Hair and Sweat Glands in Families With Hypohidrotic Ectodermal Dysplasia

Further Characterization

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Objectives: To gather and compare clinical and histologic information from individuals affected by hypohidrotic ectodermal dysplasia (HED) and unaffected control subjects and to assess the value of these data in the diagnosis of HED.

Design: Volunteer subjects attending the 20th Annual Family Conference of the National Foundation for Ectodermal Dysplasia answered a questionnaire and performed a starch-iodide sweat-function test. A subset of the subjects also donated samples of hair and 4-mm punch biopsy specimens of palmar and scalp skin. Specimens from each of these tests were assessed in a blinded fashion. Analysis was performed comparing affected and control subjects for each of the following parameters: quantification of eccrine structures in the skin biopsy specimens, analysis of hair sample trichograms for hair shaft defects, and qualitative classification of starch-iodide palm-print sweat-function test results.

Setting: An international conference for families and individuals with ectodermal dysplasias.

Subjects: A total of 40 subjects were included in the final analysis: 15 unaffected control subjects and 25 subjects with HED. Nine affected subjects and 9 unaffected subjects donated skin biopsy specimens.

Main Outcome Measure: This study was designed to assess the value of 4 simple tests in supporting the diagnosis of HED.

Results: Investigators were blinded to group during analysis of the test samples. Trichogram examination identified 3 hair shaft abnormalities, with a slightly higher prevalence in the affected group: variable shaft thickness, trichorrhexis nodosa, and pili torti. The sensitivity and specificity for each of these findings was less than 40%. Starch-iodide paper palm imprints identified a higher likelihood of diminished or absent sweat in the affected group, but this test had a low sensitivity (44%) and an imperfect specificity (93%). Examination of horizontally sectioned skin biopsy specimens from the palm were devoid of eccrine structures in a minority of affected subjects (sensitivity, 30%; specificity, 100%). In contrast, scalp biopsy specimens lacked eccrine structures in the majority of affected subjects (sensitivity, 67%; specificity, 100%). Separate analysis excluding the subjects without apparent eccrine apparatus yielded comparable numbers of eccrine ducts from control and affected groups.

Conclusions: We have defined the value of simple, easily performed tests in the morphological diagnosis of HED. Noninvasive trichogram and sweat testing results can support the diagnosis of HED, but they are not sensitive or highly specific; horizontally sectioned 4-mm punch biopsy specimens of the scalp or palms that lack eccrine structures are diagnostic of HED; scalp biopsy is much more sensitive than palmar biopsy; and a scalp biopsy specimen with detectable eccrine structures suggests that a patient does not have HED.

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HYPOHIDROTIC ECTODERMAL dysplasia (HED) is a rare genetic disorder that is characterized by fine, sparse hair; few, often pointed teeth; and diminished or absent eccrine function. The inability to sweat puts affected infants at risk for life-threatening and brain-damaging episodes of hyperthermia.1 Previous studies have used magnification of fingertips or silicone palm impressions to highlight eccrine ostia and have found a qualitative diminution.2 Case reports have also documented absent or hypoplastic eccrine glands in vertically sectioned biopsy specimens from the palms and forearms.3 These data have been frequently misinterpreted as a total absence of eccrine glands in persons with HED.4,5 More accurate quantification of eccrine structures in horizontally sectioned skin biopsy specimens has never been reported in these patients, nor have the results of histologic analysis of hair shafts, hair follicles, or eccrine structures in the scalp.
Hypohidrotic ectodermal dysplasia is most commonly transmitted in an X-linked recessive fashion; autosomal recessive and dominant transmission also occur. All the candidate genes encode proteins in a single signaling pathway. Ectodysplasin-A, a member of the tumor necrosis factor ligand superfamily, has been identified as the affected protein coded in the Xq12-q13.1 region. Ectodysplasin-A is expressed in normal fetal and adult skin and hair and in adult teeth. The autosomal recessive form, localized to 2q11-q13, has been associated with defects in the receptor for ectodysplasin-A, EDAR, a transmembrane protein. A third genotype was recently identified in families affected by HED and immunodeficiency. This X-linked defect is localized to the IKKg (NEMO) gene, encoding a protein that is a likely constituent of the ectodysplasin signaling pathway.

Despite recent advances in the genetic basis of this disorder, the diagnosis is still established clinically in the majority of patients. One diagnostic dilemma occurs when a fair-haired infant with a negative family history presents with frequent fevers of unknown origin. Another is when a healthy child presents with a chief complaint of anhidrosis. Genetic analysis is not routinely available in these cases.

This study was designed to investigate the structure and function of eccrine sweat glands as well as hair shafts and follicles in individuals with HED. The primary goals were to gather clinical information from a relatively large cohort of affected families, to compare the affected subjects with a sample of unaffected individuals, and to determine a clinicopathologic correlation that may be helpful in establishing the diagnosis.

### METHODS

Approval for this study was obtained from the institutional review board of Saint Louis University, St Louis, Mo. Subjects were enrolled at the 20th Annual Family Conference of the National Foundation for Ectodermal Dysplasia, which was held July 20 and 21, 2001, in Collinsville, Ill. All subjects gave signed, informed consent; were assigned a random number; and completed a questionnaire. The questionnaire included medical history, family history, and specific information about relevant symptoms (frequent fevers, overheating, early male pattern baldness, slow childhood hair growth, abnormal tooth development, and clogged nasal and ear secretions). This information was tabulated to determine the status of each subject: affected, carrier/unknown, or control (Table 1). The affected group included subjects who reported a previous specific diagnosis of HED, a history of frequent fevers and/or overheating, and abnormal hair and/or tooth development. The control subjects were those who had no criteria of the affected group and who were unrelated volunteers, relatives with negative results on previous genetic testing, or the fathers of families with affected sons and unaffected daughters. (The results of genetic testing were reported by the subjects. The testing was performed on an investigational basis in the laboratory of Jonathan Zonana, MD, University of Oregon Health and Sciences University, Portland.) Subjects who did not fit in either group were classified as a carrier/unknown and were excluded from final analysis. A subset of subjects donated hair samples, participated in a noninvasive starch-iodide paper test to determine sweat function, and provided 4-mm punch biopsy specimens from their scalp and palms.

Hair samples were obtained by pull test to bias the sample toward inclusion of more fragile shafts. Scissors-snipped samples were obtained from subjects whose hair did not break easily. Hair samples were qualitatively examined by light microscopy (trichogram) and digital photomicrography. The starch-iodide paper test was performed to evaluate eccrine function. It was administered by exposing a vinyl-gloved hand to an infrared light source for 5 minutes. The glove was then removed, and the palm was immediately placed on a piece of specially prepared starch-iodide paper. Patterned, purple discoloration marked sweat production. A commercially available 4-mm punch tool was used to obtain skin biopsy specimens from the hypothenar area of the nondominant palm and the palmar area of the scalp after injection of local anesthesia (0.1 mL of 1% lidocaine with epinephrine). Hemostasis was achieved with 20% aluminum chloride (Drysol). A stitch using 4-0 nylon suture was placed and secured by slipknots. The wound was dressed with antibiotic ointment and a clear, waterproof cover. Written wound care instructions were provided. The specimens were placed in formalin. After fixation, they were horizontally bisected at the level of the deep reticular dermis, serially sectioned, and processed using standard hematoxylin-eosin staining. They were then viewed microscopically to quantify the number of eccrine sweat glands and hair follicles and to identify structural malformations. Digital photomicrographs of each specimen were obtained. JPEG files were processed by digitally marking each eccrine and follicular unit using commercially available software (Adobe PhotoDeluxe; Adobe, San Jose, Calif) to facilitate a more reliable quantification (Figure 1). All data collection was performed in a blinded fashion.
RESULTS

Forty subjects were evaluated. Of these, 15 were unaffected friends or relatives and 25 had HED. In the affected group, there were 17 male subjects, 7 female subjects, and 1 person of unknown gender. In the control group, there were 10 male subjects and 5 female subjects. The control group included 8 unrelated volunteers, 2 relatives with negative results on previous genetic testing, and 5 fathers of families with affected sons and unaffected daughters. Forty subjects underwent starch-iodide paper sweat testing, 35 donated hair samples for trichogram, 18 underwent a scalp biopsy, and 20 underwent a palmar biopsy. Of the 18 scalp biopsy specimens, 9 were from subjects who were previously diagnosed with HED and 9 were from unaffected subjects. Two of the 9 biopsy specimens from unaffected subjects could not be evaluated because of processing problems; 7 were available for final analysis. Of the 20 palmar biopsy specimens, 10 were from affected individuals with HED and 10 were from unaffected individuals.

There were 22 subjects affected with HED and 13 unaffected subjects who donated hair samples. Of the

22 affected subjects, 14 were male, 7 were female, and 1 was of unknown gender. Of the 13 unaffected subjects, 9 were male and 4 were female. Trichograms revealed numerous hair shaft abnormalities in the subjects with HED as well as in the control subjects. Of the 13 unaffected subjects, 3 had variable shaft thicknesses (the diameter of individual hair shafts varied by as much as twice the size of other hair shafts), 1 had pili bifurcati (longitudinal splitting), 1 had trichorrhexis nodosa, and 3 had pili torti (twisting of the hair shaft). Of the 22 subjects affected with HED, 7 had variable shaft thickness, 1 had pili bifurcati, 3 had trichorrhexis nodosa, and 8 had pili torti. Longitudinal splitting (pili bifurcati) occurred equally in both groups, so statistical analysis was performed in the other 3 categories: (1) identification of variable shaft thickness had a sensitivity of 32%, a specificity of 23%, a positive predictive value of 70%, and a negative predictive value of 40%; (2) identification of trichorrhexis nodosa had a sensitivity of 14%, a specificity of 8%, a positive predictive value of 75%, and a negative predictive value of 39%; and (3) identification of pili torti had a sensitivity of 36%, a specificity of 23%, a positive predictive value of 73%, and a negative predictive value of 42%.

There were 25 subjects affected with HED and 15 unaffected subjects who performed the starch-iodide paper sweat test. Of the 25 affected subjects, 17 were male, 7 were female, and 1 was of unknown gender. Of the 15 unaffected subjects, 10 were male and 5 were female. The test was graded based on 4 subjective levels: intense, moderate, minimal, and no sweating (Figure 2). Of the 15 control subjects, 10 had an intense sweating response, 4 had a moderate sweating response, and 1 had a minimal sweating response. Of the 25 subjects with HED, 9 had an intense response, 5 had a moderate response, 10 had a minimal response, and 1 had no sweating. The sensitivity, specificity, and positive/negative predictive values were determined based on the following definitions: a sweat test that was positive for HED was defined as the combination of minimal and no sweating; a negative sweat test result was defined as the combination of intense and moder-
ate sweating (Figure 3). In regard to diagnosing HED, the starch-iodide paper sweat test has a sensitivity of 44%, a specificity of 93%, a positive predictive value of 92%, and a negative predictive value of 50%.

To determine the value of the starch-iodide test in predicting the number of eccrine ducts in a palmar biopsy section, the average number of eccrine ducts was calculated for the 4 qualitative groups of sweat test results (intense, moderate, minimal, and no sweating). The mean ± SD for subjects with intense sweating was 48 ± 16 (n = 22), for those with moderate sweating it was 36 ± 21 (n = 7), for those with minimal sweating it was 27 ± 31 (n = 4), and for those with no sweating it was 0 ± 0 (n = 1) (Figure 4). The wide standard deviations, especially in the groups with moderate or minimal sweating, reflect the poor correlation and predictive value of the starch-iodide paper sweat test. The poor predictive value was also demonstrated by a negative sweat test result in a volunteer-physician control subject who did not donate a palmar biopsy specimen.

To determine the variability of eccrine duct expression, the average number of ducts was calculated for both the control and affected groups. The number of eccrine ducts in a 4-mm palmar skin sample from 10 unaffected subjects was 42 ± 14 (median, 41; range, 17-63). The number of eccrine ducts in a 4-mm palmar skin sample from 10 affected subjects was 29 ± 26 (median, 28; range, 0-74); excluding the 3 subjects lacking eccrine ducts, the number was 41 ± 20 (Figure 5).

There were 10 subjects with HED and 10 unaffected subjects who performed the starch-iodide paper sweat test. Of the 10 affected subjects, 6 were male and 4 were female. Of the 10 unaffected subjects, 7 were male and 3 were female. Palmar biopsy specimens from 3 of 10 affected subjects lacked eccrine structures (all 3 subjects were male), while all 10 of the palmar biopsy specimens from control subjects showed some eccrine structures (Figure 6). The sensitivity, specificity, and positive/negative predictive values were determined based on the following definitions: a palmar biopsy specimen positive for HED was defined as one with a complete lack of eccrine structures; a negative palmar biopsy specimen was defined as one with eccrine structures. In regard to diagnosing HED, the palmar biopsy eccrine test had a sensitivity of 30%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 41%.

There were 9 subjects with HED and 7 unaffected subjects who performed the starch-iodide paper sweat test. Of the 9 affected subjects, 6 were male and 3 were female. Of the 7 unaffected subjects, 7 were male and none were female. Scalp biopsy specimens from 6 (5 male, 1 female) of 9 affected subjects lacked eccrine structures, while all 7 of the scalp biopsy specimens from control subjects showed some eccrine structures (Figure 7). The sensitivity, specificity, and positive/negative predictive values were determined as in the palmar biopsy specimens. In regard to diagnosing HED, the scalp biopsy eccrine test had a sensitivity of 67%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 70%.
Medical literature suggests that people with HED uniformly do not sweat and have diminished or absent eccrine structures. This study is the first to demonstrate widely varying sweat function test results and eccrine densities in those affected with HED. We have also defined the value of some simple clinical screening tests that can be used to support the diagnosis of HED.

Trichogram analysis is a less valuable diagnostic tool than the starch-iodide paper sweat test, but as a simple and noninvasive examination, a trichogram may support the diagnosis of HED. Identification of variable shaft thickness, trichorrhexis nodosa, or pili torti will support the diagnosis of HED in 70% to 75% of cases.

A starch-iodide paper sweat test that shows minimal or no sweat production is likely among those with HED. However, the majority of the affected subjects in the present study exhibited moderate or intense sweating, confirming that demonstrable sweat production does not rule out the condition.

In contrast to widely held belief, quantification of eccrine ducts in a 4-mm palmar skin biopsy specimen is only a valuable diagnostic tool when identifiable eccrine structures are completely absent. This finding occurred in 3 of 10 subjects with HED, all 3 of whom were male. Among the affected subjects whose palmar biopsy specimens included eccrine ducts, the average number of ducts did not differ from that in control biopsy specimens, and the SDs in the 2 groups were nearly equal. It is possible that individuals affected with HED simply have an extreme variability in the distribution of eccrine ducts. However, these results suggest that there are at least 2 subsets of individuals affected with HED: those completely lacking eccrine structures and those with a normal distribution of them. Recent studies have identified 3 unique loci for HED: 2 X-linked and 1 autosomal recessive.

Compared with the palmar biopsy findings, identification of any eccrine structures in scalp biopsy specimens is a more valuable predictor of HED, with a specificity and positive predictive value of 100% and a sensitivity and negative predictive value of 70%. We identified no false-positives, and we identified 70% true-negatives. Therefore, a scalp biopsy specimen that is bisected at the appropriate level of dermis and that lacks eccrine ducts or glands is highly suggestive of HED. Furthermore, if clinical suspicion is high for HED, and a 4-mm scalp biopsy specimen reveals identifiable eccrine structures, then there is a 70% likelihood that the individual is not affected.

In the present study, the number of palmar eccrine structures was surprisingly unrelated to the degree of palmar sweating. Of 3 subjects who lacked eccrine ducts in a single 4-mm horizontally sectioned palmar biopsy specimen, only 1 had no detectable sweating; the other 2 had minimal sweating. One of the control subjects had minimal sweating and a biopsy specimen that revealed 55 eccrine ducts. Perhaps this poor correlation simply reflects the small number of subjects in the minimal/no sweating groups, or technical difficulties with the starch-iodide test. However, it is likely that many individuals with HED have a normal amount of poorly functioning eccrine structures.

One limitation of the present study is that all of the subjects who donated biopsy specimens were adolescents or adults. Establishing objective data for early diagnosis of HED is most valuable for infants and young children, who are at highest risk from the adverse consequences of hyperthermia. However, the eccrine apparatus is fully formed by the third trimester and eccrine density diminishes with age, so we would expect scalp biopsy findings to be equally reliable in this age group.

In conclusion, we have defined the value of a few simple tests in the diagnosis of HED (Table 2). Noninvasive trichograms and sweat testing can only support the diagnosis of HED as they are not sensitive or highly specific. The results of quantification of eccrine ducts in a 4-mm palmar skin biopsy specimen are simi-

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Table 2. Sensitivities, Specificities, and Positive and Negative Predictive Values in the 4 Tests Performed

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<thead>
<tr>
<th>Test</th>
<th>Percentage</th>
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<tr>
<td></td>
<td>Sensitivity</td>
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<tr>
<td>Trichogram*</td>
<td>14-36</td>
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<tr>
<td>Sweat function</td>
<td>44</td>
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<tr>
<td>Palmar biopsy</td>
<td>30</td>
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<tr>
<td>Scalp biopsy</td>
<td>67</td>
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*The range reflects combined findings from variable shaft thickness, trichorrhexis nodosa, and pili torti.
lar in affected and control groups. A scalp or palmar biopsy specimen that lacks eccrine ducts or glands is diagnostic of HED, but the findings of a scalp biopsy are much more sensitive than those of a palmar biopsy. A scalp biopsy specimen that reveals eccrine ducts or glands suggests that the patient does not have HED. Furthermore, the scalp is a technically easier site from which to obtain a biopsy specimen and carries a lower risk of problematic scarring. For these reasons, the scalp biopsy should be the preferred test in individuals suspected of having HED. Many recent molecular studies have confirmed the interaction of ectodysplasin and its receptor protein. In the future, we hope to relate the expression of ectodysplasin to the structure and function of these affected cutaneous appendages.

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