

Most "Sporadic" Cases of X-linked Ichthyosis are not *De Novo* Mutations

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X-linked ichthyosis is an inherited disease with dark, regular and adherent scales as clinical characteristics. It is caused by a deficiency of the steroid sulphatase enzyme. Steroid sulphatase assay is a relative easy tool that enables correct diagnosis of X-linked ichthyosis patients and carriers. A large number of X-linked ichthyosis patients correspond to non-familial cases that seem to represent *de novo* mutations. In this study, we examined the X-linked ichthyosis carrier state of the mothers of 42 non-familial cases to determine whether their children corresponded to *de novo* mutations. To classify patients and carriers, a steroid sulphatase assay was performed in leukocytes using 7-[³H]-dehydroepiandrosterone sulphate as substrate. In 36 mothers (85%) we found steroid sulphatase activity compatible with the carrier state of X-linked ichthyosis. This data suggest that most of the mothers of these patients present the primary gene defect, excluding *de novo* mutations in the patients. Key words: steroid sulphatase; X-linked ichthyosis; leukocytes.

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INTRODUCTION

X-linked ichthyosis (XLI) is an inherited disease caused by a metabolic defect in the steroid sulphatase (STS) enzyme (1). The gene encoding STS is on Xp22.3 (2) and a complete deletion of the STS gene is present in most XLI cases (3). XLI patients present dark, regular and adherent skin scales as clinical characteristics (4). The extremities, trunk and neck are the main areas affected (5). Cryptorchidism in patients, delayed labour in carriers and corneal opacities in both patients and carriers are features frequently associated with the condition (6–8). Ichthyosis vulgaris shares many clinical features with XLI and a differential diagnosis must be made among both entities (9, 10). XLI diagnosis is possible by determining the STS activity in leukocytes or other cell lines (11–13). In the same way, XLI carriers can be identified through STS enzyme assay as their STS activity is lower than in normal controls (14, 15). In a previous report, 4 mothers of 5 unrelated sporadic Mexican cases of XLI showed an STS activity compatible with a carrier state, indicating that they presented the primary gene defect (16). In another report, the XLI carrier state was demonstrated in the mother of a patient classified as a *de novo* mutation (17). The aim of the present study was to corroborate in a

larger group of XLI sporadic cases that most of them do not correspond to *de novo* mutations.

MATERIALS AND METHODS

All non-familial cases of XLI referred to the General Hospital of Mexico during 1994–1997 were included in the study. The patients were informed about the details of the study and their consent to participation obtained. The study protocol was approved by the ethics committee. The presence of non-affected patients in at least three generations of the family of the propositus was the criteria for inclusion as non-familial cases. To confirm the correct diagnosis of XLI, STS activity was determined in leukocytes as follows (18): after overnight fasting 10 ml of blood was obtained with a heparinized syringe. The leukocytes were collected by centrifugation and washed 3 times with 0.9% NaCl solution. Residual erythrocytes were eliminated by adding 2 ml 0.85% NH₄Cl. The leukocyte pellet was homogenized in 0.014 M cold Tris buffer, pH 7.0, using a Polytron PT10 in two cycles of maximum speed for 20 s and 10 s.

STS assay was performed with the homogenate, using 7-[³H] dehydroepiandrosterone sulphate (7-[³H]-DHEAS, 16.3 Ci/mmol, New England Nuclear, Boston, Mass, U.S.A.) as substrate (18). Prior to the assay, 7-[³H]-DHEAS was washed with benzene (Merck, analytical grade). A volume of 5 pmol 7-[³H]-DHEAS was incubated with 200 µl homogenate for 1 h at 37°C in a shaking bath. The incubation was stopped by the addition of 1 ml cold benzene (Merck, analytical grade). The samples were mixed for 1 min and centrifuged for 3 min at 2500 rpm to achieve the correct separation of the organic and aqueous phases. Aliquots of 600 µl of the organic phase were put into glass vials for the determination of 7-[³H]-dehydroepiandrosterone (7-[³H]-DHEA) in a 3255 Packard scintillation spectrometer. All assays were done in duplicate with a blank control. The extraction of 7-[³H]-DHEA was confirmed by thin layer chromatography of the organic phase. Protein concentration in the homogenate was determined by the Bradford method (19). The validity of the STS enzymatic assay to classify XLI carriers was confirmed in 24 XLI obligated carriers. In addition, all mothers of the XLI patients initially classified as XLI carriers by this method were tested in a second time to confirm their XLI carrier condition. To confirm the XLI diagnosis in all patients, DNA was extracted from the leukocytes and amplification by PCR of the 5' and 3' ends of the STS gene was performed (20).

RESULTS AND DISCUSSION

We analysed 42 sporadic cases of XLI in a sample of Mexican patients. None of them presented an STS activity (0.00 pmol/mg protein/h) when compared with normal controls (0.84 ± 0.20 pmol/mg protein/h). The DNA from the 42 XLI patients showed no amplification of the 5' and 3' ends of the STS gene, corroborating the XLI diagnosis. These results allowed them to be classified as XLI patients. Increased blood cholesterol sulphate and electrophoretic mobility of low-density lipoprotein are two alternative methods of diagnosing XLI patients (21). Nevertheless, these procedures are not reli-

able for carrier assessment, as the values observed in XLI carriers are similar to those found in normal controls. STS activity assay in leukocytes allows identification of XLI patients and carriers (14, 15). To confirm the validity of the STS assay to discard the XLI carrier state, 24 obligated carriers were included in this study. All showed an STS activity similar to that expected for XLI carriers as reported in previous studies (14, 15). When the STS assay was performed in the mothers of the 42 XLI patients, 36 presented an STS activity compatible with an XLI carrier state (0.20 ± 0.12 versus 0.84 ± 0.20 pmol/mg protein/h). The STS assay was performed twice in all XLI carriers. This data allows us to classify 85% of the sporadic cases as inherited cases and not as *de novo* mutations, thus corroborating our previous findings. These results seem to indicate that *de novo* mutation is not a common condition in XLI.

We could only analyse 3 maternal grandmothers of the XLI sporadic cases and found normal STS activity in all of them. We also found in 7 families normal STS activity in all sisters of the XLI carriers, supporting the fact that the patients' mothers suffered the primary gene defect. In a previous report, an XLI individual case was analysed and the authors state that the gene defect of STS arose on an allele inherited from the proband's clinically normal maternal grandfather (17). It would be interesting to determine in more cases whether the maternal or paternal X-chromosome with the gene defect is transmitted to the XLI carrier mother. This data could inform us whether the recombination events that produce the STS gene deletion occur during male or female meiosis.

We conclude that in our sample, 36 of 42 mothers of XLI "sporadic" cases were carriers of the gene defect, excluding the assignment of these patients as *de novo* mutations. It is necessary to perform similar analyses in other geographic areas to identify whether the transmission pattern observed in this study is a common condition in XLI. In addition, it is important to include the STS assay as a routine test for all potential XLI carriers in order to offer adequate genetic counselling.

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