

LETTERS TO THE EDITOR

Characterization of a Novel Point Mutation (Arg432His) in X-Linked Ichthyosis

Sir,

Steroid sulfatase (STS) deficiency results in X-linked ichthyosis (XLI), an inherited disorder characterized by dark, regular and adherent scales of skin (1). STS is an ubiquitous enzyme that desulfates 3- β -hydroxysteroid sulfates (2). STS activity can be determined in several cell lines to establish XLI diagnosis (3–5). The STS gene is located on Xp22.3 (6). Most XLI patients show deletions of the entire STS locus and flanking sequences. Six patients have been identified with partial deletions of the STS gene. These deletions include: (a) a deletion within intron 7 extending over exons 8–10; (b) a partial deletion that includes exon 10; (c) an intragenic deletion spanning exons 2–5; (d) a partial deletion spanning exons 2–10 and flanking sequences DXS1131 and DXS1133; and (e) two partial deletions at the 5' end of the STS gene (7–11). A minority of XLI patients with STS-gene-encoded sequences have been identified. In these subjects, 10 point mutations in the coding and non-coding regions have also been reported (12–16). In the present study, we analyzed one XLI patient with undetectable levels of STS activity and normal amplification of the STS gene. We found in this subject a novel point mutation causing XLI.

MATERIAL AND METHODS

The XLI patient was 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. Protocol was evaluated and accepted by the ethics committee of the General Hospital of Mexico. XLI diagnosis was confirmed through STS assay. STS activity was determined in leukocytes using 7- 3 H]-dehydroepiandrosterone sulfate (16.3 Ci/mmol, NEN, Boston, Mass.) as described elsewhere (17). DNA extraction was performed with a conventional method (18). Conditions and primers to amplify exons 1–10 of the STS gene are described elsewhere (10, 15). DNA sequence analysis was performed in a ABI PRISM 310 genetic analyzer (Perkin-Elmer). All procedures were performed 3 times.

RESULTS AND DISCUSSION

STS-deficiency-patients present an unusual pattern of deletions of the entire STS gene and flanking sequences. Nevertheless,

some partial deletions and point mutations have also been identified. At the moment, 10 different point mutations have been reported, 9 in the coding region and one in the non-coding region (Table I). These point mutations are principally located at the 3' end of the STS gene. Seven mutations are missense mutations (12, 13, 15, 16). Two point mutations produce stop codons and premature termination of the STS polypeptide (14, 16). The mutation in the non-coding region affects a splice junction site between exon 8/intron 8 causing an addition of 19 bp into the STS mRNA and premature termination at 427 amino acid residue of the STS enzyme (13).

In this study, we analyzed an XLI patient with undetectable levels of STS activity (0.00 pmol/mg protein/h) and normal amplification of exons 1–10 of the STS gene. No other members of the family were affected. We found a point mutation in exon 9 causing the transition 1567G→A. This change of bases resulted in the substitution of an arginine by a histidine at amino acid residue 432 in the STS polypeptide (Fig. 1). Although both amino acids corresponded to basic hydrophilic amino acids, the R-group of histidine has a ring structure which produces a different spatial conformation.

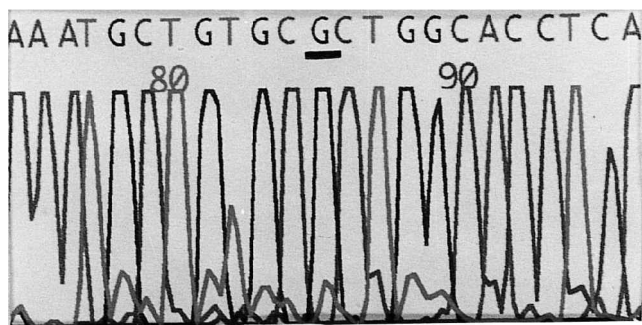
To discount a possible polymorphism, the transition 1567G→A was investigated in non-affected members of the family (n=7), in other pathologies such as Turner syndrome (n=4) and ichthyosis vulgaris (n=3), in normal males (n=15) and in normal females (n=15). We did not identify any change in the DNA sequence analysis of these subjects. So, we discounted a polymorphism. STS activity and DNA sequence analysis of the patient's mother were also analyzed and she presented a normal pattern. We concluded that the XLI patient was a *de novo* mutation. This novel mutation did not create nor abolish a site for a restriction enzyme.

This novel point mutation emphasizes the importance of the carboxyl region of the STS enzyme, as all point mutations previously reported in XLI are located in this region. Probably, the catalytic site is conformed into this region; more refined studies may confirm this hypothesis. In conclusion, we reported a novel point mutation (1567G→A) in exon 9 of the STS gene causing the substitution of arginine by histidine (Arg432His) in XLI.

Table I. Point mutations in the steroid sulfatase gene reported in the literature and the one described in the text

Exon	Amino acid	Position	Substitution	Reference
7	Gly	322	Stop	14
7	Ser	341	Leu	12
8	Gly	358	Arg	16
8	Gln	367	Stop	16
8	Trp	372	Arg	12
8	Trp	372	Pro	13
8	G-T transversion	exon8/intron8 splice donor site at nucleotide 1477	Premature termination at amino acid residue 427	13
9	His	444	Arg	13
9	Cys	446	Tyr	12
9	Arg	432	His	This paper
10	Glu	560	Pro	15

Arg
CGC



His
CAC

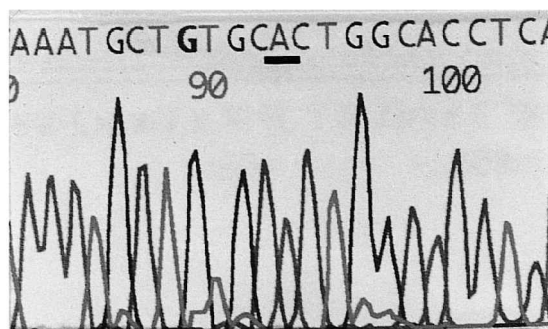


Fig. 1. Partial electropherogram showing the region of exon 9 of the steroid sulfatase gene with the point mutation. The transition 1567G→A that results in the substitution of an arginine by an histidine (Arg432His) can be seen. Normal control (top), XLI patient (bottom).

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