Current Eye Research

ISSN: 0271-3683 (Print) 1460-2202 (Online) Journal homepage: http://www.tandfonline.com/loi/icey20

Diagnosis of X-Linked Hypohidrotic Ectodermal Dysplasia by Meibography and Infrared Thermography of the Eye

Thomas Kaercher, Jasna Dietz, Christina Jacobi, Reinhold Berz & Holm Schneider

To cite this article: Thomas Kaercher, Jasna Dietz, Christina Jacobi, Reinhold Berz & Holm Schneider (2015) Diagnosis of X-Linked Hypohidrotic Ectodermal Dysplasia by Meibography and Infrared Thermography of the Eye, Current Eye Research, 40:9, 884-890, DOI: 10.3109/02713683.2014.967869

To link to this article: http://dx.doi.org/10.3109/02713683.2014.967869

Published online: 13 Oct 2014.

Current Eye Research, 2015; 40(9): 884–890
© Informa Healthcare USA, Inc.
ISSN: 0271-3683 print / 1460-2202 online
DOI: 10.3109/02713683.2014.967869

informa
healthcare

ORIGINAL ARTICLE

Diagnosis of X-Linked Hypohidrotic Ectodermal Dysplasia by Meibography and Infrared Thermography of the Eye

Thomas Kaercher[1], Jasna Dietz[2], Christina Jacobi[3], Reinhold Berz[4] and Holm Schneider[2]

[1]*Augenarztpraxis, Heidelberg, Germany,* [2]*Department of Pediatrics,* [3]*Department of Ophthalmology, University of Erlangen-Nürnberg, Germany, and* [4]*InfraMedic GmbH, Mörfelden, Germany*

ABSTRACT

Purpose: X-linked hypohidrotic ectodermal dysplasia (XLHED) is the most common form of ectodermal dysplasia. Clinical characteristics include meibomian gland disorder and the resulting hyperevaporative dry eye. In this study, we evaluated meibography and ocular infrared thermography as novel methods to diagnose XLHED.

Methods: Eight infants, 12 boys and 14 male adults with XLHED and 12 healthy control subjects were subjected to a panel of tests including the ocular surface disease index (OSDI), meibography and infrared thermography, non-invasive measurement of tear film break-up time (NIBUT) and osmolarity, Schirmer's test, lissamine green staining and fluorescein staining. Sensitivity and specificity were determined for single tests and selected test combinations.

Results: Meibography had 100% sensitivity and specificity for identifying XLHED. Infrared thermography, a completely non-invasive procedure, revealed a typical pattern for male subjects with XLHED. It was, however, less sensitive (86% for adults and 67% for children) than meibography or a combination of established routine tests. In adults, OSDI and NIBUT were the best single routine tests (sensitivity of 86% and 71%, respectively), whereas increased tear osmolarity appeared as a rather unspecific ophthalmic symptom. In children, NIBUT was the most convincing routine test (sensitivity of 91%).

Conclusions: Meibography is the most reliable ophthalmic examination to establish a clinical diagnosis in individuals with suspected hypohidrotic ectodermal dysplasia, even before genetic test results are available. Tear film tests and ocular surface staining are less sensitive in children, but very helpful for estimating the severity of ocular surface disease in individuals with known XLHED.

Keywords: Dry eye, hypohidrotic ectodermal dysplasia, infrared thermography, meibography

INTRODUCTION

X-linked hypohidrotic ectodermal dysplasia (XLHED, MIM 305100), the most frequent form of ectodermal dysplasia with an incidence of 1–2/100,000, is characterized by hypotrichosis, hypohidrosis and hypodontia. During childhood XLHED is a life-threatening disorder, because it may lead to severe hyperthermia.[1,2] Therefore, early diagnosis is of crucial importance to prevent avoidable calamities.

XLHED is caused by mutations in the gene *EDA* located on the X-chromosome. The gene product, ectodysplasin A, seems to play an important role as a signalling molecule mainly during fetal and neonatal development.[3] As in all X-linked disorders, hemizygous XLHED males are more consistently

Received 10 October 2013; revised 19 August 2014; accepted 14 September 2014; published online 8 October 2014

Correspondence: Dr. Holm Schneider, Department of Pediatrics, German Competence Center for Ectodermal Dysplasias, University of Erlangen-Nürnberg, Loschgestr. 15, 91054 Erlangen, Germany. Tel: +49 913 8533775. Fax: +49 9131 8533013. E-mail: holm.schneider@uk-erlangen.de

and severely affected than heterozygous XLHED females.

Although lack of hair and teeth are the more obvious clinical features, characteristic ocular signs in conjunction with a typical family history may also allow the diagnosis of XLHED. Besides the classical wrinkling and hyperpigmentation around the eyes, tear film disorders, lid alterations, ocular infections, and corneal disease have been reported in the literature[1,4,5] and can be detected already in young children with XLHED.[6] However, most established tests for ocular surface disease are too invasive for routine examinations of children. Furthermore, their sensitivity and specificity for XLHED is unknown. Meibography is not used routinely in dry eye patients, but has been recommended for ectodermal dysplasia patients.[7] There are no reports on the diagnostic use of infrared thermography, a fast and easy method to detect temperature variations at the ocular surface, in subjects with hypohidrotic ectodermal dysplasia.

The aim of this study was to evaluate meibography and ocular infrared thermography as novel methods to diagnose XLHED in subjects with or without dry-eye symptoms. These two methods were compared with a panel of routine tests to determine to what extent the eye could be an indicator for an underlying genetic disorder such as XLHED.

SUBJECTS AND METHODS

The ophthalmic examinations were performed on subjects with genetically confirmed XLHED during an annual meeting of the German-Swiss-Austrian patient support group in Germany or at the Competence Center for Ectodermal Dysplasias in Erlangen. The study (clinicaltrials.gov NCT 01308333) was approved by the ethics committee of the University of Erlangen-Nürnberg and was conducted according to the national regulations and GCP/ICH guidelines.

Fourteen male adults with XLHED were included (age range 18 to 58 years, mean 33.3 years) and compared with 6 healthy control subjects aged 23 to 47 years. Written informed consent was obtained from all participants. Additionally, 12 male children with XLHED (age range 6-13 years) together with 6 unaffected boys (age range 7 to 13 years) were investigated. Meibography and non-invasive measurement of tear film break-up time (NIBUT) were also performed in 8 infants with XLHED (between 0 and 35 months old). For all children, written informed consent was given by the parents.

Adults and older children were asked to fill in the ocular surface disease index (OSDI) questionnaire. For younger children, the questions if applicable were answered by their parents. The OSDI score was calculated according to Schiffman et al.[8] A score above 12 points was taken as an indicator for dry eye disease.

Slit lamp examinations were performed using a DigiPro3 system (bon Optic, Lübeck, Germany). For the younger children a hand-held slit lamp (Zeiss, Jena, Germany) was used. Then, a battery of tear film tests was applied starting with non-invasive tests and ending with the most invasive Schirmer-I test.

Tear film stability was assessed by repeated non-invasive measurement of tear film break-up time using a Tearscope Plus (Keeler Instruments, Broomall, PA).[9] Time until the first dry spot after one blink was determined, and the mean of at least two values was used for analysis. A break-up time below 10 s was interpreted as pathologic result.[7]

Tear osmolarity was measured with the Tearlab device (Bonoptic).[10] A tear sample of 50 nl was taken under the slit lamp from the temporal tear meniscus. The higher value of the two eyes was used for analysis. Osmolarity above 311 mOsml/L was considered as abnormal.[10]

Meibography, the transillumination of the lid, was used to detect a lack of Meibomian glands.[11,12] Only the lower eyelid was examined. After eversion of the lid, the architecture of the Meibomian gland was seen under the slit lamp (Figure 1). Fewer than 10 meibomian gland ducts/openings per eyelid were considered abnormal.

The ocular surface was investigated with two dyes. Lissamine green indicates areas which are not covered adequately by mucous components.[13] A cut-off value of 3 points according to van Bijsterveld[14] was taken to distinguish between normal and affected subjects. Fluorescein (5 µl of a 2% solution) as a marker for the uncovered intercellular spaces was applied especially to the corneal epithelium. A grading above 2 points is indicative for dry eye disease.

The Schirmer-I test was performed by placing a standard filter paper in the temporal lower lid and measuring how much of it was moisturized during 5 min. The lower value of the two eyes was used for analysis. A moisturized paper length under 10 mm was interpreted as pathologic result.

For routine ophthalmic tests, receiver operating characteristic (ROC) curves were used to re-evaluate given cut-off values in our study population.

Infrared thermography[15] to assess the tear film layer was performed by a representative of Infra-Medic GmbH (Mörfelden-Walldorf, Germany). Standardized thermal measurement was taken from the ocular surface and the lid apparatus with open and closed lid. The CE-approved infrared equipment included a Jenoptik Vario Cam HR infrared camera with high accuracy and sensitivity (spatial resolution 384×288 thermal sensor elements, thermal resolution better than 0.3 mK). Evaluation and interpretation of the thermal measurement data was done with Infra-Medic's expert software EXAM.

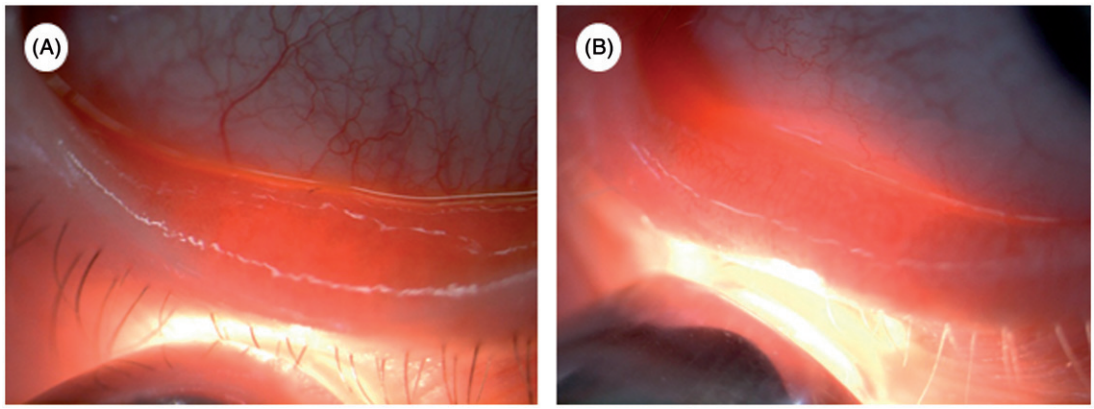

FIGURE 1 Meibography. Meibography was evaluated in 14 adults and 11 children with XLHED and in 5 control subjects. (A) Normal transillumination pattern in a healthy subject with numerous meibomian gland ducts. (B) Lack of meibomian glands in an individual with XLHED.

TABLE 1 Clinical characteristics of the study subjects.

Group	Age (years)	Body height (cm)	Weight (kg)	History of dry eye problems
Infants with XLHED ($n = 8$)	1.90 ± 1.05	83.56 ± 15.71	10.83 ± 3.37	2/8
Other children with XLHED ($n = 12$)	9.42 ± 2.47	140.25 ± 20.41	31.92 ± 13.83	4/12
	$p = 0.75$	$p = 0.74$	$p = 0.65$	$p = 0.25$
Control children ($n = 6$)	9.83 ± 2.64	143.50 ± 16.68	34.98 ± 12.60	0/6
Adults with XLHED ($n = 14$)	33.29 ± 11.08	178.50 ± 9.35	77.82 ± 13.69	10/14
	$p = 0.95$	$p = 0.31$	p = 0.05	$p = 0.01$*
Control adults ($n = 6$)	34.00 ± 10.35	184.67 ± 5.89	77.33 ± 5.47	0/6

*statistically significant.

RESULTS

Although only 10 of 14 male adults with XLHED and 4 of 12 affected male children reported a history of dry eye problems (Table 1), symptoms of xerophthalmia were more frequent, as indicated by OSDI scores >12 in 12 adults and 5 children with XLHED. Meibography was evaluated in all 14 affected adults, in all 8 infants, but only in three of the 12 other affected children, because the parents of the remaining subjects considered it as too invasive for children. Results were compared with those of five healthy control subjects. In this restricted XLHED study population, however, meibography always revealed a significant lack or complete absence of meibomian glands (Figure 1) and thus had 100% sensitivity and specificity for identifying XLHED. Only up to three meibomian gland ducts were detected in the lower eyelid of XLHED patients, whereas all control subjects showed more than 25 gland ducts/openings per eyelid. The mean number of meibomian gland openings in the lower eyelid of infants with XLHED was 0.6.

Infrared thermography of the eye, which could be evaluated in all individuals with XLHED, also yielded a typical pattern for affected subjects (Figure 2). Both in children and adults with XLHED, the average peripheral corneal temperature was lowered to 33 °C. The central region of the cornea was even colder and the difference between centre and periphery of the cornea rather large. The cold area in the central part of the ocular surface had a diameter of several millimeters. Frequently we observed a steep temperature gradient to the periphery of the eye (Figure 2). Control subjects showed a higher temperature of the peripheral zone and a much smaller central zone, the temperature of which was only slightly lower (34 °C). In general, however, infrared thermography was less sensitive (sensitivity of 85.7% for adults and 66.7% for children) than meibography or combinations of established routine tests, e.g. OSDI + NIBUT (Table 2).

In adult subjects, infrared thermography and OSDI had a sensitivity of 85.7% each for identifying XLHED, whereas increased tear osmolarity appeared as a rather unspecific ophthalmic symptom. NIBUT showed a moderate sensitivity of 71.4% with a specificity of 100%. The remaining routine tests (Lissamine green and fluorescein staining, Schirmer-I test) were of minor sensitivity. In children, NIBUT

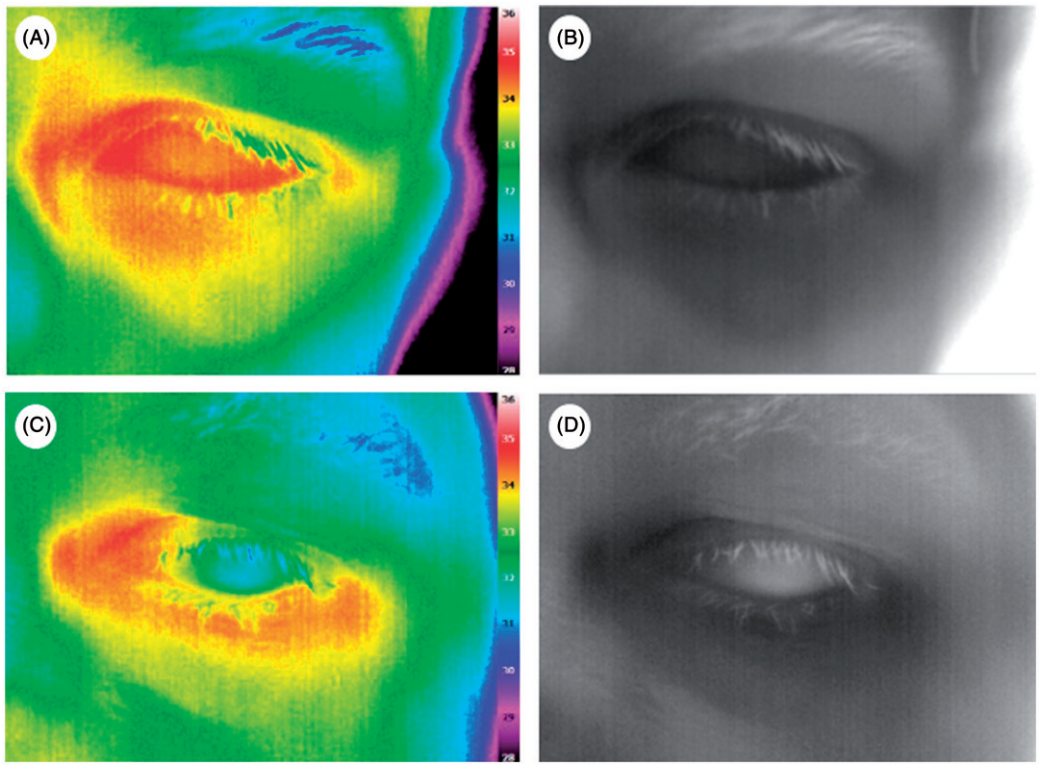

FIGURE 2 Infrared thermography of the eye. Infrared thermography was evaluated in all 14 adults and 12 children with XLHED and in 5 control subjects. Evaporation of tears which reduces the temperature of the ocular surface is indicated by local color changes. (A, B) Normal infrared thermography pattern. (C, D) Infrared thermography pattern of an individual with XLHED showing a relatively cold eye with an unusual temperature gradient between centre and periphery (temperature decline towards the cornea) and a large cold region in the central part of the ocular surface. Color bars in A and C indicate the temperature range from <28 °C (black) to 35 °C (red). Figure 2 can be viewed in color online at http://informahealthcare.com/cey.

TABLE 2 Detection of X-linked hypohidrotic ectodermal dysplasia by ocular screening tests.

Test	Sensitivity (%) / CI_w	Specificity (%) / CI_w
Meibography		
Children + Infants	100/67.6–100	No controls tested
Adults	100/78.5–100	100/56.6–100
NIBUT		
Children	72.7/46.6–89.1	100/61.0–100
Adults	71.4/45.3–88.3	100/61.0–100
OSDI		
Children	41.7/19.4–68.1	100/61.0–100
Adults	85.7/60.1–96.0	100/61.0–100
Infrared thermography		
Children	66.7/39.1–86.2	Only one control tested
Adults	85.7/60.0–100	100/51.0–100
Osmolarity		
Children	66.7/30.3–90.5	100/56.6–100
Adults	76.9/49.8–91.9	100/61.0–100
Schirmer's Test		
Adults	66.7/39.1–86.2	100/61.0–100
Lissamine green staining		
Children	33.7/6.3–79.4	100/43.9–100
Adults	42.9/21.4–67.5	100/61.0–100
OSDI + NIBUT		
Children	94.7/56.9–96.5	100/28.4–100
Adults	97.9/78.1–99.5	100/27.6–100

Confidence intervals were calculated using Wilson's interval.[30]

proved to be the only minimally invasive routine test with relatively high sensitivity (Table 2). Tear film break-up times were markedly reduced already in infants with XLHED (values between 2 and 12.5 seconds) compared to the literature.[16]

Table 3 gives the results of ROC analyses suggesting cut-offs for the routine tests which differ slightly from those reported in the literature. NIBUT with a cut-off of 18 s was found to be the best single test for adults with a sensitivity and specificity of 100% each (AUC = 1.00). For older children, OSDI with a threshold of 3 points was considered superior for identifying XLHED with a sensitivity of 91.7% and a specificity of 100% (AUC = 0.972).

DISCUSSION

This study focussed on the known association between XLHED and ocular surface disease. In relation to potentially life-threatening deficits of XLHED patients such as hypohidrosis, xerophthalmia is certainly a minor problem. The eye, however, may serve as an indicator, as it is easily accessible for diagnostic tests and could help to detect multiorgan diseases, including XLHED, before other test results are available.

TABLE 3 Results of ROC curve analyses for standard ophthalmic tests. NIBUT and OSDI are the best single tests for identifying XLHED both in children and adults.

Test	Cut-off from ROC curves	Sensitivity (%)	Specificity (%)	AUC
NIBUT				
Children	13 s	90.91	100.00	0.970
Adults	18 s	100.00	100.00	1.000
OSDI				
Children	3 points	91.67	100.00	0.972
Adults	7 points	92.86	100.00	0.976
Osmolarity				
Children	306 mOsmol/l	66.67	100.00	0.733
Adults	305 mOsmol/l	84.21	90.91	0.852
Schirmer's Test				
Adults	20 mm	83.33	100.00	0.958
Lissamine green staining				
Adults	2.5 points	85.71	100.00	0.988

A well-defined group of male individuals with genetically confirmed XLHED was investigated. As ocular manifestations of XLHED are known to be present already in early childhood,[6] the broad age spectrum of our cohort should not preclude the evaluation of particular diagnostic approaches for "screening" purposes. In addition, the course and severity of ocular surface disease might be addressed. Statistical analysis, however, was limited by the relatively small size of our cohort due to the rarity of XLHED.

To identify individuals with dry eye symptoms, the OSDI is the least invasive and easiest test to use, which may also apply to children. A questionnaire filled in by parents may provide very useful data, although it must be taken into account that children tend to report fewer dry eye symptoms than adults with similar severity of xerophthalmia.[17] This is also reflected by the results of our ROC analysis suggesting an OSDI cut-off in children of only three points. As a second step, meibography, the transillumination of the eyelid to visualize meibomian glands, seems to be the method of choice to recognize a congenital absence or a severe lack of meibomian glands in the lid, which is typical for XLHED, as specific reason for xerophthalmia. Meibography proved even more sensitive than tear film tests for detecting XLHED. This is consistent with the findings of a previous study on genetically unclassified ectodermal dysplasia patients.[5] As meibography always requires eversion of the lid, it appears less suitable for routine examinations of young children. In children with suspected hypohidrotic ectodermal dysplasia, however, it should nevertheless be considered and suggested to the parents as the best ophthalmic method to confirm the diagnosis. For young children, meibography with a hand-held device as reported by Arita et al.[18] or with an infrared video camera as described by Pult and Riede-Pult[19,20] may be preferable, which also requires eversion of the lid but avoids contact with a second instrument.

The severity of XLHED-associated ocular surface disease seems to depend mainly on the number of residual meibomian glands.[21] Functional importance of these glands was first discussed with respect to another form of ectodermal dysplasia: the EEC (ectrodactyly, ectodermal dysplasia, clefting) syndrome.[22] Later reports described the absence or reduced numbers of meibomian glands for anhidrotic and hypohidrotic ectodermal dysplasia.[23,24] The severe consequences of this anomaly are also evident in the Tabby mouse model of XLHED,[25] where ocular surface problems occur already at an age of nine weeks.

Infrared thermography of the eye visualizes the coverage of the ocular surface by an intact tear film and provides useful information about temperature gradients at the ocular surface. In XLHED subjects, the corneal temperature was found to be markedly reduced. This can be indicative for a higher evaporation as a sign of the underlying tear film disorder, while a small central zone, which is only slightly colder than the periphery, suggests an intact tear film. The typical thermography pattern described in this study may thus allow an easy and completely non-invasive distinction between XLHED subjects and healthy controls. Thermography has been used as a simple and quick screening method also in patients with other ocular conditions leading to xerophthalmia[26,27] and proved to identify dry eyes reliably. In our study, however, the method was less sensitive than meibography, particularly in children.

NIBUT can be measured easily and was found to be the most reliable among the established diagnostic approaches for adults. In infants with XLHED, tear film break-up times were much lower than those reported by Isenberg et al.[16], indicating that this test may help to detect XLHED already soon after birth.

OSDI and NIBUT may be recommended both for children and adults as the most suitable routine tests based on predefined thresholds or our ROC analysis. All tests that involve the collection of tears, e.g. measurement of tear osmolarity, are more invasive. The same applies to the use of dyes such as Lissamine green or fluorescein. For diagnostic purposes combinations of several tests have been recommended,[7] among which a combination of OSDI and NIBUT appears to be sufficient to detect XLHED with very high sensitivity.

Since the prevalence of XLHED is very low, while dry eye symptoms are common, further studies will aim at comparing XLHED patients with subjects suffering from xerophthalmia due to other conditions.

In conclusion, tear film tests and ocular surface staining certainly provide important information on severity and course of ocular surface disease in individuals with known XLHED, but meibography

is the ophthalmic test of choice in cases, where the diagnosis has not yet been established or expensive genetic tests are not available. This would facilitate prevention of life-threatening hyperthermia and possibly allow early therapeutic intervention, e.g. administration of an ectodysplasin A replacement protein as currently being evaluated in clinical trials (www.clinicaltrials.gov NCT01775462 and NCT01992289). Furthermore, if meibomian glands are missing, the use of lipid-containing tear substitutes is indicated to prevent corneal damage and impairment of vision.[28,29]

ACKNOWLEDGMENTS

We thank all the patients and healthy volunteers who participated in the study. Assistance of N. Eby and E. Kaercher in the statistical evaluation is gratefully acknowledged.

DECLARATION OF INTEREST

This study was funded partially by the German-Swiss-Austrian ectodermal dysplasia patient support group and by a grant from Edimer Pharmaceuticals (Cambridge, U.S.). R. Berz is the CEO of InfraMedic GmbH. H. Schneider has consultancy relationships and/or has received research funding from Edimer Pharmaceuticals, Novartis, Gerson Lehrman Group, pervormance GmbH, and the German-Swiss-Austrian ectodermal dysplasia patient support group in the area of potential treatments of hypohidrotic ectodermal dysplasia.

REFERENCES

1. Clarke A, Phillips DI, Brown R, Harper PS. Clinical aspects of X-linked hypohidrotic ectodermal dysplasia. Arch Dis Child 1987;62:989–996.
2. Blüschke G, Nüsken KD, Schneider H. Prevalence and prevention of severe complications of hypohidrotic ectodermal dysplasia in infancy. Early Hum Dev 2010;86: 397–399.
3. Gaide O, Schneider P. Permanent correction of an inherited ectodermal dysplasia with recombinant EDA. Nat Med 2003;9:614–618.
4. Wilson FM, Grayson M, Pieroni D. Corneal changes in ectodermal dysplasia. Am J Ophthalmol 1973; 75:17–27.
5. Kaercher T. Ocular symptoms and signs in patients with ectodermal dysplasia syndromes. Graefes Arch Clin Exp Ophthalmol 2004;242:495–500.
6. Dietz J, Kaercher T, Schneider AT, Zimmermann T, Huttner K, Johnson R, Schneider H. Early respiratory and ocular involvement in X-linked hypohidrotic ectodermal dysplasia. Eur J Pediatr 2013;172:1023–1031.
7. Lemp M. The 2007 report of the dry eye workshop (DEWS). Ocular Surface 2007;5:67–204.
8. Schiffman R, Christianson M, Jacobsen G, Hirsch DJ, Reis BL. Reliability and validity of the ocular surface disease index. Arch Ophthalmol 2000;118:615–621.
9. Yokoi N, Takehisa Y, Kinoshita S. Correlation of the tear lipid layer interference patterns with the dignosis and severity of dry eye. Am J Ophthalmol 1996;122: 818–824.
10. Lemp MA, Bron AJ, Baudouin C, Benitez del Castillo JM, Geffen D, Tauber J, et al. Tear osmolarity in the diagnosis and management of dry eye disease. Am J Ophthalmol 2011;151:792–798.
11. Robin JB, Jester JV, Nobe J, Nicolaides N, Smith RE. In vivo transillumination biomicroscopy and photography of meibomian gland dysfunction. A clinical study. Ophthalmology 1985;92:1423–1426.
12. Yokoi N, Komuro A, Yamada H, Maruyama K, Kinoshita S. A newly developed video-meibography system featuring a newly designed probe. Jpn J Ophthalmol 2007;51:53–56.
13. Norn M. Lissamine green. Vital staining of cornea and conjunctiva. Acta Ophthalmol (Copenh) 1973;51:483–491.
14. van Bijsterveld O. Diagnostic tests in the sicca syndrome. Arch Ophthalmol 1969;82:10–14.
15. Mori A, Oguchi Y, Okusawa Y, Ono M, Fujishima H, Tsubota K. Use of high-speed, high-resolution thermography to evaluate the tear film layer. Am J Ophthalmol 1997;124:729–735.
16. Isenberg SJ, Del Signore M, Chen A, Wei J, Guillon JP. The lipid layer and stability of the preocular tear film in newborns and infants. Ophthalmology 2003;110: 1408–1411.
17. Han SB, Yang HK, Hyon JY, Hwang JM. Children with dry eye type conditions may report less severe symptoms than adult patients. Graefes Arch Clin Exp Ophthalmol 2013; 251:791–796.
18. Arita R, Itoh K, Maeda S, Maeda K, Amano S. A newly developed noninvasive and mobile pen-shaped meibography system. Cornea 2013;32:242–247.
19. Pult H, Riede-Pult BH. Non-contact meibography: keep it simple but effective. Cont Lens Anterior Eye 2012; 35:77–80.
20. Pult H, Riede-Pult BH. Comparison of subjective grading and objective assessment in meibography. Cont Lens Anterior Eye 2013;36:22–27.
21. Nichols K, Foulks G, Bron A, Glasgow B, Dogru M, Tsubota K, et al. The international workshop on Meibomian gland dysfunction: executive summary. Invest Ophthalmol Vis Sci 2011;52:1922–1929.
22. Baum IJ, Bull MJ. Ocular manifestations of the ectrodactyly, ectodermal dysplasia, cleft lip-palate syndrome. Am J Ophthalmol 1974;78:211–216.
23. Ekins MB, Waring GO. Absent Meibomian glands and reduced corneal sensation in hypohidrotic ectodermal dysplasia. J Pediatr Ophthalmol Strabismus 1981;18: 44–47.
24. Allali J, Roche O, Monnet D, Brezin A, Renard G, Dufier JL. Anhidrotic ectodermal dysplasia: congenital ameibomia. J Fr Ophtalmol 2007;30:525–528.
25. Cui CY, Smith JA, Schlesinger D, Chan CC. X-linked anhidrotic ectodermal dysplasia disruption yields a mouse model for ocular surface disease and resultant blindness. Am J Pathol 2005;167:89–95.
26. Gonnermann J, Klein JP, Klamann MKJ, Maier AK, Pleyer U, Joussen AM, Bertelmann E. Dry eye symptoms in patients after eyelid reconstruction with full-thickness eyelid defects: using the Tomey TG-1000 thermographer. Ophthalmic Res 2012;48:192–198.
27. Gonnermann J, Maier AK, Klein JP, Bertelmann E, Pleyer U, Klamann MK. Evaluation of ocular surface

temperature in patients with pterygium. Curr Eye Res 2014;39:359–364.
28. Ota Y, Matsumoto Y, Dogru M, Uchino Y, Endo K, Tsubota K. Management of evaporative dry eye in ectrodactyly-ectodermal dysplasia-clefting syndrome. Optom Vis Sci 2008;85:E795–E801.
29. Kaercher T, Wickenhäuser A, Lachmann A, Welt R. The use of a lipid-containing tear substitute in patients with ectodermal dysplasia. Spektrum Augenheilkd 2004;18: 146–152.
30. Brown LD, Cai T, DasGupta A. Interval estimation for a binomial proportion. Statistical Sci 2001;16:101–133.