# Meibomian gland dysfunction and keratopathy are associated with dry eye disease in aniridia

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/bjophthalmol-2017-310927).

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Received 26 June 2017
Revised 22 December 2017
Accepted 24 February 2018



**To cite:** Landsend ECS, Pedersen HR, Utheim ØA, *et al*. *Br J Ophthalmol* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/bjophthalmol-2017-310927

## ABSTRACT

**Aims** To investigate the aetiology and characteristics of dry eye disease (DED) in a Nordic cohort of patients with congenital aniridia.

**Methods** Thirty-four Norwegian and one Danish subject with congenital aniridia and 21 healthy controls were examined. All subjects underwent an extensive dry eye examination, including evaluation of meibomian glands (MGs) by meibography, measurement of tear production and tear film osmolarity and grading of vital staining of the ocular surface. Moreover, slit-lamp biomicroscopy was undertaken, including grading of aniridia-associated keratopathy (AAK).

**Results** Mean tear film osmolarity was significantly higher (314±11 mOsmol/L) in patients with aniridia compared with the healthy control group (303±11 mOsmol/L, p=0.002). Vital staining score was higher in the aniridia group (4.3±3.0) compared with healthy controls (2.4±1.6, p=0.02). The degree of staining correlated positively with the stage of AAK (r=0.44, p=0.008) and negatively with corneal sensitivity (r=−0.45, p=0.012). Number of expressible MGs was lower in aniridia subjects (2.9±1.6) than in controls (4.0±1.3, p=0.007). MG loss, staged from 0 to 3, was higher in the aniridia group than in the control group, both in upper eyelid (0.86±0.89 vs 0.10±0.31, p=0.001) and lower eyelid (0.94±0.73 vs 0.30±0.47, p=0.003). Computerised analyses showed thinning (p=0.004) and lower density (p<0.001) of the MGs compared with the healthy population.

**Conclusions** Patients with congenital aniridia demonstrate increased tear film osmolarity, ocular surface staining, loss of MGs and lower MG expressibility. We conclude that meibomian gland dysfunction and keratopathy are related to development of DED in aniridia.

## INTRODUCTION

Congenital aniridia is a rare disorder that affects the cornea, anterior chamber angle, iris, lens, retina and optic nerve. In Norway, the prevalence is 1:76 000.[1] Iris and macular hypoplasia are characteristic features present from birth in aniridia. Glaucoma, cataract and aniridia-associated keratopathy (AAK) are commonly associated progressive ocular disorders.[1 2] Aniridia is caused by mutations in the *PAX6* gene.[3]

There are very few studies on dry eye disease (DED) in aniridia despite its high prevalence in this disease.[4 5] Previously, the degree of ocular surface disease in aniridia was explained by low production of tears.[6] Subsequently Jastaneiah and Al-Rajhi suggested that DED in these patients is related to poor tear film quality.[5]

Importantly, the prevalence of DED in patients with aniridia correlates significantly with the severity of AAK.[5] Consequently, research on DED in aniridia serves a dual purpose: to find treatments to lessen the development and symptoms of DED and to reduce the severity and/or likelihood of progression of AAK. Further, this approach may prevent visual loss, as AAK is a sight threatening complication. Herein, we aim to investigate the aetiology and characteristics of DED in aniridia, with special attention to meibomian gland dysfunction (MGD), using advanced techniques developed in the DED field during recent years.

## MATERIALS AND METHODS

### Study subjects

This study included 35 patients (21 females) with congenital aniridia and 21 healthy controls (12 females). Both eyes were examined in all participants and data analyses performed for right and left eyes separately. Patients with aniridia were recruited through the patient organisation Aniridia Norway. The patients had previously been diagnosed with aniridia after ophthalmological investigations. The diagnosis was confirmed before inclusion in the study by presence of typical iris aplasia or hypoplasia, supported by macular hypoplasia and/or associated progressive ocular disorders. The study adhered to the tenets of the Declaration of Helsinki. All participants gave written consent after receiving oral and written information about the study.

### Dry eye examination

All subjects underwent an extensive dry eye examination (online supplementary table 1). The patients with aniridia completed the Ocular Surface Disease Index (OSDI) questionnaire.[7] Increasing index number indicates more severe DED.

### Measurement of tear production

The OCULUS Keratograph 5M (OCULUS, Wetzlar, Germany) was used to measure the tear meniscus height. Tear production was further evaluated with Schirmer I tear test using Schirmer Tear Test Strips (Haag-Streit UK, Essex, UK) and then Phenol Red Thread Test (Tianjin JingMing New Technological Development, Tianjin, China). Both tests were

performed without topical anaesthetic and while the participants had their eyes closed.

### Evaluation of tear film quality

Tear film osmolarity was measured by TearLab (TearLab, San Diego, California, USA). Tear film lipid layer was evaluated through interferometry on the keratograph. The test was denoted positive if the spread of the lipid layer could be observed, or negative, if not. Fluorescein break-up time (FBUT) was measured in a slit lamp biomicroscope using blue emission light and a yellow excitation filter after placing 5 $\mu$L of 2% fluorescein sodium from a single-use container (Minims Fluorescein Sodium 2%, Bausch & Lomb House, Surrey, UK) into the conjunctival sac.

### Assessment of ocular surface staining

Punctate fluorescein vital staining of the conjunctiva and cornea was evaluated according to the Oxford grading scheme.[8] Separate numerical grades were given for the cornea, temporal conjunctiva and nasal conjunctiva, respectively (online supplementary figure 1). A total score was calculated summing these three scores.

### Quantification of corneal sensitivity

Central corneal sensitivity was measured using the Cochet-Bonnet esthesiometer (Luneau Ophthalmology, Chartres, France) and by retracting the 60 mm long monofilament in 5 mm steps from its full length, until the subject felt the contact. Length of the extending filament was then recorded.

### Evaluation of meibomian gland expressibility and meibum quality

Meibomian gland (MG) expressibility was graded according to how many of the central five glands in lower eyelid that could express secretion when pressing a swab horizontally towards the lid with a moderate force. Quality of expressed secretion (meibum) was graded according to recommendations from The International Workshop on Meibomian Gland Dysfunction.[9]

### Subjective analyses of meibography images

The keratograph was used to acquire infrared MG images from upper and lower eyelids. MG loss in each eyelid was graded subjectively according to a four-point scale (meiboscore). The area of MG loss was defined as the percentage area of MG loss in relation to the total visible tarsal area and given a score from 0 to 3. A score of 0 represented 0%–25% area of MG loss; a score of 1: 26%–50%; a score of 2: 51%–75% and score of 3: >75%.

### Digital analyses of meibography images

The meibography images were digitally analysed in ImageJ (National Institutes of Health, Bethesda, Maryland, USA). To assess MG loss, both the tarsal area and the MG area were outlined (figure 1A and B). These areas were defined according to Pult *et al*.[10] Area of MG loss was calculated by subtracting the MG area from the tarsal area, and its relation to the total tarsal area was presented as percentage of MG loss (0%–100% scale).

Additional digital morphological analyses of the MGs were performed on the upper eyelids. The number of MGs with at least one angle greater than 45° was counted (figure 1C). Further, the MG thickness (width) was measured on the three most representative glands across the eyelid, and the MG length was determined for the three most prominent MGs. Moreover, the density of the MG area was assessed by measuring the gap between two adjacent MGs at three different locations (nasal, middle and temporal part of the eyelid) (figure 1D).

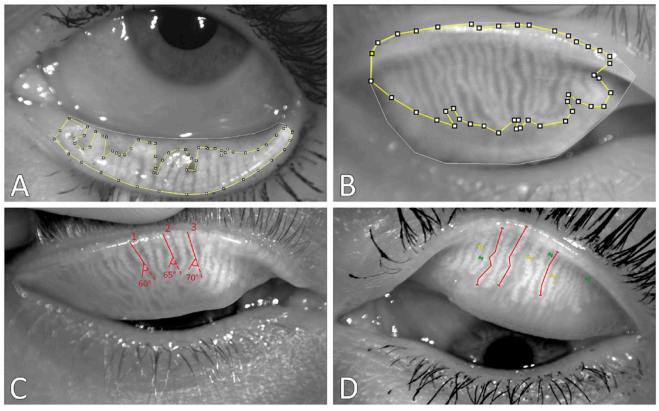


**Figure 1** Computerised analyses of infrared meibography images, demonstrating outlining of tarsal area (white line) and MG area (yellow, dotted line) in lower eyelid (A) and upper eyelid (B) and in (C) tortuous MGs with at least one angle greater than 45° and in (D) gland thickness (yellow segment), density (green segment) and length (red segment). MG, meibomian gland.

### Examination of eyelid appearance

Appearance of eyelids was evaluated based on the presence (yes/no) of the following alterations: debris, oedema and thickening, irregularity of the lid margin, hyperaemia, telangiectasia, occlusion of gland orifices at the lid margin and conjunctival papillae or hyperaemia.

### Clinical evaluation of the eye

Intraocular pressure was measured using the Icare TA01i rebound tonometer (Icare Finland Oy, Vantaa, Finland). AAK was graded according to a modification of Mackman's classification.[6] Stage 0 indicated no affect on the cornea; stage 1 referred to ingrowth of conjunctival cells and vessels in the peripheral cornea from less than 360° of the corneoscleral limbus; stage 2 related to 360° conjunctivalisation of the peripheral cornea, but clear central cornea and stage 3 described an affect as in stage 2, but included conjunctival vascularisation and/or stromal involvement of the central cornea (figure 2). Stage of cataract was graded according to the Lens Opacities Classification System III.[11]

### Quantitative and statistical analyses

Reported results are from right eyes unless otherwise specified and are presented as means±SD. Statistical analyses were performed in SPSS software V.23.0 (IBM, Armonk, New York, USA). Normal distribution was analysed using the Shapiro-Wilk test and Mann-Whitney *U*-test chosen for comparative analyses. Correlation analyses were performed with Spearman's rank correlation, except for correlations involving meiboscore, where Pearson product moment correlation was used. $P \leq 0.05$ were considered significant.

## RESULTS

### Differences in dry eye parameters in Aniridia and control group

Mean age in the aniridia group was 34.9±18.7 (range 9–72) years and in the control group 31.2±13.9 (range 19–65) years. The proportion of women was 63% and 57% in the aniridia and control group, respectively.

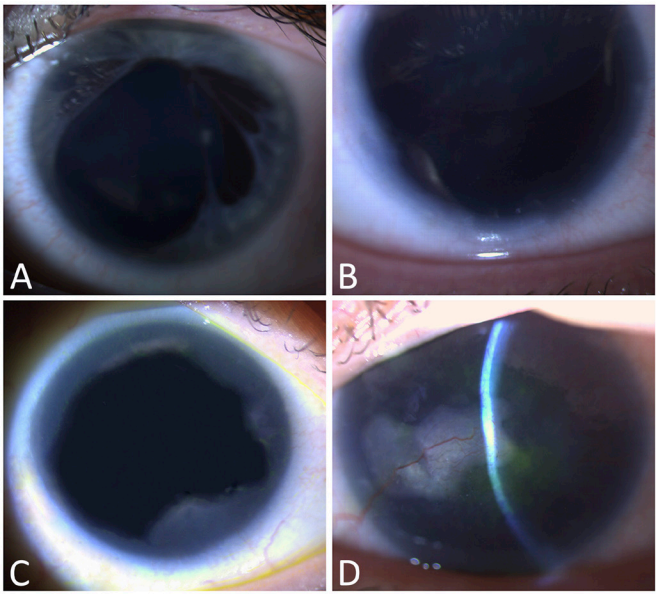


**Figure 2** Different stages of aniridia-associated keratopathy. (A), stage 0: no affect on the cornea. (B), stage 1: ingrowth of conjunctival cells and vessels in the peripheral cornea from less than 360° of the corneoscleral limbus. (C), stage 2: 360° conjunctivalisation of the peripheral cornea, but clear central cornea. (D), stage 3: affect as in stage 2, but including conjunctival vascularisation and/or stromal involvement of the central cornea.

Mean OSDI score in the aniridia group was 55.2±22.9, which indicates moderate to severe DED. Table 1 shows results from dry eye examination of right eyes.

**Table 1** Central clinical parameters in aniridia and control group (right eye)

| Parameter | Aniridia | Control | P values |
|---|---|---|---|
| Tear meniscus height (mm) | 0.30±0.11 | 0.24±0.07 | 0.049 |
| Schirmer tear test (mm) | 24.6±12.3 | 19.4±13.4 | 0.239 |
| Phenol red thread test (mm) | 21.4±8.8 | 18.2±8.5 | 0.239 |
| Tear film osmolarity (mOsmol/L) | 312±13 | 307±11 | 0.329 |
| Lipid layer interferometry (% positive) | 70.6 | 90.0 | 0.101 |
| Fluorescein break-up time (s) | 6.7±6.9 | 8.9±9.5 | 0.195 |
| Fluorescein vital staining | | | |
| Total score | 4.3±3.0 | 2.4±1.6 | 0.020 |
| Lateral conjunctiva | 1.0±1.1 | 0.9±0.8 | 0.904 |
| Cornea | 2.2±1.4 | 0.9±0.8 | 0.001 |
| Medial conjunctiva | 1.1±1.0 | 0.6±0.7 | 0.083 |
| Esthesiometry (mm) | 48.3±16.7 | 59.3±1.8 | 0.001 |
| Meibum expressibility | 2.9±1.6 | 4.0±1.3 | 0.007 |
| Meibum quality | 3.97±0.18 | 4.00±0.00 | 0.410 |
| Meiboscore upper eyelid | 0.86±0.89 | 0.10±0.31 | 0.001 |
| Meiboscore lower eyelid | 0.94±0.73 | 0.30±0.47 | 0.003 |
| Blink rate (blink/minute) | 15.7±10.9 | 19.0±10.1 | 0.065 |
| Intraocular pressure (mm Hg) | 17.6±6.9 | 15.0±3.0 | 0.186 |
| Stage of aniridia-associated keratopathy | 1.8±1.0 | 0.0±0.0 | <0.001 |
| Stage of cataract (LOCSIII) | | | |
| Nuclear | 1.5±1.2 | 1.5±0.5 | 0.247 |
| Cortical | 3.1±1.2 | 1.0±0.0 | 0.000 |
| Posterior subcapsular | 1.8±1.1 | 1.0±0.0 | 0.001 |

Data are mean±SD deviation. Group comparison with non-parametric method (Mann-Whitney U-test).
LOCSIII, Lens Opacities Classification System III.

**Table 2** Central clinical parameters in aniridia and control group (left eye, only significantly different results presented)

| Parameter | Aniridia | Control | P values |
|---|---|---|---|
| Schirmer tear test (mm) | 26.4±11.0 | 15.3±11.0 | 0.001 |
| Phenol red thread test (mm) | 28.4±14.7 | 17.3±6.9 | 0.023 |
| Tear film osmolarity (mOsmol/L) | 314±11 | 303±11 | 0.002 |
| Fluorescein vital staining | | | |
| Cornea | 2.0±1.3 | 1.1±0.9 | 0.019 |
| Esthesiometry (mm) | 49.2±14.4 | 59.3±1.8 | <0.001 |
| Meibum expressibility | 2.6±1.8 | 3.6±1.6 | 0.047 |
| Meiboscore upper eyelid | 0.8±0.8 | 0.1±0.3 | <0.001 |
| Meiboscore lower eyelid | 1.1±0.8 | 0.2±0.5 | <0.001 |
| Stage of aniridia-associated keratopathy | 1.9±1.0 | 0.0±0.0 | <0.001 |
| Stage of cataract (LOCSIII) | | | |
| Cortical | 2.8±1.2 | 1.0±0.0 | <0.001 |
| Posterior subcapsular | 1.5±0.9 | 1.0±0.0 | 0.009 |

Data are mean±SD deviation. Group comparison with non-parametric method (Mann-Whitney U-test).
LOCSIII, Lens Opacities Classification System III.

Tear meniscus height was significantly elevated in the aniridia group compared with the control group (p=0.049). We next evaluated fluorescein vital staining score. Total score (p=0.020) and corneal score (p=0.001) were increased in the aniridia compared with normal control groups. This finding demonstrates elevated epithelial damage at the ocular surface in patients with aniridia. Esthesiometry length values were lower in the aniridia than normal controls (p=0.001), confirming reduced corneal sensitivity in the aniridia cohort.

Multiple tests displayed differences in the function and anatomy of the MGs between the two groups (table 1). The number of expressible MGs was decreased in aniridia subjects (p=0.007), while meiboscore in upper eyelid and lower eyelid were raised (p=0.001 and p=0.003, respectively). Consequently, less amount of lipid material (meibum) could be expressed from MGs in the aniridia group, and MG loss was increased. Digital analyses revealed significantly reduced thickness (U=117, p=0.004) and density (U=121, p<0.001) in MGs in those with aniridia compared with controls. The MGs thus appear to be atrophic in aniridia.

Results from left eyes are presented in table 2, but only those significantly different in the two groups.

Both Schirmer (p=0.001) and phenol red test (p=0.023) results were increased in left eyes in the aniridia group. These findings agree with increased tear meniscus height found in right eye. Assessment of the tear film quality showed elevated tear film osmolarity in aniridia subjects (p=0.002).

In accordance with right eye findings, analyses of fluorescein vital staining demonstrated elevated corneal staining score in the aniridia group (p=0.019). Moreover, lower corneal sensitivity demonstrated in right eyes of patients with aniridia was supported by decreased esthesiometry length values in left eyes (p<0.001).

Results from left eyes revealed decreased MG expressibility (p=0.047) and increased meiboscore in upper and lower eyelid (p<0.001 and p<0.001, respectively), which are in agreement with the findings in right eye.

### Correlations between parameters in the aniridia group

Table 3 shows observed correlations between dry eye parameters and other clinical findings in right eye. Correlations were considered small, medium or large, according to Cohen J.[12]

**Table 3** Correlation analyses between parameters in aniridia group

| Parameter | Parameter | Correlation coefficient (large positive) | P values |
|---|---|---|---|
| Meiboscore upper lid | Irregularity lid margin | 0.819 | <0.001 |
| Age | Stage nuclear cataract | 0.663 | 0.001 |
| Corneal staining score | Stage AAK | 0.548 | 0.001 |
| Meiboscore upper lid | Oedema and thickening of eyelid | 0.532 | 0.007 |
| Meiboscore lower lid | Oedema and thickening of eyelid | 0.515 | 0.002 |
| | | **Correlation coefficient (large negative)** | |
| Age | Meibum expressibility | −0.672 | <0.001 |
| Corneal staining score | Esthesiometry | −0.565 | 0.001 |
| | | **Correlation coefficient (moderate positive)** | |
| Meiboscore lower lid | Conjunctival hyperaemia | 0.478 | 0.004 |
| Meiboscore upper lid | Telangiectasia eyelid | 0.472 | 0.02 |
| Total staining score | Stage AAK | 0.444 | 0.008 |
| MG thickness | Stage AAK | 0.407 | 0.048 |
| Age | Stage AAK | 0.346 | 0.042 |
| | | **Correlation coefficient (moderate negative)** | |
| Schirmer I test | MG loss percentage upper lid | −0.458 | 0.008 |
| Total staining score | Esthesiometry | −0.454 | 0.012 |

Correlation analyses of parameters in the aniridia group performed with Spearman's rank correlation. Exception for correlations involving meiboscore, using Pearson product moment correlation. Grouping into large positive and negative correlations and moderate positive and negative correlations.
AAK, aniridia-associated keratopathy; MG, meibomian gland.

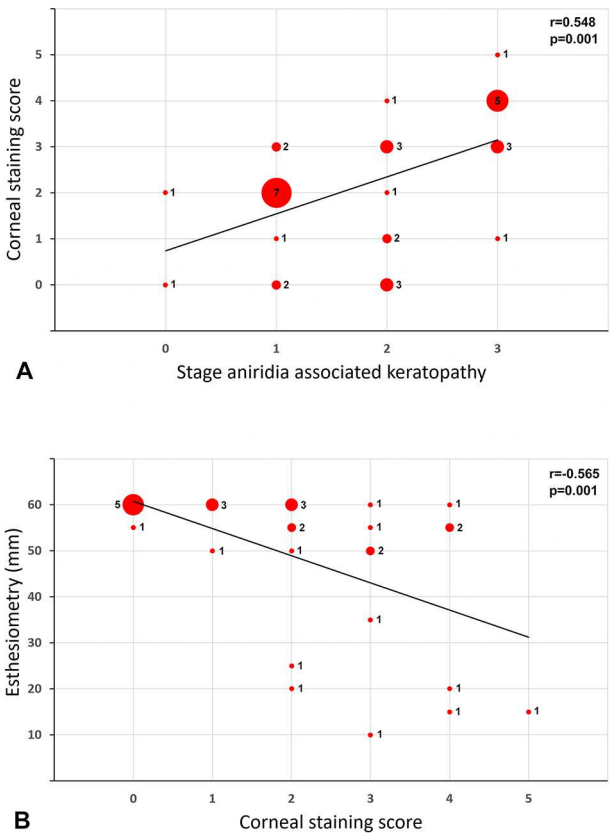


**Figure 3** Scatter plot of correlation between corneal staining score and stage of aniridia-associated keratopathy (A) and between corneal staining score and esthesiometry (B) in right eye. Patients per dot indicated with numbers.

Several correlations were found between meiboscore and structural alterations of the eyelid and are shown in table 3. A large negative correlation was observed between meibum expressibility and age ($p<0.001$). Less meibum was therefore secreted in older patients. Schirmer I tear test results correlated negatively and to a moderate extent, with MG loss calculated by digital analyses of meibography images of upper eyelid ($p=0.008$). Thus, tear production appeared to decrease with increasing loss of MG tissue in upper eyelid.

Increasing age correlated moderately with increasing severity of AAK ($p=0.042$), implicating progression of keratopathy with time. Further, epithelial damage at the ocular surface, indicated by fluorescein vital staining, was associated with stage of AAK. The strongest association was established to corneal staining, as a large positive correlation was found between corneal vital staining score and stage of keratopathy ($p=0.001$) (figure 3A). Corneal surface staining was also strongly connected to impaired corneal sensitivity, as corneal vital staining score correlated negatively with esthesiometry values ($p=0.001$) (figure 3B).

## DISCUSSION

Our study reveals higher severity of clinical findings consistent with DED in patients with aniridia compared with healthy individuals. Tear film osmolarity was elevated in the aniridia group and indicated mild to moderate DED. Furthermore, the OSDI score suggested moderate to severe disease. Loss of MG tissue was significantly greater in the aniridia group and MG expressibility significantly lower. Thus, MGD appears to be connected to development of DED in aniridia. Corneal fluorescein vital staining score was significantly higher than in control subjects and correlated with stage of AAK (positively) and corneal sensitivity (negatively). This finding suggests that corneal disease may act as a trigger for development of DED in patients with aniridia and conversely that DED could induce or exacerbate AAK.

The OSDI score has been considered a reliable tool for quantification of DED severity.[7] Mean score in the aniridia group indicated moderate to severe disease. The index, however, includes grading of visual quality and light sensitivity. Pathological features not involving the ocular surface, as cataract, might therefore influence the result.

Tear meniscus height was significantly higher in the aniridia group compared with control group in this study. In the right eye, neither phenol red thread test nor Schirmer I tear test values were significantly differently expressed in the two groups. On the contrary, both test values were significantly elevated in left eye. Taken together, the results suggest an elevated tear production in patients with aniridia.

It has been stated that tear film osmolarity is the single most effective measure of DED and a 'gold standard' in diagnosis.[13 14] In our study, tear film osmolarity was higher in the aniridia group compared with the control group, for both eyes,

but the difference was only significant for the left eye. Studies of osmolarity have suggested a cut-off value of 308 mOsmol/L for DED in general and 316 mOsmol/L for moderate to severe disease, when using the TearLab system.[14 15] In our cohort of patients with aniridia, mean tear film osmolarity was 312 mOsmol/L in the right eye and 314 mOsmol/L in the left eye, which indicate mild to moderate DED. The difference in significance between right and left eye may reflect asymmetric disease in patients with aniridia. The increase in osmolarity also indicates that even though there is an increase in tear volume, the composition of the tears is altered.

Reduced FBUT could imply tear film instability because of MGD.[16] FBUT was shorter in patients with aniridia than controls, but not significantly. However, early break-up of the tear film in aniridia might have been missed because of light sensitivity, reflexive blinking, nystagmus and irregular cornea.

Total fluorescein vital staining score and corneal staining score were significantly higher in the aniridia group compared with controls. Both scores were positively correlated with stage of AAK, with high significance. Thus, fluorescein vital staining might be the most characteristic finding of DED in aniridia. Its correlation to AAK might indicate that DED progresses parallel with keratopathy.

Decreased corneal sensitivity was shown in patients with DED.[17] Moreover, corneal hypoesthesia was correlated with degree of corneal fluorescein staining.[17 18] In accordance with these findings, the present aniridia cohort had significantly lower corneal sensitivity, with esthesiometry values correlating negatively with corneal vital staining score. Thus, in aniridia a decrease in corneal sensitivity occurs with an increase in corneal epithelial damage, suggesting a decline in corneal nerves. A reduction in number and density of sub-basal corneal nerves was observed in patients with DED.[19] The same was found in patients with aniridia.[20] Furthermore, it was reported that corneal nerves stimulate corneal epithelial growth.[21] In turn, corneal epithelial cells promote neurite extension and survival. These findings support the idea that reduced corneal nerve function in aniridia leads to epithelial damage typical of DED. This damage may lead to additional impairment of the corneal nerves, worsening of DED and probably AAK.

MGD is the major cause of evaporative DED.[16] MGD was apparent in our aniridia cohort, as the number of expressible MGs was significantly lower in this group compared with controls. On the other hand, meibum quality score was approximately the same. This result supports the belief that MGD in aniridia probably is a result of reduced production or delivery of meibum rather than changes in the quality of this lipid substance.

Our results showed significantly higher MG loss by subjective analyses and lower thickness and density of MGs by computerised analyses in aniridia subjects. This loss of glandular tissue could be an explanation for low MG expressibility and hence MGD, evident in the patients with aniridia. Lower meibum production may result in deficient tear film lipid layer, tear film instability, increased evaporation, hyperosmolarity and ocular surface alterations.[16] In our study, increased evaporation was indicated by elevated tear film osmolarity despite higher tear production than in control subjects. Raised tear production is probably a compensatory mechanism in MGD.[22] Hence, MGD appeared to be associated with DED in aniridia. However, no obvious cause of the MG tissue loss was apparent in our study. This may imply that MG atrophy could be a primary manifestation in aniridia. As *Pax6* function is evident in surface ectoderm in early embryonic development of the eye,[23] and MGs develop from the ectodermal sheet, a genetic explanation for MGD could be hypothesised.

The difference in percentage of MG loss by the objective method was not significant and might be explained by some of the tarsal area not being accessible on the images. This limitation had lower impact on the subjective grading, as the examiner could take into account that some part of the tarsal area was invisible in the image.

Our study demonstrates that patients with congenital aniridia manifest higher severity of clinical findings consistent with DED compared with healthy individuals. MGD is likely involved in the pathogenesis of DED in aniridia. Moreover, our results suggest that AAK and corneal hypoesthesia play a role. Thus, emphasis towards targeting these aetiologies may improve treatment for patients with aniridia, especially as DED might worsen AAK, a sight threatening condition.

**Acknowledgements** The authors gratefully acknowledge the support from Aniridia Norway, Dr Jon S Larsen's foundation, the Inger Holm's Memorial Fund grant, the Norwegian Association of the Blind and Partially Sighted and from the Norwegian Ophthalmological Society in terms of Professor Hjalmar Schiötz Memorial Fund grants and grants for PhD in Strabismus/ Paediatric Ophthalmology. Further, we acknowledge the support from the Department of Ophthalmology and Department of Medical Biochemistry at Oslo University Hospital, Norway and from Geir A. Qvale, Department of Ophthalmology, Oslo University Hospital, Norway in graphical editing.

**Contributors** ECSL, HRP, ØAU, RCB, TPU: Data acquisition and research execution. ECSL, HRP, ØAU, JX, MYA, BT, NL, DAD, RCB, TPU: Research design, data analysis and interpretation, manuscript preparation.

**Funding** The patient organisation Aniridia Norway, Dr Jon S Larsen's foundation, Inger Holm's memorial foundation, the Norwegian Association of the Blind and Partially Sighted, the Norwegian Ophthalmological Society and the Department of Ophthalmology and Department of Medical Biochemistry at Oslo University Hospital have financially supported part of this study. The funding organisations had no role in the design or conduct of this research.

**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** Ethics Committee approval was obtained from the Norwegian Regional Committees for Medical and Health Research Ethics (Application no. 2014/382).

**Provenance and peer review** Not commissioned; externally peer reviewed.



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