Sweat testing to identify female carriers of X linked hypohidrotic ectodermal dysplasia

Angus Clarke, John Burn

Abstract

X linked hypohidrotic ectodermal dysplasia (XHED) affects many epithelial functions, including sweat gland formation. Female carriers who manifest XHED may have defective dentition or a patchy distribution of sweating or both, as determined by starch and iodine sweat testing. Such sweat testing can be useful in assigning carrier status to at risk females in XHED families, and in obtaining an accurate diagnosis for isolated females who present with features of ectodermal dysplasia. The advantages of diagnosing female carriers of XHED include the optimisation of neonatal and paediatric care for affected male infants, who may be at substantial risk of death in infancy.

X linked hypohidrotic ectodermal dysplasia (XHED) is the commonest of the ectodermal dysplasias, and is generally much more apparent in affected males than carrier females. However, because the condition affects epithelial tissues, it is possible for females heterozygous for the disorder to manifest it patchily on their skins. This was first noted by Roberts.¹ Although dental changes in carrier females were described by Gibbs,² it was not until the report of Thadani³ that females carrying this sex linked 'recessive' disorder were noted to manifest it 'against the rules'. Kerr *et al*⁴ were the first to interpret these observations on the basis of Lyon's hypothesis of random X chromosome inactivation.⁵

It is important for carrier females to be aware of their 1 in 4 risk of having an affected child for the sake of their child's health. There is substantial mortality and morbidity associated with XHED in male infants, with about 30% dying in the first two years of life of

fever or chest infection.^{6,7} Deaths may occur in the neonatal period, often without the diagnosis of XHED being made, and may present as unexpected cot deaths.^{8,9} Previous knowledge that the child may be at risk of overheating could allow preventive measures to be taken, and the available data support the suggestion that affected boys have a better chance of survival if the family is aware of the potential problems. Thus, of 12 early childhood deaths in a recent survey of the disorder, eight occurred in the older of two affected brothers and only two boys died who were the younger of two affected brothers.⁶

The gene locus of XHED has been mapped to proximal Xq and close flanking markers are available.¹⁰ This often allows female carriers in a family to be identified with a high degree of accuracy.¹¹ Phenotypic tests, however, are still of practical importance in genetic counselling. Family structure may not be suitable for linkage studies or the probes may be uninformative. Even if DNA diagnosis is feasible, it is not yet available as a service in most centres and will continue to be expensive, and thus unattractive, for the foreseeable future.

In some carrier females, physical signs are overt with sparse, patchy scalp hair or with marked hypodontia. In Latin America,¹² patchiness of body hair may be more apparent than in the paler peoples of north-west Europe.⁴ In most cases, it is difficult to place much weight upon subjective assessments of scalp hair density, heat intolerance, breast feeding difficulties, or the appearance of the eyebrows.

Two methods of assessment of sweating have been developed to identify possible female carriers of XHED. Kerr *et al*⁴ used an elaborate sweat test protocol to illustrate the patchy sweating found in female carriers, as predicted by Lyon's hypothesis. Happle and Frosch¹³ performed sweat tests on the backs of females carrying XHED, and found a V shaped pattern of streaks that conformed to the lines of Blaschko. These lines seem not to result from metamerism segmentation, but to be epiphenomena of the complex interaction of transverse clonal proliferation of cells with the longitudinal growth and progressive flexion of the embryo, and are apparent in a number of other conditions involving mosaicism for X linked gene defects or somatic mutations.¹⁴

The other method of assessing sweat pores in

Institute of Medical Genetics for Wales, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN.
A Clarke
Department of Human Genetics, Royal Victoria Infirmary, Newcastle upon Tyne NE2 4AA
J Burn

Correspondence to Dr Clarke.

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females carrying XHED is to make counts of the sweat pores along ridges on the fingertips or palms. This has been performed by a number of authors. Frias and Smith¹⁵ examined fingers under a dissecting microscope, and found that XHED females had a reduced but uniform sweat pore count, not in conformity with the Lyon hypothesis. Verbov¹⁶ took plastic impressions of palms and fingertips, and found that most females with dental manifestations of XHED also had flattening of the ridges and a diminished sweat pore count. However, he also warned of pitfalls in the interpretation of the plastic imprints.

Crump and Danks¹⁷ found that one of five female carriers had a reduced sweat pore count, identified on fingertip impressions and confirmed on direct microscopy. This technique was subsequently used to show that sweat pores do follow the Lyon hypothesis after all.¹⁸ Another technique for assessing sweat pores is the application of 5% phthalaldehyde in xylene to the fingertips, which are photographed once the black staining appears.¹⁹ The use of carbon paper to record sweat pore patterns has also been advocated.²⁰ These three reports have found that some XHED female carriers can be identified by the examination of sweat pores on the fingertips and palms of the hands, but that there are methodological difficulties. The size of the patches of normal or absent sweat pores is variable, and to count sweat pores where they are clearly visible will bias the results obtained. Patches of skin where the ridges appear flattened and the pores reduced are found in female carriers of XHED, but may be caused by rough wear such as domestic labour, or by poor quality application of the chosen technique. Such considerations have led us to favour the first method of assessing sweating, by performing sweat tests on the whole back of the subjects in search of patchiness that might follow the lines of Blaschko.

Methods

Possible carrier females of XHED were identified as part of a clinical study of XHED in Britain.⁶ Some women have also been examined who had features of ectodermal dysplasia, but in whose families there are no known affected males. Most of the sweat tests have been carried out in the subject's own home, because of the families' geographical dispersion, and also because it is often easier to heat up a room in a domestic house than in a hospital clinic. The technique adopted is essentially that of Wada.²¹

First, a room is heated, with a gas fire if possible, or with electric fan heaters. The subject(s) and control(s) are asked to remove or loosen their clothing sufficiently to uncover their backs from waist to shoulders. A 1 to 2% solution of iodine in alcohol is applied as evenly as possible and allowed to dry. Then a suspension of corn starch in castor oil (50 to 60 g/100 ml) is applied,

also as evenly as possible. As the woman begins to sweat, a black dot appears over each sweat gland, where the starch and iodine are mixed in an aqueous environment.

The test continues until the pattern of sweat glands is apparent: this may take 20 minutes or so, varying with ambient temperature. It can be useful to give the women a hot cup of tea or coffee to drink. Better results can sometimes be obtained if the woman lies prone on a bed or blanket; this keeps her warmer, and may enhance the clarity of the sweating pattern. Precautions should be taken in case any of the reagents run down the woman's back onto clothing or bedding.

The simultaneous testing of controls is urged, particularly where the operator is unfamiliar with the normal variation over different regions of the back.²²

As sweating starts, there will often be an apparent patchiness. The test must be continued until no new areas of sweating appear. Several areas of at least 1 cm diameter clear of active sweat glands must be present before the test is regarded as positive.

The finding of streaks or large patches without sweating, with areas of clear sweating around, is clear evidence in favour of the subject carrying XHED, subject only to the remark about controls. Asymmetry between the two sides also strongly supports this conclusion, because normal variation usually gives a symmetrical pattern. The complete absence of sweating on the back is occasionally found in XHED carriers, and is also evidence in support of the diagnosis. In such a circumstance, we would recommend prolonging the test until the woman's skin has become flushed and she is moderately uncomfortable; if she is sweating elsewhere, then that is further evidence suggesting that she is a severely manifesting heterozygote.

Results

Sweat tests were performed upon 36 obligate female carriers of XHED (defined by virtue of their position in a pedigree), and were recorded as positive in 35 cases (97% apparent sensitivity). The classical appearance of mosaic hypohidrosis following the lines of Blaschko is shown in the figure. A further 47 definite carriers were tested (definite carriers being females in a family with XHED who had unequivocal dental signs of the condition), and positive results were found in 44 (pooled sensitivity 95%). A possible carrier was regarded as definitely manifesting the disorder if she had at least two missing or malformed permanent teeth, or one deciduous tooth, or generalised microdontia. The results seem clear cut, but unfortunately the interpretation of sweat tests is inherently subjective. Furthermore, two possible carrier females with normal teeth have been identified as probable carriers by recombinant DNA techniques,

using closely linked markers, when sweat testing was normal. One of these women has since given birth to an affected male infant.

Another patient referred to a peripheral genetic clinic illustrates the value of sweat testing to confirm the clinical diagnosis of an isolated female carrier manifesting XHED. This patient was seen at the age of 20 years for advice on the possible cause of her absent breast development and hypodontia. Her presenting complaint was 'tiredness' and a tendency to sleep for long periods. It was thought that this might be secondary to the psychological sequelae of her physical problems. Clinical enquiry indicated diminished sweating and a sense of 'feeling wobbly' in the heat. She had had no teeth until 2½ years, and her three deciduous maxillary incisors were malformed (and subsequently extracted); she has had no mandibular incisors, and the mandibular canines were also malformed. No secondary dentition has erupted, but dental radiography has not been performed. She had sparse body and scalp hair, eyebrows, and eyelashes with hair absent from her left arm and patchy on her left leg.

Despite a negative family history, XHED was

Lines of Blaschko shown by starch and iodine sweat test on the back of a woman manifesting mild clinical features of ectodermal dysplasia, hypodontia, and hypotrichosis, but with no family history of an affected male.

raised as a possible diagnosis. Sweat testing showed a diagnostic patchy distribution, and she was advised of a 1 in 2 risk to male offspring. Her son, born the following year, had classical features of XHED. Thanks to the diagnosis having been made before conception, her medical attendants and the clinicians responsible for the care of the child were able to institute immediate measures to avoid damage associated with hyperthermia.

Discussion

The sweat test described in this paper is unlikely to be as helpful in clinical genetic practice as the apparent 95% sensitivity might suggest for a variety of reasons. First, the families ascertained in this study will be biased towards the more severely affected, in which the female carriers are more likely to manifest signs of the disorder. Secondly, the test is likely to be most often used in situations where the diagnosis itself is uncertain, rather than situations where the diagnosis is known, but the carrier status of one female remains uncertain.

The sweat test protocol presented here is probably as good as any available. It will indicate the carrier status of many women with XHED. When it gives a clearly abnormal result, then one can be fully confident of its accuracy. When an apparently normal or an equivocal result is obtained, interpretation is harder; even in principle, such sweat testing could never be completely accurate. First, some families will inactivate the defective XHED gene on a large area of the body, such as the back. Secondly, even those patches with the defective allele may carry sufficient sweat pores to escape detection. There is considerable inter- and even intrafamilial variation in sweat pore counts in the affected males.⁶ The 'defective' patches in a woman, therefore, may well still bear some sweat glands and this will render the test misleading in such cases. We would never carry out such prolonged testing on a boy with possible XHED, because of the associated risks of iatrogenic convulsion and even death.^{6 23} We recognise no indication for performing this type of sweat test on males with possible XHED: the direct examination of sweat pores should suffice.

Before sweat testing is performed, it would be advisable to examine the woman's teeth. Dental examination and history can be very useful, and it is likely that 70 to 80% of female carriers can be identified in this way.^{6 7 24 25} However, it is necessary to recall that about 5% of females have hypodontia of their permanent dentition (excluding third molars) and that 0.3% have hypodontia of their deciduous teeth.²⁶ Only 15% of those who lack any permanent teeth lack more than two: 55% lack only a single tooth and 29% lack two.²⁷ In the interpretation of dental findings, it is useful to know that more than 90% of

the missing teeth in the normal population belong to four categories: mandibular second premolar, maxillary lateral incisor, maxillary second premolar, and mandibular central incisor. Tooth size is also significantly smaller in females carrying XHED than in the normal population,⁷ but there is an overlap between the two groups. Dental radiographs of possible female carriers can be useful in the identification of gene carriers, through recording tooth number and size, and also showing taurodontism (Crawford *et al*, in preparation).

If the sweat test is normal in the (dentally normal) mother of an isolated male case of HED whose clinical picture is compatible with the diagnosis of XHED, then there are several possibilities: (1) he has XHED, she is not a carrier; (2) he has XHED, she is a carrier but does not manifest it because of the pattern of X chromosome inactivation; (3) he has XHED, she is a carrier but does not manifest it because some sweat pores are produced even in areas with the defective XHED gene; (4) he has another type of HED, most probably autosomal recessive. We do not have the data to determine the relative likelihoods of these possibilities. However, if such a woman has a normal sweat test and normal dental history and examination, then the chance of her carrying XHED is very substantially reduced. We would be confident of detecting at least 90% of female carriers of XHED by dental examination and sweat testing in combination, when the affected male(s) in the family have virtual absence of sweat pores on their fingertips.

Some women present to genetic clinics with some features of XHED, but without a family history of an affected male. The finding of patchy sweating in such women, combined with the presenting hypodontia or hypotrichosis or both, makes the diagnosis of XHED highly probable. In our experience, the use of sweat testing in these circumstances has been very useful, because those women shown to be mosaic for hypohidrosis can be given an accurate diagnosis and can be informed of the possible hazards of overheating in any male offspring. Alternative diagnoses, however, must also be considered, in particular, incontinentia pigmenti (IP). Females carrying the gene for IP2, located at Xq28 by linkage analysis,²⁸ may also manifest patchy defects of teeth, hair, and sweating. Such women, however, will usually be recognisable because of the reticulate pattern of smooth, hairless, non-sweating skin apparent on trunk and limbs but especially on the calves of the legs.²⁹ They will also usually have a history of neonatal skin eruption, sometimes of pigmentation that may have faded by adulthood, and sometimes of recurrent miscarriages in one or more family members. Other types of ectodermal dysplasia are most unlikely to produce a patchy sweating pattern, and most such conditions

have additional clinical characteristics. Diagnostic confusion is therefore unlikely to occur.

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