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"CARACTERIZACION DE MUTACIONES INTRAGENICAS DEL GEN STS EN PACIENTES CON ICTIOSIS LIGADA AL X (ILX)"

TESIS

QUE PARA OBTENER EL GRADO DE

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PRESENTA:

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Contenido

RESUMEN

La ictiosis ligada al X (ILX) es un error innato del metabolismo que presenta una frecuencia de 1 en 2000-6000 recién nacidos varones y se caracteriza por la presencia de escamas obscuras, adherentes y generalizadas. La ILX se debe a la deficiencia de una enzima conocida como sulfatasa de esteroides (SE), cuyo locus se ubica en Xp22.3. Aproximadamente, 85-95% de los pacientes con ILX presentan deleciones génicas totales incluyendo regiones adyacentes, algunos pocos han presentado mutaciones puntuales o deleciones parciales. El propósito del presente estudio fue analizar el gen STS en 85 pacientes mexicanos con ILX (actividad de la SE de 0.00 pmoles/mg/proteína/hora). La mayoría de los pacientes (n = 81) presentaron deleción génica total. El resto de los pacientes (n = 4) mostraron mutaciones diferentes. En el primer caso, la deleción comprometía los exones 2-10 y se extendía sobre el extremo 3'a las secuencias DXS1131 Y DXS1133. El segundo paciente, mostró amplificación normal de los exones 6-10 así como de las secuencias DXS1130, DXS1139, Y DXS996 (adyacentes al extremo 5'). En el tercer sujeto la amplificación de los exones 1-10 fue normal y el análisis automatizado de la secuencia mostró una mutación puntual en el exon 9 (1567G/A). Esta mutación resultó en la sustitución de arginina por histidina en el residuo de aminoácido 432 en el polipéptido de la STS. El cuarto paciente mostró una deleción de 3 pb (AAG) en las posiciones 1252-1254 en el exon 7 del gen STS. Estos resultados indican que en la ILX pueden encontrarse diversos defectos moleculares.

ABSTRACT

X-linked ichthyosis (XLI) is an inherited inborn error of metabolism with a frequency of 1 in 2000-6000 males and is characterized by dark, regular and adherent scales of skin. XLI is due to steroid sulfatase (STS) deficiency. The STS locus is located on Xp22.3. Approximately, 85%-90% of XLI patients have large deletions of the STS gene and flanking sequences, only a few point mutations and partial deletions have been reported. The aim of the present study was to analyze the STS gene in 85 Mexican patients with XLI. These patients had no STS activity (0.00 pmol per mg protein per h). The majority of patients (n = 81) had large deletions of the STS gene and flanking sequences. The rest of patients (n = 4) showed different mutations. In the first case, the deletion involved exons 2-10 and extended over 3'flanking sequences DXS1131 and DXS1133. The second patient, showed a normal amplification of exons 6-10 and contiguous 5'flanking sequences DXS1130, DXS1139 and DXS996 of the STS gene. In the third subject the amplification of exons 1-10 was normal and the sequence analysis showed a point mutation in exon 9 (1567G/A). This mutation resulted in the substitution of arginine by histidine at amino acid residue 432 in the STS polypeptide. The four patient showed the 3bp deletion (AAG) at positions 1252-1254 within exon 7 of the STS gene. We found in this study four novel mutations indicating that all molecular defects of the STS gene are occurring in XLI.

ANTECEDENTES

Ictiosis Recesiva Ligada X

La ictiosis recesiva ligada al cromosoma X (ILX) es una genodermatosis genéticamente bien definida, se ha observado en diferentes grupos étnicos y afecta a 1 de cada 2,000-6,000 recién nacidos vivos del sexo masculino. El término ictiosis proviene de la raíz griega "ichthys" que significa pez y ha sido utilizado por más de 2000 años. La primera descripción de ictiosis que aparece en la literatura médica se encuentra en la obra "On cutaneuos diseases"escrita por Robert William en 1908. Por otra parte, existen datos que muestran que los hindúes y chinos se referían a la enfermedad como una afección en la que la piel tiene aspecto de escamas de pescado o de piel de serpiente. Alibert consideraba que vivir en áreas cercanas a corrientes de agua, mar o el ingerir pescado en estado de putrefacción originaban la enfermedad. En 1884 Fox informó el caso del "niño cocodrilo" atribuyendo su causa al ataque de un cocodrilo a la madre durante la gestación (1-6).

La ILX inicia generalmente durante los primeros meses de vida y los datos clínicos más importantes son la descamación anormal de la piel manifestada por la presencia de escamas oscuras, regulares, adherentes y de distribución generalizada. Predominan en cara anterior de abdomen, cuello y zonas de flexión, respetando generalmente palmas de las manos y plantas de los pies. Esta descamación anormal se debe a la retención del estrato córneo de la piel, debida a la deficiencia de una enzima conocida como sulfatasa de esteroides (SE) (1, 3, 7, 8).

Algunos pacientes con esta genodermatosis han presentado opacidades corneales, criptorquidia, hernia inguinal, estenosis congénita de píloro y displasia de cadera. Se ha

detectado la presencia de cáncer testicular en algunos pacientes con ILX. Las portadoras de esta enfermedad presentan algunas veces opacidades corneales, sangrado transvaginal y retardo en la labor de parto, esto último puede condicionar la presencia de hipoxia neonatal en los productos. Aproximadamente, 5% de los pacientes con ILX presentan fenotipos más complejos, es decir, además de ictiosis pueden cursar con talla baja, síndrome de Kallman, condrodisplasia punctata o retraso mental, esto se debe a la pérdida adicional de genes adyacentes al gen relacionado con la ILX (9-15).

El diagnóstico diferencial de la ILX se realiza con la ictiosis vulgar (IV), entidad autosómica dominante que muestra una frecuencia aproximada de 1: 250 recién nacidos vivos. Se ha señalado que en la IV las escamas tienden a ser más claras, muestran una distribución más regular y generalmente respetan zonas de flexión. Como datos asociados se han observado una menor gravedad del cuadro clínico, antecedentes familiares de atopia e hiperlinearidad palmo-plantar (16-19).

Gran parte de la literatura internacional indica que la mayor parte de los casos de ILX corresponden a mutaciones nuevas. Sin embargo, en nuestra población el comportamiento es diferente, ya que estudios bioquímicos y moleculares recientes en pacientes mexicanos y sus madres muestran que la mayoría de los casos de ILX de presentación aparentemente esporádica (por análisis familiar) corresponden realmente a defectos heredados. En estos casos, las madres de los pacientes muestran actividad de la enzima SE compatible con estado de portadora (menos de 25 pmoles/mg proteína/hora) y solamente una copia del gen de esta sulfatasa (gen STS) en su genoma (identificada mediante hibridación *in situ*) (8, 20, 21).

La ILX y la Sulfatasa de Esteroides

La deficiencia de SE en la ILX condiciona la acumulación de sulfato de colesterol (SC) en suero, membrana de los eritrocitos y estratos córneo y granuloso de la piel. Normalmente, en el estrato córneo la relación colesterol:sulfato de colesterol es de 10:1; sin embargo, esta proporción se pierde en los pacientes con ILX, llegando a ser de 1:1. En los estratos córneo y granuloso la actividad de la SE es alta, en contraste, los estratos dérmicos inferiores muestran una actividad enzimática muy baja, esto explicaría la acumulación del SC en las capas superficiales de la piel de pacientes con ILX. Por otra parte, se ha sugerido que la regulación de la relación colesterol:sulfato de colesterol en el complejo estrato córneo-membrana puede ser crítico en el proceso de descamación normal de la piel (22-27).

Enzima Sulfatasa de Esteroides

La SE también conocida como arilsulfatasa C (ASC) o esteril-sulfato sulfohidrolasa (EC 3.1.6.2) es una de las 6 arilsulfatasas identificadas en los tejidos humanos (A, B, C, D, E y F). Se localiza en el retículo endoplásmico rugoso, unida a la membrana microsomal y en las cisternas de Golgi. Estudios inmunológicos muestran presencia de la enzima en la membrana plasmática, especialmente en proyecciones celulares y microvellosidades. La SE tiene un peso molecular de 65492 Da, muestra una distribución ubicua y se ha detectado en próstata con hipertrofia benigna y células cancerosas de mama. Su pH óptimo oscila entre 6.5-7.5, su Km es de 0.8μM para el sulfato de estrona, de 1.7μM para el sulfato de dehidroepiandrosteona y de 0.6μM para el sulfato de testosterona. Esta arilsulfatasa cataliza la hidrólisis del radical sulfato de los 3-beta-hidroxiesteroides

sulfatados (i.e. sulfatos de colesterol, estrona, testosterona, pregnenolona y dehidroepiandrosterona). La enzima es estable a las alteraciones de pH, calor y exposición a urea (28-30).

La SE comprende dos isoformas que están codificadas por genes diferentes, localizados en los brazos cortos del cromosoma X. Tomando en cuenta su movilidad electroforética estas isoformas se clasifican en "s" o lenta (slow-migrating) y "f " o rápida (fast-migrating). La expresión de estas variantes electroforéticas es tejido específica. La forma "s" es abundante en placenta, corazón, músculo esquelético, tiroides y glándula suprarrenal y la forma "f " se encuentra presente en hígado, páncreas y riñón. Es importante señalar que ambas isoformas muestran diferente especificidad a substrato, labilidad al calor, estructura proteica, pH óptimo y propiedades antigénicas (31, 32)

Recientemente se ha sugerido que la SE juega un papel importante en la embriogénesis del ratón, sobre todo en procesos relacionados con el desarrollo de SNC. En los últimos años la actividad de la SE se ha relacionado con los procesos de formación y remodelación ósea. Por otra parte, estudios de inmunohistoquímica han identificado la presencia de SE en el citoplasma de las células epiteliales de trompas de falopio en diferentes fases del ciclo menstrual, predominando durante la fase lútea. Se ha sugerido que la SE participa en la regulación local de síntesis de esteroides y posiblemente en la función reproductiva de las trompas de falopio. Por otra parte, estudios en ratas demuestran que el uso de inhibidores de la SE (sulfamatos) modifica el perfil de presión sanguínea en estos animales de laboratorio (33-37).

La SE está compuesta por 583 aminoácidos, los primeros 22 corresponden al péptido líder el cual es escindido postranscripcionalmente para dar origen a la proteína madura. El primer aminoácido (aa) de esta proteína corresponde a una metionina y los residuos serina [21], histidina [22] o bien alanina [23] y alanina [24] representan las señales de reconocimiento de la peptidasa encargada de escindir la señal líder. Este proceso dura aproximadamente dos días y la vida media de esta enzima se ha estimado en 4 días (38).

La SE presenta las características de una glicoproteína integrada a membrana, presenta cuatro sitios potenciales de N-glicosilación (residuos de aspargina "N" en las posiciones 47, 259, 333 y 459), sin embargo, sólo dos son utilizados (N^{47} y N^{259}). Esta enzima tiene además las características de una proteína integrada a membrana y el modelo propuesto para su topología muestra la presencia de dos dominios hidrofóbicos de 53 y 30 residuos cada uno (aminoácidos 185-211 y 213-237). Se ha sugerido que el sitio activo de la enzima corresponde a un residuo de histidina ubicado en la posición 136 (H^{136}), dicho aminoácido es uno de los que muestran gran conservación evolutiva, esto con respecto a la familia génica de las arilsulfatasas. Por otra parte, se ha considerado que el sitio de unión a los diferentes ligandos se ubica en el extremo COOH de la proteína. La SE activa está formada por múltiples subunidades idénticas, cada una con un peso molecular de 63 kDa, siendo el agregado activo más pequeño un dímero de 126 kDa que incluye dos de los sitios potenciales de N-glicosilación (N^{47}, N^{259}). Aparentemente, durante el ciclo de vida de la enzima el tamaño del dímero se reduce a 61 kDa. Estudios computarizados sugieren que el primer dominio muestra una estructura secundaria de α-hélice, la cual se encuentra interrumpida por un residuo de prolina en la posición 212, generando así dos segmentos de 27 y 25 aa cada uno. El segundo dominio corresponde al de una hoja β-plegada. Los extremos amino y

carboxiló terminal se ubican en la parte luminal de la membrana, ambos contienen una cadena de oligosacáridos y se comunican por medio de un dominio hidrofóbico que comprende dos secuencias que se extienden en direcciones opuestas de la mebrana. La estabilidad de la SE en los microsomas se ha relacionado con la gran homología que sus dos dominios transmembranales guardan con otras sulfatasas lisosomales (arilsulfatasas A y B) (26, 29, 38).

Secuencia de aminoácidos de la enzima Sulfatasa de Esteroides

MPLRKMKIPF[10]	LLLFFLWEAE[20]	SHAASRPNII[30]
LVMADDLGIG[40]	DPGCYGNKTI[50]	RTPNIDRLAS[60]
GGVKLTQHLA[70]	ASPLCTPSRA[80]	AFMTGRYPVR[90]
SGMASWSRTG[100]	VFLFTASSGG[110]	LPTDEITFAK[120]
LLKDQGYSTA[130]	LIGKW[H]LGMS[140]	CHSKTDFCHH[150]
PLHHGFNYFY[160]	GISLTNLRDC[170]	KPGEGSVFTT[180]
GFKRLVFLPL[190]	QIVGVTLLTL[200]	AALNCLGLLH[210]
VPLGVFFSLL[220]	FLAALILTLF[230]	LGFLHYFRPL[240]
NCFMMRNYEI[250]	IQQPMSYDNL[260]	TQRLTVEAAQ[270]
FIQRNTETPF[280]	LLVLSYLHVH[290]	TALFSSKDFA[300]
GKSQHGVYGD[310]	AVEEMDWSVG[320]	QILNLLDELR[330]
LANDTLIYFT[340]	SDQGAHVEEV[350]	SSKGEIHGGS[360]
NGIYKGGKAN[370]	NWEGGIRVPG[380]	ILRWPRVIQA[390]
GUKIDEPTSN[400]	MDIFPTVAKL[410]	AGAPLPEDRI[420]
IDGRDLMPLL[430]	EGKSQRSDHE[440]	FLFHYCNAYL[450]
NAVRWHPQNS[460]	TSIWKAFFFT[470]	PNFNPVGSNG[480]
CFATHVCFCF[490]	GSYVTHHDPP[500]	LLFDISKDPR[510]
ERNPLPASE[520]	PRFYEILKVM[530]	QEAADRHTQT[540]
LPEVPDQFSW[550]	NNFLWKPWLQ[560]	LCCPSTGLSC[570]
QCDREKQDKR[580]	LSR[583]	

▬ = Péptido guía N= Sitios potenciales de N-glicosilación

[H] = Sitio activo de la enzima LVF.....= Región de dominios transmembranales

Los residuos en negritas corresponden a los considerados con gran conservación evolutiva

Gen STS

La actividad de la enzima SE está asociada a un gen único en humanos (gen STS). Este gen se extiende sobre una región de 146 kb en el cromosoma X (Xp22.3), muy cerca de la región pseudoautosómica. Contiene 10 exones de tamaño variable y dos regiones, una de por lo menos 206 pares de bases en el extremo 5' y otra en el extremo 3' de 668 pares de bases. Tiene además, una señal de poliadenilación (AATAAA) 13 pares de bases antes del comienzo de la cola de poli–A. Aparentemente, la región promotora del gen STS es pobre en secuencias GC y parece ser que carece de sitios de unión a factores de transcripción conocidos. Por otra parte, se ha identificado una región de aproximadamente 1.3 kb en el extremo 5' que contiene varios sitios de unión potencial para factores de transcripción, entre éstos se encuentran 3 elementos potenciadores (URE1-3) y un elemento represor (URE4). Se han identificado además tres transcritos primarios en distintas líneas celulares, cada uno con pesos moleculares diferentes (2.7, 5.2 y 7.2 Kb). Hasta este momento, no se ha definido aún la función de cada uno de estos transcritos (39-41).

El gen STS tiene su homólogo no funcional (pseudogén) en los brazos largos del cromosoma Y (Yp11.2). La homología entre el gen funcional y el pseudogén es de 90% aproximadamente y las diferencias entre ambos están dadas por sustituciones de algunos pares de bases, pequeñas adiciones y deleciones que condicionan la incapacidad del pseudogén para originar algún producto proteico funcional (42).

Recientemente, se localizaron en la región Xp22.3 los genes que codifican para las arilsulfatasas D, E y F, todos ellos con una organización genómica muy parecida, incluyendo al gen de la arilsulfatasa C. Por otra parte, trabajos recientes muestran que la

localización de estos genes es idéntica en el cromosoma X de diferentes especies de primates, mientras que la copia homóloga, presente en el cromosoma Y, es distinta en cada una de estas especies. Es posible que este agrupamiento génico de arilsulfatasas en Xp22.3 tenga su origen a partir de eventos de duplicación génica, ocurridos ancestralmente en la región pseudoautosómica. Este parecería ser un cambio reciente en el genoma de mamíferos (43-45).

El gen STS escapa al proceso de inactivación del cromosoma X, expresándose así en ambos cromosomas X en las mujeres normales. Sin embargo, la expresión de este gen es siempre menor en el cromosoma X inactivo, debido posiblemente a la presencia de genes contiguos inactivos. De esta manera, la actividad de la enzima SE en varones y mujeres no refleja el número de copias del cromosoma X presente en ambos sexos, ya que los niveles enzimáticos no guardan la esperada relación de 2:1, siendo la proporción encontrada de 1.3 –1.7:1 dependiendo de la línea celular. Unicamente en cabello se ha reportado una relación de 1:1 y en leucocitos de prepúberes de 2:1 (46-50).

Mutaciones en el gen STS

La mayoría de los pacientes con ILX (90 %) presentan pérdida total del gen STS, incluyendo parte de las regiones adyacentes hacia ambos extremos del gen. Es posible que esto se deba a la presencia de algunas familias de secuencias repetidas en número bajo de copias (G1.3 y CRI-S232) que se encuentran intercaladas y distribuidas en la región Xp22.3 y hacia ambos lados del gen STS. Muy probablemente la presencia de estas secuencias en esta región condiciona por recombinación homóloga un

apareamiento anormal durante la meiosis, explicando así la alta frecuencia de las deleciones intersticiales en esta zona del genoma (51-55).

En muy pocos casos se han presentado deleciones parciales, las cuales abarcan los exones II-V, VII-X, X, II-X y I-V, estas dos últimas reportadas recientemente por nuestro grupo de investigación. En otro caso, se ha documentado la pérdida de 3 pares de bases en el exón 7 y en 11 pacientes se han detectado mutaciones puntuales (tabla 1) (51, 53, 56-66).

Aproximadamente, 5% de los pacientes presentan pérdida del gen STS y genes adyacentes, lo que ocasiona fenotipos más complejos que incluyen además de ILX, síndrome de Kallman, retardo mental y condrodisplasia punctata (56, 67, 68).

Tabla 2. Mutaciones puntuales del gen STS documentadas en la literatura

EXON	NUCLEOTIDO	CAMBIO DE BASE	CAMBIO DE AMINOÁCIDO	POSICION DEL AMINOÁCIDO	REFERENCIA
7	1236	G-A	Gly-Stop	322	Morita et. al. 1997
7	1226	C-T	Ser-Leu	341	Basler et. al. 1992
8	1344	G-C	Gly-Arg	358	Oyama et. al 2000
8	1371	C-T	Gln-Stop	367	Oyama et. al. 2000
8	1320	T-A	Trp-Arg	372	Basler et al. 1992
8	1320	G-C	Trp-Pro	372	Alperin et. al. 1997
8	1552	G-T	Gln-Stop	422	Alperin et. al. 1997
9	1567	G-A	Arg-His	432	Valdes et. al 2001
9	1543	A-G	His-Arg	444	Alperin et. al. 1997
9	1567	G-A	Cys-Tyr	446	Basler et. al. 1992
9	1882	A-C	Glu-Pro	560	Sugawara et. al. 1999

Diagnóstico de ILX

El diagnóstico de ILX puede realizarse mediante la determinación de la actividad de la SE en tejidos como fibroblastos, piel, cabello, uñas y leucocitos. Este ensayo bioquímico permite no solamente la identificación de pacientes sino el diagnóstico de la mayoría de las portadoras de ILX. La ausencia de actividad enzimática confirma el diagnóstico de ILX y en el caso de las portadoras los niveles de actividad se encuentran por debajo de los controles masculinos y femeninos sanos. Es importante señalar que aproximadamente 10-15% de las portadoras pueden presentar actividad enzimática normal, en estos casos la realización de estudios complementarios (i.e., hibridación *in situ*) permiten establecer el diagnóstico correcto (17, 21, 69-72).

Por otra parte, considerando que la mayoría de los pacientes con ILX presentan deleciones totales del gen STS incluyendo casi siempre regiones adyacentes, la amplificación mediante PCR de los extremos 5'y 3'del gen permiten confirmar el diagnóstico bioquímico de ILX en aproximadamente el 90% de los casos. En los pacientes con ausencia de actividad de la SE y presencia parcial del gen STS se delimita la magnitud de las deleciones. Finalmente, algunos pacientes presentan amplificación de todos los exones del gen STS, en este caso, el análisis molecular mediante secuenciación automatizada ha permitido la identificación de otro tipo de mutaciones como pueden ser pequeñas deleciones, inserciones y mutaciones puntuales principalmente (52, 57, 58-60, 66).

El estudio molecular de las posibles portadoras se realiza sobre todo mediante hibridación *in situ* fluorescente, empleando para este fin una sonda específica del cDNA del gen STS. Este procedimiento se realiza casi siempre en preparaciones cromosómicas

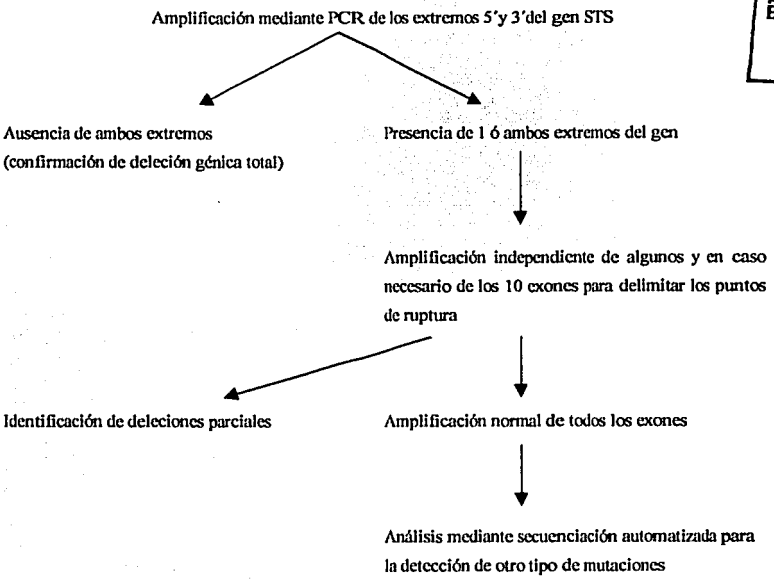
o núcleos en interfase y permite visualizar directamente el número de copias del gen STS presentes en el genoma. Las mujeres portadoras presentan solamente una copia del gen, mientras que las mujeres normales muestran dos copias. Debemos señalar que en los casos en los que la causa de la ILX no son las deleciones totales la hibridación *in situ* no es un método diagnóstico adecuado para la detección de portadoras (21).

JUSTIFICACIÓN Y OBJETIVO

Desde hace aproximadamente 7 años en el Servicio de Genética del Hospital General de México se inició el estudio de la ictiosis ligada al X desde los puntos de vista clínico, bioquímico y molecular. De entonces a la fecha, diversos trabajos han permitido identificar algunas de las características de esta genodermatósis en nuestro medio, algunas de ellas han resultado ser diferentes a las informadas en la literatura internacional. De esta manera, el objetivo del presente trabajo fue analizar el gen STS en una muestra de 85 pacientes mexicanos con diagnóstico clínico y bioquímico de ILX.

PROCEDIMIENTO

En el presente trabajo se analizaron 85 pacientes con diagnóstico clínico y bioquímico compatible con ILX. El estudio molecular se realizó de la siguiente manera.



RESULTADOS

En el presente trabajo se analizaron 85 pacientes mexicanos con diagnóstico clínico y bioquímico compatible con ILX. El análisis molecular inicial (amplificación de los extremos 5'y 3'del gen STS) mostró deleción génica total (ausencia de los exones 1-10) en 96% de los casos (n= 81), el resto (n= 4) presentaron otro tipo de mutación. De estos cuatro pacientes, uno presentó deleción de los exones 2-10 y de las regiones DXS1131 y DXS1133, las cuales se encuentran adyacentes al extremo 5' del gen. En otro de los casos, el análisis molecular reveló la pérdida de los exones 1-5 del gen. Los dos pacientes restantes mostraron amplificación normal de ambos extremos del gen así como de los 10 exones. En ambos casos, el análisis mediante secuenciación automatizada permitió identificar en uno de ellos la pérdida de 3 nucleótidos (AAG) en el exón 7 (posiciones 1252-1254) condicionando la pérdida de una glutamina en la posición 327. Esta deleción de 3 pb fue identificada también en la madre del paciente. En el otro paciente se identificó una mutación puntual en el exón 9, en el nucleótido 1567 (1567G/A). Esta transición resultó en el cambio de una arginina por una histidina en el residuo 432 de la secuencia de aminoácidos original.

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Mutation Report

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

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Abstract

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.

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

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; Shavder & Ott 1991). Several studies have estimated a frequency of 1 in 2000–6000 males (Wells &

Kerr 1966; Lykkesfeldt *et al.* 1984). XLI is due to steroid sulfatase (STS) deficiency (Shapiro & Weiss 1978); this enzyme hydrolyzes 3-beta-hydroxysteroid sulfates (Dibbelt & Kuss 1991). The STS enzyme deficiency is associated with an increase of cholesterol sulfate in the stratum corneum (Williams & 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 & 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 & 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.

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 1. 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.

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Table 1 . Primers and conditions used in the PCR analysis of the STS gene

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.

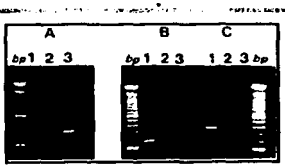

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Fig. 1. PCR analysis of the STS gene. (*a*) Exons 1 and 10 (lower and upper bands, respectively); (*b*) Exons 2, 5, and 7–9. *Line 1*, positive control; *line 2*, XLI patient with complete deletion of the STS gene; *line 3*, XLI patient described in the text with partial deletion of the STS gene; in this subject only exon 1 is observed.

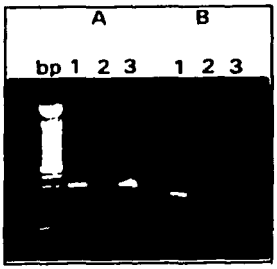

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Fig. 2 PCR analysis of the 3' flanking sequences DXS237 and DXS1133. (*a*) DXS237; (*b*) DXS1133. *Line 1*, positive control; *line 2*, XLI patient with complete deletion of the STS gene; *line 3*, XLI patient described in the text; in this subject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

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.

Acknowledgements

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Deletion of Exons 1–5 of the STS Gene Causing X-Linked Ichthyosis

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X-linked ichthyosis is an inherited disorder due to steroid sulfatase deficiency. It is clinically characterized by dark, adhesive, and regular scales of the skin. Most X-linked ichthyosis patients present large deletions of the STS gene and flanking markers; a minority show a point mutation or partial deletion of the STS gene. In this study we analyzed the STS gene in a family with simultaneous occurrence of X-linked ichthyosis and ichthyosis vulgaris. X-linked ichthyosis diagnosis was confirmed through steroid sulfatase assay in leukocytes using 7-[^{3}H]-dehydroepiandrosterone sulfate as a substrate. Exons 1, 2, 5, and 6–10, and the 5′ flanking markers DXS1130, DXS1139, and DXS996 of the STS gene were analyzed by polymerase chain reaction. X-linked ichthyosis patients of the family (n = 4 males) had undetectable levels of STS activity (0.00 pmol per mg protein per h). The DNA analysis showed that only exons 6–10 and the 5′ flanking markers of the STS gene were present. We report the first partial deletion of the STS gene spanning exons 1–5 in X-linked ichthyosis patients.

Key words: dehydroepiandrosterone sulfate/leukocytes/steroid sulfatase/STS gene/X-linked ichthyosis. J Invest Dermatol 116:456–458, 2001

Steroid sulfatase (STS, EC 3.1.6.2) is a microsomal enzyme that is capable of hydrolyzing 3-β-hydroxysteroid sulfates (Hobkirk, 1985). It shows an ubiquitous distribution in human tissues (Dibbelt and Kuss, 1991). STS deficiency results in X-linked ichthyosis (XLI), an inherited disorder characterized by dark, adhesive, and regular scales of the skin (Shapiro and Weiss, 1978). XLI patients have increased levels of cholesterol sulfate in the stratum corneum, which appears to be responsible for the scaly skin (Williams and Elias, 1981). Cholesterol sulfate produces a barrier abnormality in intact skin and extracellular anomalies in isolated stratum corneum (Zettersten *et al*, 1998), and inhibits formation of detergent-insoluble membrane domains (Xu and London, 2000). Cholesterol sulfate also acts as a transcriptional factor of the transglutaminase 1 gene in human keratinocytes (Kawabe *et al*, 1998). It has been proposed that epidermal changes in XLI could be a consequence of transglutaminase 1 dysfunction due to the accumulation of cholesterol sulfate (Nemes *et al*, 2000).

The STS gene is located on the short arm of the X-chromosome (Muller *et al*, 1981). Most XLI patients present large deletions of the entire STS gene and flanking sequences (Bonifas *et al*, 1987; Shapiro *et al*, 1989; Cuevas *et al*, 1997; Aviram *et al*, 2000), but a few point mutations and six partial deletions have also 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; Aviram *et al*, 2000; Oyama *et al*, 2000; Sugawara *et al*, 2000; Valdes *et al*, 2000). A 3 bp of homology and an incorporation of 8 bp were found at the site of the deletion junction in two unrelated cases (Bernatowicz *et al*, 1992; Yen *et al*, 1994). We previously reported a family with the simultaneous occurrence of XLI and ichthyosis vulgaris (Cuevas *et al*, 1999) and in this study we analyzed the STS gene in this family.

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MATERIALS AND METHODS

Patients The XLI patients of the family were referred to the Genetic Department of the General Hospital of Mexico. They were informed about the characteristics of the study and they agreed to participate. The protocol was accepted by the Ethics Committee of the General Hospital. Physical examination of the XLI patients (n = 4 males) showed a moderate degree of ichthyosis on the trunk, back, and extremities. Two of them had also cryptorchidism corrected surgically. No other clinical findings were observed.

STS assay STS activity was determined in leukocytes of the XLI patients 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. 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 the enzyme substrate. Assay conditions were pH 7.0 at 37°C for 1 h. The product of hydrolysis was recovered with benzene (Merck, Germany, analytical grade) and read in a scintillation spectrometer (Cuevas *et al*, 1993.). Each assay was performed twice.

Mutation detection DNA extraction was performed in the conventional way (Lench *et al*, 1998). The STS gene was analyzed by polymerase chain reaction (PCR). The conditions and primers to amplify exons 1, 2, 5, and 6–10, and contiguous 5′ flanking sequences DXS1130, DXS1139, and DXS996 of the STS gene are shown in **Table I** (Ballabio *et al*, 1990a; Schaefer *et al*, 1993; Valdes *et al*, 2000).

RESULTS AND DISCUSSION

The XLI patients of the family were shown to have undetectable levels of STS activity (0.00 pmol per mg protein per h),

17-H

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

Exon 1	F-5′ GGCCTAGAAGAAGGTTGAAGGTCCC
	R-5′ AAGAGGTTGGATGAGATGGGCATAC
Exon 2	F-5′ TCCTTTACAGGAAGATGAAG
	R-5′ CATTACCAACCTGATAGTTTT
Exon 5	F-5′ ACCACCCTTTACATCACGGC
	R-5′ CGCCTCCACCGTTAGCCTCT
Exon 6	F-5′ TTTTGCAGGAACACTGAGACTCCG
	R-5′ AACGTACCCACACTCCAGTCCATTT
Exon 7	F-5′ TCCCCTCCAGGGCAGATCTTGAAC
	R-5′ CATTTCTCACCTTTATAGATCCC
Exon 8	F-5′ GATCTTTTAGGAGGAAAAGCAAAC
	R-5′ CAGAGTACCTGTCCTCAGGCAAGG
Exon 9	F-5′ TATCCCACAGGATCATTGATGGAC
	R-5′ TTTACTCACTGTTCTGAGGTGC
Exon 10	F-5′ GAAATCCTCAAAGTCATGCAGGAAG
	R-5′ CCTCCAGTTGAGTAGCTGTTGAGCT
DXS1130	F-5′ CTCCTCAACTTGCAGACGG
	R-5′ ATGGGGCAAGGGAGCTCTC
DXS1139	F-5′ GCTTGGATCAGGGCTGGAATTAG
	R-5′ TTAAACAAGGGCCATTCTGCAAG
DXS996	F-5′ AAATTCTTGCCTAGGCCACTCTAGG
	R-5′ ACGTTGTTCTGGATCGTATGCTAGG

[a]F, forward primer; R, reverse primer. Conditions for exons 1 and 10 are: DNA 500 ng, primers 0.4 μM, deoxyribonucleoside triphosphate 0.08 mM, $MgCl_2$ 1.5 mM, buffer 1 ×, Taq Pol 1.5 U, vol. 50 μl. 94°C for 1 min, 30 cycles of 94°C for 1 min, 60°C for 30 s, and 72°C for 2 min. Conditions for exons 2, 6, 7, 8, and 9 are: DNA 500 ng, primers 0.8 μm, deoxyribonucleoside triphosphate 0.12 mM, $MgCl_2$ 1.5 mM, 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. Conditions for exon 5 are: DNA 500 ng, primers 0.8 μM, deoxyribonucleoside triphosphate 0.12 mM, $MgCl_2$ 1.5 mM, 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. Conditions for 5′ flanking sequences DXS1130, DXS1139, and DXS996 are: DNA 500 ng, primers 0.6 μM, deoxyribonucleoside triphosphate 0.12 mM, $MgCl_2$ 1.5 mM, buffer 1 ×, Taq Pol 1.5 U, Vol. 50 μl. 94°C for 5 min, 30 cycles of 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min.

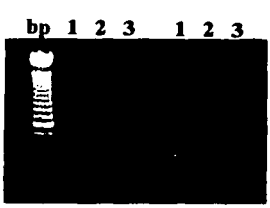

Figure 1. PCR analysis of the STS gene. (*A*) Exon 6; (*B*) exons 1 and 10 lower and upper bands, respectively. *Line 1*, positive control; *line 2*, XLI patient with complete deletion of the STS gene; *line 3*, XLI patient described in the text with the partial deletion spanning exons 1–5, in this subject exons 6 and 10 are observed.

corroborating the XLI diagnosis. DNA analysis showed a normal amplification of exons 6–10 and contiguous 5′ flanking sequences DXS1130, DXS1139, and DXS996 of the STS gene (**Figs 1** and 2). PCR amplification of exons 1, 2, and 5 failed to amplify these regions. PCR was repeated three times for all segments and identical results were obtained in each assay. These results indicated that the STS gene in our family had a partial deletion, including exons 1–5.

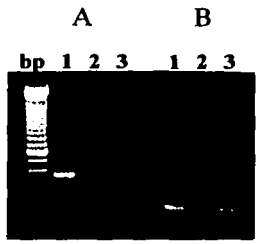

Figure 2. PCR analysis of the exon 5 and contiguous 5′ flanking sequence DXS1130. (*A*) Exon 5; (*B*) DXS1130. *Line 1*, positive control; *line 2*, XLI patient with complete deletion of the STS gene; *line 3*, XLI patient described in the text, in this subject only sequence DXS1130 is observed.

About 90% of STS deficiency patients have large deletions of the STS gene and flanking sequences. The presence of low copy number repeats on either side of the STS gene, promoting unequal crossing over, seems to play a part in the high frequency of these interstitial deletions (Ballabio *et al*, 1990b; Yen *et al*, 1990). Nevertheless, this mechanism does not apply to all STS gene deletions as in our family, the deletion involved exons 1–5 of the STS gene. DNA amplification of exons 6–10 and 5′ flanking markers indicated that the deletion breakpoints thus lay within intron 5 and between 5′ flanking sequence DXS1130 and exon 1 of the gene. Six unrelated patients with partial deletions of the STS gene have been reported: (i) one patient had a deletion that started within intron 7 and spanned exons 8–10 (Ballabio *et al*, 1989); (ii) the second had a deletion which included exon 10 (Nomura *et al*, 1995); (iii) the third showed an intragenic deletion spanning exons 2–5 (Shapiro *et al*, 1989); (iv) the fourth had a deletion involving exons 2–10 and 3′ flanking sequences DXS1131 and DXS1133 (Valdes *et al*, 2000); and (v) the fifth and sixth showed a partial deletion at the 5′ end of the STS gene (Aviram *et al*, 2000). Most of these deletions causing XLI are located at the 3′ end of the STS gene, but in our family the partial deletion was present at the 5′ end of the gene. In two previous reports and in our family, there are two intron regions in common in which the deletion breakpoints occur. These regions comprise introns 1 and 5 of the STS gene. In one case the deletion breakpoints lay within introns 1 and 5 (Shapiro *et al*, 1989), in the second one of the deletion breakpoints was located within intron 1 (Valdes *et al*, 2000), and in our family one of the deletion breakpoints lay within intron 5. Further studies could provide evidence of some specific changes occurring in these intron regions.

On the other hand, this partial deletion of the STS gene spanning exons 1–5 also indicates the importance of the amino region of the enzyme, a region that seems to be important for STS activity. In conclusion, we report the first partial deletion of the STS gene extending over exons 1–5 in XLI.

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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

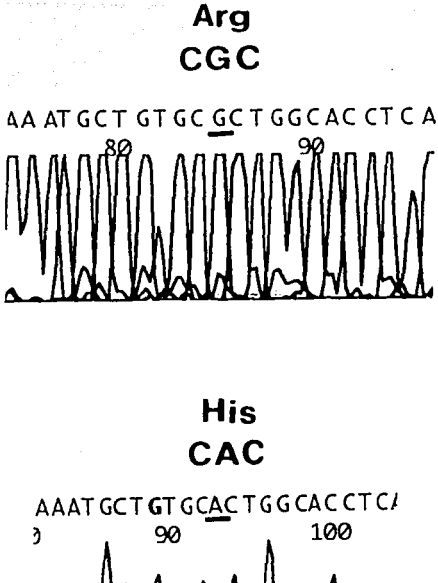

Fig. 1. Partial electropherogram showing the region of exon 9 of the steroid sulfatase gene with the point mutation. The transition 1567G→A that results in the substitution of an arginine by an histidine (Arg432His) can be seen. Normal control (top). XLI patient (bottom).

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Expression of Basal-Cell Adhesion Molecule (B-CAM) in Human Epidermis

To the Editor:

I read with interest the recent article by Schön *et al* on the expression of basal-cell adhesion molecule (B-CAM) in human skin (Schön *et al*, 2000) documenting association of B-CAM expression with activated states of keratinocytes. The authors provide convincing evidence that, besides its known function as a laminin receptor, B-CAM may be involved in cell–cell interaction or migration.

In this context I would like to bring to your attention data regarding B-CAM expression in human skin that might not have been accessible to the authors at the time their manuscript was submitted. We have been analyzing B-CAM expression in normal and diseased human skin utilizing the monoclonal antibody G253 that has also been employed in the paper by Schön *et al*. Contrary to their observation, but in line with previous findings (Garin-Chesa *et al*, 1994), the majority of specimen from normal human epidermis exhibited B-CAM positive suprabasal keratinocytes. In contrast, B-CAM expression in fetal epidermis was restricted to basal keratinocytes. The outer root sheath of hair follicles in both fetal as well as adult human skin regularly exhibited a B-CAM positive phenotype. The level of B-CAM expression was higher in psoriasis and contact dermatitis. We interpret our observations as evidence for B-CAM being a keratinocyte differentiation marker (Bernemann *et al*, 2000). This notion is supported by the effects of Ca^{2+} levels on the B-CAM phenotype of keratinocytes as described by Schön *et al*.

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Maternal Transmission of the 3 bp Deletion within Exon 7 of the STS Gene in Steroid Sulfatase Deficiency

To the Editor:

Steroid sulfatase (STS) enzyme (EC 3.1.6.2) is present in the microsomal fraction of the cell and is capable of hydrolyzing sulfated sterols (Hobkirk, 1985; Dibbelt and Kuss, 1991). STS deficiency is the biochemical defect in X-linked ichthyosis (XLI), a disease that clinically presents dark, adhesive, and regular scales of skin (Shapiro and Weiss, 1978). STS assay or FISH analyzes allow an XLI diagnosis and identify XLI-carriers (Baden *et al*, 1980; Epstein and Leventhal, 1981; Okano *et al*, 1985; Hermann *et al*, 1987; Matsumoto *et al*, 1990; Aviram-Goldring *et al*, 2000). STS enzyme is encoded by the STS gene on the short arm of the X-chromosome (Muller *et al*, 1981). Most STS enzyme deficient patients present large deletions of the entire STS gene and flanking sequences (Bonifas *et al*, 1987; Shapiro *et al*, 1989; Cuevas-Covarrubias *et al*, 1997; Aviram-Goldring *et al*, 2000). Only a few point mutations or partial deletions have been identified (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; Aviram-Goldring *et al*, 2000; Oyama *et al*, 2000; Sugawara *et al*, 2000; Valdes *et al*, 2000). In this study, we describe an XLI patient with the first 3 bp intragenic deletion of the STS gene causing XLI and demonstrate the maternal transmission of this molecular defect.

MATERIALS AND METHODS

Patient The patient was referred as having ichthyosis to the General Hospital of Mexico. He was the only child of the family. The patient and his mother were informed about the characteristics of the study and they agreed to participate. Protocol was evaluated and approved by the Ethics Committee. The patient showed a moderate degree of

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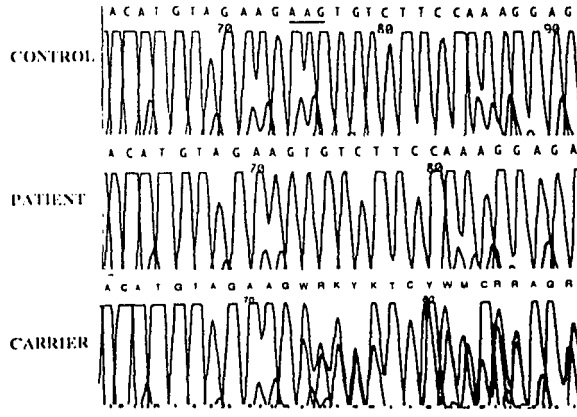

Figure 1. DNA sequence of the STS gene of the XLI patient and his mother. DNA sequence of exon 7 of the STS gene of the XLI patient (middle line), carriet mother (lower line), and normal control (upper line), only a small fragment is shown (starting at nucleotide 1241). The missing region of 3 bp observed in the XLI patient is underlining in the normal control. The heterozygote state of the mother is observed starting at nucleotide 1252. W = A + T, R = G + A, K = T + G, Y = C + T, M = A + C.

H V E E V S S K G E I H G
CAT GTA GAA GAA GTG TCT TCC AAA GGA GAA ATT CAT GGC

H V E V S S K G E I H G
CAT GTA GAA G TG TCT TCC AAA GGA GAA ATT CAT GGC

Figure 2. Amino acid sequence of the STS polypeptide. Normal sequence of exon 7 of the STS gene with the respective amino acids (upper line) and the effect observed in the XLI patient described in the text (lower line). A 3 bp deletion (shadowed area) only results in the loss of glutamine (only nucleotides 1242–1280 and amino acids 324–335 are shown). The *arrow* indicates the nucleotides conforming the new codon that corresponds to the contiguous codon in the normal amino acid sequence. H, his; V, val; E, glu; S, ser; K, lys; G, gly; I, ile.

involvement on the trunk, back, and upper extremities and more severe involvement on the lower extremities. No clinical findings were observed in his mother.

STS assay STS activity was determined in leukocytes of the XLI patient and his mother using 7-[^{3}H]-dehydroepiandrosterone sulfate as described elsewhere (Miranda-Duarte *et al*, 1990).

Mutation detection DNA extraction was performed in the conventional way (Lench *et al*, 1988). All exons of the STS gene were analyzed by polymerase chain reaction (PCR). The conditions and primers to amplify these regions are described elsewhere (Ballabio *et al*, 1990a; Oyama *et al*, 2000; Valdes-Flores *et al*, 2000). DNA sequence analysis was performed in an ABI Prism Genetic Analyzer (Perkin Elmer, CA, USA).

RESULTS AND DISCUSSION

The XLI patient had no STS activity (0.00 pmol per mg protein per h), corroborating the XLI diagnosis. STS activity of his mother was very low (0.30 pmol per mg protein per h *versus* 0.85 pmol per mg protein per h of normal control). PCR analysis of the XLI patient showed normal amplification of all exons of the STS gene. In the DNA sequence analysis, the loss of nucleotides AAG at positions 1252–1254 within exon 7 of the STS gene was observed (**Fig 1**). DNA analysis of the patient's mother showed an heterozygote state for this condition (**Fig 1**).

Most XLI patients lack the entire STS gene and flanking sequences. These large deletions appear to be due to unequal recombination between low copy number repeats on either side of the STS gene (Ballabio *et al*, 1990b; Yen *et al*, 1990). In this study, we observed AAGdel1252 of the STS gene corresponding to the last two nucleotides of the codon 327 and the first one of the codon 328 (**Fig 2**). This indicates that homologous recombination does not apply to all STS gene deletions. As STS activity of the patient's mother was very low, we analyzed her DNA and found an heterozygote state for the same molecular defect. It has also been reported that most sporadic cases in XLI correspond to inherited cases (Cuevas-Covarrubias *et al*, 1998), and we confirmed it in our family. Although the nucleotides involved in AAGdel1252 did not correspond to a specific codon, this 3 bp deletion only resulted in the absence of glutamine at position 327 of the STS enzyme (ΔE327) and not in a frameshift mutation. This amino acid is found in the C-terminal region after the second N-linked glycosylation site. It has been suggested that the N-terminal domain contains the catalytic site whereas the C-terminal region probably contains the substrate binding site (Stein *et al*, 1989). So, the mutation reported here would be affecting the substrate binding region of the STS enzyme. Additional studies will be required to elucidate if this mutation affects half-life, folding, dimerization, or post-translational processing of STS enzyme.

The majority of patients have large deletions of the STS gene and flanking sequences; however, partial deletions and point mutations in exon and intron regions have also been reported. Now, we report the first intragenic deletion of 3 bp of the STS gene observed in XLI. These data indicate that all molecular defects occur in XLI.

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Animal Model of Sclerotic Skin. IV: Induction of Dermal Sclerosis by Bleomycin is T Cell Independent

To the Editor:

Systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by excessive collagen production by activated fibroblasts resulting in the deposition in the dermis (Krieg and Meurer, 1988). Although a number of attempts have been made, the pathogenesis of SSc still remains unknown. Previous studies have suggested immunologically mediated mechanisms involving immunocytes infiltrating in the dermis. In its early stage, SSc is associated with intense mononuclear cell infiltration (Fleishmajer *et al*, 1977; Roumm *et al*, 1984), which is mainly composed of T lymphocytes, mast cells, and monocyte/macrophages, along with a smaller number of B cells and plasma cells. Several inflammatory or fibrogenic cytokines released from T cells and monocyte/macrophages are suggested to play an important role in the induction of tissue fibrosis in the affected sites. Bleomycin (BLM) is an antitumor antibiotic used for the therapy of a variety of cancers. It is well known that pulmonary fibrosis can be induced in patients treated with BLM as a side-effect. Thus, BLM-induced lung fibrosis is an established animal model, resembling human pulmonary fibrosis histologically and biochemically (Adamson and Bowden, 1974; Aso *et al*, 1976; Chandler, 1990). *In vivo* T cell depletion with anti-T cell antibodies has been shown to either reduce or completely abrogate BLM-induced pulmonary fibrosis (Thrall *et al*, 1980; Piguet *et al*, 1989). On the other hand, results of athymic nude mice lacking functional T lymphocytes have been controversial. One study showed histologically similar fibrosis in nude, euthymic mice after BLM treatment (Szapiel *et al*, 1979), whereas another study showed that BLM was not fibrogenic in nude mice (Schrier *et al*, 1983). On the contrary, a recent study has demonstrated that BLM-induced lung fibrosis occurred in C57BL/6 SCID and (C57BL/6×CB.17)F1SCID mice comparable with that seen in wild-type mice, suggesting that the initial induction of lung fibrosis is lymphocyte-independent (Lake-Bullock *et al*, 1999). We have recently established a mice model for scleroderma by repeated local injections of BLM (Yamamoto *et al*, 1999a). In this study, in order to determine the role of lymphocytes in the induction of dermal sclerotic lesions, we examined whether dermal sclerosis can be induced in SCID mice using our method. Specific pathogen-free, 6-wk-old, female BALB/C mice (wild-type) and age- and sex-matched BALB/C SCID mice (weighing about 20 g) were purchased from Clea (Tokyo, Japan), and maintained with food and water ad libitum. BLM was dissolved in phosphate-buffered saline (PBS) at a concentration of 100 μg per ml, and sterilized by filtration (0.2 μm). One hundred microliters of BLM or PBS were injected subcutaneously into the shaved back of mice every other day for 4 wk with a 27-gauge needle. The back

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CONCLUSIONES

Considerando que la mayoría de los pacientes con ILX presentan deleciones génicas totales como causa de la enfermedad el análisis molecular inicial se realizó mediante la amplificación de los extremos 5'y 3'del gen STS en todos los casos. Los primeros resultados mostraron ausencia de ambos extremos del gen en 81 de estos pacientes, lo que indicó que 96 % presentaban deleción génica total como causa de la enfermedad. Estos datos son similares a lo informado en la literatura internacional con respecto a las mutaciones del gen STS (51-53, 73).

El primero de los 4 pacientes restantes mostró amplificación normal del extremo 5'del gen STS (exón 1) y ausencia del extremo 3'(exón 10). Tomando en cuenta estos primeros resultados y con el fin de delimitar los sitios de ruptura, se procedió a la amplificación de los exones 2-5 y 7-9 así como de las regiones flanqueadoras hacia la región 3' del gen. El análisis final mostró que esta deleción comprometía además de los exones 2-10, las regiones DXS1131 y DXS1133. En este caso, la pérdida de prácticamente todo el gen STS, condicionaría la ausencia de la enzima (incluyendo sitio activo, dominios transmembranales y sitio de unión a ligando) ocasionando así la ausencia de su función. Estos resultados nos permitieron documentar en la literatura internacional la cuarta deleción parcial del gen STS y la primera que compromete la región ya descrita (58). **J Invest Dermat. 2000 Mar;114(3):591-93.**

El análisis molecular inicial de otro de los pacientes con presencia parcial del gen, mostró la presencia del exón 10, pero no del exón 1. Considerando estos hallazgos, se amplificaron los exones 2, 5 y 6-9 del gen además de las regiones adyacentes al exon 1. Los resultados revelaron que la deleción incluía solamente los exones 1-5, sin

comprometer ninguna de las regiones flanqueadoras del extremo 5'. Esta deleción de los exones 1-5 del gen STS en la cual se pierden 53.7 kb del extremo 5', produciría la presencia de una proteína truncada y no funcional, traduciéndose bioquímicamente en la pérdida de actividad enzimática. Esta deleción es la primera reportada con estas características y representa la quinta deleción parcial del gen STS documentada en la literatura (59). **J Invest Dermat. 2001 Mar 116(3):456-8.** En este caso, los estudios bioquímicos y moleculares en la familia detectaron 3 pacientes más.

El estudio del gen STS en el tercero de los cuatro pacientes reveló la presencia de todos los exones del gen, por lo que se procedió al análisis de la secuencia total del gen. Los resultados mostraron la pérdida de 3 nucleótidos (AAG) en el exon 7 (posiciones 1252-1254). Esta microdeleción incluyó los dos últimos nucleótidos del codón 327 (AA) y el primero del codón 328 (G). Este cambio no modifica la fase de lectura de la proteína, sin embargo, condiciona la pérdida de una glutamina en la posición 327 (extremo COOH). Considerando que posiblemente el sitio de unión a ligando en esta proteína se ubica en el extremo carboxilo, es posible que la pérdida de actividad enzimática en este paciente se relacione con la falta de reconocimiento e interacción entre la SE y sus substratos. Esta mutación corresponde al primer informe de una microdeleción intragénica en el gen STS (60). **J Inves Dermat. Oct; 117(4):997-999, 2001.** El estudio bioquímico en la madre de este paciente permitió identificar su condición de portadora y la secuenciación permitió confirmar el estado hetereocigoto de la madre.

El análisis del último paciente mostró amplificación de todos los exones del gen STS, procediéndose entonces a la secuenciación de los 10 exones del gen (ABI PRISM 310 de Perkin-Elmer). Este método permitió identificar una mutación puntual en el exón 9

del gen en el nucleótido 1567 (1567G→A). Esta transición resulta en el cambio de una arginina por una histidina en la posición 432 de la secuencia de aminoácidos original. A pesar de que ambos aminoácidos (arginina e histidina) son considerados como hidrofílicos, la histidina representa un iminoácido con un anillo aromático en su estructura. Con el fin de descartar un posible polimorfismo se buscó intencionadamente este cambio en miembros de la familia no afectados, en pacientes con entidades como el Sx. de Turner (n= 4), ictiosis vulgar (3) y controles masculinos (n= 15) y femeninos sanos (n= 15). En ninguno de ellos se presentó esta mutación (66). **Acta Derm Venereol 2001;81:54-55.**

Con respecto a las diversas mutaciones puntuales informadas en la ILX (incluyendo la reportada por nuestro grupo), es posible que los cambios en la secuencia original de aa de la SE ocasionadas por estas mutaciones modifiquen su estructura en regiones específicas de la proteína, condicionando así su inestabilidad, la falta de interacción con sus diferentes substratos o bien favoreciendo su rápida degradación. Por el momento, no se han documentado en la literatura mutaciones puntuales en los residuos que conforman los dominios transmembranales, tampoco en los sitios potenciales de N-glicosilación ni en el sitio activo de la enzima (H^{136}). Es importante señalar que solamente dos de las mutaciones puntuales informadas (casos 2 y 3 de la tabla 1) se presentan en residuos considerados evolutivamente conservados, esto con respecto a la familia génica de las arilsulfatasas. En los casos de deleciones totales del gen STS la falta de la proteína podría condicionar la ausencia de función de la SE. Cuando se presentan deleciones parciales intragénicas o terminales el defecto bioquímico se relaciona con la presencia de una proteína truncada, no funcional y posiblemente inestable. En los casos de mutaciones puntuales pueden ocasionarse pérdida de

actividad catalítica, o bien la falta de reconocimiento de los substratos (dependiendo del tipo y sitio del cambio).

Al analizar la distribución de las diferentes mutaciones puntuales documentadas en la literatura, llama la atención que todas se ubican en el extremo COOH de la proteína, el cual se ha considerado como el sitio de unión a ligando (s). Por el momento no se han identificado mutaciones en el extremo NH2, a pesar de que el sitio activo de la enzima se localiza en esta región (H^{136}). Consideramos que la realización de estudios complementarios (i.e., mutagénesis dirigida) permitirá conocer el efecto de las diferentes mutaciones sobre la presencia y características de la enzima (vida media, estabilidad, dimerización, actividad catalítica, procesamiento o bien modificaciones postraduccionales).

Finalmente, debemos señalar que en la ILX no se ha observado variabilidad fenotípica con respecto al tipo de mutación presente en cada caso.

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