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Genotype–Phenotype Correlation in Boys With X-Linked Hypohidrotic Ectodermal Dysplasia

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X-linked hypohidrotic ectodermal dysplasia (XLHED), the most frequent form of ectodermal dysplasia, is a genetic disorder of ectoderm development characterized by malformation of multiple ectodermal structures such as skin, hair, sweat and sebaceous glands, and teeth. The disease is caused by a broad spectrum of mutations in the gene EDA. Although XLHED symptoms show inter-familial and intra-familial variability, genotype–phenotype correlation has been demonstrated with respect to sweat gland function. In this study, we investigated to which extent the EDA genotype correlates with the severity of XLHED-related skin and hair signs. Nineteen male children with XLHED (age range 3–14 years) and seven controls (aged 6–14 years) were examined by confocal microscopy of the skin, quantification of pilocarpine-induced sweating, semi-quantitative evaluation of full facial photographs with respect to XLHED-related skin issues, and phototrichogram analysis. All eight boys with known hypomorphic EDA mutations were able to produce at least some sweat and showed less severe cutaneous signs of XLHED than the anhidrotic XLHED patients (e.g., perioral and periorbital eczema or hyperpigmentation, regional hyperkeratosis, characteristic wrinkles under the eyes). As expected, individuals with XLHED had significantly less and thinner hair than healthy controls. However, there were also significant differences in hair number, diameter, and other hair characteristics between the group with hypomorphic EDA mutations and the anhidrotic patients. In summary, this study indicated a remarkable genotype–phenotype correlation of skin and hair findings in prepubescent males with XLHED.

Key words: hypohidrotic ectodermal dysplasia; *EDA*; ectodysplasin; sweat glands; hair; genotype–phenotype correlation

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INTRODUCTION

Ectodermal dysplasias are a large and complex group of hereditary conditions, which affect ectodermal structures such as the skin and its appendages [Itin and Fistarol, 2004]. The most common form, X-linked hypohidrotic ectodermal dysplasia (XLHED), is characterized by a lack of sweat and sebaceous glands, dry and eczematous skin, sparse hair, missing or scanty eyebrows and eye lashes, oligo- or anodontia, and peg-shaped teeth [Clarke et al., 1987]. Numerous mutations in the gene *EDA* (OMIM 300451) have been identified as causes of XLHED. This gene is located on the human X chromosome and encodes ectodysplasin A, a trimeric type II transmembrane protein belonging to the tumor necrosis factor (TNF) superfamily of ligands [Kere et al., 1996; Ezer et al., 1999].

Until recently, *EDA* mutations had been considered as genetic abnormalities without clear genotype–phenotype correlation [Kobielak et al., 2001; Schneider et al., 2001]. In recent years, however, several hypomorphic mutations were reported [e.g., Tarpey et al., 2007; Li et al., 2008; Schneider et al., 2011]. Geno-

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type–phenotype correlation has been observed with respect to sweat gland function but not to the number of teeth [Schneider et al., 2011], suggesting that the various ectodermal tissues are not equally susceptible to reduced ectodysplasin A signaling activity. Intrafamilial variability may also be caused by variations in genes encoding other components of the ectodysplasin–NFkappaB signaling pathway. For example, a gain-of-function allele of the gene *EDAR* (c.1540T>C, p.V370A) was found to increase ectodysplasin-signaling potency in vitro and has been associated with increased hair thickness and shovel-shaped incisors in both mice and humans [Mou et al., 2008; Cluzeau et al., 2012].

Although the prognosis of XLHED has improved over the last decades, mortality and risk of hyperthermic brain damage are still increased [Blüschke et al., 2010]. Moreover, most children with XLHED have to deal with stares and teasing when out in public due to their special appearance [Tanner, 1988]. Skin and hair issues play an important role in body perception and self-confidence, particularly during puberty. This study aimed at a detailed characterization of the skin and hair phenotype in prepubescent males with genetically confirmed XLHED, trying to answer the question to which extent the severity of dermatologic symptoms correlates with the *EDA* genotype.

PATIENTS AND METHODS

Nineteen male children between 3 and 14 years of age with known *EDA* mutations and seven healthy controls (age range 6–14 years) were examined by non-invasive methods during a family conference of the German–Swiss–Austrian ectodermal dysplasia patient support group in Bischofsheim, Germany, or at the German Competence Center for Children with Ectodermal Dysplasias in Erlangen, Germany. Written informed consent of all parents and assent of the children was obtained. The study procedures were approved by an independent institutional ethics committee and conducted according to national regulations and GCP/ICH guidelines.

Skin Assessment

Full facial photographs were taken and evaluated semi-quantitatively with respect to signs of eczematous skin and regional hyperkeratosis, characteristic wrinkles under the eyes, and periorbital eczema or hyperpigmentation (which develops secondary to skin inflammation) by an observer blinded to the genetic background of the patients.

Confocal Laser Scanning Microscopy of the Skin

Palmar sweat ducts were visualized in an area of 36 mm^2 of the right hand by reflectance confocal microscopy with the VivaScope 1500 (Caliber Imaging & Diagnostics, New York). Microscopic images were evaluated by two independent experienced examiners blinded to the genotype of the participant. A consensus sweat duct count was obtained and extrapolated to whole body-surface area according to the Mosteller formula [Mosteller, 1987].

Quantification of Pilocarpine-Induced Sweating

Sweat was collected by a standardized procedure from an area of 57 mm^2 of the right forearm 30 min after stimulation with a pilocarpine gel disk using the Wescor 3700 device (Wescor, Logan, UT). Maximum volume that could be collected in the Wescor device was 93 μl. Pilocarpine-induced sweat production were extrapolated to whole body-surface area according to the Mosteller formula [Mosteller, 1987].

Phototrichogram

A phototrichogram of a shaved area of the occipital scalp with a diameter of approximately 1.2 cm^2 was taken using a Canfield EpiFlash camera (Canfield Scientific, Inc., Fairfield). Total hair count, number of follicles and hairs per follicular unit, number of vellus and non-vellus hairs, and mean hair width were evaluated by specialists from Canfield Scientific blinded to the genetic background of the patients.

Hair Microscopy

A few hairs of the patients clipped close to the scalp were mounted side by side, embedded in Entellan (Merck Chemicals, Darmstadt, Germany) and left to dry for 2 days. The samples were investigated under a Leica DM4000 B microscope and photographed with a DFC500 camera (Leica Microsystems, Wetzlar, Germany).

Statistical Analysis

Median and inter-quartile range (IQR) of each parameter were used as summary for continuous variables and calculated separately for each group. For group comparisons between affected and control individuals, the Wilcoxon rank-sum test was applied. A probability (P) value of less than 0.05 was considered significant. Pearson's correlation coefficient was calculated to measure the dependence between different parameters. The analysis was done and figures were created using MATLAB 7.10 for Windows (Mathworks, Natick) with the Statistics Toolbox.

RESULTS

All 19 patients with previously detected *EDA* mutations reported hypo- or anhidrosis. In facial photographs of 16 of them (84%), characteristic XLHED-related skin issues such as eczematous perioral skin, localized hyperkeratoses, periorbital eczema or hyperpigmentation, and typical wrinkles under the eyes were detected. The extent of these symptoms, however, was quite variable; 10 individuals showed more severe signs than the others (Fig. 1; Table I). Confocal laser scanning microscopy of an area of 6 × 6 mm in the hypothenar region revealed the presence of sweat ducts in six children with XLHED (IQR 5.5–36 per 36 mm^2; Fig. 2). In 11 XLHED patients, no sweat ducts were found (Fig. 2; Table I). The two remaining children with XLHED could not be investigated by confocal microscopy for technical reasons. Pilocarpine-induced sudation was observed in 8 of the 19 XLHED patients (Table I), but in none of those without sweat ducts. Therefore, the 11 anhidrotic individuals were assigned to the group "severe XLHED," the other 8 affected patients to a group with milder disease, explained by hypomorphic *EDA* mutations (Table I). The sweating ability of the second group, however, was significantly reduced in comparison

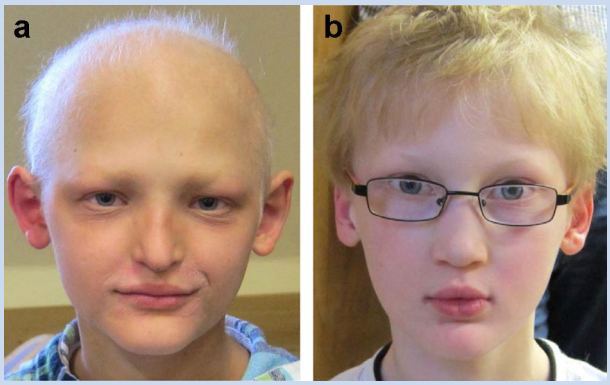

FIG. 1. Skin and hair issues in individuals with XLHED. a: Eczematous perioral skin, regional hyperkeratoses, periorbital hyperpigmentation of the skin, characteristic wrinkles under the eyes, hypotrichosis and hypopigmentation of the hair in an 11-year-old patient with the mutation p.T278LfsX2 causing the severe phenotype of XLHED. b: Skin and hair phenotype of a patient carrying a hypomorphic *EDA* mutation (in-frame deletion leading to a shortened collagen helix) associated with mild or hardly detectable clinical signs of XLHED.

with healthy control participants ($P = 0.0003$; Fig. 2d). To our surprise, a strong correlation between the number of sweat ducts at the palm and pilocarpine-induced sweat production at the forearm (Pearson's coefficient = 0.90) was observed (Fig. 2e). The sweat volume per sweat duct seemed to depend on the type of mutation (Fig. 2f). In general, the skin data summarized in Table I indicated that the *EDA* genotype correlates quite consistently with the severity of XLHED-related skin issues.

Children with a mild form of XLHED had variable hair colors (blond, ash blond, and brown), whereas the hair of the majority of patients with severe XLHED was light blond (Table II). In both XLHED groups, total hair counts and follicle numbers were significantly lower than in the control group (Fig. 3a–c; Table II). Patients with severe XLHED had fewest hairs (IQR 91–109, $P = 0.0002$). A reduced amount of vellus hairs (Table II) contributed to this difference. There was no significant correlation between number of hairs and age of the patients. Although both in XLHED group and controls one hair per follicular unit was the most common finding, the control group showed a higher proportion of follicles with two or more hairs (IQR 3.2–13.9%).

Hairs of individuals with severe XLHED were significantly thinner than control hairs ($P = 0.0002$). In contrast, the hair width of patients with mild XLHED did not differ significantly from that of healthy controls ($P = 0.0813$), except for patients ED18 and ED19, who presented with lower hair width (44.7 and 47.9 μm, respectively; IQR of the group 51.6–62.0 μm) but relatively high hair counts. Children with mild and severe XLHED had significantly fewer hairs with a diameter >74 μm (which was found to be the average width in healthy adults) than the control group ($P = 0.02$ and 0.0005, respectively). Microscopy of hairs from XLHED patients revealed typical caliber differences and trichorrhexis nodosa (Fig. 3e).

A comprehensive statistical analysis of the sweat gland and hair data is given in Table III, where the medians and IQRs of the different parameters are listed separately for mild XLHED, severe XLHED and controls. Furthermore, *P* values obtained by Wilcoxon rank-sum tests are provided for the groups with mild and severe XLHED with reference to the control group as well as for the comparison of the two XLHED groups.

In the XLHED cohort investigated in this study, 17 different *EDA* mutations were present, 7 of which are known as hypomorphic mutations, including the missense mutations R276C and G299A [Schneider et al., 2011], R153C and R384S [Dietz et al., 2013], the splice site mutation c.527G>T [Schneider et al., 2011], and the in-frame deletions K178_P184del-insTE [own unpublished data], and G180_P191del [Dietz et al., 2013]. All of these hypomorphic mutations, implying residual ectodysplasin A function during organ development, were not only associated with the presence of some sweat glands, significant sudation, and a less severe skin phenotype, but also with more and/or wider hairs (Table II; Fig. 4). There was, however, no correlation between sweat duct number and total hair count or number of follicular units (Pearson's coefficient = 0.02 and −0.01, respectively).

DISCUSSION

This study confirmed that skin and hair of prepubescent males with XLHED differ significantly from those of healthy controls, but it also revealed a noticeable difference between the phenotypes of patients with severe and hypomorphic *EDA* mutations, indicating possibly tissue-specific genotype–phenotype correlations.

Hypomorphic mutations were characterized by the presence of sweat ducts at the palm and some residual sweating ability as determined by a standard test. The less severely affected facial skin was also suggestive of partial EDA expression in this group. Interestingly, we observed a correlation between number of sweat ducts and sweat volume, which was not seen in our previous study on sweating ability and genotype in children and adults with XLHED [Schneider et al., 2011]. This difference may be explained by a greater accuracy of the sweat duct numbers determined by confocal microscopy—compared with graphite prints of the palm, which were used previously. Nevertheless, one should keep in mind that the presence of a few sweat glands does not necessarily indicate any sweating ability, as confocal microscopy may not differentiate between healthy and malfunctioning sweat glands.

One person, patient ED15, presented in repeat investigations either with zero or very few sweat ducts at the palm, but was able to produce a very small sweat volume. Irregular distribution of sweat glands in the palmar skin as in other parts of the body surface is the most likely reason for this inconsistency. XLHED patients with clinically relevant sweat production had more than 20 palmar sweat ducts per 36 mm^2, but significantly fewer than the control group. Thus, determination of the number of sweat ducts by confocal microscopy of the palmar skin appears to be a reliable diagnostic approach to identify XLHED patients.

In previous studies, different indirect methods for quantifying sweat pores and sweat gland function were investigated, including

TABLE I. Severity of the Skin Issues in XLHED Patients

Code	Age	EDA mutation	Amino acid substitution or deletion	Predicted effect of the mutation(s)	Eczematous perioral skin/ regional hyperkeratoses	Periorbital exzema or hyperpigmentation	Characteristic wrinkles under the eye	Sweat ducts per 36 mm^2	Sweat volume (μl)
ED3	13	c.466C>T	R156C	Abolished furin cleavage	+	++	++	0	0.0
ED4	3	c.467G>A	R156H	Abolished furin cleavage	+	0	+	0	0.0
ED6	11	c.206G>T/c.991C>T	R69L/Q331X	Truncated protein	+	++	++	0	0.0
ED7	3	c.911A>G	Y304C	Insufficient receptor binding	+	+	++	0	0.0
ED8	4	c.467G>A	R156H	Abolished furin cleavage	++	++	++	0	0.0
ED9	11	c.831delC	T278LfsX2	No functional protein	++	++	++	0	0.0
ED10	10	c.671G>T	G224V	Interruption of the collagen helix at a critical site	+	++	++	0	0.0
ED11	3	c.502+1G>A	Splice site mutation	Wrong mRNA splicing	+	+	+	0	0.0
ED13	6	c.659_676del	P220_P225del	Dysfunctional collagen helix	+	+	++	0	0.0
ED14	8	c.659_676del	P220_P225del	Dysfunctional collagen helix	+	+	++	0	0.0
ED16	8	c.686insC	G230WfsX10	No functional protein	++	++	++	0	0.0
ED5	14	c.1152G>C	R384S	Impaired receptor binding	0	0	+	26	6.0
ED12	8	c.527G>T	Splice site mutation	?	0	0	0	43	11.0
ED15	10	c.896G>C	G299A	Impaired receptor binding	0	+	++	0–11	1.0
ED17	12	c.533_552del-insCTGAA	K178_P184del-insTE	Shortened collagen helix	0	0	+	n.d.	5.0
ED18	5	c.533_552del-insCTGAA	K178_P184del-insTE	Shortened collagen helix	0	0	0	n.d.	12.0
ED19	14	c.826C>T	R276C	Impaired receptor binding	0	0	+	36	5.0
ED20	12	c.457C>T	R153C	Impaired furin cleavage	0	+	+	3	1.0
ED21	3	c.536_571del36	G180_P191del	Shortened collagen helix	0	0	0	21	4.0
C1	7	None	–	–	0	0	0	144	48.0
C2	8	None	–	–	0	0	0	211	56.0
C3	7	None	–	–	0	0	0	246	93.0
C4	6	None	–	–	0	0	0	n.d.	55.0
C5	8	None	–	–	0	0	0	202	82.0
C6	14	None	–	–	0	0	0	229	53.0
C7	8	None	–	–	0	0	0	n.d.	80.0

Italics indicate the subgroup with milder XLHED; n.d., not determined.

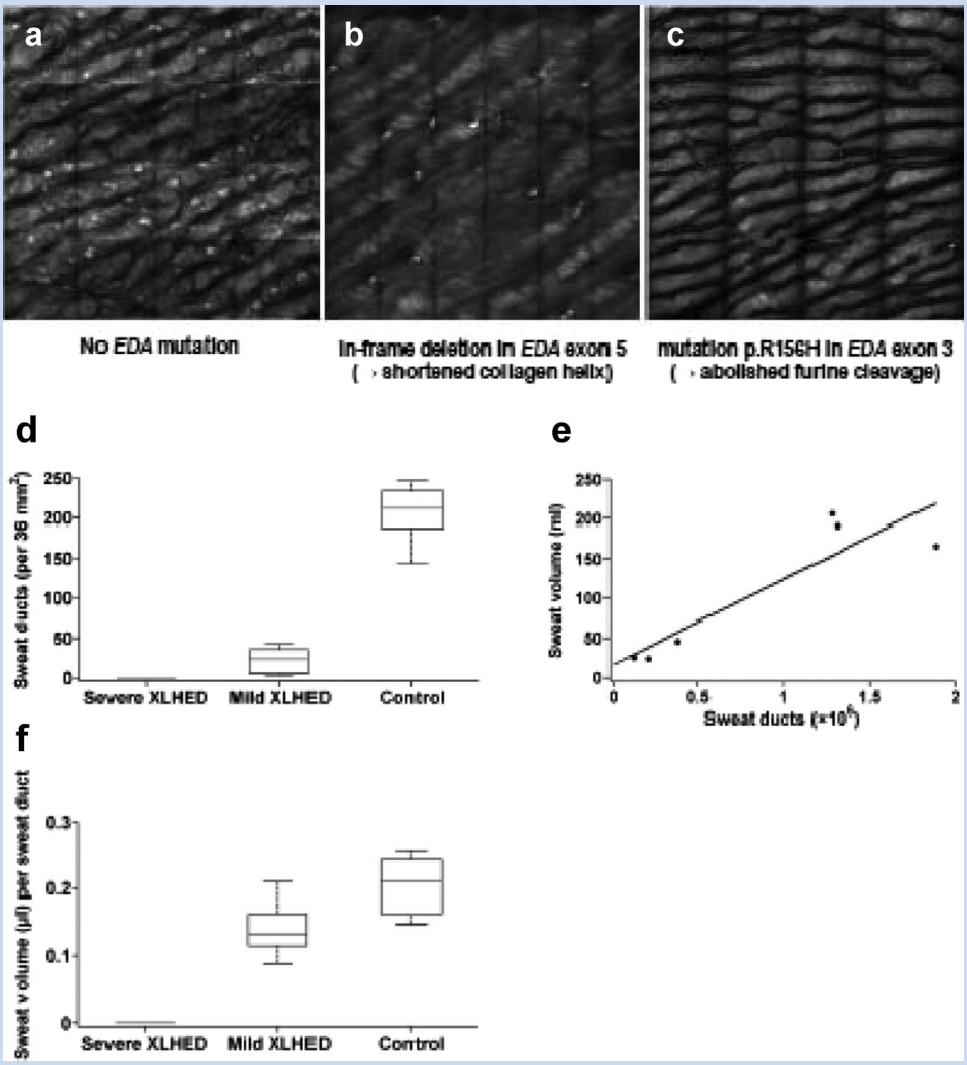

FIG. 2. Characterization of the sweat glands by confocal laser scanning microscopy and quantification of pilocarpine-induced sweating. a–c: Microscopy pictures of a 36-mm^2 area of the right palm in healthy control individuals and in patients with mild and severe XLHED, respectively. d: Boxplots showing the number of sweat ducts in a defined area of the palm in patients with severe and mild forms of XLHED and in healthy controls. The central mark indicates the median of the data points, the edges of the box denote the 25th and the 75th centile. e: Relationship between the number of sweat ducts and pilocarpine-induced sweat production in the group with mild XLHED, where a strong positive correlation (Pearson's coefficient = 0.90) is seen. Number of sweat ducts and pilocarpine-induced sweat production were extrapolated to whole body-surface area according to the Mosteller formula. f: Sweat volume per sweat duct in patients with severe and mild forms of XLHED and in healthy controls. The central mark shows the median of the data points, the edges of the box denote the 25th and the 75th centile. Whiskers indicate the range of data points.

starch–iodide paper palm imprints [Rouse et al., 2004] and measurements of palmar or plantar skin conductance before and after a needle prick [Schneider et al., 2011]. These methods are both less sensitive and less specific than confocal microscopy or evaluation of pilocarpine-induced sweat production and may, therefore, only support the clinical diagnosis of a hypohidrotic ectodermal dysplasia. As established for other genodermatoses, histological investigation of skin biopsy specimens may help to make a definitive diagnosis [Rouse et al., 2004]. Sweat ducts can be visualized nicely by immunostaining with an antibody recognizing cytokeratin-7. The non-invasive techniques applied in this study, however, should be sufficient for diagnosing most cases of XLHED.

Other studies have suggested that non-invasive assessment of hair sample trichograms by light microscopy can also help recognizing XLHED [Rouse et al., 2004; Hirano et al., 2012]. The sensitivity and specificity of such assessments, however, is relatively low, because hair samples often show unspecific pathologic variations of hair structure such as variable shaft thickness, trichorrhexis nodosa, and pili torti. We used phototrichogram analysis as a simple, non-invasive technique to determine total hair count,

TABLE II. Hair Characteristics of XLHED Patients and Healthy Controls

Code	Age	*EDA* mutation	Amino acid substitution or deletion	Hair color	Total hair count	Follicular units	Follicles with one hair	Follicles with ≥2 hairs	Vellus hairs	Non-vellus hairs	Mean hair width (μm)	Hair width >74 μm (%)
ED3	13	c.466C>T	R156C	Ash blond	104	104	104	0	0	104	57.0	1.5
ED4	3	c.467G>A	R156H	Brown	139	139	139	0	0	139	56.2	6.5
ED6	11	c.206G>T/c.991C>T	R69L/Q331X	Blond	101	101	101	0	8	93	41.2	0.0
ED7	3	c.911A>G	Y304C	Light blond	109	105	101	4	81	28	34.1	0.0
ED8	4	c.467G>A	R156H	Blond	96	96	96	0	4	92	54.9	13.4
ED9	11	c.831delC	T278LfsX2	Light blond	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ED10	10	c.671G>T	G224V	Light blond	143	136	129	7	14	129	42.5	0.0
ED11	3	c.502+1G>A	Splice site mutation	Light blond	91	91	91	0	8	83	53.2	3.6
ED13	6	c.659_676del	P220_P225del	Blond	108	108	108	0	4	104	46.8	0.0
ED14	8	c.659_676del	P220_P225del	Light blond	81	81	81	0	1	80	49.0	2.0
ED16	8	c.686insC	G230WfsX10	Light blond	79	79	79	0	10	69	42.1	0.0
ED5	14	c.1152G>C	R384S	Brown	137	128	119	9	2	135	60.9	21.9
ED12	8	c.527G>T	Splice site mutation	Blond	118	118	118	0	0	117	61.1	10.2
ED15	10	c.896G>C	G299A	Blond	100	100	100	0	0	100	62.8	23.0
ED17	12	c.533_552del-insCTGAA	K178_P184del-insTE	Ash blond	114	114	114	0	1	113	57.0	4.4
ED18	5	c.533_552del-insCTGAA	K178_P184del-insTE	Blond	182	172	162	10	26	156	44.7	0.0
ED19	14	c.826C>T	R276C	Brown	222	212	203	9	15	207	47.9	1.0
ED20	12	c.457C>T	R153C	Ash blond	221	218	215	3	12	209	55.2	1.4
ED21	3	c.536_571del36	G180_P191del	Brown	119	119	119	0	3	116	63.2	8.6
C1	7	None	—	Ash blond	342	298	254	44	101	241	71.7	47.3
C2	8	None	—	Red	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C3	7	None	—	Ash blond	324	301	278	23	20	304	70.1	43.8
C4	6	None	—	Brown	381	330	284	46	111	270	60.4	20.4
C5	8	None	—	Brown	193	187	181	6	10	183	72.1	49.2
C6	14	None	—	Chestnut	265	257	249	8	10	255	62.0	20.8
C7	8	None	—	Ash blond	326	315	304	11	122	204	57.5	11.8

Italics indicate the subgroup with milder XLHED; n.d., not determined.

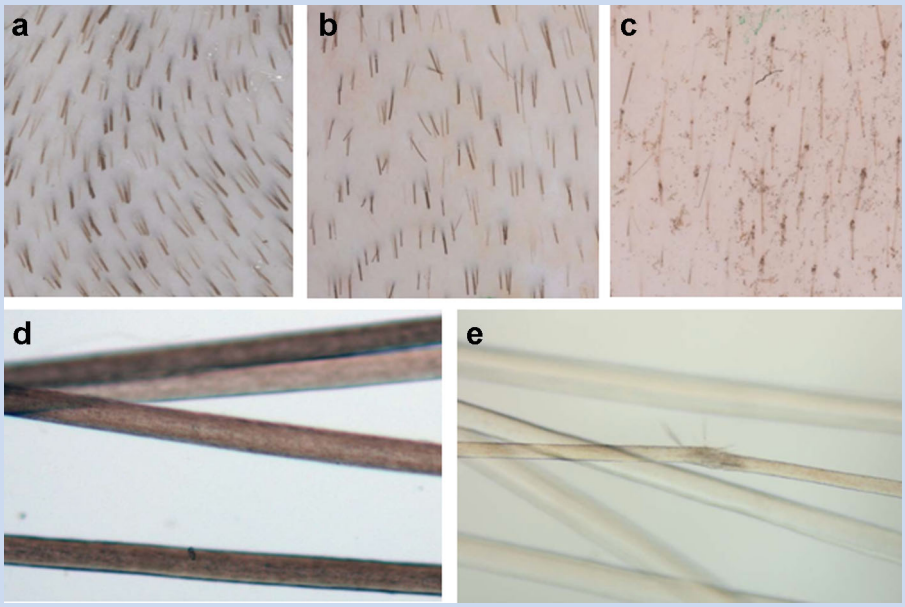

FIG. 3. Hair phenotype in boys with XLHED. a–c: Representative phototrichograms of healthy control individuals and patients with mild and severe XLHED, respectively. d: Microscopic images of control hairs, and (e) hairs from a patient with XLHED showing typical hypopigmentation, caliber differences, and trichorrhexis nodosa.

number of follicular units, number of vellus and non-vellus hairs, and hair width. In children, such phototrichogram analysis seems to be a valid method to identify individuals with the most common form of hypohidrotic ectodermal dysplasia. Typically, affected patients had a lower total hair count, a decreased number of hairs per follicle, fewer vellus hairs, and a decreased thickness of terminal hairs. Most XLHED patients who were able to sweat presented with a milder hair phenotype than XLHED patients who did not have any sweat ducts. This differs from recently reported findings in a group of older XLHED individual (between 11 and 29 years of age), where no clear genotype–phenotype correlations were observed [Jones et al., 2013].

As this study was performed in prepubescent male children, the effects of androgens on hair numbers are likely to be limited. Androgenic alopecia, the most common form of hair loss in humans, affects 80% of European males by the age of 80 [Heilmann et al., 2013]. Our study is the first to assess the XLHED hair phenotype in prepubescent males by phototrichogram analysis, which has been applied by others to evaluate hair growth in androgenic alopecia [Chamberlain and Dawber, 2003].

XLHED patients with hypomorphic *EDA* mutations had darker hair than the majority of anhidrotic XLHED patients, who presented with almost unpigmented hairs, indicating a lack of melanocytes that descend from the neuroectoderm. In contrast to

TABLE III. Statistical Analysis of the Sweat Gland and Hair Finding

	Control (n = 7)		Mild XLHED (n = 8)			Severe XLHED (n = 11)			Mild–Severe XLHED *P* value
	Median	IQR	Median	IQR	*P* value	Median	IQR	*P* value	
Sweat glands									
Number of sweat ducts (palm, 36 mm^2)	211	187.5–233.25	23.5	5.5–36	0.0043	0	0–0	0.0005	0.0002
Sweat volume (μl)	56	53.5–81.5	5	2.5–8.5	0.0003	0	0–0	0.0001	0.0001
Hair									
Total hair count	325	265–342	128	116–201.5	0.0027	102.5	91–109	0.0002	0.02
Vellus hairs (%)	17.7	5.2–29.5	2.0	0.4–6.1	0.042	6.0	1.2–9.8	0.26	0.35
Mean hair width (μm)	66.1	60.4–71.7	59.0	51.6–62.0	0.081	47.9	42.1–54.9	0.0002	0.017
Hair width >74 μm (%)	32.3	20.4–47.3	6.5	1.2–16.1	0.02	0.8	0–3.6	0.0005	0.10
Number of follicular units	299.5	257–315	123.5	116–192	0.0027	102.5	91–108	0.0002	0.02
Follicles with one hair	94.4	86.1–96.8	99.3	95.0–100	0.053	100	100–100	0.004	0.20
Follicles with ≥2 hairs	5.6	3.2–13.9	0.7	0–5.0	0.053	0	0–0	0.004	0.20

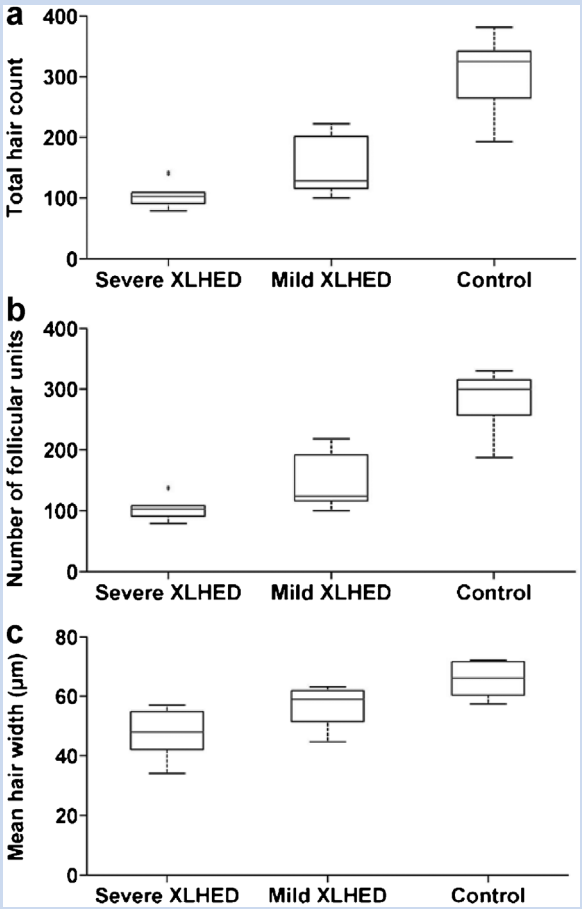

FIG. 4. Correlation of hair parameters with the EDA genotype. a: Total hair count, (b) number of follicular units, and (c) mean hair width were found to be lowest in patients predicted to have no functional *EDA* gene product, intermediate in individuals with hypomorphic *EDA* mutations (mild XLHED) and highest in healthy control patients. The central mark of the boxplot shows the median of the data points, the edges of the box denote the 25th and the 75th centile. Whiskers indicate the range of data points not considering outliers (located outside the +/− 2.7 sigma range), which are plotted individually.

individuals affected by the severe form of XLHED, children with mild XLHED had a significantly higher total hair count and thicker hairs, suggestive of partial EDA expression also in the hair follicles.

Skin appendages form during fetal development. This process starts by the appearance of focal thickenings of the epithelium (placodes), which invaginate to form buds. The hair follicle bud grows rapidly downwards and encases a cluster of dermal cells that form the dermal papilla [Mikkola and Millar, 2006]. Ectodysplasin A has been demonstrated to play an important role during these early stages of hair development [Mikkola, 2009, and papers cited therein]. Furthermore, the ectodysplasin–NFkappaB signaling pathway was found to be involved in common variations of scalp hair morphology in human populations [Fujimoto et al., 2008]. A variant of the gene *EDAR* encoding an ectodysplasin A receptor with a single amino acid substitution, V370A, has experienced strong positive selection in East Asia more than 10,000 years ago and is now near fixation [Sabeti et al., 2007]. This allele is associated with increased hair thickness [Fujimoto et al., 2008] and practically absent in European and African populations. The gene product acts by amplifying the ectodysplasin A signal to a greater degree than the European/African variant does [Mou et al., 2008].

Considering the complexity of the ectodysplasin–NFkappaB signaling pathway, lack of obvious genotype–phenotype correlations for a single component may be explained by the influence of other molecules involved and by the possibility that certain tissues are particularly susceptible to reduced ectodysplasin A signaling activity. In this study, two brothers carrying the same hypomorphic *EDA* mutation (patients ED17 and ED18) presented with different hair characteristics. Patient ED17 had fewer but thicker hairs than his younger brother, indicating that the hair phenotype depends on multiple genetic factors. A possible impact of androgens as the main reason for the hair differences between these brothers, who were 12 and 5 years of age, cannot be excluded.

Based on a previous study on XLHED patients [Dietz et al., 2013], we have further proposed a relationship between *EDA* genotype and ocular symptoms that are mainly due to meibomian gland deficiency, although the findings also pointed to an important role of factors other than *EDA* expression. The results of this study lead to a similar conclusion, supported for example by the lack of correlation between number of sweat ducts and total hair count. Despite a clear correlation between the amount of sweat ducts and sweat volume measured in children with mild XLHED, we have no doubt that there are XLHED patients with detectable sweat glands that produce no sweat. Patients ED19 and ED20 carrying the hypomorphic missense mutations R276C and R153C, respectively, had total hair counts in the range of the control group, whereas their hair width was similar to that of individuals with severe XLHED. On the other hand, all mutations expected to cause pronounced impairment of ectodysplasin A synthesis or release or insufficient binding to its receptor led to a severe skin and hair phenotype.

In summary, our data suggest a reproducible association of common *EDA* genotypes with certain XLHED phenotypes, implying that systematic mapping of *EDA* mutations together with the evaluation of quantifiable clinical data helps to distinguish functionally crucial gene defects from mutations allowing residual ectodysplasin A activity. This knowledge may prove particularly useful for a valid assessment of the efficacy of future therapeutic approaches [Gaide and Schneider, 2003; Casal et al., 2007; Cui et al., 2009; Mauldin et al., 2009; Kowalczyk et al., 2011].

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