Diadenosine polyphosphates in the tears of aniridia patients

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ABSTRACT.

Purpose: To quantify diadenosine polyphosphate levels in tears of congenital aniridia patients to estimate the ocular surface changes associated with congenital aniridia compared to normal individuals.

Methods: Fifteen patients diagnosed with congenital aniridia and a control group of forty volunteers were studied. Tears were collected to quantify the levels of diadenosine polyphosphates Ap_4A and Ap_5A by high-performance liquid chromatography (H.P.L.C). Break-up time (BUT), corneal staining, McMonnies questionnaire and the Schirmer I test were applied to both groups.

Results: Dinucleotides in congenital aniridia patients were higher than in control subjects. For the congenital aniridia group, under 15 years old, the values were 0.77 ± 0.01 μM and 0.17 ± 0.02 μM for Ap_4A and Ap_5A, respectively. The group aged from 15 to 40 years old provided concentrations of 4.37 ± 0.97 μM and 0.46 ± 0.05 μM for Ap_4A and Ap_5A, the group over 40 gave concentrations of 11.17 ± 5.53 μM and 0.68 ± 0.17 μM for Ap_4A and Ap_5A. Dinucleotide concentrations increased with age, being statistically significant different among the three age groups ($p < 0.05$). Congenital aniridia patients showed a normal tear secretion and no dry eye McMonnies scores, except for the group over 40 years old. BUT values decreased and corneal staining increased with age and correlated with the levels of diadenosine polyphosphates ($p < 0.05$).

Conclusions: The levels of dinucleotides in tears increase in aniridia patients compared with healthy subjects, and they seem to be related with the progression of corneal disorders in aniridia patients, both of which increase with ageing.

Key words: congenital aniridia – dinucleotides – keratopathy – tear film disorder

Introduction

Aniridia is a rare congenital disorder with a variety of alterations in different organs, including problems in the development of the eye. It is due to the mutation of the PAX6 gene (Ton et al. 1991; Glaser et al. 1992), located in the short arm of the chromosome 11. This pathology affects between 1:72 000 and 1:100 000 live births and presents a wide spectrum of associated ocular and non-ocular disorders (Nelson et al. 1984; Edén et al. 2008).

The main feature of aniridia is the partial or total absence of the iris, but this affliction is also associated with other ocular anomalies such as cataracts, glaucoma, nystagmus, foveal hypoplasia, limbal stem cell deficiency, tear film disorders and keratopathy (Mayer et al. 2003). Moreover, aniridia may be found alone or in association with other syndromes (Nelson et al. 1984), the latter involving systemic implications, due to the deletion of 11p13 involving both PAX6 and WT1 genes and known as WARG syndrome (Fantes et al. 1992).

Although aniridia is a congenital condition, the severity of most of the associated ocular pathologies increases with age. Interestingly, one of the most relevant pathological situations related to aniridia is keratopathy. This is produced by a stem cell deficiency, which furthermore leads to corneal opacity and to dry eye conditions (Tseng & Li 1996).

Aniridia-related corneal dystrophy, termed as aniridic keratopathy, was originally described by Mackman et al. (1979), who found a prevalence of 89%, confirmed by other studies (Kremer et al. 1993; Nishida et al., 1995). During the progress of the aniridic keratopathy, the epithelium becomes thickened and irregular (Whitson et al. 2005). Later, a peripheral pannus appears advancing towards the central cornea, and the corneal surface is replaced by conjunctiva-like epithelium (Gomes et al. 1996). All these events generate, in addition to an important visual loss, an irregular ocular surface where the tear film evaporation rate is high, giving rise to a potential dry eye disease.

Analytical techniques provide valuable information about novel components in tears that may have important biochemical and physiological functions. Among these new tear components, the diadenosine polyphosphates emerge as interesting compounds due to their intracellular and extracellular physiological actions (Miras-Portugal et al. 1999; McLennan 2000). Although the activity of these dinucleotides in ocular tissues is a matter of current research, it is already known that they modulate intraocular pressure in rabbits (Pintor et al. 2003), Ap_4A improves the rate of wound healing in the cornea of New Zealand white rabbits, in contrast to Ap_5A inhibits it (Pintor et al. 2004; Mediero et al. 2006), and Ap_4A can stimulate tear secretion after a single-dose topical application in rabbits (Pintor et al. 2002a,b). The description of the presence of diadenosine polyphosphates in human tears in 2002 (Pintor et al. 2002a,b) and the subsequent report of these substances as potential molecular markers for dry eye in 2006 (Peral et al. 2006; Carracedo et al. 2010) suggested to us the possibility of a relationship between the amounts of these substances and the development of dry eye. Besides this assertion, it has also been suggested that the shear stress over the ocular surface, due to the blink process, is one of the stimuli for release of these nucleotides into the tear film (Carracedo et al. 2013). Therefore, in the present experimental work, the level of diadenosine polyphosphates in the tears of a group of aniridia patients was examined. It is of interest to relate the changes in concentrations of these compounds with the progress of this disease, relating it to the changes in the ocular surface as it progresses with ageing.

Methods

Subjects

The study was conducted in compliance with good clinical practice guidelines, institutional review board regulations and the tenets of the Declaration of Helsinki. The subjects signed an informed consent and were free to interrupt the session at any time.

Fifteen aniridia patients (24 eyes) of both sexes, five females and 10 males, enrolled in the Spanish Association of Aniridia, participated voluntarily in the study. The ages of the participants ranged from 8 to 52 years old with an average age of 25.5 ± 15.6. The sample was divided into three groups of age: under 15 years old ($n = 7$; one female, six males), between 15 and 40 ($n = 4$; two females, two males) and over 40 years of age ($n = 4$; two females, two males).

A control group of 40 volunteers with neither aniridia nor evidence of symptoms or clinical dry eye disease participated in the present study. Controls average age of 29.0 ± 1.3 years were divided into the same age groups as the aniridia sample: under 15 years old ($n = 11$; three females, eight males), between 15 and 40 ($n = 19$; 10 females, nine males) and over 40 years of age ($n = 10$; four females, six males).

Trials

To know the dry eye symptomatology associated to the aniridia patients, the McMonnies test was performed. This is a 12-items test used as a screening tool in the dry eye clinic population. The dry eye diagnosis is based on the score obtained with the questionnaire (McMonnies et al. 1998). A score below 10 indicates a normal individual and between 10 and 20 is considered a marginal dry eye patient and a score above 20, a dry eye patient.

The tear collection was always performed after filling in the questionnaire. The volume of tears was collected using the Schirmer I test (Jones test). The Schirmer strip was located on the temporal tarsal conjunctiva of the lower lid for 5 minutes with the eyes closed. The volume of tears, as millimetres of moistened strip, was recorded and the wet part of strips were placed in Eppendorf tubes containing 500 μl of Ultrapure water and then the samples were frozen until processed for the high-pressure liquid chromatography (H.P.L.C) analysis.

After Schirmer I test, fluorescein was applied to evaluate break-up time (BUT) and corneal staining. To warrant repeatability of the staining procedure, a solution was prepared using a 10% concentration of sodium fluorescein diluted in saline (NaCl 0.9%). For each application, a micropipette with 5 μl of diluted fluorescein solution was applied to the inferior conjunctival sac, and 20 seconds later,

BUT was analysed using a chronograph to record the time to break the tear after the patient was asked to blink twice and maintain their eyes open. The cornea was divided into five areas to record the grading staining as proposed in the report of the National Eye Institute and Industry-Sponsored Dry Eye Workshop (2007), and the Cornea and Contact Lens Research Unit (CCLRU) grading scales were used.

Tear processing and H.P.L.C. analysis

The Schirmer strips collected and placed in the Eppendorf tubes were then strongly vortexed for 5 min. The strips were carefully rinsed, and the liquid in the tube was heated in a 98°C dry bath for 2 min to precipitate proteins. To pellet the proteins, tubes were submitted to centrifugation at 152 **g** for 30 min. Diadenosine polyphosphates are resistant to this treatment as demonstrated elsewhere (Pintor et al. 1991). Supernatants were chromatographed through SEP-PAK Accell QMA cartridges (Pintor et al. 1992). Briefly, 250 μl of the supernatants were passed through the cartridges which were previously equilibrated with 3 ml of ultrapure water. The elution of the nucleotides and dinucleotides was performed by applying 1 ml of a solution containing 0.2 M KCl and 0.1 M HCl. Prior to the injection in the H.P.L.C, samples were neutralized with KOH. These eluents were injected at a volume of 10–100 μl into the H.P.L.C for their analysis. Determination and quantification of diadenosine polyphosphates were performed by H.P.L.C, described by Pintor et al. (Pintor et al. 1992).

Statistical analysis

The presented data were analysed using the statistical software spss 15.0 (SPSS, Inc., Chicago, IL, USA). The values presented are the means $\pm$ SEM of the experiments performed. Normal distribution of variables was assessed by the Kolmogorov–Smirnov normality test. Sample size calculations were performed with statistical software (Granmo 6.0; Institut Municipal d'Investigacion Medica, Barcelona, Spain). With an accepted two-sided statistical significance threshold of 0.05 and a risk of 0.20 and taking into account a 2:1 group ratio (control group to Congenital Aniridia group), at least 16 subjects

were needed in the first group and 8 in the second, to find statistically significant differences.

Differences between the congenital aniridia and control group were estimated by the Student's *t*-test for independent samples between total groups. As sample sizes of aged groups were small, Mann–Whitney *U*-tests were used to compare values between the congenital aniridia and control aged groups. To correlate Schirmer values with nucleotides concentrations and McMonnies values, Pearson bivariate regression was used. $p < 0.05$ was considered statistically significant.

Results

All patients over 40 years old and one patient between 15 and 40 years of age were treated with amniotic membrane 12 months before the study. None of the patients of aniridia group had undergone limbal graft or any other corneal surgery prior to the study. Three of them (one of them belonged to 15–40 year old subgroup and two belonged to over 40 years old) were waiting for limbal graft surgery, and they were due to undergo surgery in the following 6 months after the study. The distribution of subjects and the parameters evaluated for the different groups are summarized in Table 1. Related to the volume of tears, it was considered a normal Schirmer test I above 5 mm of moistened strip (van Bijsterveld 1969).

As mentioned in materials and methods, both groups were divided into three different age groups. The scores of the McMonnies questionnaire revealed different values for each subgroup, as can be seen in Table 1. Just one subgroup in aniridia group and the same subgroup in control group that corresponding to over 40 years old showed a borderline dry eye score (McMonnies et al. 1998), while the others were not considered dry eye patients. The ANOVA test for the McMonnies scores did not show significant differences in any of the groups ($p = 0.033$).

Each subgroup of aniridia patients presented different percentages of incidence for the symptoms studied in the questionnaire. The group under 15 years of age, presented dryness and grittiness in 28%, meanwhile soreness, burning and scratchiness had a percentage of 14%. The second group, from 15 to 40, showed soreness, dryness and scratchiness with a percentage of 50% and grittiness and burning in 25%. The group over 40 years old presented all the symptoms with a percentage ranging from 25 to 75%. This group showed the highest percentage of incidence for the dryness symptom.

The volumes of tear secretion, BUT and corneal staining of the aniridia patients and control group are listed in Table 1. These results show normal tear secretion values for all the age groups, and there were no significant differences among the groups. Concerning the tear break-up time (BUT), we have found a decrease in the congenital aniridia group compared to the control group, ($p < 0.05$). On the other hand, we had statistically significant differences in the corneal staining, where the score was a third higher in the aniridia group than in controls ($p < 0.05$). Also, statistically significant differences were found between controls and aniridia patients in all age groups ($p < 0.05$).

H.P.L.C. analysis

The results obtained from the chromatographic profiles showed a gradual increase in the concentration of the substances with age, as can be seen in Fig. 1. The highest values, corresponding to the group of patients over 40 years of age, were the values for Ap_4A and Ap_5A, which were 11.18 $\pm$ 5.53 μM and 0.68 $\pm$ 0.17 μM, respectively, as can be seen in Table 1. The variations among the different groups of age were statistically significant
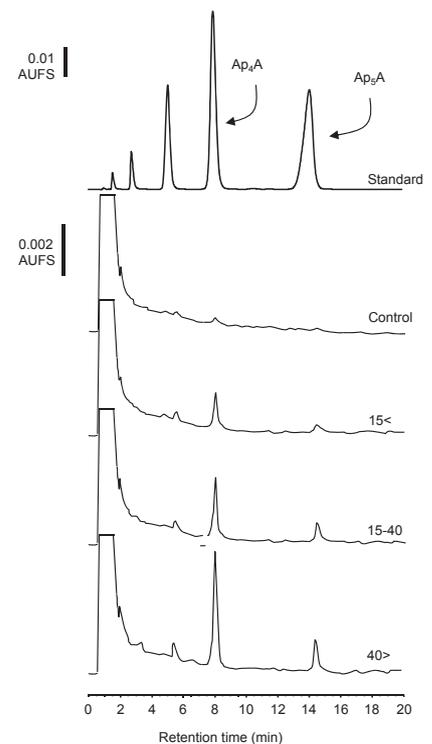

Fig. 1. Diadenosine polyphosphate detection in tears of aniridia patients. Chromatographic analysis of samples from aniridia patients showing the presence of two peaks identified as Ap_4A and Ap_5A when compared to a commercial standard (upper record). A control record (representing a healthy individual) can be compared with representative traces of aniridia patients grouped, according to methods, in three groups of age. Note that standards scale bar is different from the one for the samples. AUFS, absorbance units full scale.

Table 1. Summary of parameters evaluated for control and aniridia groups.

	Group	Control	Aniridia	p-Value
Age Mean $\pm$ SEM	Total	29.02 $\pm$ 1.30	25.25 $\pm$ 3.35	0.208
Schirmer (mm) Mean $\pm$ SEM	Total	19.93 $\pm$ 1.26	18.89 $\pm$ 1.96	0.664
BUT (seconds) Mean $\pm$ SEM	Total	16.34 $\pm$ 1.04	8.85 $\pm$ 2.55	0.002*
Corneal staining Mean $\pm$ SEM	Total	1.34 $\pm$ 0.15	3.18 $\pm$ 0.16	<0.001*
McMonnies Mean $\pm$ SEM	Total	8.30 $\pm$ 0.86	6.86 $\pm$ 1.52	0.396
Ap_4A (μM) Mean $\pm$ SEM	Total	0.15 $\pm$ 0.02	5.28 $\pm$ 0.77	<0.001*
Ap_5A (μM) Mean $\pm$ SEM	Total	0.91 $\pm$ 0.02	0.45 $\pm$ 0.03	<0.001*

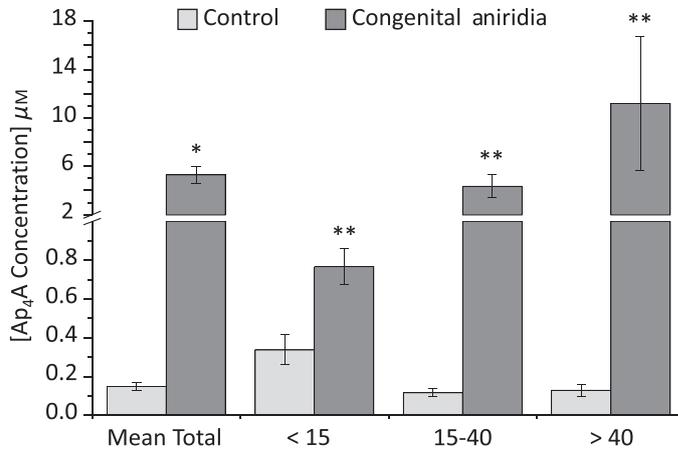*p-Value < 0.05 (Student's *t*-test independent samples; control vs. aniridia groups).

Fig. 2. Bar graph representing the concentration of diadenosine polyphosphate Ap₄A, expressed in μM, in tears of control subjects and aniridia patients for any group of age. After the chromatographic analysis of tear samples, dinucleotide concentrations were calculated by comparing the peaks in samples to the ones obtained in commercial standards of known concentrations (see Methods). The values represent the mean ± SEM. *p-Value < 0.01 (Student's *t*-test). **p-Value < 0.01 (Wilcoxon Mann–Whitney *U*-test).

(p = 0.038, ANOVA for Ap₄A and p = 0.003, ANOVA) for Ap₅A. Statistically significant differences were found among all the groups of aniridia patients and their control groups (p < 0.01, Wilcoxon Mann–Whitney *U*-test) as well as for the mean total group (p < 0.01, Student's *t*-test).

Comparing the values of Ap₄A and Ap₅A in the aniridia patients with their respective control age groups, these substances were several folds higher. Thereby, levels of Ap₄A and Ap₅A were 36 and 3.8 times more concentrated in aniridia patients for the group between 15 and 40 years old, respectively, and 86 and 17 times more for the group over 40 as is shown in Figs 2 and 3.

The Pearson's correlation coefficients for Schirmer, BUT and corneal staining scores and Ap₄A were −0.580 (p = 0.003), 0.235 (p = 0.137) and 0.692 (p = 0.001), respectively. The value of these coefficients indicate a moderate degree of correlation Ap₄A with Schirmer test and corneal staining but not with BUT.

Discussion

The present experimental work shows that patients with aniridia have elevated concentrations of diadenosine

polyphosphates, Ap₄A and Ap₅A, in their tears when compared to normal individuals. There is another study regarding a molecular analysis of the tear components in congenital aniridia (Ihnatko et al. 2013). These authors performed a proteomic tear analysis in aniridia patients revealing an elevated expression of bacteriostatic lactoferrin and a greatly elevated expression of VEGF in aniridia tears. Both findings are related to keratopathy and aniridia pathophysiology. It seems clear that further studies are needed to clarify the involvement of tear molecules in aniridia pathophysiology and their potential as targets of this severe ocular condition.

When we compared both dinucleotides, Ap₄A was always more concentrated than Ap₅A, which also happens in healthy individuals. Ap₄A and Ap₅A values in the aniridia patients who are over 40 years of age were higher than those found in younger aniridia patients. These results show a gradual increase of diadenosine polyphosphates levels with age, which could be linked to the development of the ocular surface disorders present in the aniridia patients.

The results obtained in the McMonnies tests indicate that, in general, the scores were not significantly different from normal individuals. Only the age group over 40 years old presented marginal dry eye. In the search for which symptoms were markedly different among the different groups, dryness was the one that presented the highest percentage of incidence, and it was the one that best correlated with the levels of dinucleotide. Moreover, when we compared the concentrations of the dinucleotides in the three age groups with dryness, the behaviour was similar.

Regarding the tear volume measured by the Schirmer test, it showed normal values of secretion in almost all the groups tested. While the age group between 15 and 40 presented an apparent lower value of secretion, there were no significant differences among the tested groups. As in other studies, there was no correlation between the symptoms and the volume of tear secretion (Bjerrum 1996; Schein et al. 1997; Hay et al. 1998). Regarding the tear secretion values, there is no evidence of an aqueodeficiency in the congenital aniridia patients, so we would expect normal levels of diadenosine
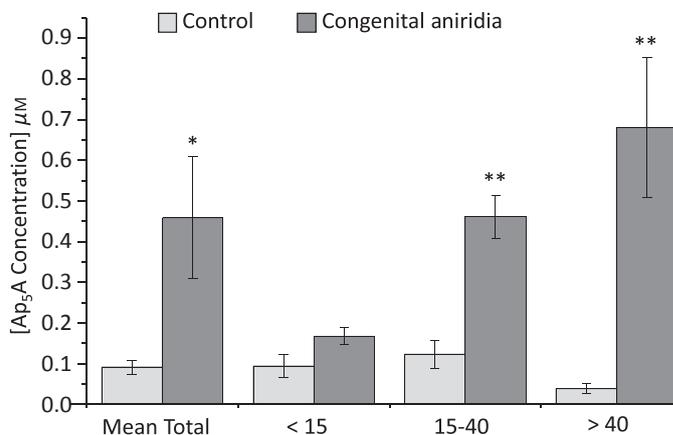

Figure 3. Bar graph representing the concentration of diadenosine polyphosphate Ap₅A, expressed in μM, in tears of control subjects and aniridia patients for any group of age. After the chromatographic analysis of tear samples, dinucleotide concentrations were calculated by comparing the peaks in samples to the ones obtained in commercial standards of known concentrations (see methods). The values represent the mean ± SEM. *p-Value < 0.01 (Student's *t*-test). **p-Value < 0.01 (Wilcoxon Mann–Whitney *U*-test).

polyphosphates (Pintor et al. 2002a,b; Peral et al. 2006), instead of the increased levels found in the present work. It is therefore reasonable to assume that there is at least one other factor in these patients that increases the levels of the diadenosine polyphosphates in their tears.

In 2004, Pintor et al. described, for the first time, the effect produced by the topical application of the diadenosine polyphosphate Ap$_4$A in the corneal wound healing of New Zealand white rabbits. This application increased the rate of corneal wound healing, enhancing epithelial integrity. In the mentioned study, Ap$_5$A did not affect the rate of re-epithelialization. *In Vitro* studies demonstrated that Ap$_4$A possesses the ability to accelerate cellular migration activating the MAPK cascade as well as the RhoA/ROK-I cascade, both of which are necessary for the cellular migration process. In contrast, Ap$_5$A would do the opposite (Mediero et al. 2006, 2008; Crooke et al. 2009).

The effect that diadenosine polyphosphates might present on corneal erosions and the fact that the patients with congenital aniridia suffer recurrent corneal erosions that increase in severity with age, suggest that the concentration of Ap$_4$A and Ap$_5$A found in the tears of aniridia patients is due to the progression of the limbal deficiency and the corneal epithelial fragility they present, more than to the dry eye these patients can suffer. Our results regarding BUT and corneal staining are the same as in other studies (Gomes et al. 1996; Jastaneiah & Al-Rajhi 2005).

Aniridia-associated keratopathy is caused by limbal stem cell deficiency, among other factors (Lee et al. 2008). It has been demonstrated that P2 purinergic receptors are associated with differentiation, proliferation and neurogenesis in neonatal and adult mouse olfactory epithelium (Jia et al. 2009; Jia & Hegg 2010). If something similar happens in the human eye, it could be the case that the increased levels of diadenosine polyphosphates act as a compensatory mechanism to stimulate the proliferation and differentiation of limbal stem cells which are deficient in aniridia disorder. This interesting possibility needs to be investigated in depth and will, indeed, be the matter of future studies.

A limiting factor of the present study was the small size of aniridia aged groups due to the condition of this rare disease. Nevertheless, the objective to divide the aniridia group in aged subgroups was not to get a final conclusion but to know the corneal keratopathy and dinucleotides progression with ageing. Although it was very difficult to grade the slit-lamp findings of the ocular surface in the whole sample, due to the discomfort the patients suffered, it is clear that the aniridia patients over 40 years of age showed more corneal staining than the other groups. Further investigation needs to be performed to correlate the concentration of the studied dinucleotides and the alteration of the others ocular surface disorders indicators as an osmolarity or mucin secretion in aniridia patients.

In conclusion, these stable molecules, Ap$_4$A and Ap$_5$A, increase in tears of aniridia patients compared with healthy subjects and are related with age and aniridia pathophysiology. Further studies are needed for an evaluation of this finding and usefulness of this potential biomarker in the development of new therapies related to symptomatology in aniridia patients.

References

van Bijsterveld OP (1969): Diagnostic tests in the Sicca syndrome. Arch Ophthalmol **82**: 10–14.

Bjerrum KB (1996): Test and symptoms in keratoconjunctivitis sicca and their correlation. Acta Ophthalmol Scand **74**: 436–441.

Carracedo G, Peral A & Pintor J (2010): Diadenosine polyphosphates in tears of Sjogren syndrome patients. Invest Ophthalmol Vis Sci **51**: 5452–5459.

Carracedo G, Guzman-Aranguez A, Loma P & Pintor J (2013): Diadenosine polyphosphates release by human corneal epithelium. Exp Eye Res **113**: 156–161.

Crooke A, Mediero A, Guzman-Aranguez A & Pintor J (2009): Silencing of P2Y2 receptor delays Ap4A-corneal re-epithelialization process. Mol Vis **15**: 1169–1178.

Edén U, Iggman D, Riise R & Tornqvist K (2008): Epidemiology of aniridia in Sweden and Norway. Acta Ophthalmol **86**: 727–729.

Fantes JA, Bickmore WA, Fletcher JM, Ballesta F, Hanson IM & van Heyningen V (1992): Submicroscopic deletions at the WAGR locus, revealed by nonradioactive *in situ* hybridization. Am J Hum Genet **51**: 1286–1294.

Glaser T, Walton DS & Maas RL (1992): Genomic structure, evolutionary conserva-tion and aniridia mutations in the human PAX6 gene. Nat Genet **2**: 232–239.

Gomes JA, Eagle RC Jr, Gomes AK, Rapuano CJ, Cohen EJ & Laibson PR (1996): Recurrent keratopathy after penetrating keratoplasty for aniridia. Cornea **15**: 457–462.

Hay EM, Thomas E, Pal B, Hajeer A, Chambers H & Silman AJ (1998): Weak association between subjective symptoms or and objective testing for dry eyes and dry mouth: results from a population based study. Ann Rheum Dis **57**: 20–24.

Ihnatko R, Edén U, Lagali N, Dellby A & Fagerholm P (2013): Analysis of protein composition and protein expression in the tear fluid of patients with congenital aniridia. J Proteomics **94**: 78–88.

Jastaneiah S & Al-Rajhi AA (2005): Association of aniridia and dry eyes. Ophthalmology **112**: 1535–1540.

Jia C & Hegg CC (2010): NPY mediates ATP-induced neuroproliferation in adult mouse olfactory epithelium. Neurobiol Dis **38**: 405–413.

Jia C, Doherty JP, Crudgington S & Hegg CC (2009): Activation of purinergic receptors induces proliferation and neuronal differentiation in Swiss Webster mouse olfactory epithelium. Neuroscience **163**: 120–128.

Kremer I, Rajpal RK, Rapuano CJ, Cohen EJ & Laibson PR (1993): Results of penetrating keratoplasty in aniridia. Am J Ophthalmol **115**: 317–320.

Lee H, Khan R & O'Keefe M (2008): Aniridia: current pathology and management. Acta Ophthalmol **86**: 708–715.

Mackman G, Brightbill FS & Optiz JM (1979): Corneal changes in aniridia. Am J Ophthalmol **87**: 497–502.

Mayer KL, Nordlund ML, Schwartz GS & Holland EJ (2003): Keratopathy in congenital aniridia. Ocul Surf **1**: 74–79.

McLennan AG (2000): Dinucleoside polyphosphates-friend or foe? Pharmacol Ther **87**: 73–89.

McMonnies C, Ho A & Wakefield D (1998): Optimum dry eye classification using questionnaire responses. Adv Exp Med Biol **438**: 835–838.

Mediero A, Peral A & Pintor J (2006): Dual roles of diadenosine polyphosphates in corneal epithelial cell migration. Invest Ophthalmol Vis Sci **47**: 4500–4506.

Mediero A, Guzman-Aranguez A, Crooke A, Peral A & Pintor J (2008): Corneal re-epithelialization stimulated by diadenosine polyphosphates recruits RhoA/ROCK and ERK1/2 pathways. Invest Ophthalmol Vis Sci **49**: 4982–4992.

Miras-Portugal MT, Gualix J, Mateo J, Diaz-Hernandez M, Gomez-Villafuertes R, Castro E & Pintor J (1999): Diadenosine polyphosphates, extracellular function and catabolism. Prog Brain Res **120**: 397–409.

Nelson LB, Spaeth GL, Nowinski TS, Margo CE & Jackson L (1984): Aniridia. A review. Surv Ophthalmol **28**: 621–642.

Nishida K, Kinoshita S, Ohashi Y, Kuwayama Y & Yamamoto S (1995): Ocular surface

abnormalities in aniridia. Am J Ophthalmol **120**: 368–375.

Peral A, Carracedo G, Acosta MC, Gallar J & Pintor J (2006): Increased levels of diadenosine polyphosphates in dry eye. Invest Ophthalmol Vis Sci **47**: 4053–4058.

Pintor J, Torres M & Miras-Portugal MT (1991): Carbachol induced release of diadenosine polyphosphates–Ap4A and Ap5A–from perfused bovine adrenal medulla and isolated chromaffin cells. Life Sci **48**: 2317–2324.

Pintor J, Diaz-Rey MA, Torres M & Miras-Portugal MT (1992): Presence of diadenosine polyphosphates–Ap4A and Ap5A–in rat brain synaptic terminals. Ca2 + dependent release evoked by 4-aminopyridine and veratridine. Neurosci Lett **136**: 141–144.

Pintor J, Carracedo G, Alonso MC, Bautista A & Peral A (2002a): Presence of diadenosine polyphosphates in human tears. Pflugers Arch **443**: 432–436.

Pintor J, Peral A, Hoyle CH, Redick C, Douglass J, Sims I & Yerxa B (2002b): Effects of diadenosine polyphosphates on tear secretion in New Zealand white rabbits. J Pharmacol Exp Ther **300**: 291–297.

Pintor J, Peral A, Pelaez T, Martin S & Hoyle CH (2003): Presence of diadenosine polyphosphates in the aqueous humor: their effect on intraocular pressure. J Pharmacol Exp Ther **304**: 342–348.

Pintor J, Bautista A, Carracedo G & Peral A (2004): UTP and diadenosine tetraphosphate accelerate wound healing in the rabbit cornea. Ophthalmic Physiol Opt **24**: 186–193.

Schein OD, Tielsch JM, Munoz B, Bandeen-Roche K & West S (1997): Relation between signs and symptoms of dry eye in the elderly. A population-based perspective. Ophthalmology **104**: 1395–1401.

Ton CC, Hirvonen H, Miwa H et al. (1991): Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region. Cell **67**: 1059–1074.

Tseng SC & Li DQ (1996): Comparison of protein kinase C subtype expression between normal and aniridic human ocular surfaces: implications for limbal stem cell dysfunction in aniridia. Cornea **15**: 168–178.

Whitson JT, Liang C, Godfrey DG, Petroll WM, Cavanagh HD, Patel D, Fellman RL & Starita RJ (2005): Central corneal thickness in patients with congenital aniridia. Eye Contact Lens **31**: 221–224.

[Not authors listed] (2007): Research in dry eye: report of the Research Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf **5**: 179–193.

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