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ORIGINAL ARTICLE

Early respiratory and ocular involvement in X-linked hypohidrotic ectodermal dysplasia

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Abstract X-linked hypohidrotic ectodermal dysplasia (XLHED; ectodysplasin deficiency) has been classically described as affecting hair, sweat glands, and dentition. What may be underappreciated is the effect ectodysplasin deficiency has on glands surrounding the airways and eyes and the resulting chronic health issues. In this study, 12 male children (age range 6–13 years) and 14 male adults with XLHED (18–58 years of age) were investigated by pulmonary function tests, measurement of fractional exhaled nitric oxide, and by ophthalmologic assessments. Twelve healthy individuals (six children, six adults) served as controls. Signs of airway constriction and inflammation were detected in eight children with XLHED, including the youngest subject, and in ten adult XLHED patients. Increased tear osmolarity, reduced tear film break-up time, and other ocular abnormalities were also present at an early age. Five of 12 XLHED subjects not reporting a history of asthma and 7 of the 12 patients not reporting a history of dry eye issues showed at least two abnormal test results in the respective organ system. The presence of residual sweat ducts, suggestive of partial ectodysplasin gene expression, correlated with milder disease in two XLHED subjects with mutations affecting the collagen-like domain of ectodysplasin. *Conclusion*: The high prevalence of asthma-like symptoms in XLHED patients as young as 6 years and a similar prevalence of dry eye problems indicate that screening evaluation, regular monitoring, and consideration of therapeutic intervention should begin in early childhood.

Keywords Ectodermal dysplasia · Ectodysplasin · Seromucous glands · Inflammation · Asthma · Dry eye

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Introduction

X-linked hypohidrotic ectodermal dysplasia (XLHED [MIM 305100], ectodysplasin deficiency), the most common of the ectodermal dysplasias, is classically described as affecting hair, sweat glands, and dentition [28]. The diagnostic hallmarks are hypotrichosis, hypohidrosis, and oligodontia. Patients display typical facial features with fine hair, sparse eyelashes and eyebrows, frontal bossing, wrinkles under the eyes, prominent lips, and abnormal teeth, and they suffer from disturbed thermoregulation [7, 11]. What may be underappreciated, however, is the effect ectodysplasin deficiency has on exocrine glands of the respiratory and ocular systems and the resulting chronic health issues. Seromucous glands were found to be lacking in the airways of patients with anhidrotic ectodermal dysplasia [5] and this condition was suspected to predispose to bronchial disease [3]. A first study in adults 40 years ago revealed a high prevalence of allergic disease among such patients [30]. In addition, XLHED patients are known to suffer from a lack of tears [12]. Insufficient function of exocrine glands may lead to chronic inflammatory processes in lung and eyes of the affected individuals, being reflected in asthma-like symptoms and dry eye disease.

XLHED is most frequently caused by mutations in the gene *EDA* [MIM 300451] located on the X chromosome. The gene product, ectodysplasin A, is a trimeric transmembrane protein belonging to the tumor necrosis factor (TNF) superfamily of ligands. Eight transcriptional variants due to alternative splicing are known. The longest transcript consists of exons 1a, 3a, and 4–9 (nomenclature according to Bayés et al. and Monreal et al. [2, 21]) encoding the ectodysplasin A1 isoform (EDA-A1), lack of which has been demonstrated to underlie XLHED. EDA-A1 comprises a small N-terminal intracellular, a transmembrane, and a large C-terminal extracellular domain with a furine cleavage site, a collagen-like domain consisting of 19 Gly-X-Y repeats with a single interruption, and a TNF homology domain. To be able to bind to its receptor EDAR, EDA-A1 has to be released by proteolytic processing at the furin recognition site as a soluble homotrimerized protein consisting of the collagen-like and TNF homology domains. Further multimerization via the collagen-like region is known to be important for protein function [25, 26].

The phenotype of individuals affected by XLHED shows both interfamilial and intrafamilial variability [13, 15, 31]. The latter may be caused by variations in genes encoding other components of the ectodysplasin-NFkappaB signaling pathway. A gain-of-function allele of the gene *EDAR* (c.1540T>C, p.V370A), which alters a highly conserved amino acid in the death domain of the ectodysplasin receptor, has been reported to increase signaling potency in vitro and has been associated with increased hair thickness and shovel-shaped incisors in both mice and humans [4, 8, 22].

Recently, we investigated the genotype–phenotype relationship in male XLHED subjects with respect to their sweating ability [24], confirming a consistent, quantifiable defect of sweat gland function as a disease biomarker. To extend the phenotypic characterization to other organ systems, this study assessed both lung function of children and adults with XLHED and chronic inflammatory processes in their airways. In addition, it addressed XLHED-associated chronic conjunctivitis and blepharitis and other signs of dry eye syndrome that are caused by quantitative and/or qualitative alterations of lacrimal fluid. The study included non-invasive, quantitative approaches to endpoint assessment in both lung and eye, as they may predict clinically relevant dysfunction and disease.

Subjects and methods

Twelve male children and 14 male adults with XLHED and 12 healthy male controls (six children, six adults) were enrolled in this study conducted alongside a family conference in Germany (clinicaltrials.gov NCT01308333). All adults gave written informed consent to participate; in the case of minors, parental consent and assent of the child were obtained. The study was approved by an independent institutional ethics committee and conducted according to national regulations and GCP/ICH guidelines. XLHED patients were included only if pathogenic *EDA* mutations had been detected prior to the study and liquid intake on the day of the study had been normal. Criteria for exclusion were acute febrile illness, acute allergic reactions, and implantable electronic devices.

Respiratory research

The medical history with respect to symptoms and signs of asthma was taken, followed by pulmonary function tests and evaluation of fractional exhaled nitric oxide (FeNO), a marker of lower airway inflammation, measured at a flow rate of 50 ml/s. FEV1 (volume of air which can be forcibly exhaled in the first second of a forced expiration), evaluated in relation to the individual's vital capacity (FVC, maximum amount of air the subject can expel from the lungs after a maximum inhalation), is commonly used to detect bronchial obstruction. In analogy to routine clinical assessments, we considered a relative FEV1 (relFEV1=FEV1/FVC) of less than 80 % as an abnormal result.

Ophthalmic investigations

The subjects were assessed for dry eye disease by Schirmer's test with a cutoff value of 10 mm, a threshold commonly accepted for ocular sicca syndrome [9], by investigation of tear osmolarity, noninvasive tear film break-up time (NIBUT; threshold of 10 s), tear fluorescein clearance and Lissamine green staining of the ocular surface, and by using an ocular surface disease index (OSDI) questionnaire with a cutoff value of 12 [19]. Schirmer's test and staining of the ocular surface were not done in children younger than 12 years.

Sweat duct imaging

Palmar sweat ducts were visualized in an area of 36 mm^2 of the right hand by reflectance confocal microscopy (VivaScope 1500; Lucid Inc., Rochester, USA). The microscopy pictures were evaluated by two independent experienced examiners blinded to the genotype of the participant. A consensus sweat duct count was obtained and calculated per square centimeter.

Statistical analysis

Descriptive statistics was calculated for each group. The standardized sweat duct counts and the EDA genotype were included in correlation analyses. Group comparisons, both between XLHED and control subjects, and between adult and pediatric subsets, were done by Mann–Whitney *U* test

using SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Fifteen individuals with XLHED had a history of eczema/atopic dermatitis indicating dryness of the skin. In 17 of 26 subjects with XLHED, complete absence of sweat ducts was confirmed in the hypothenar area investigated by confocal microscopy. Residual sweat ducts (2.8–356/cm^2) were detected in nine XLHED subjects, with significant counts only in subjects carrying known hypomorphic *EDA* mutations: ED-Ch4 (missense mutation R384S), ED-Ch9 (splice site mutation leading to aberrant splicing with expected in-frame deletion), ED-Ch12 (missense mutation R276C), and ED-A13 (in-frame deletion), who all reported to be able to sweat to some extent.

Although BMI values were relatively low in most affected boys, the BMI of subjects with and without XLHED did not differ significantly (15.5±1.9 vs. 16.5±2.0 in the children, p=0.11).

Involvement of the respiratory system

Frequent foul-smelling nasal discharge was reported by 62 % of the XLHED patients. Even more XLHED subjects (85 %) complained about a frequent hoarse voice. Seven of 12 affected boys (58.3 %) and 7 of 14 adults with XLHED (50 %) had a history of bronchial asthma, including one pair of brothers (ED-Ch2 and ED-Ch3). None of the 12 control subjects reported any of these conditions.

To obtain quantitative data on lung function and inflammation, a standardized pulmonary function test was performed and FeNO was determined in all subjects. Table 1 displays subject-specific information and the main results of the pulmonary function tests. Among children with XLHED (n=12), three of seven with known bronchial asthma and two of five without a history of asthma had relFEV1 values lower than 80 %. Peak expiratory flow measurements provided no additional information. Two of the subjects with known asthma but normal relFEV1 were on current medication for asthma (salbutamol), explaining their normal test results. In adult XLHED subjects (n=14), reduced relFEV1 values were found in five of seven subjects with known asthma and in three of seven without.

Increased FeNO values were measured both for children and adults with XLHED compared to healthy controls (Table 1; p=0.02 for children, p<0.01 for adults). Values >25 ppb in children or >33 ppb in adults are abnormal [27] and may indicate uncontrolled asthma. In the XLHED children, relFEV1 and FeNO showed an inverse correlation (Spearman r=0.432, p=0.04). Six children with XLHED (50 %) had elevated FeNO levels, but only four of them had a history of asthma. The other two children with increased FeNO but no history of asthma also presented with a reduced relFEV1, indicating that pulmonary difficulties may have been overlooked in these subjects. Only 3 of 12 children with XLHED (25 %) had no history of asthma, a normal relFEV1, and normal FeNO.

The relFEV1 of adult XLHED subjects differed significantly from that of healthy controls (75.6±11.1 vs. 84.2±4.9 %; p=0.042). Eight of 14 adult XLHED patients showed elevated FeNO values, five of the seven with asthma history, and three of seven without. However, in all individuals with newly detected pathological relFEV1, the FeNO values were also abnormal.

Involvement of the eyes

Although only four XLHED children had a history of ocular sicca syndrome, one or more dry eye issues were found in all subjects with XLHED (Table 2). Because of the anticipated discomfort associated with some tests, especially for dry eye patients, Schirmer's test was only conducted with two XLHED children and five control children, yielding normal results in each case.

Both for children and adults with XLHED, NIBUT and OSDI data differed markedly from the control values (p=0.001). Eight of the 11 tested pediatric XLHED patients showed an abnormal NIBUT (Table 2), and one had a normal NIBUT but a high OSDI score. However, 75 % of the children with pathological NIBUT had no history of dry eye. Tear osmolarity could be determined in six XLHED children and was found to be elevated in three of them. Only in one case this was correlated with a history of dry eye. Two of three children with XLHED showed abnormal Lissamine green staining of the conjunctiva (Fig. 1a).

In adults, a history of dry eye did also not correlate well with objective measures. The four adults not reporting a history of dry eye each had at least two abnormal eye findings. Tear osmolarity of adult individuals with XLHED was much higher than that of healthy controls (334.6±34.4 vs. 297.2±10.8 mOsml/L; p=0.006). In nine cases, the test value was above the threshold of 311 mOsml and could thus be considered pathologic [14], including all four XLHED subjects with no history of dry eye. Furthermore, adult XLHED patients had markedly lower Schirmer I test values (p=0.001). Among those with pathologic values were two without any history of dry eye. NIBUT was low in 10 of 14 cases including all four adult XLHED subjects who had not realized a dry eye problem so far (Table 2). Twelve adults with XLHED showed significant Lissamine green staining, whereas pathologic fluorescein staining was observed only in 3 of 14 cases. Twelve of 14 adult XLHED patients had an elevated OSDI score, reflecting relevant subjective suffering. Independent of the history of dry eye problems, there were at least two abnormal findings on dry eye assessments

Table 1 Skin issues and respiratory symptoms in pediatric and adult XLHED patients

Code	Age (years)	Body height (cm)	Weight (kg)	EDA mutation according to GenBank AF040628.1	Amino acid substitution or deletion	Sweat ducts/cm^2 (palm)	History of eczema/atopic dermatitis	Frequent smelling nasal discharge	Frequently hoarse voice	History of asthma	VCmax (L)	Relative FEV1 (% VCmax)	FeNO (ppb)
XLHED children													
ED-Ch1	10	137	27	c.831delC	p.T278LfsX2	**0**	Yes	No	Yes	**Yes**	2.32	95.8	24
ED-Ch2	8	130	23	c.659_676del18	p.P220_P225del	**0**	Yes	No	Yes	**Yes**	2.51	**67.2**	**55**
ED-Ch3	6	116	20	c.659_676del18	p.P220_P225del	**0**	Yes	Yes	Yes	**Yes**	1.53	95.0	**49**
ED-Ch4	13	177	60	c.1152G>C	p.R384S	**77.8**	Yes	No	No	**Yes**	5.17	80.6	**47**
ED-Ch5	7	122	25.5	c.686dupC		**0**	No	Yes	Yes	No	1.38	95.9	14
ED-Ch6	9	134	25	c.671G>T	p.G224V	**0**	No	No	Yes	No	2.11	**68.0**	**42**
ED-Ch7	8	128	27.5	c.871G>A	p.G291R	**0**	Yes	Yes	Yes	**Yes**	2.36	92.4	**26**
ED-Ch8	10	145	32	c.206G>T and c.991C>T	p.R69L and p.Q331X	**0**	Yes	Yes	Yes	No	2.44	95.4	9
ED-Ch9	7	128	22.5	c.527G>T	Splice site mutant	**130.6**	No	Yes	No	No	2.25	83.1	12
ED-Ch10	13	156	36.5	c.146T>C	p.L49P	**0**	No	No	Yes	No	3.68	**75.6**	**72**
ED-Ch11	9	131	24	c.467G>A	p.R156H	**2.8**	No	Yes	Yes	**Yes**	2.01	**73.9**	8
ED-Ch12	13	179	60	c.826C>T	p.R276C	355.6	Yes	Yes	Yes	**Yes**	5.10	**72.0**	14
Median (average)	*(9.42)*	*(140.25)*	*(31.92)*			*0.00**	*7/12*	*7/12*	*10/12*	*7/12*	*2.34*	*81.86*	*25.00**
Range (SD)	*(2.47)*	(20.41)	(13.83)			*355.60*	–	–	–	–	*3.79*	*28.75*	*64.00*
Control children (n = 6)													
Median (average)	*(9.83)*	*(143.50)*	*(34.98)*			*606.95*	*0/6*	*0/6*	*0/6*	*0/6*	*2.12*	*92.03*	*10.00*
Range (SD)	*(2.64)*	*(16.68)*	(12.60)			*172.20*	–	–	–	–	*1.96*	*12.82*	*20.00*
XLHED adults													
ED-A1	21	189	80.5	c.910T>A	p.Y304N	**0**	Yes	No	Yes	**Yes**	7.24	**58.4**	**113**
ED-A2	25	158	60	c.467G>A	p.R156H	**0**	Yes	Yes	Yes	**Yes**	4.53	**72.6**	20
ED-A3	43	180	89	c.467G>T	p.R156L	**0**	No	Yes	Yes	No	5.19	**79.3**	**43**
ED-A4	44	174	71.5	c.457C>T	p.R153C	**33.3**	No	Yes	Yes	No	5.84	**74.3**	**58**
ED-A5	58	180	98	c.821G>A	p.W274X	**0**	Yes	Yes	Yes	No	4.83	81.7	26
ED-A6	30	171	58	Exon3del	Exon deletion	**0**	Yes	Yes	Yes	**Yes**	4.10	87.1	**128**
ED-A7	40	196	90	c.1091T>G	p.M364R	**0**	Yes	No	No	No	6.61	85.2	30
ED-A8	34	169	72	c.457C>T	p.R153C	**2.8**	Yes	Yes	Yes	**Yes**	4.03	**46.1**	**36**
ED-A9	34	176	73	c.4G>T	p.G2C	**22.2**	Yes	No	Yes	**Yes**	4.70	82.8	**48**
ED-A10	18	184	64	c.457C>T	p.R153C	**13.9**	No	Yes	No	No	7.06	**77.6**	**53**
ED-A11	37	174	100	Exon4-9del	Exon deletion	**0**	No	Yes	Yes	**Yes**	5.40	**70.8**	**59**
ED-A12	37	184	86.5	c.707-2A>T	Splice site mutant	**0**	Yes	Yes	Yes	**Yes**	5.78	**79.7**	16
ED-A13	21	179	82	c.536_571del36	p.G180_P191del	**127.8**	No	Yes	Yes	No	6.53	81.5	20
ED-A14	24	185	65	c.467G>A	p.R156H	**0**	No	No	Yes	No	5.95	80.8	14
Median (average)	*(33.29)*	*(178.50)*	*(77.82)*			*0.00**	*8/14*	*9/14*	*12/14*	*7/14*	*5.60*	*79.49**	*39.50**
Range (SD)	*(11.08)*	*(9.35)*	*(13.69)*			*127.80*	–	–			*3.21*	*41.00*	*114.00*
Control adults (n = 6)													
Median (average)	*(34.00)*	*(184.67)*	*(77.33)*			*308.35*	*0/6*	*0/6*	*0/6*	*0/6*	*5.90*	*83.29*	*14.50*
Range (SD)	*(10.35)*	*(5.89)*	*(5.47)*			*197.20*	–	–	–	–	*1.38*	*12.15*	*18.00*

Pathologic values are in bold type; asterisks indicate statistically significant differences from the control group

SD standard deviation

Table 2 Ocular symptoms in pediatric and adult XLHED patients

Code	Age (years)	Body height (cm)	Weight (kg)	EDA mutation according to GenBank AF040628.1	Amino acid substitution or deletion	Sweat ducts/cm^2 (palm)	History of dry eye problems	OSDI score	Tear osmolarity (mOsm/L) [higher value of the two eyes]	NIBUT (s) [mean value]	Lissamine green staining [mean value]	Fluorescein staining [mean value]	Schirmer 1 test (mm) [lower value of the two eyes]
XLHED children													
ED-Ch1	10	137	27	c.831delC	p.T278LfsX2	0	No	**22.9**	n.d.	**6.3**	n.d.	n.d.	n.d.
ED-Ch2	8	130	23	c.659_676del18	p.P220_P225del	0	**Yes**	**16.6**	295	**8.0**	n.d.	n.d.	n.d.
ED-Ch3	6	116	20	c.659_676del18	p.P220_P225del	0	No	4.2	**359**	12.1	n.d.	n.d.	n.d.
ED-Ch4	13	177	60	c.1152G>C	p.R384S	77.8	No	6.3	310	**9.2**	**2**	0	13
ED-Ch5	7	122	25.5	c.686dupC		0	No	8.3	n.d.	**6.2**	n.d.	n.d.	n.d.
ED-Ch6	9	134	25	c.671G>T	p.G224V	0	No	2.1	n.d.	**5.6**	n.d.	n.d.	n.d.
ED-Ch7	8	128	27.5	c.871G>A	p.G291R	0	**Yes**	**33.3**	n.d.	n.d.	n.d.	n.d.	n.d.
ED-Ch8	10	145	32	c.206G>T and c.991C>T	p.R69L and p.Q331X	0	No	6.3	286	**7.2**	n.d.	n.d.	n.d.
ED-Ch9	7	128	22.5	c.527G>T	Splice site mutant	130.6	No	**12.5**	n.d.	**7.4**	n.d.	n.d.	n. d.
ED-Ch10	13	156	36.5	c.146T>C	p.L49P	0	**Yes**	**25.0**	**318**	10.1	**3**	0	n. d.
ED-Ch11	9	131	24	c.467G>A	p.R156H	2.8	**Yes**	2.3	n.d.	**9.6**	n.d.	n.d.	n.d.
ED-Ch12	13	179	60	c.826C>T	p.R276C	355.6	No	6.8	**328**	19.3	**5.5**	1	24
Median (average)	*(9.42)*	(140.25)	*(31.92)*			*0.00**	*4/12*	*7.55**	*314.00*	*8.00**	*3.00*	*0.00*	*18.50*
Range (SD)	*(2.47)*	(20.41)	*(13.83)*			*355.60*	–	*31.20*	*73.00*	*13.70*	*3.50*	*1.00*	*11.00*
Control children (n = 6)													
Median (average)	*(9.83)*	(143.50)	*(34.98)*			*606.95*	–	*0.00*	*297.00*	*22.15*	*0.00*	*0.00*	*27.00*
Range (SD)	*(2.64)*	*(16.68)*	*(12.60)*			*172.20*	–	*2.27*	*11.00*	*11.20*	*0.00*	*0.00*	*9.00*
XLHED adults													
ED-A1	21	189	80.5	c.910T>A	p.Y304N	0	**Yes**	**31.3**	308	**5.9**	**3**	**3**	**5**
ED-A2	25	158	60	c.467G>A	p.R156H	0	**Yes**	**63.6**	n.d.	**8.9**	**3**	0.5	n.d.
ED-A3	43	180	89	c.467G>T	p.R156L	0	**Yes**	**29.2**	**343**	**7.1**	**5**	1	**4**
ED-A4	44	174	71.5	c.457C>T	p.R153C	33.3	**Yes**	**12.5**	283	**9.1**	**3**	0	**8**
ED-A5	58	180	98	c.821G>A	p.W274X	0	**Yes**	**45.5**	**333**	17.4	**6**	**2.5**	15
ED-A6	30	171	58	Exon3del	Exon deletion	0	**Yes**	**18.2**	**400**	**5.8**	**4**	0	n.d.
ED-A7	40	196	90	c.1091T>G	p.M364R	0	No	2.1	**360**	**9.6**	2	0	**6**
ED-A8	34	169	72	c.457C>T	p.R153C	2.8	**Yes**	**25.0**	305	14.7	**3**	1	24
ED-A9	34	176	73	c.4G>T	p.G2C	22.2	**Yes**	**14.6**	**312**	**9.8**	**3.5**	1	**10**
ED-A10	18	184	64	c.457C>T	p.R153C	13.9	No	**12.5**	**385**	**7.8**	**4**	0	**2**
ED-A11	37	174	100	Exon4-9del	Exon deletion	0	**Yes**	**16.6**	**332**	11.2	**4.5**	**2**	**6**
ED-A12	37	184	86.5	c.707-2A>T	Splice site mutant	0	**Yes**	**35.4**	307	10.6	2	1	**5**
ED-A13	21	179	82	c.536_571del36	p.G180_P191del	127.8	No	6.3	**318**	**8.2**	**3**	0	26
ED-A14	24	185	65	c.467G>A	p.R156H	0	No	**25.0**	**364**	**9.2**	**3**	0	**4**
Median (average)	*(33.29)*	*(178.50)*	*(77.82)*			*0.00**	*10/14*	*21.60**	*332.00**	*9.15**	*3.00**	*0.75**	*6.00**
Range (SD)	*(11.08)*	*(9.35)*	*(13.69)*			*127.80*	–	*61.50*	*117.00*	*11.60*	*4.00*	*3.00*	*24.00*
Control adults (n = 6)													
Median (average)	*(34.00)*	*(184.67)*	*(77.33)*			308.35	–	*1.04*	*298.50*	*22.15*	*0.75*	*0.00*	*26.50*
Range (SD)	*(10.35)*	*(5.89)*	*(5.47)*			197.20	–	*6.25*	*32.00*	*6.3*	*2.00*	*0.00*	*6.00*

Pathologic values in bold type; asterisks indicate statistically significant differences from the control group

SD standard deviation

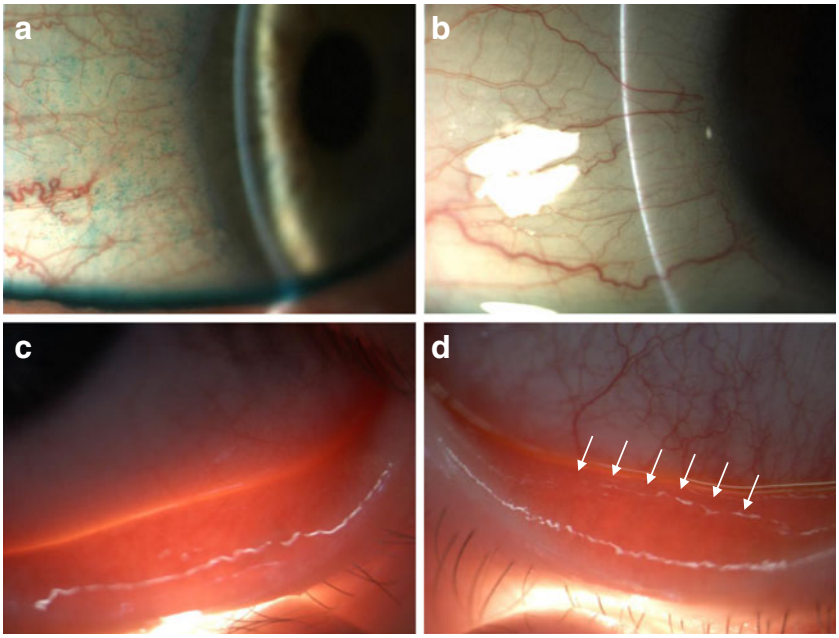

Fig. 1 Typical ocular findings in subjects with XLHED. Significant Lissamine green staining (stage 3) of the temporal conjunctiva of a subject with XLHED indicating dry eye disease (**a**) and staining in a healthy control individual (**b**). Transillumination revealing the absence of meibomian glands in an XLHED subject (**c**); meibomian glands of a healthy control (**d**)

in every adult with XLHED, and transillumination regularly revealed the lack of meibomian glands (Fig. 1c, d).

Figure 2 displays the relationship between ocular symptoms and the number of sweat glands at the palm. If the latter exceeds a certain threshold, it seems to be associated with normal ocular test results. However, the huge variance at low levels of sweat ducts indicates that factors other than ectodysplasin are involved.

Genotype–phenotype correlation

Two of the four subjects with known hypomorphic *EDA* mutations showed a milder phenotype in general. Both mutations, c.536_571del36 and c.527G>T, affect the region encoding the first part of the collagen-like domain of ectodysplasin (exon 5). The resulting in-frame deletion in that region or aberrant splicing with expected in-frame deletion [23], respectively, was associated not only with the presence of some sweat ducts and production of clinically relevant sweat volumes, but also with absence of asthma-like symptoms, normal lung function, and no history of dry eye problems. Thus, the hypomorphic character of these mutations could be confirmed, whereas the two individuals carrying the missense mutations p.R276C and p.R384S, which affect other parts of the ectodysplasin molecule, showed signs of significant pulmonary involvement.

In summary, 73 % of male XLHED subjects had either a history of asthma or test values consistent with this diagnosis, and half of the pediatric but all adult patients had either a history of dry eye or strong supporting data for the diagnosis from quantitative assessments. History alone was not a good predictor of abnormal testing in either organ system. The presence of residual sweat ducts in XLHED subjects, suggestive of partial *EDA* expression, correlated with milder disease in two subjects with genetic abnormalities in exon 5.

Discussion

In this study, bronchial asthma-like symptoms and dry eye syndrome are demonstrated to be common conditions in XLHED-affected males both in childhood and adulthood, which confirms clinical observations reported previously [3, 7, 12]. The underlying pathophysiology is likely to involve initiation of disease secondary to reduced bronchial or meibomian/tarsal gland function with an inflammatory component present in the chronic condition.

Nitric oxide produced by epithelial cells and macrophages has been considered as a reliable marker of inflammatory processes in the lower airways. Its measurement is certainly more appropriate for asthma monitoring than for asthma diagnosis because the specificity of FeNO is high, but the sensitivity is rather low. In many studies, this parameter has been used for the evaluation of anti-inflammatory treatment [20, 29]. Although the results depend to some extent on the measurement conditions [1, 27], FeNO values of 25 ppb for children and 33 ppb for adults have been suggested as upper limits of normal [27]. Thus, the mean values of the control groups of our study were clearly within the normal range and those of the XLHED groups clearly without. In our study setting, FeNO data were consistently reproducible (multiple measurements in the same patients). Since all XLHED subjects without asthma history who showed elevated FeNO levels also had a reduced relative FEV1, we considered this

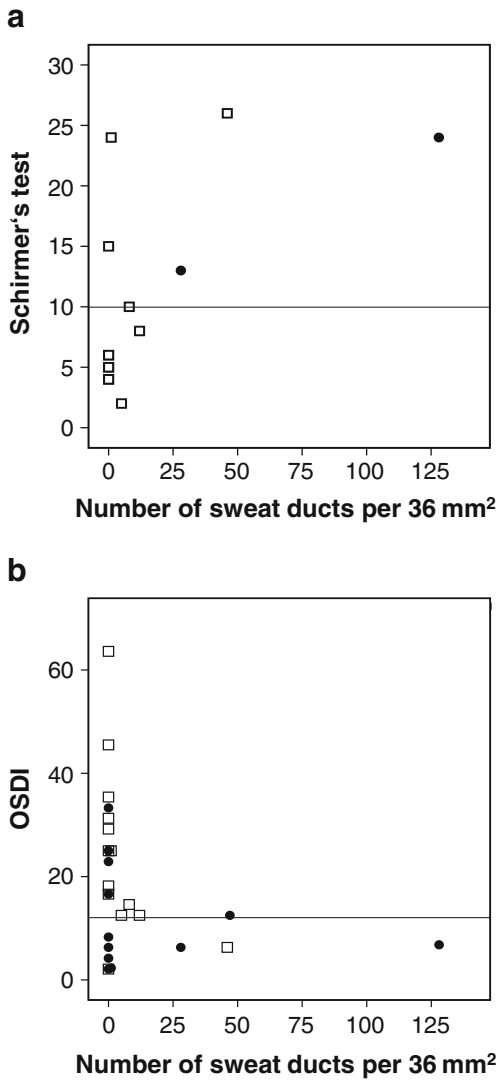

Fig. 2 Relationship between ocular symptoms and the number of sweat glands. The *horizontal lines* mark the cutoff values of 10 mm in Schirmer's test (**a**) and 12.5 in the ocular surface disease index (*OSDI*) questionnaire (**b**). If the amount of sweat glands at the palm exceeds a certain threshold, normal ocular test results seem likely. Children are displayed by *closed circles*, adults by *open squares*. The figure was created with the program SPSS

combination as indicative for a disposition to asthmatic airway constriction.

As an individual with XLHED appears to be 10–20 times more likely to develop asthma-like symptoms than a member of the normal population (referring to a reported asthma prevalence in children of around 7 % [17] and 3.7 % in adults [10]), this health issue deserves particular attention. The high prevalence observed in XLHED patients as young as 6 years suggests that screening evaluation, regular monitoring, and consideration of therapeutic intervention should begin in early childhood.

Individuals with XLHED are known to suffer from more frequent airway infections than the normal population [3, 7]. Except for the small subgroup of patients carrying *NEMO* mutations, this is not associated with a defective immune system but with the lack of seromucous glands in the respiratory tract [5, 6], which are a vital part of the mucociliary clearance mechanism. Absence of these exocrine glands is expected to result in a diminished mucous (mechanical) barrier against allergens and pathogenic microorganisms. This, together with abnormal skin and respiratory barrier functions in XLHED patients which could facilitate allergen absorption, may explain the increased asthma prevalence in XLHED subjects. Respiratory syncytial virus infections in young children may also be a risk factor for developing bronchial asthma later in life [15]. It should be interesting to explore the spectrum of pathogens causing the frequent airway infections in XLHED patients because this might help to understand the biologic background of asthma-like symptoms in individuals with and without XLHED.

Mauldin et al. [17] have shown that perinatal intravenous administration of recombinant ectodysplasin A to dogs with XLHED may lead to improved mucociliary clearance and absence of the typical respiratory infections as well as to a corrective effect on adult, permanent dentition. Although in treated XLHED dogs fewer glands were detected than in healthy control animals, a small amount of seromucous glands may well be sufficient for developing a normal mechanical barrier in the lung. A clinical trial on human neonates based on the encouraging preclinical data is being prepared.

Pediatricians aim at an early detection of asthma-like symptoms and will often consider early therapeutic intervention with bronchodilators and anti-inflammatory preparations. This also applies to pediatric XLHED patients. To stay a nonsmoker, trying to avoid noxes for the respiratory system, appears to be particularly important in that subgroup. However, some questions remain to be answered by further studies, e.g., whether there is some remodeling in the lungs of XLHED subjects similar to the findings in patients with classical asthma.

In XLHED subjects, reduced amount and/or abnormal composition of lacrimal fluid was expected. Considering the known correlation between OSDI, NIBUT, tear osmolarity, and loss of meibomian glands [23], most findings of this study may be explained by the lack of holocrine meibomian/tarsal glands. Tear abnormalities often lead to chronic eye disease. Ophthalmologists may see patients with supposed ectodermal dysplasia before a genetic classification is performed. Then, OSDI and NIBUT will provide useful and reliable first information. Among the routine examinations, NIBUT appears to be the most sensitive single test in pediatric patients. Additionally, Lissamine green is applicable in older children. Once the diagnosis of ectodermal dysplasia is specified, quantification of the ocular surface involvement will be of growing relevance. For diagnosing and staging the dry eye, a combination of OSDI and NIBUT is again recommendable, complemented

by Lissamine green staining. Schirmer's test is not practical as it causes significant discomfort.

Ocular surface disease in XLHED is known to progress with age, later involving the cornea and thus deteriorating acuity of vision. In XLHED individuals between 20 and 30 years of age, a typical deterioration of the corneal surface occurs as a result of the underlying tear film disorder [12]. For adult XLHED patients, we would therefore suggest to combine OSDI, NIBUT, and osmolarity measurements with Lissamine green and fluorescein staining. Considering the invariable progress of the disorder as seen by 100 % of adults affected, these measurements will also represent clinically meaningful endpoints for future therapies.

In the context of in-frame deletions affecting the collagen-like domain of ectodysplasin, the presence of sweat ducts, suggestive of partial *EDA* expression, appears to be predictive of milder airway or eye disease. A collagen-like domain of reduced size may still allow for some ectodysplasin multimerization and residual function of the molecule, resulting only in a mild XLHED phenotype. Further research to improve our understanding of the consequences of hypomorphic *EDA* mutations is certainly required.

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Conflict of interest Kenneth Huttner and Ramsey Johnson are employees of Edimer Pharmaceuticals. K.H. contributed substantially to the study design. R.J. performed the confocal microscopy. Holm Schneider is a member of the clinical advisory board of Edimer Pharmaceuticals and received project funding from this company. Otherwise, the company was neither involved in collection, analysis or interpretation of data, nor in the writing of the report or the decision to submit it for publication. The first draft of this manuscript was written by J.D. and H.S. None of the authors has been paid to produce this article.

References

1. American Thoracic Society and European Respiratory Society (2005) ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. Am J Respir Crit Care Med 171:912–930
2. Bayés M, Hartung AJ, Ezer S, Pispa J, Thesleff I, Srivastava AK et al (1998) The anhidrotic ectodermal dysplasia gene (EDA) undergoes alternative splicing and encodes ectodysplasin-A with deletion mutations in collagenous repeats. Hum Mol Genet 7:1661–1669
3. Beahrs JO, Lillington GA, Rosan RC, Russin L, Lindgren JA, Rowley PT (1971) Anhidrotic ectodermal dysplasia: predisposition to bronchial disease. Ann Intern Med 74:92–96
4. Bryk J, Hardouin E, Pugach I, Hughes D, Strotmann R, Stoneking M et al (2008) Positive selection in East Asians for an EDAR allele that enhances NF-kappaB activation. PLoS One 3:e2209
5. Capitanio MA, Chen JT, Arey JB, Kirkpatrick JA (1968) Congenital anhidrotic ectodermal dysplasia. Am J Roentgenol Radium Ther Nucl Med 103:168–172
6. Casal ML, Mauldin EA, Ryan S, Scheidt JL, Kennedy J, Moore PF et al (2005) Frequent respiratory tract infections in the canine model of X-linked ectodermal dysplasia are not caused by an immune deficiency. Vet Immunol Immunopathol 107:95–104
7. Clarke A, Phillips DI, Brown R, Harper PS (1987) Clinical aspects of X-linked hypohidrotic ectodermal dysplasia. Arch Dis Child 62:989–996
8. Cluzeau C, Hadj-Rabia S, Bal E, Clauss F, Munnich A, Bodemer C et al (2011) The EDAR370A allele attenuates the severity of hypohidrotic ectodermal dysplasia caused by EDA gene mutation. Br J Dermatol 166:678–681
9. de Monchy I, Gendron G, Miceli C, Pogorzalek N, Mariette X, Labetoulle M (2011) Combination of the Schirmer I and phenol red thread tests as a rescue strategy for diagnosis of ocular dryness associated with Sjögren's syndrome. Invest Ophthalmol Vis Sci 52:5167–5173
10. European Community Respiratory Health Survey (1996) Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). Eur Respir J 9:687–695
11. Hammersen JE, Neukam V, Nüsken KD, Schneider H (2011) Systematic evaluation of exertional hyperthermia in children and adolescents with hypohidrotic ectodermal dysplasia: an observational study. Pediatr Res 70:297–301
12. Kaercher T (2004) Ocular symptoms and signs in patients with ectodermal dysplasia syndromes. Graefe's Arch Clin Exp Ophthalmol 242:495–500
13. Kobielak K, Kobielak A, Roszkiewicz J, Wierzba J, Limon J, Trzeciak WH (2001) Mutations in the EDA gene in three unrelated families reveal no apparent correlation between phenotype and genotype in the patients with an X-linked anhidrotic ectodermal dysplasia. Am J Med Genet 100:191–197
14. Lemp MA, Bron AJ, Baudouin C, Benítez Del Castillo JM, Geffen D, Tauber J et al (2011) Tear osmolarity in the diagnosis and management of dry eye disease. Am J Ophthalmol 151:792–798
15. Lexner MO, Bardow A, Juncker I, Jensen LG, Almer L, Kreiborg S et al (2008) X-linked hypohidrotic ectodermal dysplasia. Genetic and dental findings in 67 Danish patients from 19 families. Clin Genet 74:252–259
16. Martinez FD (2003) Respiratory syncytial virus bronchiolitis and the pathogenesis of childhood asthma. Pediatr Infect Dis J 22:S76–S82
17. Mauldin EA, Gaide O, Schneider P, Casal ML (2009) Neonatal treatment with recombinant ectodysplasin prevents respiratory disease in dogs with X-linked ectodermal dysplasia. Am J Med Genet A 149A:2045–2049
18. Maziak W, Behrens T, Brasky TM, Duhme H, Rzehak P, Weiland SK et al (2003) Are asthma and allergies in children and adolescents increasing? Results from ISAAC phase 1 and phase 3 surveys in Münster, Germany. Allergy 58:572–579
19. Miller KL, Walt JG, Mink DR, Satram-Hoang S, Wilson SE, Perry HD et al (2010) Minimal clinically important difference for the ocular surface disease index. Arch Ophthalmol 128:94–101
20. Miraglia Del Giudice M, Marseglia GL, Leonardi S, Tosca MA, Marseglia A, Perrone L et al (2011) Fractional exhaled nitric oxide measurements in rhinitis and asthma in children. Int J Immunopathol Pharmacol 24:29–32
21. Monreal AW, Zonana J, Ferguson B (1998) Identification of a new splice form of the EDA1 gene permits detection of nearly all X-

linked hypohidrotic ectodermal dysplasia mutations. Am J Hum Genet 63:380–389
22. Mou C, Thomason HA, Willan PM, Clowes C, Harris WE, Drew CF et al (2008) Enhanced ectodysplasin-A receptor (EDAR) signaling alters multiple fiber characteristics to produce the East Asian hair form. Hum Mutat 29:1405–1411
23. Pult H, Riede-Pult BH, Nichols JJ (2012) Relation between upper and lower lids' meibomian gland morphology, tear film, and dry eye. Optom Vis Sci 89:E310–E315
24. Schneider H, Hammersen J, Preisler-Adams S, Huttner K, Rascher W, Bohring A (2011) Sweating ability and genotype in individuals with X-linked hypohidrotic ectodermal dysplasia. J Med Genet 48:426–432
25. Schneider P, Street SL, Gaide O, Hertig S, Tardivel A, Tschopp J et al (2001) Mutations leading to X-linked hypohidrotic ectodermal dysplasia affect three major functional domains in the tumor necrosis factor family member ectodysplasin-A. J Biol Chem 276:18819–18827
26. Swee LK, Ingold-Salamin K, Tardivel A, Willen L, Gaide O, Favre M et al (2009) Biological activity of ectodysplasin A is conditioned by its collagen and heparan sulfate proteoglycan-binding domains. J Biol Chem 284:27567–27576
27. Taylor DR, Pijnenburg MW, Smith AD, DeJongste JC (2006) Exhaled nitric oxide measurements: clinical application and interpretation. Thorax 61:817–827
28. Thurnam J (1848) Two cases in which skin, hair and teeth were very imperfectly developed. Proc R Med Chir Soc 31:71–82
29. Turner S (2008) Exhaled nitric oxide in the diagnosis and management of asthma. Curr Opin Allergy Clin Immunol 8:70–76
30. Vanselow NA, Yamate M, Adams MS, Callies Q (1970) The increased prevalence of allergic disease in anhidrotic congenital ectodermal dysplasia. J Allergy 45:302–309
31. Vincent MC, Biancalana V, Ginisty D, Mandel JL, Calvas P (2001) Mutational spectrum of the ED1 gene in X-linked hypohidrotic ectodermal dysplasia. Eur J Hum Genet 9:355–363