

## Point Mutation in the *STS* Gene in a Severely Affected Patient with X-linked Recessive Ichthyosis

Luz M. Gonzalez-Huerta<sup>1</sup>, Olga M. Messina-Baas<sup>2</sup>, Jaime Toral-Lopez<sup>1</sup>, María R. Rivera-Vega, Susana Kofman-Alfaro<sup>1</sup> and Sergio A. Cuevas-Covarrubias<sup>1\*</sup>

Servicios de <sup>1</sup>Genética and <sup>2</sup>Oftalmología, Hospital General de México, Facultad de Medicina, UNAM, México DF, México. \*E-mail: sergioa@servidor.unam.mx Accepted May 13, 2005

Sir,

X-linked recessive ichthyosis (XLRI), an inherited disorder characterized by scaly skin, is due to steroid sulfatase (STS) deficiency and occurs in 1 of 2000 or 6000 males (1). Onset is at birth or during the first months of life with the presence of dark, regular, adherent scales of skin. The *STS* gene is located on Xp22.3 (2). Most patients with XLRI harbour complete deletion of the entire *STS* locus and flanking markers. In some cases, abnormal pairing of the low copy number repeats G1.3 and CRI-S232, on either side of the *STS* gene, seems to be responsible for these interstitial deletions (3). Only a few patients have been reported with partial deletions (4–8) or point mutations in the *STS* gene (9–16). In the present study we analysed a severely affected XLRI patient with a missense mutation in exon 8 that seems to be a critical region in the presence of point mutations in XLRI.

### CASE REPORT AND MOLECULAR ANALYSIS

The patient and his mother were informed about the characteristics of the study and they agreed to participate. The protocol was evaluated and accepted by the Ethics Committee of the General Hospital of Mexico. The patient was a 12-year-old boy with generalized, thick, adherent, dark scales on the neck, trunk, abdomen and on the extensor areas of extremities but with relative sparing of flexures. Face, palms, soles and hair were not involved. The patient was born by caesarean section and family history revealed that his mother had delayed progression of parturition. Skin scaling first occurred on the limbs during the first year of life and successively spread to the rest of the affected areas. The child and the mother harboured small and filiform corneal opacities with no visual acuity impairment. No other clinical findings were found to be present.

XLRI diagnosis was confirmed through the STS assay. STS activity was determined in leukocytes using 7-<sup>3</sup>H]-dehydroepiandrosterone sulfate (16.3 Ci/mmol, NEN, Boston, MA, USA) as described elsewhere (17). DNA extraction from peripheral blood was performed by a saline method (17). Exons of the *STS* gene were analysed with a PCR amplification kit (Perkin-Elmer). The conditions and primers used to amplify exons are described elsewhere (7). DNA sequence analysis was performed in an ABI PRISM 310 genetic analyser (Perkin-Elmer) according to the supplier's recommendations. All procedures were performed three times.

### RESULTS AND DISCUSSION

The XLRI patient presented undetectable levels of STS activity (sensitive limit 0.15 pmol/mg protein/h) and his mother had STS activity compatible with carrier state (0.40 pmol/mg protein/h vs 0.99 pmol/mg protein/h of normal control). PCR analysis of the patient showed

normal amplification of the entire *STS* gene. After this, the coding region was searched for mutations using DNA sequencing analysis. Single point mutation was identified at exon 8 of the *STS* gene. This substitution resulted in a 1336G>C transversion (Fig. 1) with a predicted switch of a tryptophan to a serine at amino acid residue 372 (W372S). DNA analysis of the mother showed that she was heterozygous for this molecular defect. To discount a possible polymorphism, this missense mutation was investigated in non-affected members of the family ( $n=5$ ), 50 normal controls and 6 patients with ichthyosis vulgaris with negative results. To date, 14 point mutations have been reported, 13 in the coding region and 1 in the non-coding region (Fig. 2 and Table I). These point mutations are located at the 3' end of the *STS* gene in exons 7, 8, 9 and 10. Of these mutations, 11 correspond to missense mutations, two represent non-sense mutations while the one in the noncoding region affects a splice junction site (9–13). Two mutations in codon 372 within exon 8 of the *STS* gene have been reported in two subjects, one in nucleotide 1335 and the other one in nucleotide 1336

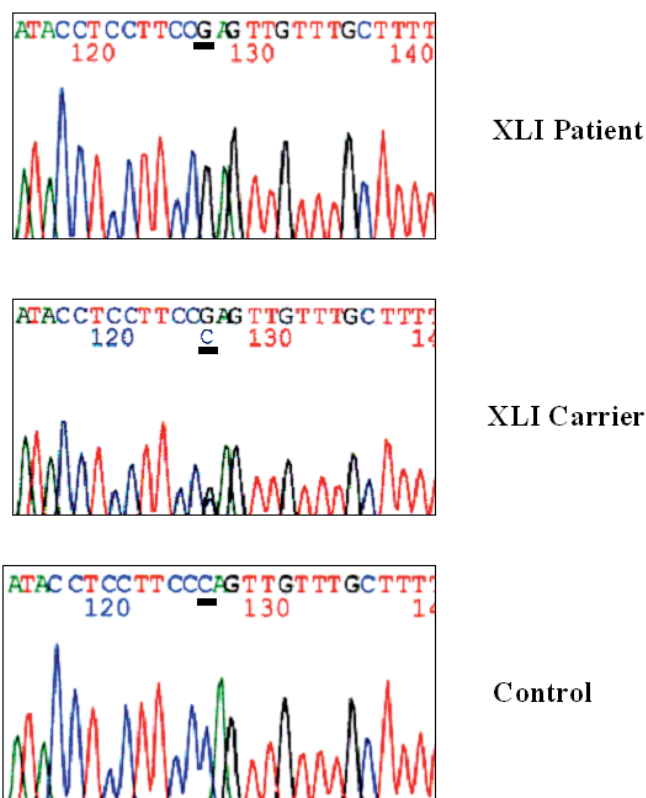


Fig. 1. DNA sequencing analysis. Identification of the point mutation within exon 8 in the steroid sulfatase gene (antisense strands). The transversion 1336G>C is underlined.

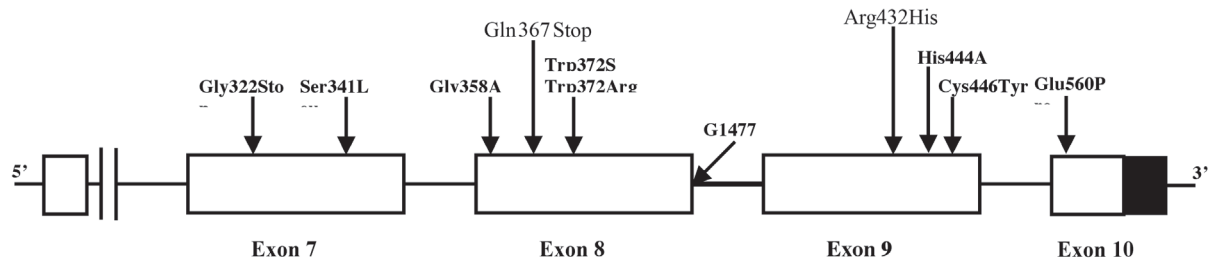


Fig. 2. Mutation of the *STS* gene. The sites for mutations are shown in the *STS* gene scheme. The arrows indicate the affected sites.

Table I. Point mutations in the *STS* gene reported in the literature.

Exon	Amino acid	Position	Substitution	Ref. no.
7	Gly	322	Stop	11
7	Ser	341	Leu	9
8	G-T transversion	splice donor site at 1477	Stop at residue 427	10
8	Gly	358	Arg	13
8	Gln	367	Stop	13
8	Trp	372	Arg	9
8	Trp	372	Ser	10, this study
9	Arg	432	His	15
9	Arg	432	Cys	16
9	His	444	Arg	10, 14
9	Cys	446	Tyr	9
10	Glu	560	Pro	12, 14

(G>C), the latter is similar to our patient. A previous study with this mutant STS polypeptide states that effects on the STS activity are probably due to a shortened half-life or a loss of substrate binding site of the STS enzyme (10).

Our patient with XLRI presented a severe skin affection that was difficult to control despite medical treatment. These data indicate the importance of environmental factors in the clinical manifestations of XLRI.

#### ACKNOWLEDGEMENT

This project was supported by Facultad de Medicina, UNAM, Hospital General de México, OD.

#### REFERENCES

- Shnyder T, Ott F. All about ichthyosis. *Pediatr Clin North Am* 1991; 38: 835–857.
- Muller CR, Wahlstrom J, Ropers HH. Further evidence for assignment of steroid sulfatase X-linked ichthyosis locus to the telomere of Xp. *Hum Genet* 1981; 58: 446.
- Yen PH, Li XM, Tsai SP, Johnson C. Frequent deletions of the human X chromosome distal short arm result from recombination between low copy repetitive elements. *Cell* 1990; 61: 603–610.
- Ballabio A, Carrozzo R, Parenti G, Gil A, Zollo M, Persico MG, et al. Molecular heterogeneity of steroid sulfatase deficiency: a multicenter study on 57 unrelated patients, at DNA and protein levels. *Genomics* 1989; 4: 36–40.
- Nomura K, Nakano H, Umeki K, Harada K, Kon A, Tamai K, et al. Study of the steroid sulfatase gene in families with X-linked ichthyosis using polymerase chain reaction. *Acta Derm Venereol* 1995; 75: 340–342.
- 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* 1989; 86: 8477–8481.
- Valdes-Flores M, Kofman-Alfaro SH, Jimenez Vaca AL, Cuevas-Covarrubias SA. A novel partial deletion of exons 2–10 of the STS gene in recessive X-linked ichthyosis. *J Invest Dermatol* 2000; 114: 591–593.
- Aviram-Goldring A, Goldman B, Netanelov-Shapira I, Chen-Shoyerman R, Zvulunov A, Tal O, et al. Deletion patterns of the STS gene and flanking sequences in Israeli X-linked ichthyosis patients and carriers: analysis by polymerase chain reaction and fluorescence in situ hybridization techniques. *Int J Dermatol* 2000; 39: 182–187.
- 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* 1992; 50: 483–491.
- 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* 1997; 272: 20756–20763.
- Morita E, Katoh O, Shinoda S, Hiragun T, Tanaka T, Kameyoshi Y, et al. A novel point mutation in the steroid sulfatase gene in X-linked ichthyosis. *J Invest Dermatol* 1997; 109: 244–245.
- Sugawara T, Shimizu H, Hoshi N, Fujimoto Y, Nakajima A, Fujimoto S. PCR diagnosis of X-linked ichthyosis: identification of a novel mutation (E560P) of the steroid sulfatase gene. *Hum Mutat* 2000; 15: 296.
- Oyama N, Satoh M, Iwatsuki K, Kaneko F. Novel point mutations in the steroid sulfatase gene in patients with X-linked ichthyosis: transfection analysis using the mutated genes. *J Invest Dermatol* 2000; 114: 1195–1199.
- Sugawara T, Fujimoto Y, Fujimoto S. Molecular analysis of X-linked ichthyosis in Japan. *Horm Res* 2001; 56: 182–187.
- Valdes-Flores M, Jimenez Vaca AL, Kofman-Alfaro SH, Cuevas-Covarrubias SA. Characterization of a novel point mutation (Arg432His) in X-linked ichthyosis. *Acta Derm Venereol* 2001; 81: 54–55.
- Gonzalez-Huerta LM, Riviera Vega MR, Kofman-Alfaro S, Cuevas-Covarrubias SA. Novel missense mutation (Arg432Cys) in a patient with steroid sulphatase deficiency. *Clin Endocrinol* 2003; 59: 263–264.
- Cuevas-Covarrubias SA, Jimenez-Vaca AL, Gonzalez-Huerta LM, Valdes-Flores M, Rivera-Vega MR, Maya-Nunez G, et al. Somatic and germinal mosaicism for the steroid sulfatase gene deletion in a steroid sulfatase deficiency carrier. *J Invest Dermatol* 2002; 119: 972–975.