#### 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, 3 with ankyloblepharon-ectodermal dysplasia-cleft lip/palate syndrome. 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

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### 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 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<sup>2</sup> 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<sup>2</sup> 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  $\mu$ l. 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 ( $\geq 20 \,\mu$ l 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

| Code         Age         Sex         Body<br>(stant)         Weight<br>(stant)         Ford winks<br>and white<br>(stant)         Total winks<br>(stant)         T   | Age<br>(pers)SerBodyWeight<br>installSport<br>servitationTrGS variant<br>conditionEvaluation<br>anstallFrom and<br>conditionFrom and<br>conditionSecuritation<br>servitationEvaluation<br>conditionEvaluation<br>anstallEvaluation<br>conditionEvaluation<br>anstallEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>conditionEvaluation<br>condition $2^{+}$ M90010.2VVPPP <td< th=""><th>Table 1</th><th>TP63</th><th>IIIutau</th><th>11 02 IIIIuauous and sweams aouith III paucius will EEC</th><th> Quin</th><th>T</th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></td<>  | Table 1  | TP63             | IIIutau   | 11 02 IIIIuauous and sweams aouith III paucius will EEC | Quin           | T  |   |           |   |                                      |  |       |      |        |
|---|--|----------|------------------|-----------|---|----------------|--|---|-----------|---|--------------------------------------|--|-------|------|--------|
| ALC partners vibrant thermoregature problem $-7275$ $-7125$ $-7225$ <td><math display="block"> \begin{array}{cccccccccccccccccccccccccccccccccccc</math></td> <td>Code</td> <td>Age<br/>(years)</td> <td></td> <td>Body<br/>height (cm)</td> <td>Weight<br/>(kg)</td> <td>Sports<br/>activities at<br/>least 2×/week</td> <td><i>TP63</i> variant according to GenBank NM_003722 <sup>a</sup></td> <td>Exon</td> <td>Amino acid<br/>substitution<br/>or deletion</td> <td>Predicted effect<br/>of the mutation</td> <td>Sweat volume,<br/>forearm (µl) <sup>b</sup></td> <td></td> <td></td> <td></td>  | $ \begin{array}{cccccccccccccccccccccccccccccccccccc$  | Code     | Age<br>(years)   |           | Body<br>height (cm)                                     | Weight<br>(kg) | Sports<br>activities at<br>least 2×/week | <i>TP63</i> variant according to GenBank NM_003722 <sup>a</sup> | Exon      | Amino acid<br>substitution<br>or deletion | Predicted effect<br>of the mutation  | Sweat volume,<br>forearm (µl) <sup>b</sup> |       |      |        |
| $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$  | EEC:         2         F         945         1(2)         - $C_{27CCF}$ 6 $\rho_{A72}^{0}(61)$ Burnho DNA huling         200         966         310         663         366         <   | EEC an   | $4 AEC p_{\ell}$ | ttients w | ithout thermo.  | regulation     | problems                                 |   |           |   |                                      |  |       |      |        |
| $35$ $F$ $1740$ $305$ $75$ $2706$ CG $6$ $h_{MMing}$ $70$ $436$ $681$ $36$ $311$ $86$ $325257$ $7$ $h_{MMing}$ $70$ $326$ $729$ $36$ $601$ $301$ $ 295557$ $7$ $h_{MMing}$ $200$ $204$ $487$ $3$ $900$ $162$ $78$ $e87386671$ $17$ $h_{MMing}$ $200$ $204$ $487$ $3$ $900$ $162$ $78$ $e9758A$ $6$ $h_{M2}^{2}96611$ $1844764$ $1863$ $200$ $200$ $400$ $483$ $1$ $110$ $82$ $e795661$ $11087746$ $11087746$ $1109$ $820$ $220$ $231$ $541$ $110$ $820$ $220$ $782661$ $11087746$ $11087746$ $1109$ $1109$ $1109$ $1109$ $1109$ $1109$ $1109$ $1109$ $1109$ $1109$ <td< td=""><td><math display="block"> \begin{array}{c ccccccccccccccccccccccccccccccccccc</math></td><td>EEC-1</td><td>5</td><td>ц</td><td>96.5</td><td>14.2</td><td></td><td>c.727C&gt;T</td><td>9</td><td>p.Arg243Trp</td><td>Disturbed DNA binding</td><td>20.0</td><td>496</td><td>3.06</td><td>36.8</td></td<>  | $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$   | EEC-1    | 5                | ц         | 96.5  | 14.2           |  | c.727C>T  | 9         | p.Arg243Trp                               | Disturbed DNA binding                | 20.0                                       | 496   | 3.06 | 36.8   |
| 34         M         1850         815         Yes $e35CST$ 7 $hArg10X_8$ Disturbed DNA binding         220         336         729           2         M         960         13.1         Ves $e35CST$ 7 $hArg10X_8$ Disturbed DNA binding         550         294         489           2         M         990         16.2         Yes $e35CST$ 7 $hArg10X_8$ Disturbed DNA binding         550         294         544           10         M         13.0         2.0         Yes $e535CST$ 6 $hArg266Gn$ Disturbed DNA binding         650         294         544           11         M         1500         2.0         Yes $e535CST$ 1 $hArg266Gn$ Disturbed DNA binding         680         532         551           5         M         11.0         8.5 $e1100$ 9.0         10.2         9.0         6.0           6.5         M         71.0         8.5 $e1000$ 9.0         10.2         2.0         574           6.5         M         11.0         8.5 $e1000$ 9.  | $ \begin{array}{cccccccccccccccccccccccccccccccccccc$  | EEC-2    | 39               | Ч         | 174.0   | 50.5           |  | c.796C>G  | 9         | p.Arg266Gln                               | Disturbed DNA binding                | 57.0                                       | 436   | 6.81 | 36.6   |
| $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$  | EEC 6 10 M 1460 314 Ye 8 e2355CT 7 pAr235(56 Disturbed DNA binding 330 420 453 570 570 453 570 570 453 570 570 570 570 570 570 570 570 570 570   | EEC-4    | 34               | Σ         | 185.0   | 81.5           |  | c.952C>T  | ٢         | p.Arg318Cys                               | Disturbed DNA binding                | 72.0                                       | 356   | 7.29 | 36.8   |
| $48$ F         1630         631         No $c_{925CT}$ 7 $p_{Arg}$ 316C/s         Disturbed DNA binding         530         244         683 $3$ $960$ 130 $-s$ $c_{925CT}$ 7 $p_{Arg}$ 318C/s         Disturbed DNA binding         550 $244$ 544 $3$ $960$ 150 $rs$ $c_{777GA}$ $6$ $p_{Arg}$ 316G/s         Disturbed DNA binding         550 $246$ $635$ 553 $11$ $M$ 1530 $420$ Yes $c_{737GA}$ $13$ $DileF$ $420$ $780$ $535$ $534$ $16.3$ $M$ $110$ $8.5$ $-1007SC$ $13$ $DileF$ $420$ $407$ $534$ $16.3$ $M$ $11.0$ $8.5$ $-1007SC$ $100$  | $ \begin{array}{cccccccccccccccccccccccccccccccccccc$  | EEC-6    | 10               | Σ         | 146.0   | 33.4           |  | c.853 855del  | ٢         | p.Ser285del                               | Disturbed DNA binding                | 28.0                                       | 420   | 4.89 | 37.0   |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$   | $ \begin{array}{cccccccccccccccccccccccccccccccccccc$  | EEC-7    | 48               | Ц         | 163.0   | 63.1           |  | c.955C>T  | ٢         | p.Arg319Cys                               | Disturbed DNA binding                | 33.0                                       | 404   | 6.83 | 37.0   |
| 3         M         900         162         Yes $c.797G_{cA}$ 6         p.Arg266Gin         Disturbed DNA binding         210         700         467           10         M         132.0         5.7         Yes $c.797G_{cA}$ 6         p.Arg266Gin         Disturbed DNA binding         6.0         700         467           11         M         155.0         5.7         Yes         c.836G_{cA}         6         p.Arg278G         3.0         4.0         4.0         5.55         5.56           11         M         155.0         42.0         Yes         c.10107-C         13         p.116577Aan         19.0         136         5.34 <td><math display="block"> \begin{array}{cccccccccccccccccccccccccccccccccccc</math></td> <td>EEC-9</td> <td>7</td> <td>Σ</td> <td>96.0</td> <td>13.0</td> <td>Ι</td> <td>c.952C&gt;T</td> <td>٢</td> <td>p.Arg318Cys</td> <td>Disturbed DNA binding</td> <td>65.0</td> <td>924</td> <td>5.44</td> <td>36.4</td>   | $ \begin{array}{cccccccccccccccccccccccccccccccccccc$  | EEC-9    | 7                | Σ         | 96.0  | 13.0           | Ι  | c.952C>T  | ٢         | p.Arg318Cys                               | Disturbed DNA binding                | 65.0                                       | 924   | 5.44 | 36.4   |
| $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$  | EEC.11         M         12.0         5.7         Yes $c.796Crd$ $b$ partyofor   | EEC-10   | б                | Σ         | 0.66  | 16.2           |  | c.797G>A  | 9         | p.Arg266Gln                               | Disturbed DNA binding                | 21.0                                       | 700   | 4.67 | 36.8   |
| 14       F       1650       567       Yes $c.866GA$ 6 $pAg279Hi$ Disturbed DNA binding       42.0       408       6.58         5       F       115.0       2.0       Yes $c.17187A$ 13       Dile577Hr       Impaired protein-protein interaction       4.0       6.53       5.47         0.5       M       71.0       8.5       - $c.1799GxA$ 14       D.GlyGOAsp       Inpaired protein-protein interaction       4.0       6.53       5.47         0.5       M       71.0       8.5       - $c.1799GxA$ 8       p.Arg343Gin       Disturbed DNA binding       8.0       5.74       0.66         ensw with relevant hypolidroxis       -       c.1028GxA       8       p.Arg343Gin       Disturbed DNA binding       8.5       2.0       2.64       1.41         2       F       8       p.Arg343Gin       Disturbed DNA binding       8.5       2.00       2.64       1.41         2       6       M       118.0       19.5       Yes       c.1028GxA       8       p.Arg343Gin       Disturbed DNA binding       8.7       2.40       2.40         2       M       118.0       19.5       Yes       c.1028  | EEC12         If         F         165.0         56.7         Yes         c.16075         13         DM3271Th         Impaired protein-protein interaction         31.0         31.3         31.0  | EEC-11   |                  | Σ         | 132.0   | 27.7           |  | c.796C>G  | 9         | p.Arg266Gln                               | Disturbed DNA binding                | 68.0                                       | 552   | 5.56 | 37.0   |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$   | AEC         II         M         IS50         4.20         Yes         C1010FC         I3         p.01673Am         Impaired protein-protein interaction         3.01         3.13         3.01  | EEC-12   | 14               | Ч         | 165.0   | 56.7           |  | c.836G>A  | 9         | p.Arg279His                               | Disturbed DNA binding                | 42.0                                       | 408   | 6.58 | 36.9   |
| 5       F       1150       2.0       Yes       c.1718T>A       13       p.lle573Asn       Impaired protein-protein interaction       340       612       5.13         16.2       M       71.0       8.5       -       c.1796D>A       14       p.Gly600Asp       Impaired protein-protein interaction       330       612       5.13         16.3       M       71.0       8.5       -       c.1796D>A       14       p.Gly600Asp       Inpaired protein-protein interaction       340       612       5.13         2       F       85.5       12.0       -       c.1028G>A       8       p.Arg343Gln       Disturbed DNA binding       0.65       237       4.38         2       F       85.0       No       c.2026C>T       8       p.Arg343Gln       Disturbed DNA binding       0.5       264       1.41         2       F       85.0       S05       yes       c.1028G>A       8       p.Arg343Gln       Disturbed DNA binding       0.5       264       1.41         11       F       1420       S05       yes       c.1028G>A       8       p.Arg343Gln       Disturbed DNA binding       0.5       264       1.06         11.1       F       1420       S   | AEC6         5         F         1150         220         Yes         C1718T-A         13         p.10505A         14         p.01600Asp         Impained protein interaction         430         612         513         537         360         363         360         363         360         363         360         363         360         363         360         361         360         361         360         361         360         361         360         361  | AEC-1    | 11               | Σ         | 155.0   | 42.0           |  | c.1610T>C   | 13        | p.Ile537Thr                               | Impaired protein-protein interaction | 23.0                                       | 432   | 5.81 | 36.1   |
| 0.5       M       71.0       8.5       -       c.1799G>A       14       p.Gly600Asp       Inpaired protein-protein interaction       4.0       1336       5.47 $16.2$ 16.2       16.2       234.6       0.66       5.74       0.66 $16.3$ 16.3       -       c.1799G>A       14       p.Gly600Asp       Inpaired protein-protein interaction       4.0       5.74       0.66 $2.3$ F       85.5       12.0       -       c.1028G>A       8       p.Arg343GIn       Disturbed DNA binding       0.5       5.74       1.41 $2.3$ F       15.70       6.50       No       c.925C>T       7       p.Arg343GIn       Disturbed DNA binding       0.5       2.64       1.41 $2.3$ F       15.50       5.05       yes       c.1028G>A       8       p.Arg343GIn       Disturbed DNA binding       0.5       2.66       2.37 $11.1$ F       14.20       3.88       No       c.1789/S       Inpaired protein-protein interaction       1.0       8.4       1.06 $11.1$ F       155.0       50.5       yes       c.1789/S       Inpaired protein-protein interaction       1.0       8.7  | AEC9         0.5         M         7.10         8.5         -         c.1799C>A         14         p.Gly600Asp         Impaired protein-protein interaction         4.30         1336         5.47         3.69         3.73         3.69         3.73         3.69         3.61         3.73         3.69         3.73         3.69         3.73         3.69         3.73         3.69         3.73         3.69         3.73         3.66         0.66         0.36         3.61         3.66         0.33         3.65         3.66         0.66         0.36         3.66         0.33         3.66         3.73         3.66         3.75         3.75         3.75         3.75         3.75         3.75         3.75         3.75 <td>AEC-6</td> <td>5</td> <td>ц</td> <td>115.0</td> <td>22.0</td> <td>Yes</td> <td>c.1718T&gt;A</td> <td>13</td> <td>p.Ile573Asn</td> <td>Impaired protein-protein interaction</td> <td>34.0</td> <td>612</td> <td>5.13</td> <td>37.0</td>  | AEC-6    | 5                | ц         | 115.0   | 22.0           | Yes                                      | c.1718T>A   | 13        | p.Ile573Asn                               | Impaired protein-protein interaction | 34.0                                       | 612   | 5.13 | 37.0   |
| $16.2$ $42.2$ $539.7$ $574$ $16.3$ $16.3$ $16.3$ $120$ $284.6$ $0.66$ $16.3$ $157.0$ $65.0$ No $c.1028C>A$ $8$ $p.Arg343GIn$ $Disturbed DNA binding$ $0.5$ $284.6$ $1.41$ $2$ $F$ $157.0$ $65.0$ No $c.952C>T^{\circ}$ $7$ $p.Arg343GIn$ $Disturbed DNA binding$ $0.5$ $264$ $1.41$ $2$ $F$ $157.0$ $65.0$ No $c.952C>T^{\circ}$ $7$ $p.Arg343GIn$ $Disturbed DNA binding$ $0.5$ $264$ $1.41$ $2$ $F$ $157.0$ $65.0$ No $c.1028C>A$ $8$ $p.Arg343GIn$ $Disturbed DNA binding$ $2.72$ $4.58$ $28$ $F$ $155.0$ $30.5$ $yes$ $c.1028C>A$ $8$ $p.Arg343GIn$ $Disturbed DNA binding       9.5 2.72 4.58 1.11 2.14 D.16507Pa 14 D.16507Pa 14 P.16507Pa 4.3 2.18.4 2.40 1.11 1.12 $  | Arrange 16.2<br>Space 16.2<br>Space 16.2<br>Space 16.3<br>EEC particles with relevant hypohidrosis<br>EEC 3 2 F 1570 65.0 $-$ c.1028G>A 8 p.Arg345Gin Disturbed DNA binding 05 2.847 0.46 0.66 0.3<br>EEC 3 2 F 1570 65.0 $-$ c.1028G>A 8 p.Arg345Gin Disturbed DNA binding 05 2.272 4.58 37.5<br>EEC 4 2 F 1550 9.5 $-$ c.1028G>A 8 p.Arg345Gin Disturbed DNA binding 0.5 2.272 4.58 37.5<br>EEC 4 2 F 1550 9.5 $-$ c.1028G>A 8 p.Arg345Gin Disturbed DNA binding 0.5 2.272 4.58 37.5<br>EEC 4 2 F 1550 9.5 $-$ c.1028G>A 8 p.Arg345Gin Disturbed DNA binding 0.5 2.272 4.58 37.5<br>EEC 4 2 F 1550 9.5 $-$ c.1028G>A 8 p.Arg345Gin Disturbed DNA binding 0.5 2.272 4.58 37.5<br>Arrange 14.2 $-$ 2.100 $-$ 2.1028G>A 8 p.Arg345Gin Disturbed DNA binding 0.5 $-$ 2.37 $-$ 2.40 $-$ 37.5<br>Arrange 14.2 $-$ 2.100 $-$ 2.1028G>A 8 p.Arg345Gin Disturbed DNA binding 0.5 $-$ 2.37 $-$ 3.66 $-$ 37.5<br>Arrange 14.2 $-$ 2.100 $-$ 2.1028G>A 8 p.Arg345Gin Disturbed DNA binding 0.5 $-$ 2.37 $-$ 3.66 $-$ 37.6<br>Arrange 14.2 $-$ 2.100 $-$ 2.1028G>A 8 p.Arg345Gin Disturbed DNA binding 0.5 $-$ 2.37 $-$ 3.66 $-$ 37.6<br>Arrange 14.2 $-$ 2.100 $-$ 2.1028G>A 14 p.Pro590L38 impaired protein interaction 1.0 $-$ 2.16 $-$ 37.6 $-$ 2.16 $-$ 37.6 $-$ 2.16 $-$ 37.6 $-$ 2.16 $-$ 2.16 $-$ 2.16 $-$ 2.17 $-$ 2.10 $-$ 2.16 $-$ | AEC-9    | 0.5              | Σ         | 71.0  | 8.5            | Ι  | c.1799G>A   | 14        | p.Gly600Asp                               | Impaired protein-protein interaction | 43.0                                       | 1336  | 5.47 | 36.9   |
| $16.3$ $19.0$ $28.46$ $0.66$ $2$ $F$ $85.5$ $12.0$ $ c.1028G>A$ $8$ $p.Arg^343GIn$ $Disturbed DNA binding$ $0.5$ $264.6$ $0.66$ $23$ $F$ $8.5.5$ $12.0$ $ c.1028G>A$ $8$ $p.Arg^343GIn$ $Disturbed DNA binding$ $0.5$ $264$ $1.41$ $2.3$ $F$ $13.0$ $0.5$ $Ves$ $c.1028G>A$ $8$ $p.Arg^343GIn$ $Disturbed DNA binding$ $8.0$ $2.72$ $4.58$ $2.37$ $12.7$ $F$ $14.2$ $D$ $P$ $D$ $D$ $296$ $2.37$ $28$ $F$ $155.0$ $30.5$ $yes$ $c.1028G>A$ $8$ $P.Arg^343GIn$ $Disturbed DNA binding       50 2.49 2.40 243 11.0 S_{11} P.Arg^343GIn Disturbed DNA binding       50 176 2.60 11.41 I.1200 38.6 Vrg^343GIn D D 296 2.34 11.1 F 14.7 $  | Distribution  | Average  |                  |           |   |                |  |   |           |   |                                      | 42.2                                       | 589.7 | 5.74 | 36.8   |
| ents with relevant hypohidrosis       ents with relevant hypohidrosis $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.8$ $0.5$ $0.5$ $0.8$ $0.5$ $0.5$ $0.5$ $0.8$ $0.5$ $0.5$ $0.5$ $0.8$ $0.5$ $0.5$ $0.5$ $0.8$ $0.5$ $0.5$ $0.5$ $0.8$ $0.5$ <td>EEC partents with relevant hypohidrosis         EEC partents with relevant hypohidrosis         EEC partents with relevant hypohidrosis         <math>(-1, -1, -1, -1, -1, -1, -1, -1, -1, -1, </math></td> <td>SD</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>19.0</td> <td>284.6</td> <td>0.66</td> <td>0.3</td>   | EEC partents with relevant hypohidrosis         EEC partents with relevant hypohidrosis         EEC partents with relevant hypohidrosis $(-1, -1, -1, -1, -1, -1, -1, -1, -1, -1, $  | SD       |                  |           |   |                |  |   |           |   |                                      | 19.0                                       | 284.6 | 0.66 | 0.3    |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$   | EEC-3 2 F 85.5 12.0 - c0028C>A 8 p.Arg343Gin Disturbed DNA binding 05 264 1.41 372 EEC-3 2 F 1570 65.0 No c.022Cr <sup>e</sup> 7 p.Arg343Gin Disturbed DNA binding 70 296 2.37 366 EEC-13 12 F 1490 38. No c.17895>T 14 p.LeS97Phe Impaired protein protein interaction 1.0 84 1.06 37.5 4.58 $M_{\rm eff}$ end 18.0 0.5 7.0 296 2.37 366 EEC-13 12 F 155.0 50.5 yes c.1028C>A 8 p.Arg343Gin Disturbed DNA binding 5.0 176 2.60 37.5 4.58 $M_{\rm eff}$ end 18.0 0.7 176 2.40 37.1 3.4 87.8 1.37 0.4 37.1 S. Arerage 14.2 3.0 No c.17895>T 14 p.LeS97Phe Impaired protein protein interaction 1.0 84 1.06 37.5 $M_{\rm eff}$ end 19.0 38.8 No c.17895>T 14 p.LeS97Phe Impaired protein protein interaction 1.0 84 2.40 37.1 S. Arerage 14.2 3.0 No c.17895>T 14 p.HeS9128 Impaired protein protein interaction 3.0 176 2.34 36.9 Arerage 14.2 3.0 No c.17667>T 14 p.Pro590138 Impaired protein protein interaction 3.0 196 2.34 36.9 AEC-3 44 F 163.0 87.5 No c.17667>T 14 p.Pro590138 Impaired protein protein interaction 3.0 196 2.34 36.9 AEC-3 44 F 163.0 87.5 No c.17667>T 14 p.Pro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AEC-3 46 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AEC-3 6 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AEC-3 6 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AIC-3 6 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AIC-3 6 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AIC-3 6 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AIC-3 6 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AIC-3 6 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.12 3.48 37.6 AIC-3 6 F 170.0 79.0 Yes c.17996>A 14 p.Rro590138 Impaired protein protein interaction 1.0 2.23 37.6              | EEC par  | tients wit.      | h releva  | nt hypohidros.  | is             |  |   |           |   |                                      |  |       |      |        |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$   | $ \begin{array}{cccccccccccccccccccccccccccccccccccc$  | EEC-3    | 7                | ц         | 85.5  | 12.0           | Ι  | c.1028G>A   | 8         | p.Arg343Gln                               | Disturbed DNA binding                | 0.5  | 264   | 1.41 | 37.2   |
|   | $ \begin{array}{cccccccccccccccccccccccccccccccccccc$  | EEC-5    | 23               | ц         | 157.0   | 65.0           | No                                       | c.952C>T <sup>e</sup>   | 7         | p.Arg318Cys e                             |                                      | 8.0  | 272   | 4.58 | 37.5   |
| 12         F         149.0         38.8         No <b>c.1789A&gt;T</b> 14 <b>p.He597Phe</b> Impaired protein-protein interaction         1.0         84         1.06           28         F         155.0         50.5         yes         c.1028G>A         8         p.Arg343GIn         Disturbed DNA binding         5.0         176         2.60           14.2 $4.3$ $2.18.4$ $2.40$ $1.37$ $3.4$ $87.8$ $1.37$ 11.1         F         142.0 $36.0$ No <b>c.1769C&gt;T</b> 14 <b>p.Pro590Lys</b> Impaired protein-protein interaction $3.6$ $2.40$ $1.37$ <i>init vith relevant hypohidrosis</i> $3.0$ No <b>c.1769C&gt;T</b> $14$ $p.Pro590Lys$ Impaired protein-protein interaction $0.5$ $2.60$ $2.34$ $44$ F $163.0$ $87.5$ No <b>c.1769C&gt;T</b> $14$ $p.Pro590Lys$ Impaired protein-protein interaction $0.5$ $2.60$ $2.34$ $50$ $67.9$ No <b>c.1769C&gt;T</b> $14$ $p.Pro590Lys$ Impaired protein-protein interaction $0$   | EEC-13         12         F         149.0         38.8         No         c.1789A>T         14 <b>p.lke97Phe</b> Impaired protein-protein interaction         10         84         1.06         36.6           EEC-13         28         F         155.0         50.5         yes         c.10280>A         8 $p.Arg3436$ In         Disturbed DNA binding         5.0         17.4         2.40         37.1           SD         11.1         F         14.20         36.0         No         c.1769C>T         14 <b>p.Pro5901.ys</b> Impaired protein-protein interaction         3.4         87.8         1.37         0.4           AEC         11         F         142.0         36.0         No         c.1769C>T         14 <b>p.Pro5901.ys</b> Impaired protein-protein interaction         3.0         36.9         36.9           AEC         30         8.0         c.1769C>T         14 <b>p.Pro5901.ys</b> Impaired protein-protein interaction         3.0         36.9         36.9           AEC         8.0         67.9         No         c.1769C>A         14 <b>p.Pro5901.ys</b> Impaired protein-protein interaction         3.0         36.9         36.9         36.9         36.9 <t< td=""><td>EEC-8</td><td>9</td><td>Σ</td><td>118.0</td><td>19.5</td><td>Yes</td><td>c.1028G&gt;A</td><td>8</td><td>p.Arg343Gln</td><td>Disturbed DNA binding</td><td>7.0</td><td>296</td><td>2.37</td><td>36.6</td></t<>   | EEC-8    | 9                | Σ         | 118.0   | 19.5           | Yes                                      | c.1028G>A   | 8         | p.Arg343Gln                               | Disturbed DNA binding                | 7.0  | 296   | 2.37 | 36.6   |
| 28       F       155.0       50.5       yes       c.1028G>A       8       p.Arg343GIn       Disturbed DNA binding       5.0       176       2.60         14.2 $1.4.2$ $3.4$ $8.7.8$ $2.18.4$ $2.40$ $1.37$ $11.1$ $F$ $1.20$ $3.6$ $No$ $c.1769C>T$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.34$ $11.1$ $F$ $142.0$ $36.0$ $No$ $c.1769C>T$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.34$ $11.1$ $F$ $142.0$ $36.0$ $No$ $c.1769C>T$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.34$ $50$ $F$ $168.0$ $57.7$ $No$ $c.1769C>T$ $14$ $p.Ho590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.56$ $50$ $60$ $F$ $170.0$ $79.0$ $70$ $156$ $2.55$ $50$ $2.76$ $2.77$ $7.7$ $7.9$ $2.20$ <  | $ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$  | EEC-13   | 12               | ц         | 149.0   | 38.8           | No                                       | c.1789A>T   | 14        | p.Ile597Phe                               | Impaired protein-protein interaction | 1.0  | 84    | 1.06 | 36.6   |
| 14.2 $4.3$ $218.4$ $2.40$ $11.1$ $3.4$ $87.8$ $1.37$ ems with relevant hypohidrosis $3.6$ $87.8$ $1.37$ $11.1$ $F$ $142.0$ $36.0$ No $c.1769C>T$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.34$ $11.1$ $F$ $163.0$ $87.5$ No $c.1769C>T$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.34$ $50$ $F$ $163.0$ $87.5$ No $c.1766C>T$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.34$ $50$ $F$ $153.0$ $67.9$ No $c.1766C>A$ $14$ $p.Ho590Lys$ Impaired protein-protein interaction $1.56$ $2.25$ $50$ $7.7$ $Yes$ $c.1768C>A$ $14$ $p.Gly600Asp$ Impaired protein-protein interaction $7.0$ $129.2$ $2.55$ $7.7$ $F$ $129.0$ $25.7$ $Yes$ $c.1799G>A$ $14$ $p.Gly60$   | Average $I_{11}$ $3.4$ $2.18.4$ $2.40$ $37.1$ SD $11.1$ $3.4$ $8.7.8$ $1.37$ $0.4$ Abscale $11.1$ $3.6$ $8.7.8$ $1.37$ $0.4$ AEC patients with relevant hypohidrosis $5.7$ $80$ $5.7.7$ $0.4$ $3.6.9$ $6.9$ $3.6.9$ <t< td=""><td>EEC-14</td><td>28</td><td>ц</td><td>155.0</td><td>50.5</td><td>yes</td><td>c.1028G&gt;A</td><td>~</td><td>p.Arg343Gln</td><td>Disturbed DNA binding</td><td>5.0</td><td>176</td><td>2.60</td><td>37.5</td></t<>  | EEC-14   | 28               | ц         | 155.0   | 50.5           | yes                                      | c.1028G>A   | ~         | p.Arg343Gln                               | Disturbed DNA binding                | 5.0  | 176   | 2.60 | 37.5   |
| 11.1 $3.4$ $87.8$ $1.37$ $2$ patients with relevant hypohidrosis $2$ patients with relevant hypohidrosis $2$ $36.0$ No $c.1769CrT$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.34$ $C-2$ $11$ $F$ $142.0$ $36.0$ No $c.1769CrT$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $3.0$ $196$ $2.34$ $C-3$ $44$ $F$ $163.0$ $87.5$ No $c.1766TrSd$ $14$ $p.Pro590Lys$ Impaired protein-protein interaction $0.5$ $80$ $1.59$ $C-4$ $50$ $F$ $168.0$ $57.7$ No $c.1766TrSd$ $14$ $p.Hro590Lys$ Impaired protein-protein interaction $0.5$ $80$ $1.59$ $C-5$ $60$ $F$ $153.0$ $67.9$ $No$ $c.1790CrSd$ $14$ $p.Hro590Thr$ Impaired protein-protein interaction $0.5$ $2.55$ $C-7$ $36$ $F$ $170.0$ $79.0$ $70.0$ $2.57$ $2.55$ $2.56$ $2.66$   | SD         11.1 $3.4$ $87.8$ $1.37$ $0.4$ AEC patiens with relevant hypohidrosis $3.69$ $87.8$ $1.37$ $0.4$ AEC-2         11         F $142.0$ $36.0$ $N_0$ $c.1769C>T$ $14$ $p.$ ProS90Lys         Impaired protein-protein interaction $3.0$ $166$ $2.34$ $36.9$ AEC-3         11         F $162.0$ No $c.1760C>T$ $14$ $p.$ ProS90Lys         Impaired protein-protein interaction $3.0$ $166.9$ $2.34$ $36.9$ AEC-3         60         F $153.0$ $67.9$ No $c.1760C>T$ $14$ $p.$ ProS90Lys         Impaired protein-protein interaction $10.0$ $2.02$ $3.69$ $3.69$ AEC-3         60         F $13.0$ $67.9$ No $c.1790G>A$ $14$ $p.Gly600Asp$ Impaired protein-protein interaction $10.0$ $70.0$ $70.0$ $70.0$ $70.0$ $70.0$ $2.57$ $37.4$ AEC-7 $36$ $7$ $71.00$ $79.0$  | Average  |                  |           |   |                |  |   |           |   |                                      | 4.3  | 218.4 | 2.40 | 37.1   |
| C patients with relevant hypohidrosis       C patients with relevant hypohidrosis         C-2       11       F       142.0       36.0       No       c.1769C>T       14       p.Pro590Lys       Impaired protein-protein interaction       3.0       196       2.34         C-2       11       F       142.0       36.0       No       c.1769C>T       14       p.Pro590Lys       Impaired protein-protein interaction       0.5       80       1.59         C-3       44       F       163.0       87.5       No       c.1766T>G       14       p.Ine590Lys       Impaired protein-protein interaction       0.5       30       1.59         C-4       50       F       168.0       57.7       No       c.1766T>G       14       p.Ine590Lys       Impaired protein-protein interaction       10.0       212       3.48         C-5       60       F       153.0       67.9       No       c.1768C>A       14       p.Ine590Thr       Impaired protein-protein interaction       70       122       2.55         C-7       36       F       170.0       79.0       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       0.5       2.26       2.11         7.48       <  | AEC patients with relevant hypohidrosisAEC patients with relevant hypohidrosis $AEC 2$ 11F142.036.0Noc.1769C>T14p.Pro590LysImpaired protein-protein interaction0.5801.5936.9 $AEC-3$ 44F163.087.5Noc.1769C>T14p.Pro590LysImpaired protein-protein interaction0.52123.4837.6 $AEC-4$ 50F168.057.7Noc.1766C>A14p.Ile889SerImpaired protein-protein interaction801.5936.9 $AEC-4$ 50F153.067.9Noc.1766C>A14p.Ile889SerImpaired protein-protein interaction801.562.5536.9 $AEC-5$ 60F153.067.9Noc.1799G>A14p.Gly600AspImpaired protein-protein interaction701322.5537.4 $AEC-8$ 7F129.02.57Yesc.1799G>A14p.Gly600AspImpaired protein-protein interaction602.2537.4 $AEC-8$ 7F129.02.57Yesc.1799G>A14p.Gly600AspImpaired protein-protein interaction602.2537.8 $AEC-8$ 7F129.02.57Yesc.1799G>A14p.Gly600AspImpaired protein-protein interaction602.253.6 $AEC-8$ 772.919.79.33.13.13.13.1 $AEC$   | SD       | I.I              |           |   |                |  |   |           |   |                                      | 3.4  | 87.8  | 1.37 | 0.4    |
| C-2       11       F       14.0       36.0       No <b>c.1769C&gt;T</b> 14 <b>p.Pro590Lys</b> Impaired protein-protein interaction       3.0       196       2.34         C-3       44       F       163.0       87.5       No <b>c.1769C&gt;T</b> 14 <b>p.Pro590Lys</b> Impaired protein-protein interaction       0.5       80       1.59         C-3       44       F       163.0       87.5       No <b>c.1766C&gt;T</b> 14       p.Ile580Ser       Impaired protein-protein interaction       0.5       80       1.59         C-4       50       F       168.0       57.7       No <b>c.1766C&gt;A</b> 14       p.Ile580Ser       Impaired protein-protein interaction       8.0       1.59       3.48         C-5       60       F       153.0       67.9       No <b>c.1766C&gt;A</b> 14       p.Ile580Ser       Impaired protein-protein interaction       7.0       132       2.55         C-7       36       F       170.0       79.0       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       0.5       2.26       2.11 <i>C-8</i> 7       F       129.0       2.57       Yes       c.1799G>A <td>AEC-2         I1         F         142.0         36.0         No         c.1769C&gt;T         14         <b>p.Pro590Lys</b>         Impaired protein interaction         3.0         196         2.34         36.9</td> <td>AEC pa</td> <td>tients wit.</td> <td>h releva</td> <td>nt hypohidros.</td> <td>is</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> | AEC-2         I1         F         142.0         36.0         No         c.1769C>T         14 <b>p.Pro590Lys</b> Impaired protein interaction         3.0         196         2.34         36.9   | AEC pa   | tients wit.      | h releva  | nt hypohidros.  | is             |  |   |           |   |                                      |  |       |      |        |
| C-3       44       F       163.0       87.5       No $c.1769C>T$ 14 <b>p.Pro590Lys</b> Impaired protein-protein interaction       0.5       80       1.59         C-4       50       F       168.0       57.7       No $c.17661>G$ 14       p.Ile589Ser       Impaired protein-protein interaction       11.0       212       3.48         C-4       50       F       168.0       57.7       No $c.17661>G$ 14       p.Ile589Ser       Impaired protein-protein interaction       11.0       212       3.48         C-5       60       F       153.0       67.9       No $c.1768C>A$ 14       p.Pro500Thr       Impaired protein-protein interaction       8.0       156       2.25         C-7       36       F       170.0       79.0       Yes $c.1799G>A$ 14       p.Gly600Asp       Impaired protein-protein interaction       0.5       22.0       22.11         C-8       7       F       129.0       25.7       Yes $c.1799G>A$ 14       p.Gly600Asp       Impaired protein-protein interaction       0.5       22.0       22.11         rage       3.4.7       5.0       166.0       2.25       2.25       2   | AEC-3 44 F 163.0 87.5 No <b>c.1769C&gt;T</b> 14 <b>p.Pro590Lys</b> Impaired protein-protein interaction 0.5 80 1.59 36.9 $36.9$ AEC-4 50 F 168.0 57.7 No <b>c.1766</b> T>G 14 p.Ile5895er Impaired protein-protein interaction 11.0 2.12 3.48 37.6 AEC-5 60 F 153.0 67.9 No <b>c.1766</b> C>A 14 p.Pro590Thr Impaired protein-protein interaction 8.0 1.56 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-7 36 F 129.0 25.7 Yes c.1799G>A 14 p.Gly600Asp Impaired protein-protein interaction 6.5 2.00 2.11 36.8 Areage 34.7 $X_{Verage}$ 37.4 $X_{Verage}$ 34.7 $X_{Verage}$ 34.0 $X_{Verage}$ 37.1 $X_{Verage}$ 37.1 $X_{Verage}$ 37.4 $X_{Verage}$ 34.0 $X_{Verage}$ 34.0 $X_{Verage}$ 34.0 $X_{Verage}$ 37.9 $X_{Verage}$     | AEC-2    | 11               | Ч         | 142.0   | 36.0           | No                                       | c.1769C>T   | 14        | p.Pro590Lys                               | Impaired protein-protein interaction | 3.0  | 196   | 2.34 | 36.9   |
| C-4       50       F       168.0       57.7       No       c.1766T>G       14       p.Ile589Ser       Impaired protein-protein interaction       11.0       212       3.48         C-5       60       F       153.0       67.9       No       c.1768C>A       14       p.Pro590Thr       Impaired protein-protein interaction       8.0       156       2.65         C-7       36       F       170.0       79.0       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       7.0       132       2.55         C-8       7       F       129.0       25.7       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       0.5       220       2.11         cage       34.7       F       129.0       25.7       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       0.5       220       2.11         rage       34.7       5.0       166.0       2.25       2.13         21.4       21.4       p.Gly600Asp       Impaired protein-protein interaction       0.5       2.00       2.11         rage       34.7       5.0       166.0       2.25       2.11  | AEC-4         50         F         168.0         57.7         No         c.1766T>G         14         p.lle589Ser         Impaired protein-protein interaction         1.0         2.12         3.48         37.6           AEC-5         60         F         153.0         67.9         No         c.1766T>A         14         p.Pro590Thr         Impaired protein-protein interaction         8.0         156         2.65         36.9           AEC-5         60         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         0.0         132         2.25         37.1           Average         34.7         S         2.12         9.6         0.66.0         2.25         37.1           SD         21.4         p.Gly600Asp         Impaired protein-protein interaction         0.5         2.20         2.11         36.8           SD         21.4         p.Gly600Asp         Impaired protein-protein interaction         0.5 <td< td=""><td>AEC-3</td><td>44</td><td>ц</td><td>163.0</td><td>87.5</td><td>No</td><td>c.1769C&gt;T</td><td>14</td><td>p.Pro590Lys</td><td>Impaired protein-protein interaction</td><td>0.5</td><td>80</td><td>1.59</td><td>36.9</td></td<>   | AEC-3    | 44               | ц         | 163.0   | 87.5           | No                                       | c.1769C>T   | 14        | p.Pro590Lys                               | Impaired protein-protein interaction | 0.5  | 80    | 1.59 | 36.9   |
| C-5       60       F       153.0       67.9       No <b>c.1768C&gt;A</b> 14 <b>p.Pro590Thr</b> Impaired protein-protein interaction       8.0       156       2.65         C-7       36       F       170.0       79.0       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       7.0       132       2.55         C-8       7       F       129.0       25.7       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       0.5       220       2.11         cage       34.7       F       129.0       25.7       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       0.5       220       2.11         rage       34.7       5.0       166.0       2.25       2.25       2.14         21.4       2.1.4       p.Gly600Asp       Impaired protein-protein interaction       0.5       2.20       2.11         rage       34.7       5.0       166.0       2.65       2.25       2.25         21.4       21.4       21.4       p.Gly600Asp       Impaired protein-protein interaction       0.72       2.25         21.4       21.4       21.4  | AEC-5 60 F 153.0 67.9 No <b>c.1768C&gt;A</b> 14 <b>p.Pro590Thr</b> 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 $SD$ 21.4 $Average$ 34.7 $f$ 129.0 25.7 Yes c.1799G>A 14 p.Gly600Asp Impaired protein-protein interaction 0.5 220 2.11 36.8 $J.7$ Average 34.7 $f$ 129.0 25.7 Yes c.1799G>A 14 p.Gly600Asp Impaired protein-protein interaction 0.5 220 2.11 36.8 $J.7$ Average 34.7 $f$ 129.0 25.7 Yes c.1799G>A 14 p.Gly600Asp Impaired protein-protein interaction 0.5 2.25 37.1 $J.4$ Average 34.7 $J.4$                 | AEC-4    | 50               | ц         | 168.0   | 57.7           | No                                       | c.1766T>G   | 14        | p.Ile589Ser                               | Impaired protein-protein interaction | 11.0                                       | 212   | 3.48 | 37.6   |
| C-7       36       F       170.0       79.0       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       7.0       132       2.55         C-8       7       F       129.0       25.7       Yes       c.1799G>A       14       p.Gly600Asp       Impaired protein-protein interaction       0.5       220       2.11         rage       34.7       5.0       166.0       2.25       2.25       2.25         rage       34.7       5.0       166.0       2.25       2.14       2.14         21.4       21.4       p.Gly600Asp       Impaired protein-protein interaction       0.5       220       2.11         rage       34.7       5.0       166.0       2.25       2.25       2.25         21.4       2.1.4       p.Gly600Asp       Impaired protein-protein interaction       0.5       2.0       2.25         21.4       2.1.4       p.Gly600Asp       Impaired protein-protein interaction       0.5       2.20       2.25         21.4       2.1.4       p.Gly600Asp       Impaired protein-protein interaction       0.5       2.26       2.25         21.4       2.1.4       p.Gly600Asp       Impaired protein-protein       4.3       <  | 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<br>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<br>Average 34.7 5.0 $166.0$ 2.25 37.1 $3.6.8$<br>3.0.225 37.1 $3.6.83.0.225$ 37.1 $3.6.83.0.225$ 37.1 $3.6.83.0.225$ 37.1 $3.6.83.0.225$ 37.1 $3.6.83.0.225$ 37.1 $3.6.83.0.225$ 37.1 $3.6.83.0.225$ 37.1 $3.6.83.0.225$ $3.7.1$ $3.6.83.0.225$ $3.7.1$ $3.6.83.0.225$ $3.7.1$ $3.6.83.0.225$ $3.7.1$ $3.6.8$ $3.7.1$ $3.6.8$ $3.7.1$ $3.2.2$ $3.7.1$ $3.6.8$ $3.7.1$ $3.6.8$ $3.7.1$ $3.6.8$ $3.7.1$ $3.6.8$ $3.7.1$ $3.6.8$ $3.7.1$ $3.6.8$ $3.7.1$ $3.7.1$ $3.6.8$ $3.7.1$ $3.7.1$ $3.6.8$ $3.7.1$ $3.6.8$ $3.7.1$ $3.7$ $3.7.1$ $3.6.8$ $3.7.1$  | AEC-5    | 60               | ц         | 153.0   | 67.9           | No                                       | c.1768C>A   | 14        | p.Pro590Thr                               | Impaired protein-protein interaction | 8.0  | 156   | 2.65 | 36.9   |
| C-8 7 F 129.0 25.7 Yes c.1799G>A 14 p.Gly600Asp Impaired protein-protein interaction 0.5 220 2.11<br>5.0 166.0 2.25<br>21.4 21.4 6.121.1  | 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<br>Average 34.7 $5.0 	 166.0 	 2.25 	 37.1$<br>SD 21.4 $4.3 	 54.0 	 0.42 	 0.3$<br>$a^{-3}$ Previously unreported mutations in bold type; <sup>b</sup> upper detection limit = 93 µl; <sup>c</sup> within a 1-cm <sup>2</sup> area of the palm; <sup>d</sup> relative to total body-surface area as calculated with the Mosteller method; <sup>e</sup> carries heterozyously an additional pathogenic <i>EDA</i> variant; <i>M</i> , male; <i>F</i> , female; <i>SD</i> , standard deviation   | AEC-7    | 36               | ц         | 170.0   | 79.0           | Yes                                      | c.1799G>A   | 14        | p.Gly600Asp                               | Impaired protein-protein interaction | 7.0  | 132   | 2.55 | 37.4   |
| rage 34.7 5.0 166.0 2.25<br>21.4 4.3 54.0 0.42  | Average 34.7 5.0 $166.0$ 2.25 $37.1$<br>SD 21.4 $4.3$ 54.0 $0.42$ $0.3$<br><sup>a</sup> Previously unreported mutations in bold type; <sup>b</sup> upper detection limit = 93 µl; <sup>c</sup> within a 1-cm <sup>2</sup> area of the palm; <sup>d</sup> relative to total body-surface area as calculated with the Mosteller method; <sup>e</sup> carries heterozygously an additional pathogenic <i>EDA</i> variant; <i>M</i> , male; <i>F</i> , female; <i>SD</i> , standard deviation  | AEC-8    | 7                | Ц         | 129.0   | 25.7           | Yes                                      | c.1799G>A   | 14        | p.Gly600Asp                               | Impaired protein-protein interaction | 0.5  | 220   | 2.11 | 36.8   |
| 21.4 4.3 54.0 0.42  | $SD_{21.4}$ $2.1.4$ $0.42$ $0.3$ $0.3$ $ 0.42$ $0.3$ $ 0.42$ $0.3$ $ 0.42$ $ 0.3$ $         -$   | Average  |                  |           |   |                |  |   |           |   |                                      | 5.0  | 166.0 | 2.25 | 37.1   |
|   | <sup>a</sup> Previously unreported mutations in bold type; <sup>b</sup> upper detection limit = 93 $\mu$ l; <sup>c</sup> within a 1-cm <sup>2</sup> area of the palm; <sup>d</sup> relative to total body-surface area as calculated with the Mosteller method; <sup>e</sup> carries heterozygously an additional pathogenic <i>EDA</i> variant; <i>M</i> , male; <i>F</i> , female; <i>SD</i> , standard deviation  | SD       |                  |           |   |                |  |   |           |   |                                      | 4.3  | 54.0  | 0.42 | 0.3    |
|   | <sup>a</sup> Previously unreported mutations in bold type; <sup>b</sup> upper detection limit = 93 $\mu$ l; <sup>c</sup> within a 1-cm <sup>2</sup> area of the palm; <sup>d</sup> relative to total body-surface area as calculated with the Mosteller method; <sup>e</sup> carries heterozygously an additional pathogenic <i>EDA</i> variant; <i>M</i> , male; <i>F</i> , female; <i>SD</i> , standard deviation  |          |                  |           |   |                |  |   |           |   |                                      |  |       |      |        |
|   |  | heterozy | /gously          | an addi   | tional pathog   | enic EDA v     | variant; M, male                         | F, female; $SD$   | , standai | rd deviation                              |                                      |  |       |      | (more) |

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 splithand phenotype (Fig. 1, upper panel). In general, there was no correlation between the severity of sweat gland maldevelopment and the extent of splithand 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 splithand 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.

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 proteinprotein 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.

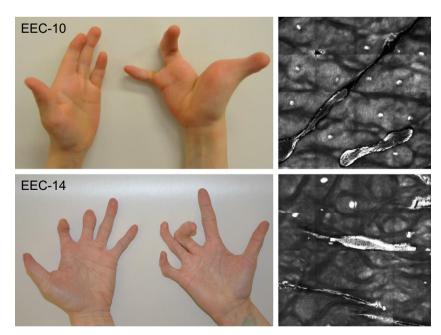
Although for p63-associated syndromes rather strong genotype-phenotype correlations have been observed [1, 11], there are exceptions with overlapping symptoms. In this study, all but one mutations affecting the sterile alpha motif (SAM) domain of p63 resulted in AEC syndrome. Patient EEC-13 who carried 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

# Discussion

This study shows that hypohidrosis is rather uncommon in EEC syndrome, as stated recently also by Sutton and van

**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 p63associated disorders may be attributable to certain genotypes.

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Authors' contributions P.F. and H.S. conceived the study, investigated the patients, and wrote the first draft of the manuscript. Most of the work was performed by P.F. in fulfillment of the requirements for obtaining the degree "Dr. med." from the Friedrich-Alexander-Universität Erlangen-Nürnberg. S.W. provided essential assistance with the confocal laser scanning microscopy. All authors reviewed the results and approved the final version of the manuscript.

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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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