbal stem cells and their microenvironment has modified the therapeutic approach. The use of autologous serum eye drops, amniotic membrane transplantation, limbal transplantation or cultivated limbal cell transplantation have all been reported as a treatment for several

RESUMEN

Objetivo: Revisar la patogenia de la queratopatía por aniridia y establecer un protocolo de tratamiento según su gravedad.

Métodos: Revisión bibliográfica y experiencia personal en el manejo de pacientes con queratopatía por aniridia.

Resultados: Las manifestaciones oculares de esta anomalía incluyen defectos a nivel corneal, glaucoma, subluxación del cristalino y cataratas, hipoplasia de iris, fovea y nervio óptico, ambliopía y nistagmus. La queratopatía por aniridia ocurre en un 20% de los pacientes. La correcta expresión del gen PAX6 es necesaria para el normal desarrollo corneal y actividad de las células limbares así como la correcta migración y adhesión de las células epiteliales.

Conclusiones: El manejo de los trastornos oculares debidos a la deficiencia limbal asociada a la aniridia es complejo pero ha cambiado en los últimos años. El concepto de célula madre limbal y de su microambiente ha modificado la aproximación terapéutica de estos pacientes. El empleo del suero autólogo, trasplante de membrana amniótica, trasplante de

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limbo o trasplante de células limbares cultivadas han sido utilizados en el tratamiento de distintos trastornos de la superficie ocular.

Palabras clave: Aniridia, insuficiencia limbal, suero autólogo, superficie ocular, ojo seco, epitelio corneal.

INTRODUCTION

Aniridia is an infrequent disease (1:65.000-95.000) caused by a bilateral alteration in the eye's development. It has no gender or racial preference. It is a well-documented genetic anomaly which may appear sporadically or within families, exhibiting a dominant autosomic inheritance pattern with variable expression amongst the members of a family. The gene which accounts for the disease (PAX6) is located in the short arm of chromosome 11 (1) and finds broad expression in the development of various eye structures including the cornea, lens, cameral angle, ciliar body and all retina layers. Therefore, aniridia is a global eye disorder in which iridian hypoplasia (which gives its name to the disease) is only the most evident clinical sign. The alterations in the anterior segment include keratopathy due to limbus dysfunction, dry eye, glaucoma, cataract, lens subluxation and a number of abnormalities in the cameral angle. The posterior segment alterations include macular and optic nerve hypoplasia with a frequent association of strabismus and nystagmus, which worsen the visual prognosis since an early age (2) (fig. 1).

The first description of the disease was made by Barratta in 1818, who gave it the name of irideremia in relation to the most apparent clinical sign. In the early twentieth century the current denomination began to be used.

In humans, PAX6 gene mutations were found not only in patients with aniridia, but also in some case of Peter's anomaly. Said mutations can be intragenic or deletions of the 11p chromosome. No correlation was found between the mutation site of the PAX6 gene and variations in the expression of the aniridia phenotype expression. In fact, a broad range of phenotypes can occur as a consequence of the same mutation.

Among the most important extraocular alterations we find the development in sporadic cases of Wilms tumor, frequently as part of the WAGR

syndrome (Wilms tumor, aniridia, genital-urinary abnormalities and mental retard). The appearance of these extraocular alterations in aniridia is more common in sporadic cases. When aniridia is diagnosed in a child whose family does not have antecedents of the disease, it is important to establish via chromosome analysis whether or not the deletion extends to the domain of the gene which predisposes to Wilms tumor in order to establish a monitoring regime for the period of risk of expression of this tumor, which is usually up to age 8.

Although the phenotype may vary considerably, even among members of the same family, the visual defect appears in the first decade of live with visual acuity values ranging between 10 and 20% due to foveal hypoplasia combined with the possible existence of congenital cataract, nystagmus and amblyopia (3). In contrast with the stability of retinal alteration, the progressive deterioration of the eyesight is mainly due to the development of

glaucoma (50-75%), cataract and keratopathy.

Keratopathy associated to aniridia (KAA) affects 20% of patients, although alterations in the eye sur-

Fig. 1: Keratopathy associated to aniridia. The illustration shows the superficial neovascularization and subepithelial fibrosis.

face are found in up to 90% of cases (3). Expressions can begin in the first decade of life with irregular thickening of the peripheral epithelium followed by superficial neovascularization which advances centripetally for years to ultimately involve the entire corneal surface. Patients suffer recurring erosions, ulcers and chronic pain and, if not treated, ultimately exhibit serious corneal opacity due to subepithelial fibrosis and stromal scarring (fig. 2).

GENETIC BASES AND PATHOGENIC MECHANISMS IN KERATOPATHY ASSOCIATED TO ANIRIDIA

PAX6 is the gene responsible for aniridia. It is found in the 11p13 chromosome segment (4-6). The majority of recent studies on the genetic bases of hereditary aniridia are based on animals, particularly mice. The gene PAX6 homozygotic alteration models give rise to mice with complete absence of eyes and nasal cavities which die soon after birth. The heterozygotic defect of PAX6 produces the Sey (Small eye) mouse model with microphthalmus and ocular alterations similar to those seen in humans with congenital aniridia, therefore regarded as a good experimental model for the disease.

PAX6 is contained in a region of DNA considered to be of an extraordinary phylogenetic importance because it has been conserved in numerous species of vertebrates and invertebrates throughout the evolutionary process (7). This gene is con-

sidered to be an important regulator in ocular morphogenesis, controlling cellular proliferation, differentiation and apoptosis processes in normal eye development. The normal expression of PAX6 is deemed essential for the development of ocular tissue such as the corneal epithelium, iris, ciliary body, lens and retina (8-10).

The regulatory role of PAX6 continues in adult life and occurs at several levels.

Regulation of the proliferation and differentiation of the corneal epithelium

The absence of a selective marker for the limbus stem cells (LSC) hinders the study of the epithelial proliferative compartment in animal models. The results obtained with the p63 nuclear transcription factor, suggested as a possible marker for epithelial stem cells and for other, more differentiated cells such as the amplified transient cells, doesn't seem to exhibit alterations in the PAX6 mutation models. In addition, conclusive results were not obtained with cell proliferation markers such as BrdU (11-13). The expression of CK-12 and CK-3 through the epithelial cells is considered to be a differentiation marker more specific for the cornea and besides it is regulated by PAX6. These are water insoluble cell cytoskeleton proteins which make up the intermediate filaments of the epithelial cells. Their main function is to stabilize the corneal surface. In aniridia experimental models we find a reduced expression of these cytokeratins, which translates in vacuolization and fragility of the corneal epithelium (14,15).

Regulation of cell adhesion

The corneal epithelial cells have numerous adhesion mechanisms, both intra- and inter-cellular as well as with the extra-cellular matrix. Said mechanisms include «tight-junctions», «gap-junctions», desmosomes and adhering unions. In addition, there is a large variety of adhesion molecules such as catenines, integrines, demogleine and desmocholine which make the corneal epithelium highly resistant to external attacks.

In experimental aniridia models we find a reduction in desmogleine as well as beta- and alpha-catenine, the synthesis of which seems to be regulated

Fig. 2: Corneal opacity and cataract in patient with hereditary aniridia.

by gene PAX6, which gives rise to spaces between epithelial cells (16). These findings, together with the cytokeratine CK-3 and CK-12 deficit, make the corneal surface very fragile. The clinical expression of this is the presence of recurring erosions or persistent epithelial defects (17).

Regulation of extra-cellular matrix activity

The extra cellular matrix gives the cornea its structural organization. In normal conditions, the extra cellular matrix is subject to a slow but continuous remodeling which gathers pace in the process of repairing corneal damage (18,19). There is a balance between the production of collagen by keratocytes and their degradation by a group of enzymes called metalloproteinases (MMP). These enzymes are produced by epithelial cells and fibroblasts, and their expression is influenced by genes, growth factor, tissue inhibitors of metalloproteinases and elements of injuries. In turn, said enzymes are involved in the activation of cytokines, in the rupture of adhesion molecules and the creation of biologically active fragments (20,21). Three types of metalloproteinases are known: MMP-1

or collagenase type 1, MMP-3 or stromelysine and gellatinase. All these are secreted as proenzymes and need zinc as a cofactor (22). MMP1 is produced mainly by keratocytes and degrades collagen type 1, 2 and 3. MMP-3 exhibits an intense proteolytic activity vis-à-vis caseine, fibronectine and proteoglycanes. In turn, gellatinase is more selective for gellatin and collagen type 4, 5 and 7. Two types of gellatinases are differentiated: MMP-2 is more active in the presence of collagen type 4, whereas MMP-9 is more active in the presence of collagen type 5. The regulation of Gel-B or MMP-9 depends on PAX6. In PAX mutation animal models with Gel-B deficiency we find an accumulation of fibrine and infiltration by inflammatory cells in relation to an increase in the levels of IL-1 (23). The accumulation of fibrine alters the orderly arrangement of collagen fibres, producing loss of corneal transparency. On the other hand, cellular infiltration translates into an important neovascular proliferation stimuli.

Figure 3 shows the different actions of gene PAX6 on the eye surface and its clinical consequences.

ALTERATIONS OF THE OCULAR SURFACE IN ANIRIDIA PATIENTS

It is increasingly frequent to find in our daily practice alterations of the eye surface caused by a limbal insufficiency syndrome which in most cases is caused by external agents, either traumatic or inflammatory. In the case of KAA, it involves an intrinsic limbal involvement which qualitatively alters the LSC functions. The alterations of the eye surface which appear in aniridia are due, at least initially, to a LSC dysfunction (24). As with other cases of partial limbal insufficiency, patients with aniridia can remain without symptoms until an external factor acts upon the limbus, overthrowing the fragile balance which maintained the integrity of the corneal epithelium.

Relevant worsening of KAA symptoms are described after surgery with excessive manipulation of the limbus or application of topical antimetabolites for controlling the frequently associated glaucoma (25). Other authors underline the asymmetry they observed in corneal alterations in these patients and, in many cases, relate it to traumatic or surgical events in the eye with the worst evolution.

Therefore, the clinical treatment will depend on the degree of involvement. Thickness of the central cornea is usually very increased (26), frequently involving superficial neovascularization, subepithelial fibrosis, progressive corneal opacity, high degree of instability and irregularity in the ocular surface which, at the clinical level, is translated in persistent or recurring epithelial defects, corneal conjunctivalization, hyperplasia of the limbal epit-

Fig. 3: Pathogeny of keratopathy associated to aniridia.

helium, chronic inflammation, conjunctival hyperemia, changes in Bowman's membrane and keratinization. Symptoms include crying, pain, eyesight reduction, dry eye, photophobia and blepharospasm, with the frequent addition of bacterial infections and the risk of eye perforation (27,28).

The instability of the ocular surface which is caused by the deficient regeneration of the corneal epithelium will determine, among other things, the appearance of a secondary dry eye and the loss of the corneal epithelium's barrier function (fig. 4). This chronic aggression on the eye surface epithelium will produce a reaction of the latter in the form of metaplastic transformation. Accordingly, squamous metaplasia is a process utilized by humid epithelia to provisionally overcome attacks. This process involves both corneal and conjunctival epithelial cells as well as the conjunctiva's secreting cells (calliciform cells) (29). This is a pathological and reversible transition of the normal, non-keratinized stratified epithelium to keratinized epithelium.

Clinically, the keratinization process is easily recognizable via microscopy but squamous metaplasia which arises before keratinization can go unnoticed, above all in the initial stages. In these cases, impression cytology provides an objective estimate of the degree of involvement of the ocular surface, allowing an improved therapeutic approach to the disease (30). In addition to assessing the degree of squamous metaplasia, cytology will allow the identification of the presence of calliciform cells in the corneal epithelium, a characteristic conjunctivalization sign and therefore signalling limbal

insufficiency (fig. 5). Impression cytology also facilitates the epithelial phenotype study via marking with monoclonal antibodies of selective cytokeratins of each cellular lineage (31,32). Accordingly, the conjunctival epithelium cells present cytokeratine CK19, whereas the corneal and limbal epithelial cells present cytokeratins CK3 and CK12 (33). The cornea of patients with LSC deficiency present a reduction of cytokeratins CK3 and CK12 and an increase of cytokeratine CK19 (34). Furthermore, impression cytology allows a study of the ocular surface after interventions such as limbus transplant and amniotic membrane transplant (35,36).

It is important to classify all the above signs and symptoms in order to plan the therapeutic handling of these patients. We have differentiated four levels or stages of development. A patient is considered to exhibit slight limbal insufficiency (phase 1) when he/she refers a maximum of two recurring ulcer or erosion events in the past 6 months, slight photophobia and epiphora and also exhibit slight vascular pannus not exceeding 1 mm from the limbic arch and small disorders in the absorption of fluorescein. A patient is considered to exhibit moderate limbal insufficiency (phase 2) when the number of recurring ulcer or erosion events equals or exceeds 3 in the past 6 months, exhibits permanent instability of the lacrimal film and vascular pannus (with or without sub-epithelial fibrous tissue which involves at least the peripheral half of the cornea, with photophobia, epiphora and red eye being the norm. The third phase of the disease is considered to be reached when a patient exhibits corneal vascularization involving the centre of the cornea as well as perma-

Fig. 4: Tincture with sodium fluorescein, showing conjunctivalization of the corneal epithelium.

Fig. 5: Impression cytology. The presence of calliciform cells in corneal impression cytology defines the instability of the lacrimal film and an epithelial defect.

nent clinical signs of corneal erosions and instability of the lacrimal layer. Photophobia, epiphora and red eye are the norm, together with the loss of vision due to involvement of the visual axis. Finally, in phase «0» or sub-clinical limbal insufficiency we include patients with etiological processes liable to limbal insufficiency who do not express associated clinical signs (fig. 6).

TREATMENT

One of the causes of progressive loss of vision and morbidity in aniridia patients is keratopathy derived from the dysfunction of LSCs. The progress in recent years in the understanding of the mechanisms involved in cellular renewal of the cornea have allowed an adequate therapeutic approach of these patients in any phase of the disease (37,38). However, to date it hasn't been possible to demonstrate in KAA patients the existence of a LSC deficiency due to the lack of specific markers allowing for their identification in histological studies (39,40), as the clinical findings in these patients coincide with those described in the limbal insufficiency syndrome.

Until about 3 years ago, the approach to KAA was based on supporting treatment with topical lubricants, therapeutic contact lenses or tharso-rraphy and, when patients developed severe corneal opacity, lamellar or penetrating keratoplasty was adopted with very negative results due to recurrence of pre-surgical corneal alterations on the graft (27,41).

As with the rest of limbal insufficiency patients, treatment should aim at repopulating the sclerocorneal limbus of the LSC and/or to restore the micro-

environment surrounding them in order to ensure their expansion and survival.

The therapeutic management of these patients will depend on the degree of involvement of the ocular surface. Thus, in patients exhibiting sub-clinical or slight limbal insufficiency, treating with artificial tears without preservatives could suffice. Although there are many tear drops in the market, we prefer to use sodium hyaluronate preparations free of preservatives. It is also important to apply with these patients a number of measures aimed at improving their symptoms, such as protection against sunlight by means of dark glasses, or a preference for humid environments. The corneal erosion events shall be treated just like in any other patients, by means of occlusion and topical antibiotics. In patients with slight keratopathy, we have tried treatment cycles with autologous serum and found a subjective improvement and a reduction in the number of corneal erosions (unpublished results).

In patients with moderate keratopathy, treatment with artificial tears is not enough. In these patients autologous serum or amniotic membrane transplant can be utilized. The former has proved to be a highly interesting therapeutic weapon for handling various pathologies involving the ocular surface (42,43). As for autologous serum, it involves many factors such as the epithelial growth factor, fibronectine, vitamina A, the fibroblast growth beta transforming factor, α 2macroglobuline and neural factors such as the P substance, which act upon the proliferation, migration and differentiation of corneal epithelial cells (44,45). In this regard, autologous serum would have an effect similar to that produced by the amniotic membrane, contributing to improve the environment and facilitating the mechanisms involved in the cellular renewal and maintenance of the ocular surface epithelium (46). The amniotic membrane transplant (AMT) exhibits important clinical advantages as it improves considerably the environment of the extracellular matrix of the limbal epithelial cells (47). The therapeutic usefulness of the AM is derived from its mechanical and above all biological properties (48,49). This makes it very useful for patients with partial limbal insufficiency where the aggregation of multiple factors enhances the development and expansion of the surviving LSCs. AMT has been utilized for limbal insufficiencies associated to various etiologies (50,51) as well as in the treatment of aniridia-asso-

Fig. 6: Subclinical keratopathy in aniridia patient.

ciated keratopathy (52). However, medical treatment by means of AMT in these patients provides only temporary results, as we proved in aniridia patients (52) (fig. 7).

In patients with severe stage 3 keratopathy, the stabilization of the corneal surface involves supplying LSC by means of a limbus transplant (53,54). Aniridia being a bilateral disease excludes a self-transplant, which are substituted by allografts with tissue from healthy relatives having high HLA compatibility, or from cadavers (55). In these cases, the risk of rejection requires the application of a postop oral immunosuppressor treatment with potential side effects. The limbus transplant from relatives allows for greater HLA compatibility, thus partially reducing the rejection risk although it does not allow for obtaining grafts as large as those obtained when the limbal tissue comes from cadaver donors.

Homologous lamellar limbokeratoplasty has been performed in patients with bilateral aniridia with quite acceptable results (56). The combination of limbus transplant and AMT increases the benefit of each separately (35,57). In addition to the typical limbus transplant, at present we can supply LSCs grown artificially on amniotic membrane (58-60). The main advantage of this technique is that an important number of cells can be transplanted from a small limbus sample obtained from the same patient. This considerably reduces surgical trauma in the eye and the risk of rejection of the limbus allotransplant. Although good results have been described with these techniques for handling various ocular surface pathologies (61,62), in practice there are important issues to be resolved concerning the principles of this technique in the specific case of aniridia patients. On the one hand, there is no selective and specific method for identifying the LSCs and on the other, as mentioned above, aniridia seems to involve primarily an alteration of the environment surrounding the LSCs, with the quantitative alteration being secondary. Therefore, it is necessary to study the survival, mobility and adherence of epithelial cells after transplanting them in the eye of aniridia patients.

Figure 8 schematically illustrates the handling of the eye surface of KAA patients.

In addition to keratopathy, these patients frequently exhibit other anterior segment alterations such as cataract, glaucoma or corneal opacity requiring on some occasions surgical procedures such as trabeculectomy (63,64), corneal transplant (41) or

Fig. 7: Amniotic membrane transplant. The membrane covers the limbicorneal surface and is sutured with 10/0 nylon.

phacoemulsification, with or without intraocular diaphragm implants (65,66). Particular attention should be given to treatment of glaucoma in these patients; whenever possible, preservative-free anti-glaucomatous agents should be utilized to avoid the damages caused to the ocular surface by some preservatives included in pharmacological preparations routinely used for treating glaucoma.

Although it does not appear in all patients, KAA is a frequent cause of morbidity. Therefore, an adequate therapeutic approach could be extremely useful to improve the quality of life of these patients. Finally, it can be said that aniridia patients are an important challenge for ophthalmologists due to the

Fig. 8: therapeutic scheme of the ocular surface handling of aniridia patients.

frequent association of the macula, optic nerve, nystagmus and amblyopia disorders to the above mentioned anterior segment disorders, which considerably reduce their visual capacity (67). On the other hand, photophobia can be highly invalidating, to the extreme that many patients require the iris prostheses (68,69). Lastly, we must not forget that in many cases aniridia is associated to other systemic disorders which also require our full attention (70,71).

REFERENCES

1. Zolog I, Belengeanu V, Marinca S, Soim A. Familial congenital aniridia. *Oftalmologia* 1997; 41: 326-328.
2. Ndoeye Roth PA, de Medeiros Quenum ME, Wane Khouma AM, Dieng M, Ndiaye PA, Diane MH, et al. Aniridia: five case studies. *J Fr Ophthalmol* 2005; 28: 845-849.
3. Nishida K, Kinoshita S, Ohashi Y, Kuwayama Y, Yamamoto S. Ocular surface abnormalities in aniridia. *Am J Ophthalmol* 1995; 120: 368-375.
4. Hanson IM, Fletcher JM, Jordan T, Brown A, Taylor D, Adams RJ, et al. Mutations at the PAX6 locus are found in heterogeneous anterior segment malformations including Peters' anomaly. *Nat Genet* 1994; 6: 168-173.
5. Jordan T, Hanson I, Zaletayev D, Hodgson S, Prosser J, Seawright A, et al. The human PAX6 gene is mutated in two patients with aniridia. *Nat Genet* 1992; 1: 328-332.
6. Chauhan BK, Yang Y, Cveklova K, Cvekl A. Functional properties of natural human PAX6 and PAX6(5a) mutants. *Invest Ophthalmol Vis Sci* 2004; 45: 385-392.
7. Callaerts P, Halder G, Gerhring WJ. PAX-6 in development and evolution. *Annu Rev Neurosci* 1997; 20: 483-532.
8. Collinson JM, Hill RE, West JD. Different roles for Pax6 in the optic vesicle and facial epithelium mediate early morphogenesis of the murine eye. *Development* 2000; 127: 945-956.
9. Collinson JM, Quinn JC, Buchanan MA, Kaufman MH, Wedden SE, West JD, et al. Primary defects in the lens underlie complex anterior segment abnormalities of the Pax6 heterozygous eye. *Proc Natl Acad Sci USA* 2001; 98: 9688-9693.
10. Koroma BM, Yang JM, Sundin OH. The Pax-6 homeobox gene is expressed throughout the corneal and conjunctival epithelia. *Invest Ophthalmol Vis Sci* 1997; 38: 108-120.
11. Collinson JM, Chanas SA, Hill RE, West JD. Corneal development, limbal stem cell function, and corneal epithelial cell migration in the Pax6 ($\pm$) mouse. *Invest Ophthalmol Vis Sci* 2004; 45: 1101-1108.
12. Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, et al. p63 identifies keratinocyte stem cells. *Proc Natl Acad Sci U S A* 2001; 98: 3156-3161.
13. Ramaesh K, Dhillon B. Ex vivo expansion of corneal limbal epithelial/stem cells for corneal surface reconstruction. *Eur J Ophthalmol* 2003; 13: 515-524.
14. Liu CY, Zhu G, Westerhausen-Larson A, Converse R, Kao CW, Sun TT, et al. Cornea-specific expression of K12 keratin during mouse development. *Curr Eye Res* 1993; 12: 963-974.
15. Chiambaretta F, Blanchon L, Rabier B, Kao WW, Liu JJ, Dastugue B, et al. Regulation of corneal keratin-12 gene expression by the human Kruppel-like transcription factor 6. *Invest Ophthalmol Vis Sci* 2002; 43: 3422-3429.
16. Davis J, Duncan MK, Robison WG Jr, Piatigorsky J. Requirement for Pax6 in corneal morphogenesis: a role in adhesion. *J Cell Sci* 2003; 116: 2157-2167.
17. Ramaesh T, Collinson JM, Ramaesh K, Kaufman MH, West JD, Dhillon B. Corneal abnormalities in Pax6+/- small eye mice mimic human aniridia-related keratopathy. *Invest Ophthalmol Vis Sci* 2003; 44: 1871-1878.
18. Fini ME. Keratocyte and fibroblast phenotypes in the repairing cornea. *Prog Retin Eye Res* 1999; 18: 529-551.
19. Jester JV, Petroll WM, Barry PA, Cavanagh HD. Expression of alpha-smooth muscle (alpha-SM) actin during corneal stromal wound healing. *Invest Ophthalmol Vis Sci* 1995; 36: 809-819.
20. Sivak JM, Mohan R, Rinehart WB, Xu PX, Maas RL, Fini ME. Pax-6 expression and activity are induced in the re-epithelializing cornea and control activity of the transcriptional promoter for matrix metalloproteinase gelatinase B. *Dev Biol* 2000; 222: 41-54.
21. Sivak JM, Fini ME. MMPs in the eye: emerging roles for matrix metalloproteinases in ocular physiology. *Prog Retin Eye Res* 2002; 21: 1-14.
22. Brown D, Chwa M, Escobar M, Kenney MC. Characterization of the major matrix degrading metalloproteinase of human corneal stroma. Evidence for an enzyme/inhibitor complex. *Exp Eye Res* 1991; 52: 5-16.
23. Mohan R, Chintala SK, Jung JC, Villar WV, McCabe F, Russo LA, et al. Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. *J Biol Chem* 2002; 277: 2065-2072.
24. Dua HS, Saini JS, Azuara-Blanco A, Gupta P. Limbal stem cell deficiency: concept, aetiology, clinical presentation, diagnosis and management. *Indian J Ophthalmol* 2000; 48: 83-92.
25. Nelson LB, Spaeth GL, Nowinski TS, Margo CE, Jackson L. Aniridia. A review. *Surv Ophthalmol* 1984; 28: 621-642.
26. Brandt JD, Casuso LA, Budenz DL. Markedly increased central corneal thickness: an unrecognized finding in congenital aniridia. *Am J Ophthalmol* 2004; 137: 348-350.
27. Gomes JA, Eagle RC Jr, Gomes AK, Rapuanao CJ, Cohen EJ, Laibson PR. Recurrent keratopathy after penetrating keratoplasty for aniridia. *Cornea* 1996; 15: 457-462.
28. Jastanciah S, Al-Rajhi AA. Association of aniridia and dry eyes. *Ophthalmology* 2005; 112: 1535-1540.
29. Rivas L, Murube J, Rivas A, Murube E. Estudio del ojo seco en pacientes con aniridia congénita, mediante citología de impresión. *Arch Soc Esp Oftalmol* 2003; 78: 615-622.
30. Sridhar MS, Vemuganti GK, Bansal AK, Rao GN. Impression cytology-proven corneal stem cell deficiency in patients after surgeries involving the limbus. *Cornea* 2001; 20: 145-148.
31. Lavker RM, Tseng SC, Sun TT. Corneal epithelial stem cells at the limbus: looking at some old problems from a new angle. *Exp Eye Res* 2004; 78: 433-446.
32. Buck RC. Ultrastructure of conjunctival epithelium replacing corneal epithelium. *Curr Eye Res* 1986; 5: 149-159.

33. Lauweryns B, van den Oord JJ, Missotten L. The transitional zone between limbus and peripheral cornea. An immunohistochemical study. *Invest Ophthalmol Vis Sci* 1993; 34: 1991-1999.
34. Tseng SC, Li DQ. Comparison of protein kinase C subtype expression between normal and aniridic human ocular surfaces: implications for limbal stem cell dysfunction in aniridia. *Cornea* 1996; 15: 168-178.
35. Lopez-Garcia JS, Rivas L, Garcia-Lozano I. Tratamiento de la insuficiencia limbal grave mediante cirugía combinada de trasplante de limbo y trasplante de membrana amniótica. *Arch Soc Esp Oftalmol* 2005; 80: 405-412.
36. Prabhasawat P, Tseng SC. Impression cytology study of epithelial phenotype of ocular surface reconstructed by preserved human amniotic membrane. *Arch Ophthalmol* 1997; 115: 1360-1367.
37. Dua HS, Azuara-Blanco A. Limbal stem cells of the corneal epithelium. *Surv Ophthalmol* 2000; 44: 415-425.
38. Tseng SC. Concept and application of limbal stem cells. *Eye* 1989; 3: 141-157.
39. Schlotzer-Schrehardt U, Kruse FE. Identification and characterization of limbal stem cells. *Exp Eye Res* 2005; 81: 247-264.
40. Ramaesh K, Ramaesh T, Dutton GN, Dhillon B. Evolving concepts on the pathogenic mechanisms of aniridia related keratopathy. *Int J Biochem Cell Biol* 2005; 37: 547-557.
41. Tiller AM, Odenthal MT, Verbraak FD, Gortzak-Moors-tein N. The influence of keratoplasty on visual prognosis in aniridia: a historical review of one large family. *Cornea* 2003; 22: 105-110.
42. Geerling G, MacLennan S, Hartwig D. Autologous serum eye drops for ocular surface disorders. *Br J Ophthalmol* 2004; 88: 1467-1474.
43. Kojima T, Ishida R, Dogru M, Goto E, Matsumoto Y, Kaido M, et al. The effect of autologous serum eyedrops in the treatment of severe dry eye disease: a prospective randomized case-control study. *Am J Ophthalmol* 2005; 139: 242-246.
44. Poon AC, Gerling G, Dart JK, Fraenkel GE, Daniels JT. Autologous serum eyedrops for dry eyes and epithelial defects: clinical and in vitro toxicity studies. *Br J Ophthalmol* 2001; 85: 1188-1197.
45. Alvarado Valero MC, Martinez Toldos JJ, Borrás Blasco J, Alminana Alminana A, Pérez Ramos JM. Tratamiento de los defectos epiteliales persistentes mediante suero autólogo. *Arch Soc Esp Oftalmol* 2004; 79: 537-542.
46. Noble BA, Loh RS, MacLennan S, Pesudovs K, Reynolds A, Bridges LR, et al. Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular disease. *Br J Ophthalmol* 2004; 88: 647-652.
47. Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. *Exp Eye Res* 2000; 70: 329-337.
48. Koizumi NJ, Inatomi TJ, Sotozono CJ, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res* 2000; 20: 173-177.
49. Sonnenberg A, Calafat J, Janssen H, Daams H, van der Raaij-Helmer LM, Falcioni R, et al. Integrin alpha 6/beta 4 complex is located in hemidesmosomes, suggestin a major role in epidermal cell-basement membrane adhesion. *J Cell Biol* 1991; 113: 907-917.
50. Sridhar MS, Bansal AK, Sangwan VS, Rao GN. Amniotic membrane transplantation in acute chemical and thermal injury. *Am J Ophthalmol* 2000; 130: 134-137.
51. Pires RT, Tseng SC, Prabhasawat P, Puangsricharern V, Maskin SL, Kim JC, et al. Amniotic membrane transplantation for symptomatic bullous keratopathy. *Arch Ophthalmol* 1999; 117: 1291-1297.
52. López-García JS, Rivas L, García-Lozano I. Trasplante de membrana amniótica en el tratamiento de la insuficiencia limbal moderada de pacientes con aniridia congénita. *Arch Soc Esp Oftalmol* 2005; 80: 517-523.
53. Kruse FE, Reinhard T. Limbus transplantation for reconstruction of the ocular surface. *Ophthalmologie* 2001; 98: 818-831.
54. Holland EJ, Djalilian AR, Schwartz GS. Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique. *Ophthalmology* 2003; 110: 125-130.
55. Meisler DM, Perez VL, Proudfit J. A device to facilitate limbal stem cell procurement from eye bank donor tissue for keratolimbal allograft procedures. *Am J Ophthalmol* 2005; 139: 212-214.
56. Sundmacher R, Reinhard T. Homologous lamellar central limbokeratoplasty in severe limbal stem cell deficiency. *Klin Monatsbl Augenheilkd* 1998; 213: 254-255.
57. Tseng SC, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without allografts for corneal surface reconstruction in patients with limbal stem cells deficiency. *Arch Ophthalmol* 1998; 116: 431-441.
58. Koizumi N, Inatomi T, Quantock AJ, Fullwood NJ, Dota A, Kinoshita S. Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. *Cornea* 2000; 19: 65-71.
59. Schwab IR, Reyes M, Isseroff RR. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. *Cornea* 2000; 19: 421-426.
60. Tsai RJ, Li LM, Chen J. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med* 2000; 343: 86-93.
61. Shimazaki J, Shinozaki N, Tsubota K. Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. *Br J Ophthalmol* 1998; 82: 235-240.
62. Nakamura T, Kinoshita S. Ocular surface reconstruction using cultivated mucosal epithelial stem cells. *Cornea* 2003; 22: S75-S80.
63. Arroyave CP, Scott IU, Gedde SJ, Parrish RK 2nd, Feuer WJ. Use of glaucoma drainage devices in the management of glaucoma associated with aniridia. *Am J Ophthalmol* 2003; 135: 155-159.
64. Chen TC, Walton DS. Goniosurgery for prevention of aniridic glaucoma. *Arch Ophthalmol* 1999; 117: 1144-1148.
65. Mavrikakis I, Casey JM. Phacoemulsification and endocapsular implantation of an artificial iris intraocular lens in traumatic cataract and aniridia. *J Cataract Refract Surg* 2002; 28: 1088-1091.

66. Esquenazi S, Amador S. Bilateral cataract surgery combined with implantation of a brown diaphragm intraocular lens after trabeculectomy for congenital aniridia. *Ophthalmic Surg Lasers* 2002; 33: 514-517.
67. McCulley TJ, Mayer K, Dahr SS, Simpson J, Holland EJ. Aniridia and optic nerve hypoplasia. *Eye* 2005; 19: 762-764.
68. Menezes JL, Martinez-Costa R, Cisneros A, Desco MC. Implantation of iris devices in congenital and traumatic aniridias: surgery solutions and complications. *Eur J Ophthalmol* 2005; 15: 451-457.
69. Mavrikakis I, Mavrikakis E, Syam PP, Bell J, Casey JH, Casswell AG, et al. Surgical management of iris defects with prosthetic iris devices. *Eye* 2005; 19: 205-209.
70. Fischbach BV, Trout KL, Lewis J, Luis CA, Sika M. WAGR syndrome: a clinical review of 54 cases. *Pediatrics* 2005; 116: 984-988.
71. Yasuda T, Kajimoto Y, Fujitani Y, Watada H, Yamamoto S, Watarai T, et al. PAX6 mutations as a genetic factor common to aniridia and glucose intolerance. *Diabetes* 2002; 51: 224-230.