A Novel Partial Deletion of Exons 2–10 of the STS Gene in Recessive X-Linked Ichthyosis

Margarita Valdes-Flores, Susana H. Kofman-Alfaro,* Ana L. Jimenez Vaca,* and Sergio A. Cuevas-Covarrubias*

Servicio de Genetica, Instituto Nacional de Ortopedia; *Servicio de Genetica, Hospital General de Mexico, Facultad de Medicina, UNAM, Mexico D.F., Mexico

X-linked ichthyosis is an inherited disease due to steroid sulfatase deficiency. Onset is at birth or early after birth with dark, regular, and adherent scales of skin. Approximately 85%–90% of X-linked ichthyosis patients have large deletions of the STS gene and flanking sequences. Three patients have been identified with partial deletions of the gene. Two deletions have been found at the 3′ extreme and the other one implicating exons 2–5. This study describes a novel partial deletion of the STS gene in an X-linked ichthyosis patient. The subject was classified through steroid sulfatase assay in leukocytes using 7-[^{3}H]-dehydroepiandrosterone sulfate as a substrate. Exons 1, 2, 5, and 7–10, and 3′ flanking sequences DXS1131, DXS1133, DXS237, DXS1132, DXF22S1, and DXS278 of the STS gene were analyzed through polymerase chain reaction. The DNA analysis showed that exon 1 and 3′ flanking sequences from DXS237 to DXS278 were present. In this study we report the fourth partial deletion of the STS gene and the first spanning exons 2–10 in X-linked ichthyosis patients. ***Key words: dehydroepiandrosterone sulfate/leukocytes/steroid sulfatase/STS gene/X-linked ichthyosis. J Invest Dermatol 114:591–593, 2000***

X-linked ichthyosis (XLI) is a relatively common inherited inborn error of metabolism characterized by dark, adhesive, and regular scales of skin. It is present at birth or soon after birth (Okano *et al*, 1988; Shayder and Ott, 1991). Several studies have estimated a frequency of 1 in 2000–6000 males (Wells and Kerr, 1966; Lykkesfeldt *et al*, 1984). XLI is due to steroid sulfatase (STS) deficiency (Shapiro and Weiss, 1978); this enzyme hydrolyzes 3-beta-hydroxysteroid sulfates (Dibbelt and Kuss, 1991). The STS enzyme deficiency is associated with an increase of cholesterol sulfate in the stratum corneum (Williams and Elias, 1981). This defect appears to be the cause of the delay in the normal process of skin desquamation. The STS enzyme assay allows the correct diagnosis of XLI to be established (Baden *et al*, 1980; Epstein and Leventhal, 1981; Matsumoto *et al*, 1990). The STS gene locus is located on Xp22.3 (Muller *et al*, 1981). Molecular studies have revealed that most XLI patients present large deletions of the STS gene (Bonifas *et al*, 1987; Shapiro *et al*, 1989; Cuevas *et al*, 1997). Only a few point mutations and three partial deletions have been reported (Bonifas *et al*, 1987; Ballabio *et al*, 1989; Shapiro *et al*, 1989; Basler *et al*, 1992; Nomura *et al*, 1995; Alperin and Shapiro, 1997; Morita *et al*, 1997). A 3 bp of homology at deletion breakpoints in the sequence analysis of a partial deletion of the 3′ end of the STS gene has also been reported (Bernatowicz *et al*, 1992). In this study, we describe the presence of a novel partial deletion of the STS gene in an XLI patient.

Manuscript received July 2, 1999; revised November 29, 1999; accepted for publication December 14, 1999.

Reprint requests to: Dr. Sergio Cuevas Covarrubias, Servicio de Genética, Hospital General de México, Fac. Medicina, UNAM, Dr. Balmis 148 Col. Doctores C.p. 06726, México D.F., México. Email: sergioa@servidor.unam.mx

Abbreviations: STS, steroid sulfatase; XLI, X-linked ichthyosis.

MATERIALS AND METHODS

Patient An XLI patient was initially referred to the Genetic Department of the General Hospital of Mexico. He was informed about the characteristics of the study and he agreed to participate. The protocol was evaluated and accepted by the Ethics Committee of the General Hospital of Mexico. The patient was an 18-y-old Mexican male. He was the product of an uncomplicated pregnancy with normal spontaneous vaginal delivery. There was no history of cryptorchidism. He developed ichthyosis during the second month. Physical examination showed ichthyosis on the trunk and extremities with a moderate degree of affection. No corneal opacities were found to be present. XLI diagnosis was confirmed through the STS assay.

STS assay STS activity was determined in leukocytes as follows: 10 ml of blood was obtained with a heparinized syringe. The leukocyte pellet was obtained through centrifugation and washed three times with 0.9% NaCl. Residual erythrocytes were eliminated with 0.85% NH_4Cl solution. The STS assay was performed in the leukocyte pellet, which was homogenized in chilled 0.014 M Tris(hydroxymethyl)-aminomethane buffer with a polytron in two cycles of 20 s and 10 s, respectively. 7-[^{3}H]-dehydroepiandrosterone sulfate (16.3 Ci per mmol, NEN, Boston, MA) was used as enzyme substrate. Assay conditions were pH 7.0, 37°C, 1 h. The product of hydrolysis was recovered with benzene (Merck, analytical grade) and read in a scintillation spectrometer (Cuevas *et al*, 1993).

Mutation detection DNA extraction was performed in the conventional way (Lench *et al*, 1988). The exons 1, 2, 5, and 7–10, and 3′ flanking sequences DXS1131, DXS1133, DXS237, DXS1132, DXF22S1, and DXS278 of the STS gene were analyzed through polymerase chain reaction (PCR) with a PCR amplification kit (Perkin-Elmer). The conditions and primers to amplify exons 1, 2, 5, and 7–10 are shown in **Table I**. The conditions and primers to amplify the 3′ flanking sequences of the STS gene are described elsewhere (Schaefer *et al*, 1993). All procedures were performed three times.

Table I. Primers and conditions used in the PCR analysis of the STS gene

Exon 1
F-5′GGCCTAGAAGAAGGTTGAAGGTCCC
R-5′AAGAGGTTGGATGAGATGGGCATAC
DNA 500 ng, primers 0.4 μM, dNTP's 0.08 mM, Mg buffer 1×, Taq Pol 1.5 U, Vol. 50 μl
94°C for 1 min, 30 cycles of 90°C for 1 min, 60°C for 30 s, and 72°C for 2 min
Exon 2
F-5′ TCCTTTACAGGAAGATGAAG
R-5′ CATTACCAACCTGATAGTTTT
DNA 500 ng, primers 0.8 μM, dNTP's 0.12 mM, Mg buffer 1×, Taq Pol 1.5 U, Vol. 50 μl
94°C for 3 min, 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min
Exon 5
F-5′ ACCACCCTTTACATCACGGC
R-5′ CGCCTCCACCGTTAGCCTCT
DNA 500 ng, primers 0.8 μM, dNTP's 0.12 mM, Mg buffer 1×, Taq Pol 1.5 U, Vol. 50 μl
94°C for 5 min, 25 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min
Exon 7
F-5′ TCCCCTCCAGGGCAGATCTTGAAC
R-5′ CATTTCTCACCTTTATAGATCCC
DNA 500 ng, primers 0.8 μM, dNTP's 0.12 mM, Mg buffer 1×, Taq Pol 1.5 U, Vol. 50 μl
94°C for 3 min, 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min
Exon 8
F-5′ GATCTTTTAGGAGGAAAAGCAAAC
R-5′ CAGAGTACCTGTCCTCAGGCAAGG
DNA 500 ng, primers 0.8 μM, dNTP's 0.12 mM, Mg buffer 1×, Taq Pol 1.5 U, Vol. 50 μl
94°C for 3 min, 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min
Exon 9
F-5′ TATCCCACAGGATCATTGATGGAC
R-5′ TTTACTCACTGTTCTGAGGTGC
DNA 500 ng, primers 0.8 μM, dNTP's 0.12 mM, Mg buffer 1×, Taq Pol 1.5 U, Vol. 50 μl
94°C for 3 min, 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min
Exon 10
F-5′GAAATCCTCAAAGTCATGCAGGAAG
R-5′CCTCCAGTTGAGTAGCTGTTGAGCT
DNA 500 ng, primers 0.4 μM, dNTP's 0.08 mM, Mg buffer 1×, Taq Pol 1.5 U, Vol. 50 μl
94°C or 1 min, 30 cycles of 90°C for 1 min, 60°C for 30 s, and 72°C for 2 min

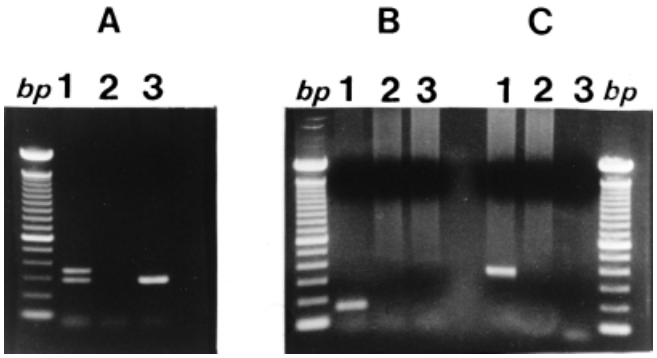

Figure 1. PCR analysis of the STS gene. (*a*) Exons 1 and 10 (lower and upper bands, respectively); (*b*) Exon 2; (*c*) Exon 5. *Line 1*, positive control; *line 2*, XLI patient with complete deletion of the STS gene; *line 3*, XLI patient described in the text with the partial deletion spanning exons 2–10; in this subject only exon 1 is observed.

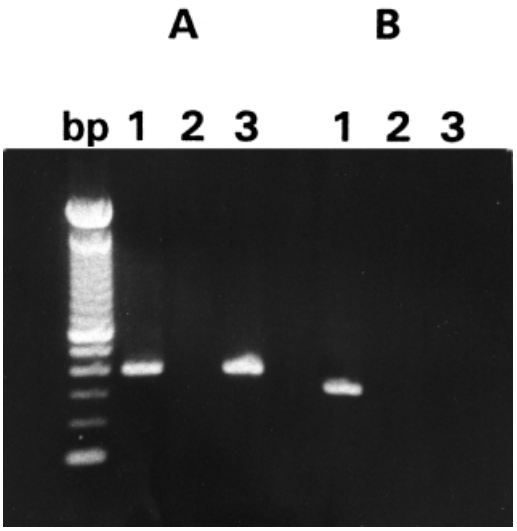

Figure 2. PCR analysis of the 3′ flanking sequences DXS237 and DXS1133. (*a*) DXS237; (*b*) DXS1133. *Line 1*, positive control; *line 2*, XLI patient with complete deletion of the STS gene; *line 3*, XLI patient described in the text; in this subject only sequence DXS237 is observed.

RESULTS AND DISCUSSION

The XLI patient was shown to have undetectable levels of STS activity (0.00 pmol per mg protein per h). This result corroborated the XLI diagnosis. Initial DNA analysis of both extremes of the STS gene (Ballabio *et al*, 1990) showed a normal amplification of the 5′ segment (**Fig 1*a***, *line 3*). Subsequent PCR amplification of exons 2, 5, and 7–9 (**Fig 1*b***), and the 3′ flanking sequences DXS1131 and DXS1133 (**Fig 2**) failed to amplify these segments. Only exon 1 and 3′ flanking sequences from DXS237 to DXS278 were normally amplified. So, in this XLI patient the partial deletion of the STS gene involved exons 2–10. PCR was repeated three times for all exons and identical results were obtained on each assay.

The molecular basis of XLI presents an unusually high frequency of complete STS gene deletions. Analysis of flanking DNA markers closely linked to the STS gene indicates that these sequences are also frequently involved. Only a few patients with STS-gene-encoded sequences have been reported and they presented point mutations in different exon and intron regions. Three subjects have been identified with partial deletions of the STS gene. Most partial deletions have been found at the 3′ end of the gene. In one of these cases, the deletion started within intron 7 of the STS gene and extended over 150 kb, involving the last three exons of the gene

(Ballabio *et al*, 1989), whereas the other case had a partial deletion that included exon 10 (Nomura *et al*, 1995). The third patient had an intragenic deletion of about 40 kb spanning exons 2–5 (Shapiro *et al*, 1989). At this time, we have examined the STS gene of 74 XLI Mexican patients. Most of them (n = 72) have had complete deletion of the STS gene similar to results in other geographic areas previously reported in the literature. The patient presented here initially showed normal amplification of the 5′ end of the STS gene indicating the presence of a partial deletion at the 3′ end of the gene. The subsequent DNA analysis of our patient showed only amplification of exon 1 and of 3′ flanking sequences from DXS237 to DXS278. The breakpoints of the deletion thus lie within intron 1 and between 3′ flanking sequences DXS1133 and DXS237, indicating that the STS gene in our patient lacks exons 2–10. This partial deletion also lies within intron 1, similar to the deletion previously reported that lacked exons 2–5 (Shapiro *et al*, 1989). In our case, the deletion involves exons 2–10 and extends over 3′ flanking sequences DXS1131 and DXS1133. It would be very interesting to know if there is a homology region at the deletion breakpoint as was reported at the 3′ end of the STS gene. A more rigorous and precise study will be required to detail the changes involved at deletion breakpoints. On the other hand, only one of our 74 patients has presented a normal amplification of these segments and he is now being analyzed to discard a possible point mutation in the STS gene.

In conclusion, we report the fourth partial deletion of the STS gene in XLI patients and the first spanning exons 2–10.

This study was supported by CONACYT, México, project number 26362-M.

REFERENCES

Alperin ES, Shapiro LJ: Characterization of point mutations in patients with X-linked ichthyosis. Effects on the structure and function of the steroid sulfatase protein. *J Biol Chem* 272:20756–20763, 1997

Baden HP, Hooker PA, Kubilus J, Tarascio A: Sulfatase activity of keratinizing tissues in X-linked ichthyosis. *Pediatr Res* 14:1347–1348, 1980

Ballabio A, Carrozzo R, Parenti G, *et al*: Molecular heterogeneity of steroid sulfatase deficiency: a multicenter study on 7 unrelated patients, at DNA and protein levels. *Genomics* 4:36–40, 1989

Ballabio A, Ranier JE, Chamberlain JS, Zollo M, Caskey CT: Screening for steroid sulfatase (STS) gene deletions by multiplex DNA amplification. *Hum Genet* 84:571–572, 1990

Basler E, Grompe M, Parenti G, Yates J, Ballabio A: Identification of point mutations in the steroid sulfatase gene of three patients with X-linked ichthyosis. *Am J Hum Genet* 50:483–491, 1992

Bernatowicz LF, Li XM, Carrozzo R, Ballabio A, Mohandas T, Yen PH, Shapiro LJ: Sequence analysis of a partial deletion reveals 3 bp of homology at deletion breakpoints. *Genomics* 13:892–893, 1992

Bonifas JM, Morley BJ, Oakey RE, Waikon Y, Epstein EH: Cloning of cDNA for steroid sulfatase: frequent occurrence of gene deletions in patients with recessive X-chromosome-linked ichthyosis. *Proc Natl Acad Sci (USA)* 84:9248–9251, 1987

Cuevas-Covarrubias S, Juarez-Oropeza M, Miranda-Zamora R, Diaz-Zagoya J: Comparative analysis of human steroid sulfatase activity in pre and postpubertal males and females. *Biochem Mol Biol Inter* 30:691–695, 1993

Cuevas-Covarrubias S, Kofman-Alfaro S, Maya G, Diaz-Zagoya J, Orozco E: X-linked ichthyosis in Mexico: high frequency of deletions in the steroid sulfatase encoding gene. *Am J Med Genet* 72:415–416, 1997

Dibbelt L, Kuss E: Human placental sterylsulfatase: interaction of the isolated enzyme with substrates, products, transition-state analogues, amino-acid modifiers and anion transport inhibitors. *Biol Chem Hoppe-Seyler* 372:173, 1991

Epstein EH, Leventhal ME: Steroid sulfatase of human leukocytes and epidermis and the diagnosis of recessive X-linked ichthyosis. *J Clin Invest* 67:1257–1262, 1981

Lench N, Stainer P, Williamson R: Simple non-invasive method to obtain DNA for gene analysis. *Lancet* 18:1356–1358, 1988

Lykkesfeldt G, Nielsen MD, Lykkesfeldt A: Placental steroid sulfatase deficiency: biochemical diagnosis and clinical review. *Obstet Gynecol* 64:49–54, 1984

Matsumoto T, Sakura N, Ueda K: Steroid sulfatase activity in nails: screening for X-linked ichthyosis. *Pediatr Dermatol* 4:266–269, 1990

Morita E, Katoh O, Shinoda S, Hiragon T, Tanaka T, Kameyoshi Y, Yamamoto S: A novel point mutation in the steroid sulfatase gene in X-linked ichthyosis. *J Invest Dermatol* 109:244–245, 1997

Muller C, Walhstrom J, Rogers H: Further evidence for assignment of steroid sulfatase X-linked ichthyosis locus to the telomere of Xp. *Hum Genet* 58:446, 1981

Nomura K, Nakano H, Umeki K, *et al*: Study of the steroid sulfatase gene in families with X-linked ichthyosis using polymerase chain reaction. *Acta Derm Venereol* 75:340–342, 1995

Okano M, Kitano Y, Yoshikawa K, Nakamura T, Matsuzawa Y, Yuasa T: X-linked ichthyosis and ichthyosis vulgaris: comparison of their clinical features based on biochemical analysis. *Br J Dermatol* 119:777–783, 1988

Schaefer L, Ferrero GB, Grillo A, *et al*: A high resolution deletion map of human chromosome Xp22. *Nature Genet* 4:272–279, 1993

Shapiro LJ, Weiss R: X-linked ichthyosis due to steroid sulphatase deficiency. *Lancet* 14:70–72, 1978

Shapiro LJ, Yen P, Pomerantz D, Martin E, Rolewic L, Mohandas T: Molecular studies of deletions at the human steroid sulfatase locus. *Proc Natl Acad Sci (USA)* 86:8477–8481, 1989

Shayder T, Ott F: All about ichthyosis. *Pediatr Clin North Am* 38:835–857, 1991

Wells RS, Kerr CB: Clinical features of autosomal dominant and sex-linked ichthyosis in an English population. *Br Med J* 1:947–950, 1966

Williams MI, Elias PM: Stratum corneum lipids in disorders of cornification. *J Clin Invest* 65:1404–1410, 1981