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Abstracts and case reports



SUMMARY

- Pg. 04** Aniridia: Guide for its genetic study, based on the experience at Hospital Fundación Jiménez Díaz.
F. Blanco-Kelly, M. Corton, C. Villaverde, C. Vélez, I. Lorda, M.J. Trujillo, E Vallespín, J.M. Millan, and C. Ayuso.
- Pg. 05** New colored diaphragm intraocular lens (CDIOL) with active pupillary zone (APZ) in the treatment of aniridia.
D. Casanave, E.V. Chentsova, K.V. Lugovkina.
- Pg. 06** Foveal Hypoplasia Grade Correlates To Visual Acuity In Congenital Aniridia.
Pilar Casas-Llera, José María Ruiz-Moreno, Jorge L Alió, Ana Siverio.
- Pg. 07** PAX6 mutation in French patients: an update
N. Chassaing, J.M. Rozet, P. Calvas, with the french rare diseases networks (AnDDI-RARES & SENSGENE)
- Pg. 08** Evaluation of chromosome 11p13 deletions in patients with syndromic aniridia in Spain.
M. Corton, F. Blanco-Kelly, C. Villaverde, E. Vallespin, M. Palomares, C. Vélez, M.J. Trujillo, I. Lorda, J. Nevado, P. Lapunzina, and C. Ayuso.
- Pg. 09** Cultivated oral mucosa epithelium transplantation (COMET) procedure in corneal epithelium restoration of aniridia patients
Dariusz Dobrowolski, Edward Wylęgała, Bogusława Orzechowska-Wylęgała, Bogumił Wowra, Maria Grolik, Krzysztof Szczubialka, Maria Nowakowska
- Pg. 10** Aniridia associated keratopathy – is it time to evaluate treatment?
Fagerholm, P; Edén, U; Lagali, N; Dellby, A; Ihnatko R; Utheim, T; Chen, X; Riise, R.
- Pg. 11** Modality of glaucoma treatment on adult patients with aniridia.
Kliuiev G.
- Pg. 12** "Living with aniridia" – One voice!
Inskog, W., Akerli, A., Hansen, H., Ørstenvik, S., von der Lippe, C.
- Pg. 13** Treatment of aniridic glaucoma in pediatric patients.
M. Mazza; E. Piozzi.
- Pg. 14** Visual prognosis in 13 children with congenital aniridia: our experience.
Mazzarolo M., Pinello L..
- Pg. 15** Central corneal thickness in aniridics and relatives. A pilot study.
Javier Paz Moreno-Arrones, Alberto Rodero Sánchez, Francisco Avilés Rogel, Miguel A. Teus.
- Pg. 16** Outcomes of surgical correction of congenital aniridia
Pozdeyeva N.A.
- Pg. 17** Genetic evaluation in subjects with aniridia clinically defined.
Primignani P, Manfredini E, Romitti L, Mauri L, Patrosso MC, Franzoni A, Del Longo A, Piozzi E, Gesu GP, Damante G, Penco S.
- Pg. 18** Effect of Storage Temperature on the Viability, Phenotype, Metabolism and Morphology of Cultured Human Oral Keratinocytes.
Tor P. Utheim, Catherine Jackson, Jon R. Eidet, Edward B. Messelt, Rima Maria Corraya, Torstein Lyberg, May Griffith, Darlene A. Dartt, and Rakibul Islam.
- Pg. 19** Novel large deletions of the PAX6 gene in aniridia patients from Russia.
Vasilyeva T.A., Khlebnikova O.V., Pozdeyeva N.A., Voskresenskaya A.A., Zinchenko R.A.
- Pg. 20** Evaluation of corneal changes in confocal microscopy and OCT Visante in congenital
Bogumił Wowra, Edward Wylęgała, Dariusz Dobrowolski, Bogusława Orzechowska-Wylęgała, Maria Grolik
- Pg. 21** Biomechanical properties of the cornea measurement using Scheimpflug noncontact tonometry in aniridia patients
Bogumił Wowra, Dorota Tarnawska, Dariusz Dobrowolski, Edward Wylęgała

F. Blanco-Kelly*^{1,4}, M. Corton^{1,4}, C. Villaverde^{1,4}, C. Vélez^{1,4}, I. Lorda^{1,4}, M.J. Trujillo^{1,4}, E Vallespín^{2,4}, J.M. Millan^{3,4}, and C. Ayuso*^{1,3}.

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Introduction

Aniridia is a congenital panocular disease (1/50000-100000) characterized by partial or total absence of the iris. Aniridia can appear in an isolated way or as part of more complex trait (syndromic) with a mayor implication of ocular structures and / or systemic alterations (WARG syndrome among other).

The most frequent cause of aniridia are *PAX6* gene mutations. It is located on the chromosomal region 11p13, and encodes for a transcriptional regulator key of the embryonic eye development.

It has been published that 2/3 of aniridia cases are familial (autosomal dominant inheritance) and 1/3 are "de novo" (sporadic).

Nowadays molecular many genetic techniques are used for the diagnosis of aniridia (Sanger sequencing, karyotype, MLPA, FISH, CGH array). The application of the different techniques varies depending on the individual and familial characteristics of each aniridia case (age, inheritance, systemic implication).

Objectives

This study aimed to establish a guide for aniridia diagnosis and to study its efficacy on clinical practice during 16 years, in order to allow for an efficient application of the new genetic diagnostic techniques and an improved clinical management and integral care of the patients and their families.

Methods

Fifty-four families referred to the Department of Genetics at Hospital Fundación Jiménez Díaz for the study of Aniridia between 1997 and 2013 were included in this study. Clinical and genetic data, informed consent and biological samples for genetic study were collected during a first interview in the genetic medical visit. A further genetic analysis individually adapted was performed, considering several variables (familial *vs* sporadic cases, newborn *vs* adult, presence or absence of extra-ocular symptoms).

Results

A guide for the genetic study of aniridia, based on more than 15 years of experience in the evaluation and diagnosis of aniridia cases, that has allowed for the diagnostic of 89% of the familial cases and 77% of "de novo" cases.

Conclusions

The establishing of a protocol for the management and study of patients with aniridia has allowed the diagnosis of almost 80% of patients referred for study of aniridia.

A global and efficient system allows an early diagnosis and appropriate genetic counseling, providing not only a better individualized treatment and monitoring of patients, but access to the benefits of an accurate diagnosis.

The collaboration between the parties involved in the process (patients, clinicians, researchers, associations, ...), allows the development of protocols that transcend interdisciplinary in better overall care of these patients.



New colored diaphragm intraocular lens (CDIOL) with active pupillary zone (APZ) in the treatment of aniridia.

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The aim was to evaluate the clinical results of optical - reconstructive surgery with the implantation of a new model of colored diaphragm intraocular lens (CDIOL) with an active pupillary zone (APZ) in patients with posttraumatic total or partial aniridia.

Eight patients from 17 to 57 y.o. with posttraumatic aniridia underwent implantation of CDIOL APZ with our special surgical technique that includes a new method of fixation using transcleral elements of support in case of capsular absence or deficiency.

Besides of good cosmetic results, clinically we achieved the disappearance of photophobia, and mean visual acuity of 0,3. In patients with secondary glaucoma and CDIOL APZ fixation using transcleral elements (three cases), normalization of the IOP has been achieved up to 18-22 mm Hg throughout the observation period (16 months). In patients with intracapsular implantation of CDIOL APZ (five cases), IOP was within 14 to 19 mm Hg. In both types of cases, there was no use of hypotensive medication during the whole observation period at any time. The average visual field $92^{\circ} - 54^{\circ}$ horizontally and $45^{\circ} - 65^{\circ}$ vertically (Humphrey HFA. Sita standard C24-2).

The CDIOL APZ used in the reconstruction of the anterior chamber with large defects of the iris or complete aniridia is an acrylic-based polymer artificial foldable colored diaphragm intraocular lens with an "active pupillary zone" able to recover the intraocular outflow.

Total diameter: 10 mm

Pupil's diameter: 2,5 mm

Diameter of the optical part: 5,0 mm

Gap between the optical part and haptic part: 1,0mm

The transscleral elements of support are made of PMMA.

Total length: 5,5 mm

Length upper part: 4,5 mm, bent 35°

1.5 mm from the inner edge, is connected with the edge of the bottom plate of 2,5 mm in length.

Width at the base: 3,3 mm

The distance between the two plates is 0,2 mm. The upper plate has an oval opening for suturing the support element to the sclera. The prosthesis was designed in the trauma department of Moscow Helmholtz Research Institute of Eye Diseases and manufactured by Reper NN, Russia.

The benefit of our model is the gap between the optical part and the artificial iris that provides a functional artificial pupil and allows the normal outflow of aqueous humor.

The method of fixation using special elements of support, reduces the risk of ectopy of CDIOL, decreases the formation of gonio synechiae and prevents the development of secondary glaucoma while working as a complementary system of drainage.

Our next step is to implant this prosthesis in cases of congenital aniridia.

Keywords: colored diaphragm intraocular lens (CDIOL), active pupillary zone (APZ), aniridia, normalization of the outflow of the aqueous humor, posttraumatic glaucoma, elements of support.



Foveal Hypoplasia Grade Correlates To Visual Acuity In Congenital Aniridia.

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Purpose

To analyze the foveal morphology assessed by spectral-domain OCT as a prognostic indicator for best corrected visual acuity (BCVA) in congenital aniridia patients.

Methods

This is an observational single-center study performed between January 2012 and September 2013 in the pediatric ophthalmology department at Vissum Corporation, Alicante, Spain. A total of 12 eyes from 6 patients with congenital aniridia were included. After a complete ophthalmological examination, a high-resolution spectral-domain OCT (Topcon Medical Systems, Tokio, Japan) with a 3-dimensional scan program (512x128 resolution) macular protocol was used. A morphological grading system of foveal hypoplasia was used varying from grade 1 in which there is a presence of outer nuclear layer (ONL) widening and presence of outer segment (OS) lengthening to grade 4 in which none of these processes occur.

Results

No correlation between central, mid-peripheral and peripheral macular thickness and logMar BCVA was found. Grade 3 foveal hypoplasia was associated with better BCVA with a median BCVA, 0.13 logMAR, whereas grade 4 was associated with poorer VA with a median BCVA of 0.7 logMAR.

Conclusion

Foveal hypoplasia morphology can predict the BCVA. The presence of the outer nerve layer widening is a prognostic visual function indicator.



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Introduction

Due to early expression in the developing eye, the *PAX6* gene is associated with various congenital ocular malformations, including aniridia, Peters' anomaly, keratitis, congenital cataract, optic nerve malformations, and microphthalmia. Aniridia is considered as a haploinsufficiency ocular disorder, and is typically associated with *PAX6* nonsense or frameshift mutations introducing a premature termination codon, whereas *PAX6* missense mutations, of which the majority are located in the paired domain and result in impaired DNA binding, lead to non-aniridia phenotypes. The purpose of this study was to provide a comprehensive survey of *PAX6* mutations in a large cohort of patients recruited throughout the French territory and to better delineate the ophthalmological and extra-ocular expression of mutations in this homeobox gene.

Material and methods: We perform mutational screening of *PAX6* exons and intron-exon boundaries (Sanger sequencing), search for small intragenic microrearrangements (QMPSF/qPCR) and CNV analysis (array CGH) in a cohort of 172 index cases fulfilling the minimal diagnostic criteria of aniridia. The consequence of variants affecting splice-site changes was assessed by sequencing the mRNA when samples were available. Genotype-phenotype correlations were searched by reviewing the clinical files of patients.

Results

The screening of the *PAX6* gene in our cohort resulted in the identification of 120 disease alleles : including -in order of frequency- 51 nonsense mutations (29%), 34 splice-site variants (19%), 30 small deletions (17 %), 22 large deletions (13 %), 18 missense mutations (11 %), 11 small insertion (6%) and variants of unknown significance (VOUS, 2 %). Familial segregation analysis and review of clinical data in patients with *PAX6* mutations indicated full penetrance of mutations. Yet, a high inter- and intra-familial variability of clinical expression was noted. Data regarding the extra-ocular expression in patients was sparse but we noted that neurodevelopmental and genital anomalies was not uncommon. Glycaemia dysregulation including neonatal hypoglycaemia was noted in around 3%. Variant phenotypes (e.g. Peters anomaly) were rather linked to missense mutations. Papillary and optic nerve anomalies were observed in both patients with haploinsufficiency or missense mutations. Heterozygous missense mutations (Asn64, Gly65, Cys66) involving Paired-Domain medial 3rd helix (constitutive of the DNA 1st linkage domain) were identified in severe phenotypes including microphthalmia / anophthalmia, brain and cerebellar malformations. We observed that large deletions involving both *PAX6* and *WT1* are more prone to trigger isolated aniridia than WAGR syndrome. Finally 3 unrelated cases harbouring compound heterozygous mutations were affected with severe malformations involving constantly eye and CNS {i.e. c.del[(?_316)_(*64_?)]:[56G>C], c.[256G>A];[117_118insC]}

Discussion

Combining the search for point mutations, microrearrangements and CNVs allowed the identification of *PAX6* mutations in more than 90 % of the patients of the French aniridia cohort. Consistent with previous studies, mutations introducing a premature stop codon and gene rearrangements made up the majority of mutations. Some genotype-phenotype correlations could be drawn from the available clinical data of patients but in the majority of cases the data were sparsely especially with respect to extra-ocular expression. It appears quite necessary to better delineate the ocular and extraocular expression of *PAX6* mutations. Hopefully this will allow improving genotype-phenotype correlations. These correlations are essential to provide parents and affected children with a prognosis as well as to set up a tailor-made follow-up of ocular and extraocular functions.



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Introduction

Aniridia is a rare congenital panocular disease with an incidence of 1:50000-100000. It is characterized by congenital absence or hypoplasia of the iris associated with other ocular changes. Aniridia appears either as an isolated ocular abnormality without systemic alterations or as part of syndromic presentation such as the WAGR (Wilms' tumor, Aniridia, Genitourinary anomalies, and mental Retardation) syndrome.

Isolated and syndromic aniridia are caused by mutations or chromosomal rearrangements of the *PAX6* gene at chromosome 11p13. About two-thirds of all aniridia cases are familial showing autosomal dominant inheritance with high penetrance. The remaining sporadic cases carry *de novo* mutations that will be dominantly inherited in further generations. Specifically, *de novo* deletions of the chromosomal region 11p13 encompassing *PAX6* and *WT1* cause WAGR syndrome.

Nowadays, different molecular and cytogenetic techniques are used for the diagnosis of WAGR syndrome (MLPA, FISH, karyotype and high resolution CGH arrays). The application of these techniques varies depending on the individual and familial characteristics of each case (age, inheritance, systemic implication).

Objectives

This study aimed to determine the incidence and mutational spectrum of chromosomal defects on 11p13 in our Spanish patients with syndromic aniridia, to establish its frequency and diagnostic rate among our cohort of Aniridia cases, and finally to provide an exact molecular diagnosis.

Methods

A cohort of 54 unrelated cases referred to the Department of Genetics at the Hospital Fundación Jiménez Díaz for the genetic study of Aniridia between 1997 and 2013 was included in this study. Genetic assessment of chromosomal region 11p13 was performed using molecular (MLPA, commercial and custom CGH arrays) and/or cytogenetic (FISH and karyotype) techniques.

Results

From a total of 54 patients with aniridia, three (6%) presented aniridia and systemic abnormalities. In all cases, a large *de novo* 11p13 deletion encompassing *PAX6* and other contiguous genes was identified mainly by MLPA analysis. In addition, genetic studies allowed for the identification of a contiguous gene deletion syndrome in four additional newborn cases with isolated aniridia. In most cases, chromosomal breakpoints were further characterized using a customized CGH array (WAGR array), specifically designed for the analysis of the chromosomal region 11p13.

The 11p13 deletion included both *PAX6* and *WT1* genes in two cases with Aniridia and other features of WAGR syndrome and in three newborn with sporadic aniridia. In the remaining two characterized cases, the chromosomal deletion affected only *PAX6* and several downstream genes, being *WT1* not deleted.

Conclusions

In our cohort of Spanish patients with aniridia, 13% (7/54) of them carry large chromosomal 11p13 deletions, encompassing *PAX6* and other contiguous genes. In all patients suffered with clinically diagnosed with syndromic Aniridia, the causative genetic defect was identified. In addition, four additional patients with Aniridia at risk of developing systemic anomalies could be identified through genetic analysis.

The implementation of new genomic techniques such as CGH arrays in the clinical practice will enhance the molecular diagnostic of Aniridia, WAGR and other contiguous gene deletion syndromes, improving the diagnostic accuracy and rate through a more accurate analysis of the chromosomal 11p13 region.



Cultivated oral mucosa epithelium transplantation (COMET) procedure in corneal epithelium restoration of aniridia patients

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Purpose

Efficacy of COMET procedure in corneal epithelium restoration of aniridia patients.

Methods

27 aniridia patients (30 eyes) with irregular, vascular conjunctival pannus underwent autologous cultivated oral mucosa epithelial transplantation. Single oral mucosa epithelial cells were seeded on denuded amniotic membrane. Cultures were carried in presence of 3T3 fibroblasts for 7 days. Corneal surface, epithelial regularity, visual acuity were evaluated.

Results

6 month after surgery 82,7% of eyes had regular transparent epithelium, 17,2 % of eyes developed central corneal haze, in 62,2 % of eyes visual quality increased from mean 0,02 to 0,12.

Conclusion

COMET restores regular epithelium and improves vision



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Background

Aniridia associated keratopathy is a common and feared complication in aniridia ridden eyes. Loss of corneal epithelial stem cell function results in conjunctivalisation of the cornea resulting in reduced visual acuity and chronic irritation. Eye surgery for glaucoma and cataract can accelerate conjunctivalisation but not always.

Materials and methods: A Swedish and a Norwegian cohort of aniridia patients were examined. The stem cell niche was examined using in vivo confocal microscopy. The keratopathy was graded into four stages. Tears were assembled in Shirmer strips and analysed using proteomics.

Results

The mechanisms underlying progressive corneal pathology, known as aniridia associated keratopathy (AAK) involve abnormal corneal healing responses, anomalous extracellular matrix metabolism, fragility of epithelial cells, and progressive corneal thickening and vascularization. The increase in AAK parallels the morphology of the deteriorated stem cell niche. Our immunoblot analysis revealed elevated levels of 80 kDa isoform corresponding to VEGF-C in aniridia patients. Using two-dimensional gel electrophoresis coupled with liquid chromatography tandem mass spectrometry, another 7 proteins were identified differentially expressed with $P < 0.01$ between aniridia patients and control subjects. Five of them were more expressed in healthy subjects, particularly α -enolase, peroxiredoxin 6, cystatin S, gelsolin and apolipoprotein A-1. Two other proteins were more expressed in aniridia patients and were identified as zinc- α 2-glycoprotein and lactoferrin.

Conclusion

The differentially expressed proteins in patient tears may be new candidate molecules involved in the pathophysiology of aniridia and may be helpful for development of novel treatment strategies for the symptomatic therapy of this vision threatening condition. Anti VEGF therapy should be evaluated as a means to stop or reduce aniridia associated keratopathy



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Secondary glaucoma is often attends by congenital or trauma's acquired aniridia. There are few such patients, but their treatment represents considerable difficulties.

Aim

To provide results of laser and surgical treatment on 2 patients with congenital aniridia and glaucoma.

Patients and methods

The research included 2 cases of a congenital aniridiya (4 eyes) at adult patients.

In the first case on 1 eye with aphakia and avitria (after vitrectomy and lensectomy) Ahmed implant have been provided. On pair eye IOP compensation was reached by medicament treatment: timolol 0,5%, brinzolamid 1%.

In the second case, on 1 eye was provided the transscleral contact compression cyclocoagulation by infrared diode laser of 810 nanometers, with follow-up additional transpupillar cyclocoagulation by green laser of 532 nanometers. On pair eye IOP compensation was reached by medicament treatment: brimonidin 0,2%, timolol 0,5%, brinzolamid 1%.

Results

In the first case initial intraocular pressure was 36 mm Hg.

Ahmed implant allowed to reduce intraocular pressure from 34 to 16 mm. However in 1,5 months, in connection with recurrence of increase of intraocular pressure to 27 mm. the patient was refered timolol 0,5%.

In the second case initial intraocular pressure under brimonidin 0,15%, timolol 0,5%, brinzolamid 1%. The latanoprost was 31 mm Hg across Goldman.

After carrying out the transscleral contact compression cyclocoagulation by the infrared diode laser of 810 nanometers, IOP decreased to 29 mm Hg. In 6 days for additional IOP decrease the transpupillar coagulation of ciliary processes by means of Goldman's lens and green laser of 532 nanometers have been provided. Was reached IOP decreasing up to 21 mm. Latanoprost was canceled. The patient continued get instillations of brimonidin 0,15%, timolol 0,5%, brinzolamid 1%.

Conclusions

On adult patients with aniridia and secondary glaucoma it is reasonable to combine hypotensive drops instillations with a transpupillar cyclocoagulation, as the least traumatic procedure.

In cases where corneal opacities and lens does not have sufficient visualization do not let to see ciliary processes, it is expedient to carry out a transscleral diodlaser cyclocoagulation in microimpulse mode.

The filtrational surgery, including Ahmed implant leads to complications more often and not always led to avoid recidivation of pressure increase therefore it should be used in extreme cases.



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Congenital aniridia is a rare disorder. There are approximately 60 persons living in Norway with aniridia, with a prevalence of 1:76.000. Published information on aniridia and the challenges of living with the disorder is meagre. In order to better understand the challenge this diagnosis poses for those living with the disorder, information is essential. We will present a project, based on collaboration between professionals and lay people, which culminated in the handbook ”Living with aniridia”.

In 2007 the Norwegian Association of Aniridia expressed the need for a handbook on living with aniridia. The Norwegian ExtraFoundation for Health and Rehabilitation supported the project financially. The target group for the handbook was persons living with aniridia and their families, but the handbook was also intended for professionals. A formal collaboration between the association and Centre for Rare Disorders (CRD) (www.sjeldnediagnoser.no) was established in 2009. CRD is an interdisciplinary, nationwide competence centre, which offers information, counselling and seminars on a selected range of rare disorders. The centre obtains and coordinates specialist knowledge on, and experience with, designated rare disorders. This information is distributed via counselling, seminar activities, information booklets, videos and internet. The service is aimed at the appropriate patients and their families, and at professionals working with these disorders. The centre is a national undertaking and works on behalf of the Norwegian Directorate for Health Affairs. The centre is a division of Oslo University Hospital.

A steering committee consisting of two members from The Norwegian Association of Aniridia and two professional councillors from CRD collaborated in editing the handbook. One member from the association was project leader. All involved experienced equal status and good collaboration throughout the process. The association felt their thoughts and requirements were accommodated. Individual opinions were heard and respected, and designated areas of responsibility were clarified and followed. Ophthalmologists, geneticists and ocular therapists contributed to chapters in the book. It was difficult to find professionals with specialist knowledge of aniridia. Each chapter begins with a personal story from a patient or parent. Professionals and lay people express themselves differently and it was challenging to find a common form throughout the handbook. The handbook was published in 2011. The layout of the handbook is loose-leaf. This allows pages to be removed or added for easier editing, and also facilitates using a light box. The handbook is also published on the association’s and CRD’s webpages (www.aniridi.no/www.sjeldnediagnoser.no) and is available as an audio book.

Via this project, the Norwegian Association of Aniridia and Centre for Rare Disorders established contact with many professionals and patients/parents, making this a win-win situation for all involved. Collaboration between professionals and lay people was the major factor when compiling this handbook, which provides unique information neither professionals or lay people could provide alone. Important factors which contributed to the success of the collaboration were equal status, respect, economy, enthusiasm, priority given to the project and personal chemistry.



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Aniridia is a severe congenital panophthalmic disorder characterized by almost complete absence of the iris.

It is commonly familial and is transmitted in an autosomal dominant manner with high penetrance and variable expressivity.

Glaucoma occurs in 50% to 75% of aniridic patients and typically presents in adolescence.

Most aniridic glaucomas are considered refractory to medical therapy alone, and surgery is thought to be the mainstay of therapy for many.

The surgical options include trabeculotomy, goniosurgery, trabeculectomy with antimetabolic agents, trabeculotomy with trabeculectomy, glaucoma drainage devices, and cyclodestructive procedures.

We present a case in pediatric age of aniridic glaucoma in pseudophakic with artificial iris where it was made before a trabeculectomy with MMC, and subsequently applied to a system of draining Molteno 3.



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Purpose

To study the following in children with congenital aniridia: age at first examination, frequency of family history of aniridia, frequency of ocular and general diseases associated with aniridia and to investigate visual outcome and rehabilitation.

Methods

A retrospective case review was performed and data were collected, including family history, incidence of associated keratopathy, glaucoma, cataract, macular or optic nerve hypoplasia and poor vision. 20 cases were evaluated and 13 of them were followed up.

Results

The mean age at first evaluation was 4,8 years , with 61% sporadic and a 39% familial cases and 4 patients with systemic abnormalities :2 case with WAGR syndrome (Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation), 1 case with microcephaly and microphthalmia, 2 cases with Gillespie syndrome characterized by partial aniridia, cereberall ataxia , mental retardation. 6 children had keratopathy (46%), all of them had foveal hypoplasia (100%). 5 patients developed glaucoma (38%) and 9 cataract (69%). Developmental delay was reported in 4 cases. At last follow-up the best-corrected visual acuity (BCVA) ranged from 7/10 to light perception (3/10 or more in 38 %) and was count fingers or worse in 6 eyes (23%). A total of 69 % of patients had congenital cataract, 38% glaucoma, 61,5% nystagmus, 46% corneal opacifications.

The best-corrected visual acuity (BCVA) ranged from 7/10 to light perception and was count fingers or worse in 6 eyes (25%). 5 eyes were treated with patch therapy, none is worsened, in 3 there was an improvement of two lines.

Vision damage was treated with spectacle correction of refractive errors, tinted or photochromic lenses to reduce light sensitivity, occlusion therapy for amblyopia, and low-vision aids such as closed-circuit television and adaptive technology. Cataract extraction was performed in 3 cases. Glaucoma was treated with topical anti-glaucoma medication. Corneal disease was treated with lubricants. Conclusions: The incidence of the various ophthalmic problems found in patients in our study are consistent with those found in patients with aniridia reported in the ophthalmic literature. In this study, the visual prognosis in congenital aniridia varies from patient to patient: unmonitored and untreated complication of aniridia may damage vision. An early diagnosis, a careful treatment of complications and a specific rehabilitation approach can improve visual functional outcome.



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Purpose

To compare central corneal thickness (CCT) measured by ultrasound corneal pachymetry (US) and intraocular pressure (IOP) measured by Goldmann applanation tonometry (GAT) in patients suffering from congenital aniridia and in first-degree relatives not affected.

Methods

We designed a transversal and observacional study. We performed five measurements of CCT by US and three IOP measurements by GAT in 16 eyes suffering from congenital aniridia with no glaucoma nor remarkable corneal pathology and in also in healthy 14-eyes of parents and 10-eyes of brothers. We used an ANOVA test as statistical analysis.

Results

Average CCT in aniridic group was of $651,81 \pm 50,40 \mu\text{m}$, whereas in aniridia parents group was of $588,07 \pm 29,58 \mu\text{m}$ and $576 \pm 47,92 \mu\text{m}$ in aniridia brothers group ($P < 0.0001$). GAT in affected eyes was $15,25 \pm 2,01 \text{ mmHg}$, $15,64 \pm 2.43 \text{ mmHg}$ in parents and of $16,1 \pm 1,52 \text{ mmHg}$ in brothers ($P < 0.5$).

Conclusions

Patients suffering from congenital aniridia develop thicker CCT than general population included first-degree relatives. In addition, IOP in this scenario seems to be significantly overestimated in this patients and relatives. This finding might be intimately related to the gene PAX6, that codifies for aniridia. Moreover, a thicker ultrasound pachymetry in first-degree relatives could be a diagnosis of a form fruste aniridia in not symptomatic population



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Thirty eyes of 21 consecutive patients operated for congenital aniridia from 2004 to 2013 were studied. Nine patients had bilateral surgery, 14 patients were female. The mean age was 34 years (range 12 to 68 years) that corresponded to the time of cataract progression and significant visual impairment to less than 0.1. Artificial iris-lens diaphragm (ILD) manufactured by «Reper-NN» (Nizhniy Novgorod, Russia) was implanted in all cases. In 14 eyes ILD was placed intracapsularly, in 13 eyes - on the capsule. In 3 cases artificial iris without the optical part was placed on the surface of IOL, which had been implanted earlier.

Preoperatively UCVA averaged 0.03 ± 0.08 (SD) and BCVA – 0.05 ± 0.08 (range L.P. to 0.1). Postoperative mean UCVA was 0.16 ± 0.14 ($p=0.000009$), mean BCVA improved to 0.19 ± 0.17 (range 0.03 to 0.6) ($p=0.000026$). Secondary glaucoma before ILD implantation was diagnosed in 7 eyes, four of which had previous glaucoma surgery. In the postoperative period in these 3 nonoperated eyes decompensation of IOP occurred necessitating Ahmed valve implantation. New-onset secondary glaucoma developed in 6 eyes after ILD implantation, requiring glaucoma surgery on 3 of them. In 19 cases evident signs of secondary dry eye syndrome were noted. Punctual plugs deployed simultaneously with ILD implantation in 11 eyes helped to improve or eliminate dry eye symptoms. Keratopathy evolved in the postoperative period within 1 month to 2 years in 9 eyes, six of which developed pronounced neovascularization, and 3 eyes – endothelial decompensation.

Confocal microscopy revealed characteristic changes: increased epithelium desquamation, a decrease in nucleus-cytoplasm ratio, swelling and polymorphism of wing-shaped and basal epithelial cells, opacification, thickening, «shagreen» appearance of Bowman's membrane, Langerhans' cells in the anterior stroma, insufficient number of nerve endings, their irregularity and pronounced tortuosity, diminished transparency of the extracellular matrix.

Impression cytology and confocal laser microscopy identified migration of goblet cells to the cornea, manifestation of which increased with keratopathy progression.

Level of IL-4 in the tear fluid prevailed over the amount of IL-1 β preoperatively. Up to 1 month after ILD implantation there was a significant increase of concentration of pro-inflammatory cytokine IL-1 β (more than 5.5 times) and IL-4 (3.2 times) relative to the normal values. The increase of IL-4 against a background of normal values of IL-1 β in patients, whose tear fluid was taken in more than 1 month after the surgery, can be interpreted as a decrease in severity of local inflammation and development of fibrosis in the implant zone.

Thus, surgical treatment of cataract in case of congenital aniridia is feasible only when there is a significant decrease in visual acuity with the location of opacities in the optical zone at the oldest age possible because of high risks of corneal decompensation in the postoperative period. Intracapsular ILD implantation through corneoscleral tunnel is optimal, but in case of large-swinging nystagmus implantation on the capsule is recommended because of the risk of ILD dislocation from the capsular bag as a result of jerky eye movements. In the presence of secondary dry eye it is recommended to complete its treatment prior to ILD implantation. It is reasonable to perform a single-step punctual occlusion and instillation of autologous serum. After ILD implantation lubricants should be continued for up to 1 year after the surgery.



Genetic evaluation in subjects with aniridia clinically defined

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Hereditary aniridia is a rare disease transmitted as autosomal dominant trait; it is caused by mutation in *PAX6* gene on chromosome 11p13 or by mutation in *PAX6* cis-regulatory element residing in an intron of the adjacent *ELP4* gene. The disease may also be part of the WAGR syndrome (Wilms tumor, Aniridia, Genitourinary anomalies and mental Retardation) caused by deletions of 11p13 involving *PAX6* and *WT1* gene.

Molecular diagnostic approach consists in genetic counseling first followed by sequencing analysis of the *PAX6* coding region (detection mutations 90%) in isolated aniridia either sporadic or familiar, whereas FISH and/or MLPA analysis involving 11p13 (detection mutations up to 30%) in individuals with no family history.

Eleven patients affected by isolated (8) or syndromic (3) aniridia were recruited through the Genetic Service and the Department of Pediatric Ophthalmology of the Niguarda Ca' Granda Hospital of Milan. We identified 6/8 mutations in *PAX6* gene: 4 coding mutations and 2 deletions involving *PAX6* and *ELP4/DCDC1* genes respectively. In syndromic patients we found: a deletion including *PAX6*, *WT1* and the surrounding genes; an interstitial deletion of chromosome 11: 46,XX,del(11)(p12p15.1) and a reciprocal translocation involving the long arm of chromosome 2 and the short arm of chromosome 6, 46,XY,t(2;6)(q24.3;p24). The first two patients had a clinical picture compatible with WAGR syndrome, while the last had bilateral aniridia, glaucoma and cataracts, frontal hypertrichosis, single transverse palmar crease and intellectual disability.

In conclusion we were able to identify genetic cause of the disease in 9 out 11 aniridia patients.



Effect of Storage Temperature on the Viability, Phenotype, Metabolism and Morphology of Cultured Human Oral Keratinocytes

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Purpose/Aims

Aniridia-associated keratopathy secondary to limbal stem cell deficiency can result in constant red eye, epiphora, photophobia, pain and loss of vision, thus significantly reducing quality of life. Although limbal stem cell deficiency can be treated by transplantation of cultured oral mucosal cells, patient access is limited, as there are few centers in the world that provide the treatment. The present study aims to make the treatment of severe aniridia associated keratopathy more readily available by providing a short-term storage option for cultured autologous cell sheets. Storage of cultured oral mucosal keratinocytes in hermetically sealed containers offers a number of advantages with regard to the treatment of aniridia-associated keratopathy, including: 1) possibility of transportation from culture units to transplantation clinics worldwide; 2) sufficient time to allow for quality assessment and sterility control of the cultured cells prior to surgery; and 3) increased flexibility in scheduling operations.

Materials and Methods

Cultured human oral mucosal cells were stored in HEPES- and sodium bicarbonate-buffered Minimum Essential Medium at nine temperatures from 4°C to 37°C for seven days. Viability was assessed by calcein fluorescence, metabolic parameters (glucose, lactate, pH, and pO₂) in the storage medium by blood gas analysis, and morphology by scanning electron microscopy. The phenotype was assessed by immunocytochemistry using three markers for undifferentiated cells (ABCG2, Bmi-1, C/EBPδ) and a marker for proliferation (PCNA), human oral keratinocytes (cytokeratin 18) and apoptosis (caspase-3).

Results

Relative to the cultured, but non-stored control, cell viability was best conserved in the 12°C and 16°C storage groups (85%±13% and 68%±10%, respectively). Expression of ABCG2, Bmi-1, C/EBPδ, PCNA, cytokeratin 18, and caspase-3 were maintained after storage between 4°C and 20°C, compared to the control. Glucose, pH and pO₂ in the storage medium declined, whereas lactate increased progressively with increasing storage temperature. Morphology was best preserved following storage between 12°C and 20°C.

Conclusion

Overall, storage temperatures between 12°C and 16°C for one-week storage were found to be optimal for maintenance of cell viability, phenotype and morphology of cultured human oral keratinocytes. Importantly, the storage method described in the present study may be applicable for other cell types and tissues; thus its significance may extend beyond human oral keratinocytes and the field of ophthalmology.



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Aniridia is a congenital ocular disorder caused by the mutations of the PAX6 gene coding a paired box DNA-binding protein. We carried out an investigation of 21 aniridia patients from Russia and determined a specific PAX6 mutations spectrum with a significant rate of de novo mutations. Missense, nonsense, indel mutations, small insertions and deletions were identified by sequencing in 16 out of 21 cases. Here we focus on revealed by MLPA large deletions (in 4 cases). The latter type of mutations makes up a quarter of all identified in our study PAX6 changes. Large deletions of 11p13 region just outside the PAX6 gene and/or invading the PAX6 gene cause phenotype of aniridia with complications of a broad range: from uncomplicated aniridia to one with associated ocular abnormalities and/or disorders of other body systems. Lack of the close to the PAX6 gene portion of DNA sequence leads to similar defects as known intragenic point mutations do. Defects seem to be more complex and severe in patients with a lack of an area downstream of the PAX6 gene including the RCN gene and a part of the WT1 gene.

Four patients with revealed rearrangements have bilateral total iris absence. One of them has aniridia combined with microcornea and cataract, three patients do not have any other ocular associated pathology. One of these three patients has aniridia combined with bilateral nephroptosis, other one with nephropylitis and adiposis, and one of the patients without auxiliary ocular associated pathology has an uncomplicated isolated aniridia. The patients are from unrelated families; all of them undergo ophthalmic examination and DNA-testing. The DNA was extracted from peripheral blood leucocytes with Promega DNA isolation kit according to the manufacturer protocol. DNA-testing includes initial sequencing of PAX 6 gene 14 exons and subsequent MLPA analysis with the help of the MRC Holland SALSA MLPA probmix P219-B2 PAX6.

MLPA analysis revealed in 4 patients without identified by sequencing point mutations large deletions invading PAX6 and/or close neighbor genes: a) 31307603_31650221del, spanning from DCDC1 gene exon 4 to exon 9 ELP4 gene not including the PAX6 gene (approximately 342 618 bp deletion distal of the PAX6 gene), b) 31650221_32417549del, spanning from ELP4 gene exon 9 and including genes PAX6, RCN, gene WT1 exon 5 (approximately 767 328 bp deletion distal & proximal of the PAX6 gene), c) c.(-316-?)_724+?del inside the PAX6 gene from intron 1 to exon 8 (approximately 23 000 bp), d) 31817459_31369675del, invading distal of PAX6 genes: DCDC1, IMMP1L, ELP4 and PAX6 gene intron 1 (approximately 450 000 bp). The exact borders of identified large deletions are to be determined.

All but one detected mutations are novel (the deletion inside PAX6 gene from intron 1 to exon 8 was described earlier). Together with novel identified point mutations, high frequency of de novo mutations inclusively revealed large rearrangements, specific large deletions of chromosome 11 make up the peculiarity of the mutations spectrum in patients from Russian Federation.



Evaluation of corneal changes in confocal microscopy and OCT Visante in congenital aniridia patients

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Purpose

To evaluate of corneal changes in congenital aniridia patients.

Patients and methods

40 patients (79 eyes) suffered from congenital aniridia underwent confocal microscopy and SD OCT Visante examination. Aniridia related keratopathy corneal structure and corneal pannus thickness was evaluated.

Results

Mean epithelial thickness performed in 5 different points was $54,4 \pm 16,6 \mu\text{m}$. Structure changes of cornea epithelium and subbasal nerves plexus was observed in confocal microscopy examination.

Conclusion

SD OCT Visante and confocal microscopy examination are useful to evaluate corneal changes in congenital aniridia patients.



Biomechanical properties of the cornea measurement using Scheimpflug noncontact tonometry in aniridia patients

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Purpose

To evaluate biomechanical properties of the cornea in patients with congenital aniridia

Methods

26 patients (16 women, 10men) with congenital aniridia aged from 6 to 55 underwent corneal examination using Scheimpflug noncontact tonometry. CCT, deformation amplitude, first applanation time, second applanation time, highest concavity and IOP were evaluated

Results

CCT ranged from 496 μm to 718 μm (mean 623 μm). Deformation amplitude range from 0.36 to 1.01 mm (mean 0.62 mm) correlated with IOP result range from 15.6 mmHg to 42.8 mmHg (mean 22.82 mmHg).

Conclusion

Evaluation of corneal biomechanical properties is useful in estimation real IOP in aniridia patients.



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