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A Study of the Steroid Sulfatase Gene in Families with X-linked Ichthyosis Using Polymerase Chain Reaction

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We have studied the steroid sulfatase (STS) gene in three Japanese families with X-linked ichthyosis (XLI), using polymerase chain reaction (PCR). PCR was performed using three sets of intraexonic primers covering exons 1, 5 and 10. In affected individuals from two of the families, DNA was not amplified in any of the three exons, suggesting that XLI in these families was due to the complete deletion of the STS gene. In affected individuals in the remaining family, DNA was amplified in predicted sizes in exons 1 and 5, but not in exon 10, suggesting that XLI in this family was due to partial deletion of the STS gene including exon 10. These results suggested that STS gene deficiency is heterogeneous in Japanese families with XLI. PCR is useful for the rapid diagnosis of XLI, the differentiation of XLI from ichthyosis vulgaris, and genetic counseling of XLI families. The PCR method was not applicable for carrier detection. *Key word: gene deletion.*

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X-linked ichthyosis (XLI) occurs in approximately 1 out of 5,000–6,000 males (1). In 1978, Shapiro et al. described steroid sulfatase (STS) deficiency as the biochemical basis of XLI and demonstrated markedly reduced STS activity in fibroblasts from XLI patients (1, 2). Subsequently, measurement of STS activity in lymphocytes (3), fibroblasts (2) and plantar callus (4) has been used to distinguish XLI from ichthyosis vulgaris, and to detect carriers. In 1987, STS cDNA was isolated simultaneously by two groups and mapped to chromosome Xp 22.3 (5, 6). Subsequent Southern hybridization studies revealed that more than 90% of XLI patients had complete deletion of the STS gene from the X chromosome (5, 6). Only a very few patients showed a partial deletion or point mutation of the STS gene (7–9). A few studies then demonstrated the application of PCR amplification of the STS gene for the search for the molecular defect in XLI (10, 11).

The purpose of this study was to examine the STS gene in three Japanese families with XLI using the polymerase chain reaction (PCR). We found that XLI in two of the three families was associated with complete deletion of the STS gene, suggesting that STS gene deficiency is heterogeneous in Japanese families with XLI. The reliability of the method in diagnosis, genetic counseling and carrier detection is discussed.

PATIENTS AND METHODS

Families

Family A (Fig. 1a). The proband was an 18-year-old boy. His 76-year-old maternal grandfather had the same skin condition. The diagnosis in this pedigree had been confirmed by increased electromobility rate of β-lipoprotein (β-LP) in affected compared with unaffected individuals (12). His mother was judged as a carrier. His sister, however, could not be confirmed as a carrier using the β-LP mobility technique.

Family B (Fig. 1b). The proband was a 15-year-old boy. His STS activity, measured using dehydroepiandsteronesulphate (DHEA) as substrate (assayed at Shionogi laboratories, Japan) in peripheral blood lymphocytes was 25 pmol DHEA/mg/h, approximately 20% of the normal male value. The STS activity of his sister and mother was 205 and 97, respectively, and on the basis of this test, his mother was judged to be a carrier (3).

Family C (Fig. 1c). The proband was a 10-year-old boy. His maternal grandmother's brothers and nephew had ichthyosis. His STS activity was below the limit of detection of the assay. All affected individuals in the three families had typical XLI without other phenotypic abnormalities, for example corneal opacities, undescended testis, and anosmia.

DNA isolation

Genomic DNA was prepared from affected and unaffected individuals in the pedigrees. Briefly, high molecular weight DNA was isolated from peripheral leucocytes with proteinase K digestion following phenol/chloroform extraction and ethanol precipitation (13).

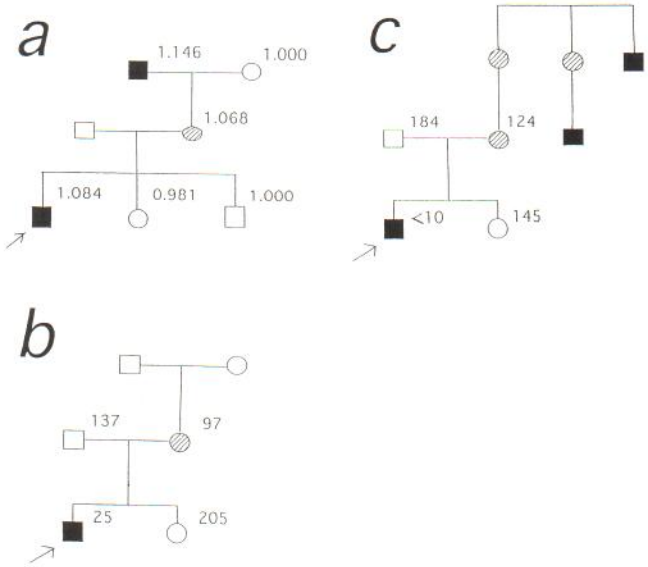

Fig. 1. Pedigrees of the three families analyzed in this study, with values of β-lipoprotein mobility rate (family A) or steroid sulfatase activity (families B and C) (DHEA/mg/h). The values are shown above each individual. *a*; family A, *b*; family B and *c*; family C. *Filled symbols* represent affected, *open symbols* unaffected individuals. *Hatched symbols* are probable carriers. The *arrow* indicates the proband.

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Table I. *PCR oligonucleotide primers*

Primer set	Sequence	Amplified
exon 1	F-5' GGCCTAGAAGAAGGTTGAAGGTCCC 3'	
	R-5' AAGAGGTTGGATGAGATGGGCATAC 3'	292 bp
econ 5	F-5' ACCACCCTTTACATCACGGC 3'	
	R-5' CGCCTCCACCGTTAGCCTCT 3'	359 bp
exon 10	F-5' GAAATCCTCAAAGTCATGCAGGAAG 3'	
	R-5' CCTCCAGTTGAGTAGCTGTTGAGCT 3'	363 bp

Polymerase chain reaction (PCR)

Three sets of primers were designed in order to cover exons 1, 5 and 10 in the STS gene. The method of Ballabio et al. (10) was followed in preparing the oligonucleotide primer sequences of exons 1 and 10. Oligonucleotide primers in exon 5 were prepared according to the published STS sequence (14). Particular care was taken to ensure that oligonucleotide pairs would not amplify the highly homologous STS pseudogene on the Y chromosome (14). The nucleotide sequences of the primers and the size of the amplified regions are listed in Table I. PCR was performed in a 100 µl reaction volume by adding 100 pmol of both primers, 500 ng template DNA and 2.5 U Taq polymerase (Takara, Japan). The PCR conditions were 25 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min. After completion of PCR, 5 µl of the product was electrophoresed on 1% agarose gel containing ethidium bromide and photographed.

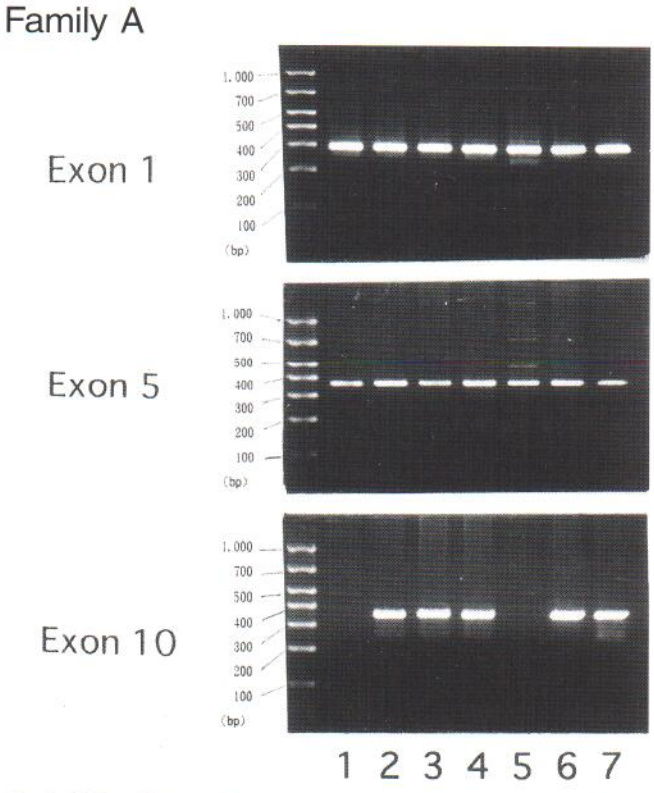

Fig. 2. PCR of family A. *1*; grandfather, *2*; grandmother, *3*; father, *4*; mother, *5*; proband, *6*; sister, *7*; brother. In exons 1 and 5, DNA was amplified in predicted sizes (292 bp and 359 bp, respectively) in all members. In exon 10, however, DNA was not amplified in the affected individuals (grandfather and proband).

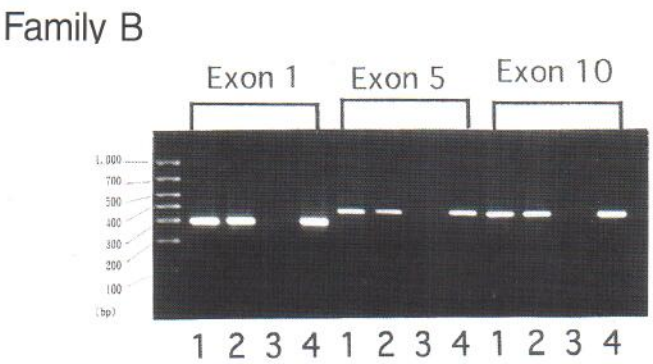

Fig. 3. PCR of family B. *1*; father, *2*; mother, *3*; proband, *4*; sister. DNA was amplified in predicted sizes (292 bp, 359 bp and 363 bp, respectively) in the father, mother and sister. In the proband, DNA was not amplified in exons 1, 5 or 10.

RESULTS

In family A, DNA was amplified in exons 1 and 5 at the predicted sizes in all individuals (Fig. 2). In exon 10, however, the DNA was not amplified in the proband and grandfather, both of whom were affected. The mother, who was considered to be a carrier, showed a normal amplifying pattern in the three exons. In families B and C (Figs 3, 4), DNA was not amplified in any of the three exons in each proband. The other members, including the mother, showed a normal amplifying pattern with predicted sizes. The integrity of DNA of probands in families B and C was confirmed by normal PCR amplification with keratin K14-specific primers (data not shown), indicating that the lack amplification was due to a lack of the STS gene in the template and not to degraded DNA. The PCR was repeated three times in each family and identical results were obtained on each occasion.

DISCUSSION

The isolation of STS cDNA enabled us to search for a molecular defect of the STS gene in patients with XLI. Southern hybridization studies using STS cDNA revealed that 14 out of 15 (5) and 8 out of 10 (15) patients with XLI had gross deletion of the STS gene from the X chromosome. Furthermore, Ballabio et al. studied 38–57 European XLI patients at STS DNA and protein levels and found that 84% of patients had complete deletion of

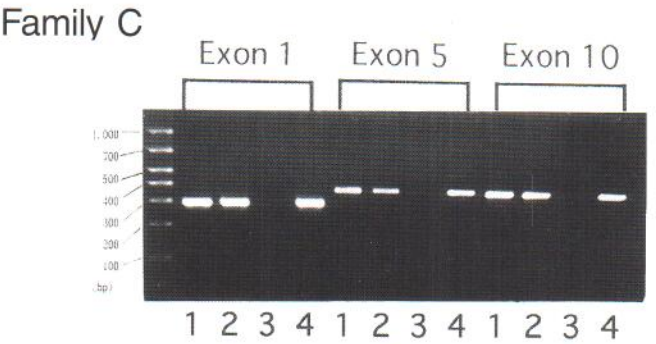

Fig. 4. PCR of family C. *1*; father, *2*; mother, *3*; proband, *4*; sister. DNA was not amplified in exons 1, 5 and 10 in the proband.

the STS gene (8,10). Southern hybridization using STS cDNA and anonymous flanking DNA markers closely linked to the STS gene also revealed complete deletion of the STS gene in two XLI families (16). Ballabio et al. first analyzed the STS gene using PCR and obtained results consistent with their previous Southern hybridization study (10). Sugawara et al. (11) found 6 Japanese XLI patients to have complete deletion of the STS gene.

In this study, we examined the STS gene in three Japanese families with XLI. The PCR results suggested that the STS gene in two families was completely deleted, while one family had a partial deletion including exon 10. Only two XLI patients with partial deletion of the STS gene have been reported so far. One showed deletion of exons 2–5 (7) and the other showed deletion at the 3' end of the gene (8). Although further examination will be required to demonstrate a break point in the STS gene in our case, it will be of interest to learn whether mutations without gross deletions are more common in Japan. Three XLI patients with point mutations in the STS gene have been reported by Basler et al. (9). It would be of interest to investigate whether disease severity correlates with the type of mutation in XLI patients with STS gene point mutations.

A further question was whether the PCR method used here is applicable for carrier detection, since the identification of carriers is important for the genetic counseling of XLI families. In all families, each mother of the proband was judged as a carrier from the inheritance pattern, STS activity or both. The sisters of families A and C could not be confirmed as a carrier using STS activity or LDL mobility. Their PCR amplification pattern was similar to that of normal individuals, because the STS gene was amplified in the one normal allele by PCR in the carrier DNA templates. Therefore, PCR amplification of the STS gene is not applicable for carrier detection. Bonifas & Epstein described an STS gene dosage study using Southern hybridization for carrier detection (17), and this method seems to be the most reliable method for carrier detection at present.

Finally, our results suggest that STS gene deficiency is heterogeneous in Japanese families with XLI. The PCR method is useful for the rapid diagnosis of XLI, the differentiation of XLI from ichthyosis vulgaris, and genetic counseling of XLI families.

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