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SHORT COMMUNICATION

Sweating ability of patients with p63-associated syndromes

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Abstract

Sweating deficiency has been reported to represent a cardinal symptom of ectrodactyly-ectodermal dysplasia-cleft lip/palate syndrome and ankyloblepharon-ectodermal dysplasia-cleft lip/palate syndrome, two rare p63-associated disorders. According to online resources, hypohidrosis may lead to most life-threatening complications in affected patients. Thus, counseling on the prevention of hyperthermia would be indispensable in case of such syndromes, although detailed information on this issue is missing in the literature. We investigated 14 individuals with ectrodactyly-ectodermal dysplasia-cleft lip/palate syndrome (age range 2–48 years) and 9 individuals with ankyloblepharon-ectodermal dysplasia-cleft lip/palate syndrome (0.5–60 years of age) by confocal laser scanning microscopy to determine their palmar sweat duct density and by quantification of pilocarpine-induced sweating. Genotype-phenotype correlations were assessed. In 12 of 23 patients (52%), a normal amount of sweat ducts was detected. These individuals (9 with ectrodactyly-ectodermal dysplasia-cleft lip/palate syndrome, 3 with ankyloblepharon-ectodermal dysplasia-cleft lip/palate syndrome) produced sufficient sweat volumes (≥ 20 μl) in response to pilocarpine. All other patients had clearly reduced sweating ability and fewer sweat glands, but no anhidrosis. Alteration of a specific proline residue (Pro590) of p63 was consistently linked to impaired perspiration.

Conclusion: Hypohidrosis in p63-associated syndromes is less common and potentially less severe than previously thought and may be attributable to certain genotypes.

What is Known:
• *Hypohidrosis which has been listed as a cardinal symptom of AEC and EEC syndromes may lead to life-threatening hyperthermia.*

What is New:
• *Patients with EEC and AEC syndromes often can sweat normally.*
• *Hypohidrosis seems to be attributed to certain TP63 genotypes.*

Keywords Ectodermal dysplasia · Sweat glands · p63 · AEC syndrome · EEC syndrome · Hypohidrosis

Introduction

Sweating matters, particularly during summer sun exposure or intense physical activity when insufficient perspiration (hypohidrosis), may lead to heat exhaustion and heatstroke. Ectrodactyly-ectodermal dysplasia-cleft lip/palate (EEC) syndrome [OMIM 604292] and ankyloblepharon-ectodermal dysplasia-cleft lip/palate (AEC) syndrome [OMIM 106260] are very rare genetic disorders caused by mutations in the gene *TP63* encoding the transcription factor p63 [1, 3]. In several textbooks, e.g., *Andrews' diseases of the skin: clinical dermatology*, and online resources such as Orphanet and Wikipedia, hypohidrosis is listed as a cardinal symptom of these two p63-associated disorders. The current Orphanet entry on EEC syndrome (ORPHA1896), for example, states that hypohidrosis would lead to most life-threatening complications in affected patients. If that was true, the diagnosis of

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EEC or AEC syndrome would require early postnatal counseling of affected families on the prevention of overheating, as recommended and routinely provided to the parents of infants with hypohidrotic ectodermal dysplasia [6, 9]. To date, however, not many details on the sweating ability of patients with EEC or AEC syndrome have been reported in the literature.

Sweat pore densities can be estimated on graphite prints of the palm [9] provided that the staining is applied evenly across the dermal ridges. In more recent studies, reflectance confocal laser scanning microscopy has allowed more accurate determination of sweat pore densities at the palms or soles, and data from individuals with hypohidrotic ectodermal dysplasia as well as from healthy control subjects of various age groups have been collected [2, 4, 10]. Sweat gland function can be evaluated by quantification of pilocarpine-induced sweating with well-established devices [9, 10] that are used worldwide in infants for the laboratory diagnosis of cystic fibrosis [7]. To clarify whether *TP63* mutations that underlie EEC or AEC syndrome are indeed associated with severe sweating deficiency, we measured number and function of sweat glands and assessed genotype-phenotype correlations in a group of individuals with known or yet unreported *TP63* mutations.

Subjects and methods

Fourteen individuals with EEC syndrome between 2 and 48 years of age and 9 individuals with AEC syndrome (age range 0.5 to 60 years) were enrolled in this study conducted alongside a family conference of the German-Swiss-Austrian ectodermal dysplasia patient organization. All adult subjects gave written informed consent to participate; in the case of minors, parental consent and if possible 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. Subjects were included only if pathogenic *TP63* variants 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, pregnancy, implantable electronic devices, and known plaster allergy.

The medical history of each subject was taken (including finger and toe numbers at birth, extent of orofacial clefting, and genetic data), followed by routine clinical assessments, photo-documentation of split-hand and foot malformations, and standardized evaluation of palmar sweat ducts and sweating ability.

Sweat duct imaging

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, NY). Microscopic images were evaluated by an independent experienced examiner blinded to the genotype of the subject. The sweat duct count was calculated per square centimeter. As infants—due to their smaller body surface—have a much higher sweat pore density than schoolchildren and adults, sweat duct counts were compared after being extrapolated to whole-body surface area according to the Mosteller formula.

Quantification of pilocarpine-induced sweating

Sweat was collected by a standardized procedure from an area of 57 mm^2 of the right forearm for 30 min after stimulation with a pilocarpine gel disk using the Wescor 3700 device (Wescor, Logan, USA). Maximum volume that could be collected in the disposable microbore tubing spiral (Macroduct Sweat Collector) placed over the stimulated area of the skin was 93 μl. A small amount of blue dye facilitated quantification of the sweat volume in the tubing by comparison with a spiral template marked with appropriate lines.

Statistical analysis

Descriptive statistics were calculated for each group. Group comparisons between EEC/AEC patients without thermoregulation problems and EEC or AEC patients with relevant hypohidrosis were done by Mann-Whitney *U* test using SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Twelve of 23 subjects with AEC or EEC syndrome investigated in this study (52%) had a medical history without thermoregulation problems, as reported by the patients themselves or by their parents, and were found to have both normal sweat pore densities at the palm and sufficient sweat production on the forearm (≥ 20 μl within 30 min) in response to stimulation with pilocarpine. None of them had ever been hospitalized for unexplained hyperthermia, and 8 practiced sports regularly at least two times per week. For 5 patients with EEC syndrome (1 male, 4 female) and 6 patients with AEC syndrome (all female), mild to moderate thermoregulation problems were reported including the occurrence of hyperthermic episodes during childhood, in the summer months, or when exercising for more than 30 min. In all 11 affected subjects, palmar sweat pore density and pilocarpine-induced sweat production were diminished, but none of these patients suffered from anhidrosis. Individual data for each subject are shown in Table 1.

Since in each case the pathogenic *TP63* variant had been identified prior to the study, including six previously unreported missense mutations (Table 1), genotype-phenotype

Table 1 *TP63* mutations and sweating ability in patients with EEC or AEC syndrome

Code	Age (years)	Sex	Body height (cm)	Weight (kg)	Sports activities at least 2×/week	*TP63* variant according to GenBank NM_003722 [a]	Exon	Amino acid substitution or deletion	Predicted effect of the mutation	Sweat volume, forearm (μl) [b]	Sweat pore counts, palm [c]	Extrapolated sweat ducts in total (×10^6) [d]	Body temperature (°C)
EEC and AEC patients without thermoregulation problems													
EEC-1	2	F	96.5	14.2	–	c.727C>T	6	p.Arg243Trp	Disturbed DNA binding	20.0	496	3.06	36.8
EEC-2	39	F	174.0	50.5	Yes	**c.796C>G**	6	**p.Arg266Gln**	Disturbed DNA binding	57.0	436	6.81	36.6
EEC-4	34	M	185.0	81.5	Yes	c.952C>T	7	p.Arg318Cys	Disturbed DNA binding	72.0	356	7.29	36.8
EEC-6	10	M	146.0	33.4	Yes	**c.853_855del**	7	**p.Ser285del**	Disturbed DNA binding	28.0	420	4.89	37.0
EEC-7	48	F	163.0	63.1	No	c.955C>T	7	p.Arg319Cys	Disturbed DNA binding	33.0	404	6.83	37.0
EEC-9	2	M	96.0	13.0	–	c.952C>T	7	p.Arg318Cys	Disturbed DNA binding	65.0	924	5.44	36.4
EEC-10	3	M	99.0	16.2	Yes	c.797G>A	6	p.Arg266Gln	Disturbed DNA binding	21.0	700	4.67	36.8
EEC-11	10	M	132.0	27.7	Yes	**c.796C>G**	6	**p.Arg266Gln**	Disturbed DNA binding	68.0	552	5.56	37.0
EEC-12	14	F	165.0	56.7	Yes	c.836G>A	6	p.Arg279His	Disturbed DNA binding	42.0	408	6.58	36.9
AEC-1	11	M	155.0	42.0	Yes	c.1610T>C	13	p.Ile537Thr	Impaired protein-protein interaction	23.0	432	5.81	36.1
AEC-6	5	F	115.0	22.0	Yes	**c.1718T>A**	13	**p.Ile573Asn**	Impaired protein-protein interaction	34.0	612	5.13	37.0
AEC-9	0.5	M	71.0	8.5	–	c.1799G>A	14	p.Gly600Asp	Impaired protein-protein interaction	43.0	1336	5.47	36.9
Average	*16.2*									*42.2*	*589.7*	*5.74*	*36.8*
SD	*16.3*									*19.0*	*284.6*	*0.66*	*0.3*
EEC patients with relevant hypohidrosis													
EEC-3	2	F	85.5	12.0	–	c.1028G>A	8	p.Arg343Gln	Disturbed DNA binding	0.5	264	1.41	37.2
EEC-5	23	F	157.0	65.0	No	c.952C>T [e]	7	p.Arg318Cys [e]	Disturbed DNA binding [e]	8.0	272	4.58	37.5
EEC-8	6	M	118.0	19.5	Yes	c.1028G>A	8	p.Arg343Gln	Disturbed DNA binding	7.0	296	2.37	36.6
EEC-13	12	F	149.0	38.8	No	**c.1789A>T**	14	**p.Ile597Phe**	Impaired protein-protein interaction	1.0	84	1.06	36.6
EEC-14	28	F	155.0	50.5	yes	c.1028G>A	8	p.Arg343Gln	Disturbed DNA binding	5.0	176	2.60	37.5
Average	*14.2*									*4.3*	*218.4*	*2.40*	*37.1*
SD	*11.1*									*3.4*	*87.8*	*1.37*	*0.4*
AEC patients with relevant hypohidrosis													
AEC-2	11	F	142.0	36.0	No	**c.1769C>T**	14	**p.Pro590Lys**	Impaired protein-protein interaction	3.0	196	2.34	36.9
AEC-3	44	F	163.0	87.5	No	**c.1769C>T**	14	**p.Pro590Lys**	Impaired protein-protein interaction	0.5	80	1.59	36.9
AEC-4	50	F	168.0	57.7	No	c.1766T>G	14	p.Ile589Ser	Impaired protein-protein interaction	11.0	212	3.48	37.6
AEC-5	60	F	153.0	67.9	No	**c.1768C>A**	14	**p.Pro590Thr**	Impaired protein-protein interaction	8.0	156	2.65	36.9
AEC-7	36	F	170.0	79.0	Yes	c.1799G>A	14	p.Gly600Asp	Impaired protein-protein interaction	7.0	132	2.55	37.4
AEC-8	7	F	129.0	25.7	Yes	c.1799G>A	14	p.Gly600Asp	Impaired protein-protein interaction	0.5	220	2.11	36.8
Average	*34.7*									*5.0*	*166.0*	*2.25*	*37.1*
SD	*21.4*									*4.3*	*54.0*	*0.42*	*0.3*

[a] Previously unreported mutations in bold type; [b] upper detection limit = 93 μl; [c] within a 1-cm^2 area of the palm; [d] relative to total body-surface area as calculated with the Mosteller method; [e] carries heterozygously an additional pathogenic *EDA* variant; *M*, male; *F*, female; *SD*, standard deviation

correlations with respect to number and function of sweat glands could be assessed.

Most interestingly, in-frame deletion of the serine residue at position 285 of p63 (DNA binding domain) did not cause hypohidrosis, albeit being associated with a severe split-hand phenotype (Fig. 1, upper panel). In general, there was no correlation between the severity of sweat gland maldevelopment and the extent of split-hand malformation. Subject EEC-14, for example, had all ten fingers but very few sweat glands (Fig. 1, lower panel). The three subjects with the *TP63* variant p.Arg266Gln showed normal sweat duct densities and sweating ability but highly variable split-hand and foot malformation.

All patients with the *TP63* missense mutation c.1028G>A (p.Arg343Gln) known to cause EEC syndrome had fewer sweat ducts and lower sweating ability than patients with upstream mutations (Table 1). Subject EEC-5 was an exception, most likely explained by the additional *EDA* mutation p.Arg69Leu that impairs sweat gland development [9]. In our patients with AEC syndrome, alteration of a specific proline residue (Pro590) always led to hypohidrosis, and the missense mutations c.1766T>G (p.Ile589Ser) or c.1799G>A (p.Gly600Asp) were in all cases but one associated with relatively few sweat ducts and reduced sweating ability, whereas two mutations upstream which also caused the classical phenotype of AEC syndrome did not appear to have affected sweat gland number and function.

Discussion

This study shows that hypohidrosis is rather uncommon in EEC syndrome, as stated recently also by Sutton and van Bokhoven in GeneReviews [*TP63*-related disorders]. However, reduced sweating ability seems to be a more frequent issue in patients with AEC syndrome, which may be due to pathogenetic differences. *TP63* mutations that cause AEC syndrome are known to lead to impaired protein-protein interaction; protein aggregation of the p63 transcription factor was shown to underlie severe skin fragility in AEC syndrome [8]. Taking into account that none of our 23 patients suffered from anhidrosis and more than half could sweat normally, the importance of disturbed thermoregulation in both syndromes seems to be overestimated in current textbooks and online resources such as Orphanet.

Although for p63-associated syndromes rather strong genotype-phenotype correlations have been observed [1, 11], there are exceptions with overlapping symptoms. In this study, all but one mutations affecting the sterile alpha motif (SAM) domain of p63 resulted in AEC syndrome. Patient EEC-13 who carried a previously unreported mutation in the same region, however, displayed a distinct phenotype of EEC syndrome including split-foot malformation, extremely sparse hair, lack of eyebrows, hypoplastic nails, and severe hypohidrosis. We found no correlation between the extent of hand or foot malformation and maldevelopment of sweat glands, but identified two sites in the gene *TP63*, mutation of which was regularly associated with fewer sweat glands and impaired perspiration. This indicates some relevant, previously unrecognized genotype-phenotype correlation with respect to the sweating ability.

Interestingly, 10 of 11 patients with thermoregulation problems due to hypohidrosis were female. This might reflect in part the general perception that, beyond a certain requirement for heat loss, men have a larger sweat output per gland than women [5] but would be fully explained by the lower sweat

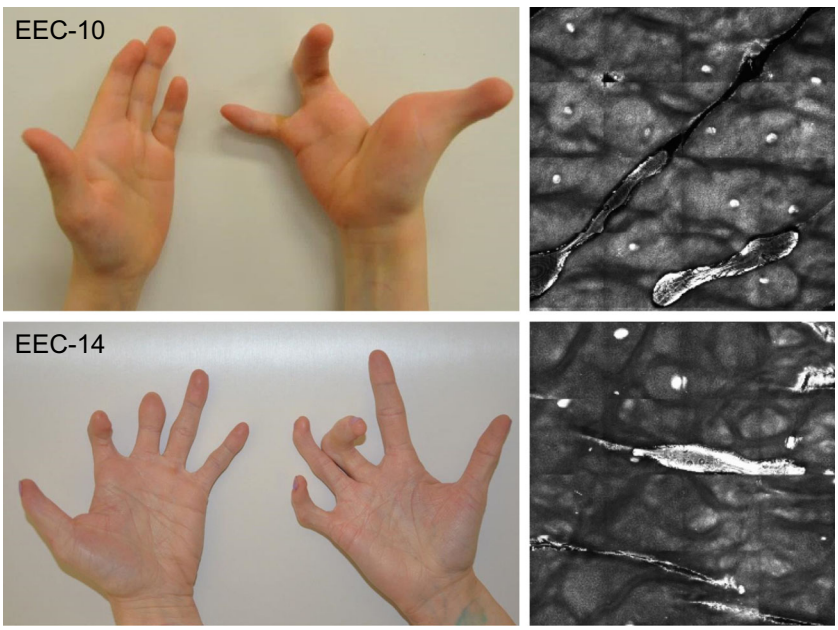

Fig. 1 Lack of correlation between the severity of sweat gland maldevelopment and the extent of split-hand malformation. Sweat pores (brighter circular spots in the middle of dermal ridges) were visualized at the palm

gland density and sweat production observed in the affected individuals. The patients with relevant hypohidrosis also showed a tendency for higher body temperature at rest compared with those EEC/AEC patients who were able to sweat normally. Nevertheless, thermoregulation problems did not seem to affect their daily life too much and did not prevent 4 of 11 hypohidrotic patients (36%) from practicing sports at least two times per week. Although the risk of overheating therefore does not approximate that of X-linked hypohidrotic ectodermal dysplasia, we think it is reasonable and probably cost-effective to recommend confocal microscopy and/or sweat volume assessment in all infants with EEC or AEC syndrome.

Thus, hypohidrosis in EEC/AEC syndromes is less common and potentially less severe than previously thought, but a postnatal discussion with the family regarding risk of hyperthermia is still warranted. Reduced sweating ability as a facultative symptom of p63-associated disorders may be attributable to certain genotypes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent All adult participants provided written informed consent; in the case of minors, parental consent was obtained.

Abbreviations AEC, Ankyloblepharon-ectodermal dysplasia-cleft lip/palate; EEC, Ectrodactyly-ectodermal dysplasia-cleft lip/palate; SAM, Sterile alpha motif; DNA, Deoxyribonucleic acid

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