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# Carrier Identification in Steroid Sulphatase Deficiency and Recessive X-Linked Ichthyosis

GERT LYKKESFELDT[1] and ANNE E. LYKKESFELDT[2]

[1]*Department of Obstetrics and Gynaecology Y, and Department of Dermatology H, Rigshospitalet, University of Copenhagen, and* [2]*The Fibiger Institute, Copenhagen, Denmark*

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Steroid sulphatase (STS) activity was measured with tritiated dehydroepiandrosterone sulphate (DHEAS) and oestrone sulphate (OES) in leucocytes as well as in skin fibroblasts from 31 women who were presumed to be carriers of STS deficiency and recessive X-linked ichthyosis. Overall, 30 of the 31 women (96.8%) could be identified as heterozygotes in at least one of the four assay systems used, i.e. on the basis of having an STS activity below the 2.5 percentile calculated for normal control females. In the individual assay systems, the highest carrier detection rate was achieved with OES in leucocytes (96.2%), followed by DHEAS in leucocytes (80.8%), whereas a more pronounced overlap was present in the fibroblast systems. In leucocytes as well as in fibroblasts, the STS activity determined with DHEAS was positively correlated with the STS activity determined with OES ($p<0.001$) suggesting that a single sulphatase is responsible for the hydrolysis of both steroid sulphates. *Key words: Steroid sulphatase deficiency; Recessive X-linked ichthyosis; Carrier detection.* (Received August 30, 1985.)

Gert Lykkesfeldt, Skolevej 15, DK-2820 Gentofte, Denmark.

The biochemical basis of recessive X-linked ichthyosis (RXLI) is a deficiency of the microsomal enzyme, steroid sulphatase (STS) (reviewed in 1, 2). The STS deficiency has been documented in all hitherto examined tissues from affected patients, e.g. placental tissue, skin fibroblasts, hair roots, leucocytes, and testicular tissue (1, 2, 3). An early prenatal diagnosis of RXLI can be obtained by amniocentesis with a subsequent demonstration of the STS deficiency in cultured amniotic fluid cells (2, 4, 5).

RXLI males have been shown to accumulate abnormally high quantities of cholesterol sulphate, a natural substrate of the STS enzyme, in their stratum corneum (6). Moreover, convincing evidence exists that the scale formation in RXLI is a consequence of the lack of the STS catalysed convertion of cholesterol sulphate to unconjugated cholesterol (7), resulting in a disruption of the so-called epidermal cholesterol sulphate cycle (8). The issue of the relative importance of increased cholesterol sulphate or decreased cholesterol in RXLI stratum corneum to the pathogenesis of scaling remains to be resolved (7). In some patients, however, the scaling has been shown to diminish significantly or even to clear after topical application of a cholesterol-containing cream (9).

In addition to ichthyosis, STS deficiency might cause other deleterious effects to the affected patients. Thus a high occurrence of testicular maldescent has been found in RXLI males (10, 11), and an increased risk of testis cancer cannot be excluded (11, 12, 13). The link between the STS deficiency and the development of gonadal diseases might be found in the recent uncovering of an abnormal androgen and oestrogen metabolism in RXLI men (14).

Female relatives of patients with RXLI will often express their desire to know whether or not they themselves could have an affected boy. Such information can be given with authenticity, if a dependable test to identify heterozygotes, i.e. the women who are carriers of a defect STS gene, is available. The possibility of carrier detection in RXLI

using STS determinations was first indicated by a short report demonstrating that all of five investigated heterozygotes had STS activity below the normal female range in skin fibroblasts (15), and, secondly, by a corresponding study with leucocytes involving six carriers (16). More recently, however, a briefly reported investigation of STS activity in leucocytes from 18 RXLI heterozygotes revealed that four had enzyme values overlapping the normal control range (17).

None of the previous studies have engaged the issue of carrier identification on a more comprehensive basis by determining the efficacy of detection in a large group of heterozygotes using various types of tissues and STS substrates. We have therefore studied leucocytes as well as skin fibroblasts from normal women and from 31 women presumed to be carriers of RXLI. The STS enzyme activity was determined with two different substrates, dehydroepiandrosterone sulphate (DHEAS) which was also applied in the above mentioned reports, and oestrone sulphate (OES) which have not previously been used for this purpose.

## MATERIALS AND METHODS

The 31 women were presumed to be carriers on the basis of being mothers of male patients with RXLI. In these males, the RXLI diagnosis was verified by the absence of STS activity in leucocytes and/or skin fibroblasts determined with both $^3$H-DHEAS and $^3$H-OES (2, 18). Twenty-nine of the 31 women could be classified as obligate heterozygotes since they were related to at least one additional affected male. In the remaining two instances, i.e. cases 12 and 24 (Table 1), the possibility of a new mutation in the offspring could not be ruled out by pedigree analysis. Leucocytes from 20, and skin fibroblasts from 10 normal healthy women with a negative family history of ichthyosis were used as control material.

Leucocytes were isolated from peripheral blood according to a previously reported procedure (18), and skin biopsy specimens were used to obtain fibroblasts (2). Leucocytes and fibroblasts were stored at −80°C until the time of assay.

STS activity was determined with two different steroid substrates, $^3$H-DHEAS and $^3$H-OES (New England Nuclear); and the assay for leucocytes as well as for fibroblasts was performed exactly as previously reported (18). In both leucocytes and fibroblasts, aliquots of the same homogenate were used to determine the STS activity with the two substrates, and carriers and controls had their enzyme activity measured in the same experiment.

## RESULTS

The distributions obtained for STS activity with the four assay systems are depicted in Fig. 1. Enzyme activities of carriers and controls were seen to overlap in all four systems. Ratios between geometric means of carrier and control values were : *leucocytes with DHEAS*- 13.31/4.87 (2.73), *leucocytes with OES*- 20.50/7.20 (2.85), *fibroblasts with DHEAS*- 18.26/7.53 (2.42), and *fibroblasts with OES*- 33.14/16.34 (2.03). In all four instances, the enzyme activities of carriers and controls were significantly different ($p<0.001$, Wilcoxon's two-sample rank sum test).

In each assay system, STS values of carriers were considered in the heterozygote range (and thus detectable) when they were below the 2.5 percentile calculated for the normal control subjects. The carrier detection rates obtained with the four assay systems were (Table 1): *leucocytes with DHEAS*- 21/26 (80.8%), *leucocytes with OES*- 25/26 (96.2%), *fibroblasts with DHEAS*- 15/22 (68.2%), and *fibroblasts with OES*- 14/22 (63.6%). The overall detection rate achieved with the four systems was 30/31 (96.8%), which is identical to that of OES in leucocytes.

In leucocytes as well as in fibroblasts, the STS activity determined with DHEAS was positively correlated with the STS activity determined with OES ($p<0.001$). *Leucocytes:* $r=0.87$ (carriers, $n=26$); $r=0.90$ (controls, $n=20$). *Fibroblasts:* $r=0.94$ (carriers, $n=22$);

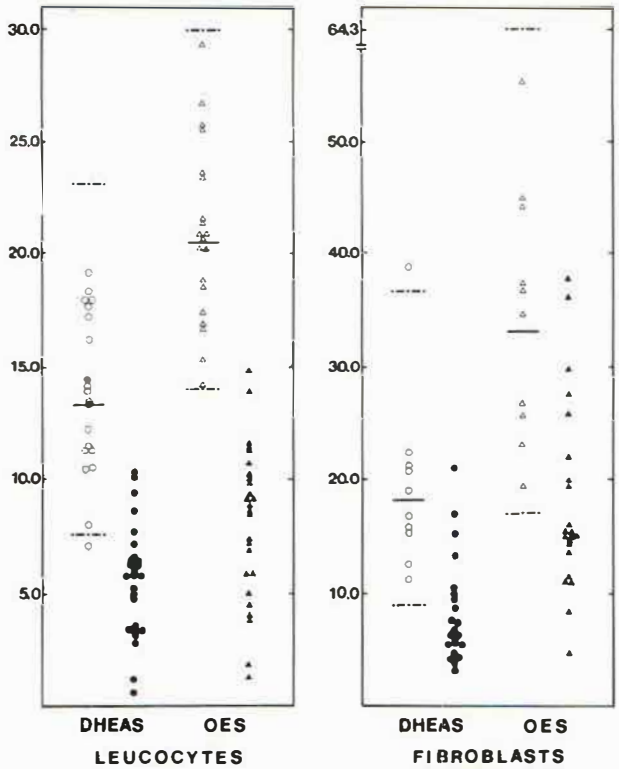


*Fig. 1.* Distribution of steroid sulphatase activity in leucocytes and skin fibroblasts determined with $^{3}$H-dehydroepiandrosterone sulphate (DHEAS) and $^{3}$H-oestrone sulphate (OES). Presumed carriers of RXLI (leucocytes: $N=26$, fibroblasts: $N=22$) are denoted by closed symbols, and normal females (leucocytes: $N=20$, fibroblasts: $N=10$) by open symbols. The geometric means calculated for the normal females are indicated by *solid* horizontal lines, and the 95% confidence limits by *broken* horizontal lines. *Ordinate*: pmol hydrolysed substrate per mg protein per 20 min.

$r=0.92$ (controls, $n=10$). A weaker positive correlation between leucocyte STS activity and fibroblasts STS activity could also be demonstrated in the carriers for both substrates. *DHEAS:* $r=0.62$ ($n=17$, $p<0.01$); *OES:* $r=0.49$ ($n=17$, $p<0.05$). A similar estimation was not possible for the control subjects because of an insufficient number of corresponding (leucocyte-fibroblast) samples.

## DISCUSSION

The present study confirms the feasibility of carrier identification in RXLI by STS determinations. However, leucocytes were clearly of much greater use in this respect than skin fibroblasts. The detection rate achieved with leucocytes and OES (96.2%) was actually equal to the detection rate yielded by all four assay systems together. More generally, the significant correlations between the STS activities obtained with these systems seem to indicate that the simultaneous application of several tissues and substrates, in order to enhance detection, may prove unprofitable.

The only presumed carrier (case 12, Table I) who could not be identified as a heterozygote with OES in leucocytes also escaped detection with the three other systems. As this woman had no relatives with RXLI, apart from her only boy, the possibility remains that she might not be a carrier of the defect STS gene. At present this uncertainty cannot be resolved; it serves, however, to emphasize that the STS activities of heterozygotes and normal women tend to overlap. This conclusion is in harmony with an earlier report observing that four out of 18 carriers had STS activities overlapping the normal control range (17).

Table I. *Steroid sulphatase activity in 31 presumed carriers for recessive X-linked ichthyosis (pmol/mg protein/20 min)*

DHEAS = $^3$H-dehydroepiandrosterone sulphate, OES = $^3$H-oestrone sulphate, * = heterozygote range, ND = not done

| | Leucocytes | | Skin fibroblasts | |
|---|---|---|---|---|
| Case no. | DHEAS | OES | DHEAS | OES |
| 1 | 6.37* | 10.66* | 10.07 | 22.01 |
| 2 | 3.26* | 5.03* | 9.60 | 19.25 |
| 3 | 2.88* | 4.11* | 6.92* | 13.57* |
| 4 | 4.97* | 6.91* | ND | ND |
| 5 | 3.38* | 3.85* | ND | ND |
| 6 | 5.88* | 10.20* | 13.25 | 27.45 |
| 7 | 7.70 | 11.37* | 10.58 | 25.80 |
| 8 | 7.24* | 9.23* | 6.22* | 14.95* |
| 9 | 4.83* | 7.21* | ND | ND |
| 10 | 10.13 | 13.92* | 16.98 | 29.72 |
| 11 | 6.48* | 8.58* | 4.25* | 11.41* |
| 12 | 9.38 | 14.81 | 15.17 | 37.73 |
| 13 | 5.77* | 5.89* | ND | ND |
| 14 | 5.83* | 8.91* | ND | ND |
| 15 | 6.25* | 9.20* | 6.64* | 15.39* |
| 16 | 3.45* | 4.48* | 7.74* | 14.27* |
| 17 | 5.17* | 8.54* | 4.86* | 11.09* |
| 18 | 5.78* | 11.30* | 3.94* | 8.46* |
| 19 | 8.62 | 9.76* | 6.64* | 15.26* |
| 20 | 3.56* | 7.39* | 5.77* | 14.46* |
| 21 | 10.22 | 9.31* | 20.95 | 36.06 |
| 22 | 6.42* | 9.96* | 4.63* | 4.85* |
| 23 | ND | ND | 7.61* | 16.01* |
| 24 | ND | ND | 3.62* | 11.05* |
| 25 | ND | ND | 8.81* | 19.86 |
| 26 | ND | ND | 5.91* | 15.07* |
| 27 | ND | ND | 5.79* | 14.95* |
| 28 | 3.38* | 5.86* | ND | ND |
| 29 | 0.66* | 1.40* | ND | ND |
| 30 | 1.25* | 1.86* | ND | ND |
| 31 | 6.25* | 11.63* | ND | ND |
| Controls | $n=20$ | | $n=10$ | |
| Mean | 13.31 | 20.50 | 18.26 | 33.14 |
| 2.5% | 7.67 | 14.03 | 9.10 | 17.07 |

The results with fibroblasts showed considerably more overlap than with leucocytes. Reasons for this are not evident, but one might draw the inference that fibroblasts do not reflect, as accurately as leucocytes, the actual situation in vivo. Possibly, some condition of skin fibroblast culture may exert a capricious influence on the STS activity. While this issue is in need of further clarification, it goes without saying that leucocytes are far more convenient and less time-consuming to obtain than skin fibroblasts. In relation to the choice of substrate for the STS determinations, OES seems preferable to DHEAS because it offers somewhat better assay conditions, due to very low background values and negligible non-enzymatic hydrolysis.

It deserves to be mentioned that whereas DHEAS is an alkylsulphate, OES is an arylsulphate. Nevertheless, the presence of a strong correlation between STS activities

determined with the two substrates argues in favour of a single enzyme being responsible for the hydrolysis of both steroid sulphates. Furthermore, the positive correlation between STS activities of leucocytes and fibroblasts, as demonstrated for the carrier group, could suggest that the expression of STS activity in different tissues may be subject to coordinate genetic regulation.

In conclusion, we suggest that leucocytes should be chosen instead of skin fibroblasts for the identification of carriers among female relatives of patients with RXLI. Moreover, excellent detection can be accomplished with only a single assay system using OES as substrate for the STS determinations.

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