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ESTUDIO DE LAS CARACTERISTICAS CLINICAS, ENZIMATICAS Y MOLECULARES DE LA ICTIOSIS LIGADA AL X.

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ESTUDIO DE LAS CARACTERISTICAS CLINICAS, ENZIMATICAS Y MOLECULARES DE LA ICTIOSIS LIGADA AL X.

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RESUMEN

La ictiosis recesiva ligada al X (XLI) es una genodermatosis causada por la deficiencia de la enzima sulfatasa de esteroides (STS). Tiene una frecuencia de 1:2000-6000 recién nacidos (RN) varones, inicia al nacimiento o en el primer año y se caracteriza por presentar escamas oscuras, adherentes y regulares con predominio en extremidades y tronco. El diagnóstico diferencial de la ILX se realiza con la ictiosis vulgar (IV), autosómica dominante, que tiene una frecuencia de 1:250 RN y un cuadro clínico muy similar. La STS se localiza en el retículo endoplásmico rugoso, actúa conformada como pentámero y tiene como substratos a los sulfatos de 3-beta-hidroxiesteroides. El gen que codifica para la STS se localiza en Xp22.3 y escapa al proceso de inactivación del cromosoma X. Las alteraciones moleculares del gen STS informadas en la ILX son pérdida total del gen en 85-90% de los casos, 5 mutaciones puntuales que modifican la composición de aminoácidos de la STS, 1 mutación en el exón 7 que produce un codón de terminación prematura, 3 deleciones parciales intragénicas y 2 mutaciones en intrones que forman codones de terminación prematura. De los pacientes con ILX, 5% presentan deleciones génicas mayores que producen fenotipos más complejos conocidos como síndromes de genes contiguos y que incluyen los genes del síndrome de Kallmann (SK), condrodisplasia punctata, retardo mental y/o corta estatura. El objetivo del presente trabajo fue analizar clínica, enzimática y molecularmente una muestra de pacientes mexicanos con ILX para conocer su comportamiento en nuestra población e identificar

características de este padecimiento no informadas en la literatura. Se incluyeron a todos los pacientes que fueron referidos por ictiosis durante 1994-1997 al Hospital General de México. La clasificación de pacientes y portadoras se llevó a cabo determinando la actividad de la STS en leucocitos utilizando el sulfato de 7-[^{3}H]-dehidroepiandrosterona como substrato y la identificación de deleciones del gen STS amplificando mediante PCR sus extremos 5' y 3'. Se identificó la secuencia de los exones 1, 2, 5 y 10 del gen STS mediante el método de fluorescencia con un secuenciador automatizado. Los resultados mostraron grandes diferencias con lo informado previamente. Se analizaron 66 pacientes referidos por ictiosis, 60 correspondieron a ILX y 6 a IV, lo que representa una relación de 10:1 de la ILX con respecto a la IV, un dato contrario a lo informado en la literatura para ambas entidades. Dos hallazgos clínicos no reportados previamente fueron la presencia de hernia inguinal en 7% de los casos con ILX y de sangrado transvaginal durante el primer trimestre del embarazo en 15% de las madres de los pacientes. La consideración de ambos datos clínicos en la revisión del paciente pudieran orientar al diagnóstico de ILX. Los casos no familiares representaron 70% de la muestra, sin embargo, 85% de éstos resultaron ser hereditarios al detectarse en la madre el defecto enzimático inicial. Estos datos indican que la mayoría de los pacientes con ILX no son mutaciones *de novo* como se había informado en estudios previos. Es importante considerar este hallazgo ya que el asesoramiento genético es diferente en ambos casos. Otro dato interesante fue que 95% de los pacientes referidos por ictiosis (ILX o IV) fueron

hombres, un hallazgo que parecería ser una característica especial observada en nuestra población. En el estudio molecular se observó que 98% de los pacientes con ILX tuvieron deleción total del gen STS, encontrándose sólo en un paciente la presencia del gen STS. Sin embargo, en este caso, en los exones secuenciados no fue posible identificar una mutación puntual. En 2 pacientes se presentó la asociación de ILX y SK, ambos casos con pérdida del gen STS. Sin embargo, un paciente amplificó todos los exones (1-14) y el otro los exones 4-14 del gen KAL, descartándose un síndrome de genes contiguos. La asociación de ambas entidades, observadas en este estudio, podría sugerir condiciones de recombinación y segregación génicas interesantes, muy poco frecuentes y no informadas hasta ahora en la literatura.

SUMMARY

X-linked ichthyosis (XLI) is an inherited disease due to steroid sulfatase (STS) deficiency with a frequency of 1:2000-6000 new born (NB) males. Onset is at birth or early after birth with dark, regular and adherent scales in extremities and trunk. Ichthyosis vulgaris (IV), with a frequency of 1:250 NB, shares many clinical characteristics with XLI, and differential diagnosis must be performed among both entities. STS is present as a pentameric enzyme in the rough endoplasmic reticulum. Its substrates are the 3-beta-hydroxy-steroid sulfates. STS gene is on Xp22.3 and escapes the X-inactivation process. Most of the XLI patients (85-90%) have a complete deletion of the STS gene. Six point mutations, 3 intragenic deletions and 2 intronic mutations have also been reported. Less than 5% of the XLI patients have a more complex phenotype that includes Kallmann syndrome (KS), chondrodysplasia punctata, short stature and/or mental retardation. The association of these entities is known as a contiguous gene syndrome. The aim of the present study was to analyze XLI at clinical, enzymatic and molecular level to identify characteristics of the disease in Mexican patients and findings not previously reported. All patients referred by ichthyosis during 1994-1997 to the Hospital General de México were included. XLI patients and carriers were classified through STS assay in leukocytes using the 7-[^{3}H]-dehydroepiandrosterone sulfate as substrate. To identify possible deletions, amplification of the 3' and 5' ends of the STS gene was performed through PCR. Sequence of the 1, 2, 5 and 10 exons were done in a

fluorescence automatic form. Of the 66 patients referred by ichthyosis (XLI or IV), 60 corresponded to XLI and 6 to IV. This data represents a 10:1 ratio, that is very different to that previously reported where IV is more predominant than XLI. It was also observed inguinal hernia in patients and transvaginal bleeding in mothers. These clinical findings, not observed previously, could be considered when XLI diagnosis is proposed. Sporadic cases were found to be present in 70% of the sample. Nevertheless, 85% of them were really inherited cases as their mothers were identified as XLI carriers. This data is different to that reported in the literature and must be taken into account to offer an adequate genetic counseling. Another finding observed in this study was the fact that 95% of the sample were males. This seems to represent an special characteristic of the Mexican population. The DNA from 59 patients showed not amplification of the 5' and 3' ends of the STS gene. Only one patient presented normal amplification of these segments. Nevertheless, in this case the sequences of the 1, 2, 5 and 10 exons were normal. Two patients had XLI and KS with a complete STS gene deletion. When amplification of the all exons (1-14) of the KAL gene in these two patients was performed, one case presented a normal amplification of all the exons while in the other patient, the amplification was observed from exon 4 to 14, demonstrating a deletion of exons 1-3, excluding the classic form of the contiguous gene syndrome in both cases. The association of these entities, observed in this study, could represent an special form of recombination and segregation not reported previously in the literature.

INTRODUCCION

La ictiosis es una genodermatosis conocida desde el año 200 A.C. El término ictiosis ha sido

utilizado durante más de 2000 años y proviene de la raíz griega IXOYE (ictios) que significa

pez. Los hindúes y los chinos hacían referencia de la enfermedad como una afección con piel

de serpiente o escamas de pescado y el médico árabe Avicenna como "albarras nigra". Alibert

atribuía sus causas a vivir cerca del mar o de agua estancada o por comer pescado en estado

de descomposición. En 1884 Fox reportó "el niño cocodrilo" relacionando su presencia con el

ataque de un cocodrilo sufrido por la madre durante su gestación. La primera descripción

médica de la ictiosis se encuentra en la obra "On cutaneous diseases" por Robert Willan (1),

siendo Cockaine el primero en realizar una clasificación genética, modificándola

posteriormente Greither, Touraine y Wells & Kerr (2-4).

Las ictiosis son un grupo de dermatosis que presentan engrosamiento del estrato córneo,

debido a una retención o hiperproliferación de la capa córnea de la piel y se manifiestan

clínicamente por presentar una descamación anormal. Actualmente, las 5 variedades

principales de ictiosis son ictiosis vulgar (IV), ictiosis ligada al X (ILX), ictiosis lamelar,

eritrodermia ictiosiforme congénita e ictiosis bular, siendo la ILX y la IV las más importantes

por su frecuencia. Existen además otras condiciones ictiosiformes aún no bien definidas genéticamente (5).

La ILX es una entidad que afecta a 1 de cada 2,000-6,000 hombres (1:13,500 recién nacidos vivos, RN) (6,7). En 1965, Kerr y cols., estudiando sus manifestaciones clínicas y genealogía, la consideraron como una enfermedad distinta a las demás ictiosis (8). La ILX se presenta generalmente al nacimiento o en el primer año de vida. Los signos principales son escamas generalizadas, adherentes, regulares y oscuras que afectan pabellones auriculares, cuello, zonas de flexión, cara anterior de abdomen y espalda respetando cara, palmas de las manos y plantas de los pies. Aproximadamente 7% de los pacientes presenta criptorquidia como hallazgo clínico asociado (5). Además, se han observado opacidades corneales en algunos pacientes y portadoras y en estas últimas retardo en el trabajo de parto (9,10). Histológicamente se han reportado hipertrofia y cambios no específicos en la zona granular de la epidermis (5)

El diagnóstico diferencial de la ILX se realiza con la IV, entidad autosómica dominante que tiene una frecuencia de 1:250 RN (5). Se ha considerado que a diferencia de la ILX, en la IV las escamas son irregulares, claras y respetan las zonas de flexión. Para la IV se han

observado como datos asociados una hiperlinearidad palmo-plantar con hiperqueratosis, antecedentes familiares de atopia y una menor gravedad del cuadro clínico (11).

En 1978, Shapiro y cols. encontraron en fibroblastos de piel de pacientes con ILX, una deficiencia de la actividad de la sulfatasa de esteroides (STS) (12). Paralelamente, estudios en madres con deficiencia placentaria de esta enzima reportaron niveles bajos de estriol y retardo en la labor del parto asociados a niveles bajos de la actividad de la STS (13). El seguimiento de las madres demostró la presencia de la ILX en los productos. De esta manera, se descubrió en forma independiente la deficiencia de la STS en pacientes con ILX y su asociación con las madres que presentaban dicha alteración a nivel placentario.

La STS (E.C.3.1.6.2) o arilsulfatasa C es una enzima de distribución ubicua que se encuentra unida a la membrana microsomal. Evidencias químicas y genéticas parecen indicar que la arilsulfatasa C comprende dos isoenzimas "s" y "f" (s de slow y f de fast) caracterizadas por una movilidad electroforética diferente, codificadas por genes distintos, en cuyo caso únicamente la forma s correspondería a la STS propiamente dicha (14). La STS está compuesta de 583 aminoácidos, tiene un PM de 63 Kda y una vida media de 4 días (15). Esta enzima es procesada mediante la escisión de un péptido guía de 22 aminoácidos hidrofóbicos para finalmente dar origen a la STS madura de 61 Kda. La STS presenta oligosacáridos

unidos a residuos de asparaginas y se encuentra unida a la membrana microsomal como un pentámero. La STS hidroliza los sulfatos de 3-beta-hidroxiesteroides siendo sus principales substratos con sus respectivas Km los sulfatos de colesterol 2.0 μM (SC), estrona 0.8 μM, dehidroepiandrosterona 1.7 μM (DHEAS), pregnenolona 0.6 μM y testosterona 40 μM. Su pH óptimo oscila entre 6.5 y 7.5 (16).

La deficiencia de la STS observada en la ILX produce una elevación del SC en piel, suero y membrana de los eritrocitos, modificándose de manera importante la relación colesterol/sulfato de colesterol (17). La determinación del SC puede ser utilizada con fines diagnósticos. El SC es transportado por las lipoproteínas de baja densidad (LDL) del plasma, y su incremento en esta lipoproteína produce un aumento en la movilidad electroforética de las LDL debido a la carga negativa del radical sulfato. De esta manera, la electroforesis de lipoproteínas también permite realizar el diagnóstico de ILX (18). Otros cambios observados en las LDL de los pacientes con ILX son la disminución del contenido de los ésteres de colesterol y el aumento de las relaciones entre triacilgliceroles/colesterol y apoproteína B/colesterol (19). Además de la acumulación de SC en la epidermis de los pacientes con ILX, se ha reportado una modificación en la composición relativa de los lípidos con una disminución marcada de esteroles libres y lípidos neutros totales (20).

La determinación de la actividad de la STS en distintos tejidos como piel, uñas, pelo, callo plantar, fibroblastos y leucocitos permite establecer el diagnóstico correcto de la ILX, diferenciándola así de la IV (21-23). Asimismo, determinando la actividad de la STS es posible conocer si la madre o alguna familiar del paciente es portadora de la ILX, ya que el valor de esta enzima es menor en las portadoras de ILX y no se traslapa con los valores observados en los controles sanos masculinos o femeninos (24).

El gen que codifica para la STS se localiza en los brazos cortos del cromosoma X en la región Xp22.3, cercano a la región pseudoautosómica (25). Tiene su contraparte (gen homólogo) no funcional en el brazo largo del cromosoma Y conocido como pseudogén de la STS (26). Ambos genes presentan una homología del 85%. El pseudogén STS tiene pequeñas adiciones, deleciones y sustituciones de algunos pares de bases, por lo que no codifica para un producto proteico funcional. El gen STS se extiende sobre una región de 146 Kb, tiene 10 exones, 9 intrones y presenta dos regiones que no se transcriben, una en el extremo 5' de por lo menos 206 pb y otra región 3' de 668 pb intercaladas por una secuencia de marco de lectura abierta de 1683 pb. Tiene además una señal de poliadenilación (AATAAA) 13 pb antes del comienzo de la cola poli-A. Se han identificado 3 transcritos de RNAm de pesos moleculares diferentes (2.7, 5.2 y 7.2) en distintas líneas celulares. Esto parece ser consecuencia de la adición de colas poli-A de longitud variable y no resultado de empalmes alternativos (27). Aún falta por

definir si estos transcritos tienen propiedades distintas. El gen STS se expresa en los cromosomas X activo e inactivo escapando así al proceso de inactivación de este cromosoma. Sin embargo, estudios de la actividad enzimática de la STS en distintas poblaciones celulares indican una relación de la actividad hombre/mujer que va de 1.3:1 a 1.8:1 y no la esperada de 2:1 por tratarse de un gen que escapa al proceso de inactivación del X. Este efecto de dosis génica incompleta sugiere que el gen STS localizado en el cromosoma X inactivo escapa de manera parcial al proceso de inactivación, posiblemente por la presencia de genes contiguos inactivos (28). Este hallazgo y el proceso de inactivación al azar del X podrían ser la explicación de los valores tan bajos de la actividad de STS encontrados en portadoras de ILX. La mayoría de los pacientes con ILX (85-90%) tienen deleciones submicroscópicas que abarcan el gen STS y regiones flanqueadoras (29). En 3 pacientes se han observado deleciones intragénicas que abarcan los exones 2-5, 7-10 y 10, respectivamente (30,31). Se han reportado 8 mutaciones puntuales resultando en la sustitución de prolina o arginina por triptófano-372, arginina por histidina-444, tirosina por cisteína-446, leucina por cisteína-341 y en el exón 7 donde se forma un codón de terminación prematura. Las otras 2 mutaciones se localizan en sitios de unión intrón-exón y forman codones de terminación prematura (32-35).

Existen familias de secuencias repetidas en número bajo de copias (G1.3 y CRI-S232) que se encuentran intercaladas en la región Xp22.3 incluyendo ambos lados del gen STS. La

presencia de estas repeticiones podría explicar las deleciones intersticiales en esta región debido a un apareamiento anormal durante la meiosis y a la recombinación homóloga entre estas unidades de repetición (36).

Algunos pacientes (-5%) tienen un fenotipo más complejo, conocido como síndrome de genes contiguos, con deleciones génicas mayores que pueden incluir los genes del síndrome de Kallmann (SK), retardo mental, talla baja y/o condrodisplasia punctata. En algunos de estos pacientes se han observado anormalidades citogenéticas como deleciones parciales o terminales en Xp (37).

Por todos estos antecedentes, el estudio del gen STS en pacientes con ILX, resulta útil e interesante para detallar los cambios moleculares ocurridos en este padecimiento y sus consecuencias clínicas y enzimáticas en pacientes mexicanos. De esta manera, se establece la posibilidad de que existan alteraciones clínicas, enzimáticas y moleculares en general, no informadas hasta ahora en la literatura.

CONCLUSIONES

La muestra incluyó 66 pacientes, 64 hombres y 2 mujeres, que acudieron a consulta referidos por ictiosis de 1994 a 1997. La determinación enzimática de la STS permitió la clasificación de 60 casos como ILX y 6 como IV al presentar los primeros una actividad de 0.0 pmol/mg proteína/hr vs 0.84 ± 0.10 pmol/mg proteína/hr de controles sanos y pacientes con IV. La presencia de más casos de ILX que de IV difiere de manera importante a lo informado en la literatura mundial. La frecuencia reportada para la IV es de 1:250 RN y para la ILX de 1:2000-6000 RN varones que representa una relación 8-24:1 de la IV sobre la ILX, considerando únicamente la presencia de la enfermedad. En la muestra de pacientes mexicanos analizada se encontró una relación de 10:1 de la ILX con respecto a la IV, lo que representa un hallazgo contrario a lo esperado (38). Si consideramos lo informado en la literatura, esperaríamos una presencia de cuando menos 480 casos de IV por los 60 de ILX observados en nuestro estudio. Aún considerando la posibilidad de un sesgo de concentración, el número de pacientes revisados y el tiempo de inclusión apoyan el hecho de que la relación de ambos tipos de ictiosis en nuestro medio es muy diferente a lo reportado para otras áreas geográficas. Esta información resalta la importancia de realizar estudios de distintos padecimientos genéticos en pacientes mexicanos para conocer adecuadamente como se comportan en nuestra población.

Otro de los aspectos importantes observados fue la dificultad de realizar un diagnóstico clínico correcto (39). En el presente estudio, 50% de los pacientes enviados como IV correspondieron a ILX y de los 4 pacientes hombres diagnosticados enzimáticamente como IV, 2 habían sido referidos inicialmente por ILX. Esto podría explicarse por la similitud de los cuadros clínicos y la falta de información de la prevalencia de ambas ictiosis en nuestro país, así como por la carencia de estudios que permitan al médico especialista conocer el comportamiento de los padecimientos genéticos en nuestro medio.

Otro de los hallazgos clínicos que no habían sido reportados previamente fue la presencia de hernia inguinal en 7% de los pacientes y de sangrado transvaginal en el primer trimestre del embarazo en 15% de las madres, independientemente de presentar o no el estado de portadora de la enfermedad (40). Estos datos no informados previamente, pudieran apoyar el diagnóstico clínico si se piensa en una probable ILX. Por otra parte, los antecedentes de atopia familiar e hiperlinearidad palmo-plantar con hiperqueratosis parecían ser datos compatibles con el diagnóstico de IV. Sin embargo, de los 4 pacientes que presentaron estos datos clínicos, 2 fueron diagnosticados como ILX y 2 como IV. Esto nos indica que estas características clínicas no son exclusivas de la IV y que pueden estar presentes en ambos padecimientos. El considerar estas características clínicas inicialmente como parte de la IV pudo deberse a la falta de la determinación de la actividad de la STS, la cual permite establecer correctamente el diagnóstico entre ambas ictiosis. Otro de los hallazgos

encontrados en este estudio, fue que la mayoría de los pacientes referidos a consulta por ictiosis (IV o ILX) eran hombres (95%), un dato interesante que indica algunas de las características especiales de ambos padecimientos en nuestro medio.

La determinación de la actividad de la STS permitió la clasificación adecuada de los pacientes (0.0 pmol/mg proteína/hr) y portadoras (0.20±0.06 vs 0.84±0.10 pmol/mg proteína/hr de los controles sanos) de ILX. De esta manera, se pudo reconocer que en los casos no familiares (n=42) 85% de las madres presentaron el defecto enzimático inicial, concluyéndose que los pacientes no correspondían a una mutación *de novo*, sino a un defecto heredado de la madre (41). Hallazgo que difiere a lo reportado en la literatura, en la cual se había considerado a las mutaciones *de novo* como la causa más frecuente en los casos esporádicos de ILX. El estudio enzimático en 7 familias que incluyeron a la madre y hermanas de la mamá del paciente apoyaron estas conclusiones, ya que en estos casos todas las familiares, a excepción de la portadora, presentaron una actividad normal de la STS. Estos resultados son demasiado importantes y deben tomarse en cuenta cuando se proporciona asesoramiento genético, ya que los riesgos de recurrencia son distintos en los casos heredados y en las mutaciones *de novo*.

El estudio molecular de los 60 pacientes mostró en 98% de los casos deleciones totales del gen STS al no amplificarse sus extremos 5' y 3' (42). Una frecuencia mayor a lo conocido en la literatura. Sin embargo, corresponde a lo informado previamente donde se considera a la pérdida total del gen STS como la causa más frecuente a nivel molecular de la ILX. Sólo un paciente de los 60 amplificó ambos extremos. En este paciente también se amplificaron los exones 2 y 5 para llevar cabo la secuencia de los exones 1, 2, 5 y 10, dada la factibilidad y el costo del estudio por tratarse de un sólo paciente. Cuando se identificó la secuencia de estos exones en la búsqueda de mutaciones puntuales, no se encontraron diferencias con la secuencia normal del gen STS. Probablemente, la mutación se encuentre en cualquiera de los otros exones o bien en algún intrón o región promotora.

Dos pacientes presentaron la asociación de SK e ILX. Ambos casos con deleción total del gen STS. Sin embargo, cuando se amplificaron los exones correspondientes al gen de KAL, en uno la amplificación fue normal y en otro la deleción se encontró en los exones 1-3 (43). En ambos casos se descartó la forma clásica del síndrome de genes contiguos, ya que la deleción no abarcó ambos genes. En el primer caso, una explicación podría ser la presencia de una mutación puntal en el gen KAL o bien pudiera tratarse de una forma autosómica del SK. En el segundo caso, la presencia de los exones 4-14 del gen KAL descarta de igual manera el síndrome de genes contiguos ya que ambos genes (KAL y STS) se encuentran ubicados en

dirección opuesta, es decir, el exón 10 del gen STS se continúa con el exón 14 del gen KAL. Esta deleción de los exones 1-3 pudiera explicarse por la presencia de dos eventos independientes de segregación para ambos genes, o bien, por un mecanismo muy especial de recombinación que condicionara una inversión del gen KAL para posteriormente producirse una deleción continua abarcando los exones mencionados. De cualquier forma, ambos pacientes representan casos muy interesantes de recombinación o segregación génicas que no habían sido reportados anteriormente en la literatura.

Los hallazgos encontrados en el presente estudio, resaltan la importancia de analizar entidades genéticas en nuestro medio. En el presente trabajo se observan grandes diferencias en comparación a lo descrito para otras poblaciones, así como características no informadas en la literatura. La relación de la 10:1 de la ILX sobre la IV es un dato completamente opuesto a lo informado en otras áreas geográficas, indicando un predominio de la ILX con respecto a la IV en nuestro medio. Sin embargo, también podría indicarnos que la relacion ILX:IV conocida anteriormente, pudo haber estado sesgada al no utilizar la actividad de la STS como estándar de oro en el diagnóstico diferencial de ambas entidades. Lo mismo parecería ocurrir ante la presencia de atopia e hiperlinearidad palmo-plantar con hiperqueratosis atribuidos únicamente a la IV y que fueron observados en la ILX en nuestra muestra. Los hallazgos clínicos de hernia inguinal en pacientes y sangrado transvaginal en portadoras informados en

este estudio, enfatiza la necesidad de realizar una buena semiología en el estudio de cualquier entidad y aporta nuevos datos que permitirán orientar más adecuadamente hacia el diagnóstico clínico de la ILX. Por otra parte, la dificultad de realizar un diagnóstico clínico correcto observada en este estudio obedece a la gran similitud entre la ILX e IV y a la carencia de datos nacionales acerca de ambos padecimientos. Otro dato interesante fue que 95% de los pacientes referidos por ictiosis fueron hombres, una característica que parecería ser distintiva de nuestro medio. La deleción total del gen STS observada en nuestro estudio fue más frecuente que la infomada previamente. Esta incidencia mayor (98%) refuerza la necesidad de llevar a cabo estudios en nuestra población. Finalmente, la asociación observada entre ILX y SK no fue debida a un síndrome de genes contiguos. En el primer caso, el gen KAL estaba presente sugiriendo una alteración probablemente autosómica o bien una mutación puntual. En el segundo caso se observaron los exones 4-14, lo que nos indicaría un evento poco frecuente de recombinación o bien la asociación de eventos independientes, ya que ambos genes se encuentran en dirección opuesta. En ambos casos la asociación de ILX y SK observada en la muestra analizada representó eventos poco frecuentes e interesantes no informados en la literatura.

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March 2, 1998

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RE: 97-273

Dear Dr. Cuevas-Covarrubias:

I am pleased to inform you that your paper, Higher Prevalence of
X-Linked Ichthyosis vs Ichthyosis Vulgaris in Mexico, has been
accepted for publication in the International Journal of Dermatology.

Future correspondence pertaining to the manuscript should be directed
to Will Wilcox, Blackwell Science Ltd, Osney Mead, Oxford OX2 OEL
United Kingdom to whom the enclosed copyright form should be returned.

Thank you for your interest in the International Journal of
Dermatology.

Sincerely,

Lawrence Charles Parish, MD

LCP:cjc

Enclosure

HIGHER PREVALENCE OF X-LINKED ICHTHYOSIS *vs* ICHTHYOSIS VULGARIS IN MEXICO.

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TO THE EDITOR

Dominant ichthyosis vulgaris (IV) and recessive X-linked ichthyosis (XLI) are the most frequent types of ichthyosis. Previous reports have shown a frequency of 1:250 newborn for IV (1,2) and 1:2000-6000 males for XLI (1,3), which represents an IV prevalence of 6-7:1 over XLI. Although both types of ichthyosis are the consequence of a different genetic defect, they share many clinical features (4). The most frequent molecular defect in XLI is a complete deletion of the steroid sulfatase (STS) gene (5), which codifies for the STS enzyme (EC 3.1.6.2). The determination of the enzymatic activity confirms XLI diagnosis (6). IV seems to be caused by a keratohyalin defect that may affect the matrix protein of the stratum corneum (7). However, the gene defect in IV has not been identified. In the present study we determined the prevalence of both types of ichthyosis in a sample of the Mexican population, comparing the results with those reported in the literature.

MATERIAL AND METHODS

Patients referred because of ichthyosis during 1993-1997 to Centro Dermatologico Pascua and Hospital General de Mexico were included in this study. The protocol was approved by the ethic committee of the Hospital General de Mexico. All patients were informed about the characteristics of the study and invited to participate. To identify the type of ichthyosis, STS assay in leucocytes using 7-[3H]-dehydroepiandrosterone sulfate as a substrate was performed as described elsewhere (8). Patients were classified as XLI when STS activity was no detectable. Conversely, a normal activity of the STS enzyme allowed to classify IV patients. In all cases amplification by PCR of the 5' and 3'ends of the STS gene was performed (9).

RESULTS

Sixty six patients (2 females and 64 males) were referred for ichthyosis and all of them were included in this study. In 60 male patients the STS activity was absent (0.0 pmol/mg protein/h), confirming the XLI diagnosis. Four males and the 2 females showed normal STS activity (1.2±0.3 pmol/mg protein/h), indicating that they had IV. Amplification of the 3'-5' ends of the STS gene was present in all IV patients but not in 59 of the 60 XLI patients. These results indicate an XLI prevalence of 10:1 over IV.

COMMENTS

Wells and Kerr (2) reported in caucasoid population an incidence of 1 per 6,190 males for XLI and 1 per 250 for IV (1:24 ratio). Okano et al., (4) reported a clinical comparison with 30 XLI and 32 IV patients (1:1 ratio) and Yoshiike et al., (10) reported the misdiagnosis of 16 XLI and 5 IV cases (3:1 ratio). This data could indicate that XLI in mongoloid population is more frequent than in caucasoid population. In this study, we found a higher ratio of XLI over IV (10:1) in the Mexican sample than that previously reported. This seems to be an special characteristic of both diseases in this region that could be attributed to ethnic factors. Other countries in Latin America should be analyzed to discard an analogous situation. Clinical diagnosis is very difficult between both types of ichthyosis and it is very important for dermatologists to know the prevalence of both diseases in different regions (4,10,11).

Although the possibility of a slope is present in the sample selection, this is low considering that both Centers are the main National Health Institutions for reference of dermatological affections. Besides, the study included a four year period which is enough to include a

considerable and representative number of patients and the severity of the diseases is similar for both types of ichthyosis and most patients ask for medical advise.

Another fact observed in this study was the low frequency of affected females, 2 in 64 patients. It is not clear, why males were more predominant than females in this study. These are interesting findings observed in both diseases in the Mexican population.

ACKNOWLEDGMENTS

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Accuracy of the Clinical Diagnosis of Recessive X-Linked Ichthyosis *vs* Ichthyosis Vulgaris

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Accuracy of the Clinical Diagnosis of Recessive X-Linked Ichthyosis *vs* Ichthyosis Vulgaris

Sergio A. Cuevas-Covarrubias, Susana H. Kofman-Alfaro, Angélica Beirana Palencia* and
Juan C. Díaz-Zagoya**

Abstract

The present study analyzes the accuracy of the clinical diagnosis of X-linked ichthyosis (XLI) *vs*
ichthyosis vulgaris (IV), in a sample of Mexican patients. The study was double blind, using
steroid sulfatase (STS) activity as the golden standard. Twenty male patients were included; 16
corresponded to XLI and 4 to IV. The clinical diagnosis was correct in 9 of the 16 XLI cases (56%)
and in 2 of the 4 IV cases (50%). Some clinical findings in XLI, such as cryptorchidism in patients
and delayed labor in their mothers, were important features for diagnosis. Statistical analysis of
the results showed: among physicians (n=2) Kappa value 0.50, specific concordance 0.40, and
absolute concordance 0.75; other values were sensibility 0.56, specificity 0.50, positive predictive
value 0.82, negative predictive value 0.22, accuracy 0.55, prevalence 0.80. In conclusion, the
differential diagnosis of XLI and IV is very difficult, and we consider that this is not explained
either by personal skills or by other conditions. It could be attributed to the similarities in skin
manifestations of these two diseases. The performance of the STS assay is imperative in order to
correctly diagnose the disease and offer adequate genetic counseling.

Key words: X-linked ichthyosis; ichthyosis vulgaris; human steroid sulfatase

Introduction

In 1976, Kerr et al. (1) classified the different
types of ichthyosis. In 1978, Shapiro et al. (2, 3)
demonstrated the steroid sulfatase (STS) defi-
ciency in X-linked ichthyosis (XLI). Since then,
several clinical features have been proposed to
distinguish among the types, especially between
XLI and ichthyosis vulgaris (IV), as both types
appear at a high frequency (4-6). IV shows
fine, white and irregular scales; the extremities
are usually more involved than the trunk.
Sometimes the palms and soles show hyper-
linearity and hyperkeratosis. XLI presents dark,
large, adhesive, and regular scales affecting the

extensor and flexor regions of the limbs. The
abdomen is more involved than the back, and
no palm or sole abnormalities are observed.
Corneal opacities and cryptorchidism in pa-
tients (7, 8) and delayed labor in mothers of
affected fetuses (9), are frequently associated
features. It is well known that XLI is due to STS
gene defect; 90% of the cases (10–12) present a
complete deletion of the gene, mapped in
Xp22.3, while 10% point show mutations or
partial deletions (13).

The clinical distinction between IV and XLI
can be very difficult, because their skin mani-
festations resemble each other. In fact, when
Cockayne (14) outlined XLI, he described
another type of ichthyosis, very similar to IV,
but present only in males. Nevertheless, there
are very few studies examining the difficulty of
an adequate clinical diagnosis (5, 15). The
present study was done double blind to try to
determine the accuracy of the clinical diagnosis
between XLI and IV by determining the STS
activity in leucocytes as a golden standard in a
sample of Mexican patients diagnosed as

Table 1.   Clinical criteria for XLI and IV diagnoses

	Inheritance	Incidence	Onset	Scale	Distribution	Associated features
IV	AD	1:300	3–12M	Fine, bran like flakes adherent	Extensor extremities hyperlinear palms/soles face spared	Keratosis pilaris, atopy
XLI	XL	1:2000	0–3M	Large, dark, adherent	Extensor and flexor extremities, trunk, lateral face, neck, palms and soles spared	Corneal opacities, cryptorchidism

IV=Ichthyosis vulgaris, AD=Autosomal dominant, XLI=X-linked ichthyosis, XL=X-linked, M=Months

having ichthyosis.

Material and Methods

The sample included only male patients referred with ichthyosis during 1993–1994 to the General Hospital and the Pascua Dermatological Center, both in Mexico City. Patients were examined by a dermatologist from each institution during the same season. Their ages at the moment of the examination ranged from 1 to 38 years old. None had received treatment before the initial clinical diagnosis, and all were Mexican mestizos living in Mexico City. The clinical criteria to classify XLI and IV were based on previous reports which included: familial pedigree, age of onset, affected areas of the skin, characteristics of the scales, and associated features (Table 1). Concordance between the dermatologists of both hospitals was calculated as the Kappa value. Patients were informed about the characteristics of the study and its implications and invited to participate in it. The study was evaluated and accepted by the ethics committee of the General Hospital of Mexico.

Definitive diagnosis of the patients and carrier mothers of XLI were determined by STS activity in peripheral leucocytes, using 7-[³H]-dehydroepiandrosterone sulfate as the substrate (NEN, Boston, Mass., USA), as described elsewhere (16, 17). Protein concentration was determined by the Bradford method (18). All the assays were done in duplicate, and a positive control was always included in each experiment.

Results

The differential diagnosis of IV and XLI was determined by STS assay. Clinical data of the patients and definitive diagnoses are shown

in Table 2. Of the 20 patients studied, 16 corresponded to XLI and 4 to IV. The clinical diagnosis was correct in 9 XLI (56%) and 2 IV (50%) cases. In XLI, six mothers had delayed labor, and 3 patients presented with cryptorchidism. Ten XLI and 3 IV cases were not familial cases. Various levels of skin alteration, from mild to severe, were present in both types of ichthyosis. The clinical concordance between the two physicians was moderate, showing a Kappa value of 0.50 with acceptable specific and absolute concordances of 0.40 and 0.75, respectively. Other statistical values of the clinical diagnosis were sensibility, 0.56; specificity, 0.50; positive predictive value, 0.82; negative predictive value, 0.22; accuracy, 0.55; and prevalence, 0.80.

Conclusion

XLI and IV share many clinical features; consequently, the correct diagnosis is difficult. Yoshiike et al. (15) found an accurate diagnosis for XLI and a low percentage of misdiagnosis for IV with a considerable percentage of uncertain cases (20%). Okano et al. (5) reported correct diagnosis in 61% for IV and 63% for XLI. In our study, it was observed that 50% of IV and 56% of XLI patients were correctly diagnosed. These data reflect the difficulty of differentially diagnosing these two diseases. The fact that climatic conditions can modify the skin manifestations in both types of ichthyosis complicates the appropriate diagnosis even more. All our patients have lived in Mexico City under the same weather conditions, which usually are temperate and dry. This affects the complex process of desquama-

Table 2. Differential diagnosis of IV and XLI

	In HGM	DX CDP	Familial pedigree	Carrier mother	DEL LAB	Genitalia	Onset	STS ACT	DEF DX
1	IX	IX	+	YES	+	NL	6M	0.0	IX
2	IX	IX	−	YES	−	NL	6M	0.0	IX
3	IX	IX	−	YES	+	CRYP, IH	6M	0.0	IX
4	IV	IV	+	YES	−	IH	1A	0.0	IX
5	IV	IX	−	YES	−	IH	4M	0.0	IX
6	IV	IV	+	YES	−	NL	3M	0.0	IX
7	IX	IV	−	YES	−	NL	1A	0.0	IX
8	IX	IX	+	YES	+	CRYP	2A	0.0	IX
9	IV	IX	−	ND	−	NL	RN	1.5	IV
10	IV	IV	+	YES	−	NL	9M	0.0	IX
11	IV	IV	−	NO	−	NL	1A	0.0	IX
12	IX	IX	−	ND	−	NL	2A	1.7	IV
13	IX	IV	−	YES	−	NL	1A	0.0	IX
14	IV	IV	−	NO	−	NL	8M	0.0	IX
15	IX	IX	+	YES	+	IH	RN	0.0	IX
16	IX	IV	+	ND	−	NL	6M	1.6	IV
17	IV	IV	−	YES	−	NL	4M	0.0	IX
18	IX	IX	−	YES	+	CRYP	3M	0.0	IX
19	IV	IV	−	ND	--	NL	8M	1.6	IV
20	IX	IX	−	NO	+	NL	RN	0.0	IX

DX=Diagnosis, GHM=General Hospital of Mexico, PDC=Pascua Dermatological Center, STS ACT=Steroid sulfatase activity, IX=X-linked ichthyosis, IV=Ichthyosis vulgaris, ND=Not determined, NL=Normal, CRYP=Cryptorchidism, IH=Inguinal hernia, NB= Newborn, A=annus, year, Del Lab=delayed labor. STS activity is reported in pmol/mg protein/h. Ichthyoses vulgaris are enclosed. Carrier mother was determined through the STS assay.

tion and skin involvement in both diseases. On the other hand, the concordance among our two institutions was acceptable, leading us to consider the importance of this kind of study in different populations. This also indicates that the difficulty of diagnosis is due to the clinical similarity of these two diseases and not to their different diagnostic interpretations.

Although the positive predictive value for the clinical diagnosis of XLI was good (0.82), the negative predictive value was very low (0.22) indicating the difficulty in discarding XLI. This could be explained, in part, because the prevalence of XLI (0.80) in this population differs from other samples in the literature. Instead of 1 XLI for 6 to 20 IV cases, as has been reported (19, 20), in this sample there were 4 XLI for 1 IV. This sample could have affected the

medical interpretation. A more appropriate approximation of the frequency of this entity must be done in the Mexican population. However, in 1975, Yoshiike et al. reported a similar prevalence with a low misdiagnosis (15). Cryptorchidism in patients and delayed labor in the mothers were considered important for the correct diagnosis of XLI. Pedigree analysis could be a very important tool for an adequate diagnosis, although in the present study, 13 of the subjects had no familial history of the disease. In conclusion, in the present study we corroborated that the correct clinical identification between XLI and IV is very difficult and that medical expertise is not the determinant. This argument is supported by the low accuracy and acceptable concordance observed in this study. Accurate diagnosis is only possible using

the STS assay. This enzymatic determination must be taken into consideration as a routine test in differential diagnosis between XLI and IV.

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Inguinal Hernia in Recessive X-Linked Ichthyosis

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Inguinal Hernia in Recessive X-Linked Ichthyosis

To the Editor: Recessive X-linked ichthyosis (XLI) is a genetic entity which affects the skin. Its principal features consist of dark and adhesive scales on the neck, trunk and extremities (1). Patients and carriers of XLI may have corneal opacities (2). Cryptorchidism in patients (3) and delayed labor with low serum steroid levels in carriers (4) are also reported.

Currently, we are interested in studying the clinical and biochemical features of XLI patients. We observed ten unrelated patients with XLI in the Genetics Department of the General Hospital of Mexico that had been referred for ichthyosis. The initial diagnosis was based on the characteristics of the scales and affected areas of the skin and confirmed through the steroid sulfatase (STS) assay, which was performed as described elsewhere (5) in leucocytes from the patients and their possible carrier mothers. The enzymatic activity was undetectable in all XLI cases (pmol ^{3}H-DHEA/mg protein/h) and was deeply reduced in nine of the ten mothers from the levels in normal female controls as previously reported (6). The carriers ranged from 0.17 to 0.25 pmol/mg protein/h, and the control average is reported to be 1.21 ± 0.23 pmol/mg protein/h. The STS assay confirmed the XLI diagnosis and identified the carrier state.

The clinical features in the XLI patients and a review of the medical reports of the pregnancy of their mothers showed the presence of inguinal hernia in three patients, two on the left and one bilateral, which is a higher incidence than in the normal population. These three cases needed surgical treatment and, in two patients, the inguinal hernia was the initial reason for requesting medical assistance. The medical reports showed that four mothers had transvaginal bleeding during the first trimester of their pregnancies, which is a very high incidence of this complication. In fact, complete rest was prescribed in two cases. Nevertheless, one of these mothers was not a carrier.

We postulate that the occurrence of transvaginal bleeding in the mothers during pregnancy and the presence of inguinal hernia in the patients must be considered when a XLI diagnosis is proposed.

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THE BIOCHEMICAL IDENTIFICATION
OF CARRIER STATE IN MOTHERS OF SPORADIC
CASES OF X-LINKED RECESSIVE ICHTHYOSIS

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Summary: *The biochemical identification of carrier state in mothers of sporadic cases of X-linked recessive ichthyosis:* X-linked recessive ichthyosis (XLI) is an inherited inborn error of metabolism due to steroid sulfatase (STS) deficiency. The STS activity was studied in 13 families that were referred to the Genetic Department, General Hospital of Mexico City, as being affected by ichthyosis. The study was specially focused on five apparently non familial cases and their mothers, in order to identify carrier status and provide adequate genetic counseling. STS activity was determined in leucocytes using 7-[3H]-dehydroepiandrosterone sulfate as substrate. None of the XLI patients showed STS activity (pmol/mg protein/h), four mothers had an activity compatible with a carrier state (0.19±0.02 vs 0.66±0.14 males or 0.90±0.30 females pmol/mg protein/h, p<0.001) and only one mother showed a normal pattern, indicating that her son had a de novo mutation. It is important to determine the STS activity in the propositus mother of apparently non familial cases of XLI to identify the carrier state and provide an accurate genetic counseling, as most of these seem to correspond to inherited cases.

Key-words: X-linked ichthyosis · Steroid sulfatase – Genetic counseling.

Résumé: *Identification biochimique du status de l'Ichtyose récessive liée à l'X chez les mères de cas sporadiques:* La forme d'ichtyose récessive liée à l'X (ILX) est une erreur innée du métabolisme causée par la déficience en sulfatase stéroidienne (SE). Treize familles avec ichtyose adressées au Département de Génétique de l'Hôpital Général de Mexique, ont été étudiées pour la détermination de la sulfatase stéroidienne. En particulier, notre intérêt s'est rapporté au niveau de cinq cas non familiaux et leurs mères, dans le but d'identifier les sujets porteurs et d'apporter un conseil génétique adéquat. L'activité de la SE a été déterminée dans les leucocytes en utilisant comme substratum le sulfate de 7-(³H)-dehydriepiandrosterone. Aucun des patients avec ILX a présenté activité de la SE (0.00 pmol/mg protéine/h), quatre mères ont présenté une activité compatible avec l'état de sujet porteur (0.19±0.02 vs 0.66±0.14 hommes ou 0.90±0.30 femmes pmol/mg protéine/h, p<0.001) et seulement une mère a eu un résultat normal qui indique que son enfant a présenté une mutation de novo. Dans les cas apparemment non familiaux de l'ILX, il est donc important de mesurer l'activité de la SE pour identifier les sujets porteurs et donner un conseil génétique, en considérant que la plupart des cas résultent de formes héréditaires.

Mots-clés: Ichtyose liée a l'X – Sulfatase stéroidienne – Conseil génétique.

INTRODUCTION

X-linked recessive ichthyosis (XLI) is an inherited inborn error of metabolism caused by steroid sulfatase (STS) defi-

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ciency. The main features of this syndrome are dark and adhesive scales on the trunk, extremities and neck, while palms, soles and face are generally spared (14,8). Cryptorchidism and corneal opacities in XLI patients and delayed labour as a clinical manifestation of an affected foetus are sometimes observed (18,3). Since 1978 (16), this entity was associated with the STS activity deficiency and the accuracy of the diagnosis has been possible through the enzymatic assay in sev-

eral cell lines (15,5,9) It is well known that the STS gene is one of the few genes that escapes X-inactivation (6). Nevertheless, females are indeed not exactly twice the value of males, as would be expected from the non-inactivation of the STS gene, but still higher in females. This suggests an incomplete inactivation of one of the two alleles (10,11). Otherwise, studies indicate a lower STS activity in carriers than in normal controls (7,12). The biochemical identification of the carrier status of the mother in XLI non familial cases is very important for genetic counseling. In addition, there are no previous studies exploring the possibility of carrier mothers in these cases. The aim of this study was to determine the STS activity in the propositus mother in non familial cases, to discard a carrier status in order to provide an adequate genetic counseling.

characteristics of the study and invited to participate in it. Their main clinical features are shown in Table I.

STS assay

Steroid sulfatase activity assay was determined in leucocytes (4,5). Ten ml of peripheral blood were obtained with a heparinized syringe from overnight fasting patients. Leucocyte fraction was obtained by centrifugation and washed three times with 0.9% NaCl. The cells were torn in chilled 0.014 mol/l buffer Tris, pH 7.0, with a polytron in two cycles of 20 and 10 s, respectively. The enzymatic assay was carried out in the leucocyte homogenate in a final volumen of 200 µl, with 33 nmol/l 7-[3H]-dehydroepiandrosterone sulfate as substrate (New England, Boston, Mass.) at 37°C for 1 h. The final

Table I: Main clinical features of the patients with XLI.

Patient	Age yr	Onset	Delayed labour	Cryptorchidism	scale	main affected areas
1	16	6m	yes	no	dark	legs, arms, back
2	14	8m	yes	yes	dark	legs, abdomen
3	19	4m	no	no	dark	back, legs
4	9	3yr	no	yes	dark	legs, abdomen
5	1*	NB	yes	no	dark	legs, arms, back

*cesarea.   NB = newborn.

MATERIAL AND METHODS

Patients

From 13 families that were referred to the Genetic Department, General Hospital of Mexico City, as being affected by ichthyosis, five corresponded to apparently non inherited cases. The analyses of the pedigree of these 5 families showed the absence of other affected members on three generations. Patients were informed about the

product of the hydrolytic enzyme was extracted adding 1 ml of bencene, vortexed 30 s and 0.6 ml were recovered from the upper phase to be read in a Beckman scintillation spectrometer. A blank without enzyme and samples from normal male and female controls and also from the possible carrier mother were always included in each patient assay. All samples were analyzed by duplicate. Protein concentration in the homogenate was determined by the Bradford method (1).

STS activity results are expressed as the mean ± SD and evaluated by Student's t-test for non paired samples.

RESULTS

The biochemical pedigree analyses of the five families are shown in figure 1. There were no maternal uncles and grandfathers affected by ichthyosis. The mothers of cases 1-4 presented 50% STS activity or less compared to male or

with the STS analyses of ten obligated XLI carrier mothers identified by familial pedigree (values ranged from 0.17 to 0.25 pmol/mg/protein/h).

CONCLUSION

X-linked recessive ichthyosis is one of the most frequent genetic entities affecting the skin and it is due to STS deficiency (16). Clinically it is similar to ichthyosis vulgaris (13) so, it is absolutely necessary
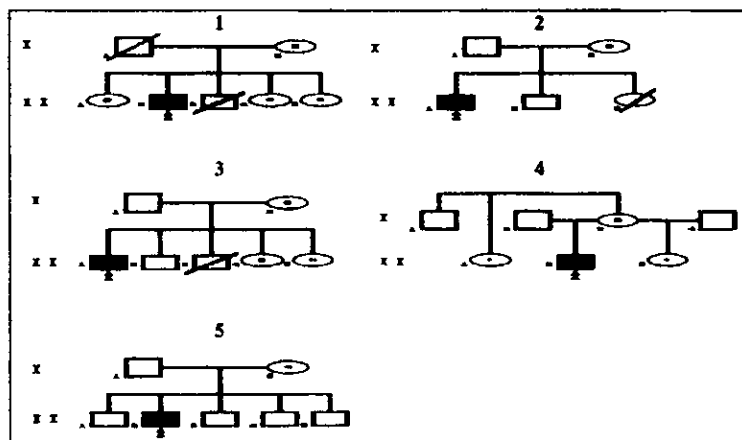

Figure 1: Biochemical pedigree analyses of the five apparently non familial cases. Arrows and closed squares indicate propositus, O STS activity with a carrier state, * normal STS activity, + STS activity not determined.

female controls (0.19± 0.02 vs 0.66±0.14 (males) or 0.90±0.30 (females) pmol/mg protein/h, p<0.001). Five of 7 daughters in the studied families could be analyzed (table II). A female in family 1 (II$_1$) and another in family 3 (II4) showed low STS activity as their mothers, confirming the existence of a carrier state (0.19 and 0.17 pmol/mg protein/h, respectively). In family 5 the mother showed a normal pattern of STS activity (1.51 pmol/mg protein/h). The accuracy of STS enzymatic assay to determine a carrier state was confirmed

to establish the differential diagnosis by the STS activity assay. In this study, five apparently non familial cases of XLI were analyzed and none of them showed STS activity. On the other hand, the STS activity of the propositus mother was also determined using the same assay, trying to identify their possible carrier status, as carriers present a much lower activity than the controls and do not overlap with those values found in normal women. The biochemical analyses of the five apparently non familial cases of XLI showed

...f Steroid sulfatase activities in the five cases of XLI

N°	Patient	Mother	Sisters+	Control	
				male	female
1	0.33¹	0.50	0.19⁺	0.4⁺	0.51
2	0.00	0.19	NS	0.74	0.66
3	1.00	0.17	0.17*	0.5⁷	0.73
4	0.00	0.21	ND	0.79	1.01
5	0.00	1.53**	NS	0.7⁶	1.9⁷

STS activity is expressed as pmol/mg protein/h *One carrier sister **Mother of the patient 5 showed normal STS activity ND = not done NS = no sisters. +Only 5 of 7 daughters were analyzed

that 4 mothers were carriers and could bear other affected boys or carrier daughters while the fifth had a normal STS activity with no family risk. Therefore, in this entity the hereditary pattern is a frequent condition and also the family risk must be taken into consideration principally in apparently non familial cases. This proved to be true in families 1 and 3 where two daugthers were found to be XLI carriers. On the contrary, in family 5 the mother showed a normal STS activity and only the patient had the enzymatic defect. The latter case represents a de novo mutation with a low risk for his parents to have other affected children, since germ line mosaicism is not excluded, and is a possibility (as for Duchenne muscular dystrophy). On the other hand, DNA studies of STS gene in several groups indicate a complete deletion of STS gene in approximately 90% of cases (2,17). Although, enzymatic assay is a reliable and non expensive method to identify correctly XLI and the carrier state, DNA studies must be performed to recognize gene deletions or gene mutations in this population. In conclusion, it is necessary to determine the STS activity in the XLI propositus, his mother and sisters to provide an adequate genetic counseling, specially in apparently non familial cases as they could also correspond to an inherited form.

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X-Linked Ichthyosis in Mexico: High Frequency of Deletions in the Steroid Sulfatase Encoding Gene

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The present study analyzes the frequency of molecular deletions in the steroid sulfatase (STS) encoding gene in a sample of 50 Mexican subjects with biochemical diagnosis of X-linked ichthyosis (XLI). To establish the correct diagnosis, STS activity was determined in leukocytes using 7-³H-dehydroepiandrosterone sulfate as the substrate. No amplification of the 3′ and 5′ ends of the STS gene by PCR was detected in the DNA of 49 patients, whereas only one sample of 50 presented a normal amplification. This report shows a very high frequency of deletions in the human STS encoding gene in a representative sample of the Mexican population, and it defines the characteristics of XLI in patients whose STS gene has a complete deletion as a major molecular defect. Am. J. Med. Genet. 72:415–416, 1997.
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KEY WORDS: human leukocytes; X-linked ichthyosis; STS gene deletion

INTRODUCTION

X-linked ichthyosis (XLI) is a disease characterized by dark, adhesive, and regular scales of skin, and it is present at birth or soon after birth [Shayder and Ott, 1991; Okano et al., 1988]. The primary defect of XLI is the deficiency of steroid sulfatase enzyme (STS), which hydrolyzes the 3-beta-hydroxysteroid sulfates [Shapiro and Weiss, 1978]. The gene locus is on Xp22.3 [Muller et al., 1981]. The STS enzyme deficiency is associated with an increase of cholesterol sulfate in the stratum corneum [Williams and Elias, 1981]. This defect appears to be the cause of the delay in the normal process of skin desquamation. ILX shares many clinical characteristics with ichthyosis vulgaris (IV), and differential diagnosis with this entity is difficult [Shayder and Ott, 1991]. The activity of the STS enzyme can be determined in leukocytes and other cell lines to establish the correct diagnosis [Epstein and Leventhal, 1981; Matsumoto et al., 1990]. Previous reports indicate a complete deletion of STS gene in 85–90% of XLI patients, while a few point mutations or intragenic deletions have also been reported [Yen et al., 1987; Bonifas et al., 1987; Shapiro et al., 1989; Basler et al., 1992]. The aim of this study was to identify the presence of STS gene deletions of XLI patients in a representative Mexican sample to determine their frequency.

METHODS

The sample included 50 XLI patients referred for ichthyosis to the General Hospital of Mexico. They were informed about the characteristics of the study and invited to participate in it. To exclude ichthyosis vulgaris, the definitive diagnostic test for XLI was undertaken to determine the STS activity in leukocytes as following: 10 ml of blood were obtained with a heparinized syringe. Leukocytes were obtained through centrifugation, and were washed three times with 0.9% NaCl. The STS assay was performed on the leukocyte pellet that was homogenized with a polytron in two cycles of 20 and 10 sec, respectively. 7-[³H]-dehydroepiandrosterone sulfate (NEN, Boston, MA) was used as enzyme substrate, and the product of hydrolysis was recovered with benzene and read in a scintillation spectrometer [Cuevas et al., 1995]. DNA extraction was performed through a conventional method [Lench et al., 1988]. The 5′ and 3′ extremes of the STS gene were amplified through PCR, using the following conditions with a PCR amplification kit (Perkin Elmer, San Francisco, CA): 30 cycles were carried out for 1 min at 94°C, for 30 sec at 60°C annealing, and for 2 min at 72°C extension. The primers were: 5′ STS gene primers, F-5′GGCCTAGAAGAAGGTTGAAGGTCCC, and R-5′AAGAGGTTGGATGAGATGGGCATAC; and 3′ STS gene primers, F-5′GAAATCCTCAAAGTCATG-
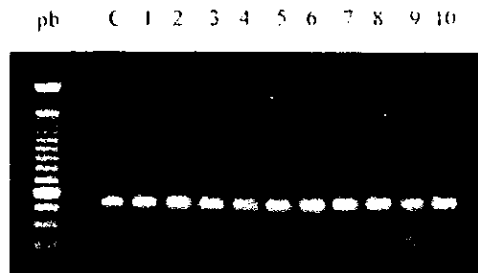
ph   C   1   2   3   4   5   6   7   8   9   10

Fig. 1 Amplification of the 5' and 3' ends of the steroid sulfatase gene in 10 patients of the 50 analyzed. A fragment of the Duchenne muscular dystrophy gene (upper band) was used as an internal control. Only patient 9 showed normal amplification of the 5' and 3' ends of the steroid sulfatase gene as the control male (lane C).

CAGGAAG, and R 5'CCTCCAGTTGACTAGCTGTT GAGCT. The amplification of a fragment of the Duchenne muscular dystrophy gene was used as an internal control [Ballabio et al., 1990]

RESULTS AND DISCUSSION

None of the 50 patients showed STS activity (0.0 pmol/mg protein/hr). The DNA from 49 XLI patients showed no STS amplification of the 5' and 3' ends of the STS gene. Only one of 50 patients (2%) presented normal amplification of these segments (Fig. 1). These data show that the STS gene deletion is the main cause of XLI at a molecular level in the Mexican population. This type of analysis is necessary in Latin America to determine the frequency of STS gene deletions along with the prevalence of XLI relative to the other ichthyoses. In previous studies which included populations from several geographic areas, complete deletion of STS gene was present in about 85–90% of the cases. In this study, 98% of XLI patients had a complete STS deletion. This is a higher percentage than that previously observed. This means that a point mutation or an intragenic deletion may be present in only one out of 50 XLI patients, a lower frequency than that reported for other populations [Bonifas et al., 1987; Shapiro et al., 1989; La 1992; Fan et al., 1993]. The patient with normal amplification of STS gene ends is being analyzed to

determine the molecular defect. DNA amplification is a quick and easy test to perform for a correct diagnosis. Amplification of the STS gene does not rule out the XLI diagnosis, and in these cases an STS enzyme assay must be done to be sure of the diagnosis.

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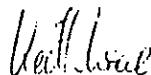CP 06725 Mexico

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Contiguous Gene Syndrome Due to Deletion of the First Three Exons of the KAL Gene and Complete Deletion of the STS Gene

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short title: Contiguous gene syndrome

SUMMARY

BACKGROUND AND OBJECTIVE Large terminal or interstitial deletions of the 22.3 region, of the short arm of the X chromosome cause contiguous gene syndromes. Kallmann syndrome (hypogonadotropic hypogonadism + anosmia or hyposmia) associated with X-linked ichthyosis, due to a contiguous gene syndrome, is an uncommon finding. Genetic defects have been demonstrated on the Xp22 region explaining the presence of one or both entities in affected individuals. In this report we describe the molecular findings of a patient with Kallmann syndrome and X-linked ichthyosis.

PATIENT A 20 year old subject with hypogonadism, anosmia and generalizad ichthyosis was studied endocrinologically, biochemically and molecularly.

MEASUREMENTS Levels of LH, FSH, GH, testosterone, estradiol and cortisol were determined basally and after specific stimulation tests. Enzymatic activity of steroid sulphatase was measured in leucocytes. Polymerase chain reaction of the 14 exons of the Kallmann gene and of the 5′ and 3′ extremes of the steroid sulphatase gene was performed in genomic DNA.

RESULTS A partial deletion from exon 1 to exon 3 of the Kallmann gene, as well as a complete deletion of the steroid sulphatase gene were observed.

CONCLUSION A patient bearing a contiguous gene syndrome with a partial deletion of the Kallmann syndrome gene and a completed deletion of the steroid sulphatase gene is described. This is the first time a mutation in the conserved cysteine rich N-terminal region which corresponds to the whey acidic protein motif of the Kallmann gene is characterized, thus demonstrating the importance of this specific region for the function of the gene.

INTRODUCTION

Large terminal or interstitial deletions of the 22.3 region, on the short arm of the X chromosome, have been described in patients affected with various associations of several diseases, so called contiguous gene syndromes (Bouloux et al., 1993; Meindl. et al., 1993; Klink et al., 1994; Martul et al., 1995). Genes responsible for these diseases have been ordered from the telomere to the centromere: short stature, chondrodysplasia punctata, mental retardation, X-linked ichthyosis (XLI) due to steroid sulphatase (STS) deficiency and Kallmann syndrome (KS) (Ballabio et al., 1989; Petit et al., 1990; Ballabio & Andria, 1992).

Kallmann syndrome is characterized by the association of hypogonadotropic hypogonadism caused by GnRH deficiency and anosmia or hyposmia. This disorder is due to a neuronal migration defect which involves both the olfactory and the GnRH producing neurons (Hardelin et al., 1993a). Migration of both olfactory axons and GnRH neurons is arrested within the meninges above the cribriform plate (Rugarli et al., 1996) . KAL gene spans 210kb of genomic DNA in Xp22.3 and has 14 coding exons, escapes X inactivation and has a non-functional homologue at Yqll.2 (Franco et al., 1991; Legouis et al., 1991). Mutations of the human KAL gene which cause the X-linked form of KS have been described in exons 4-14 (Ballabio et al., 1989; Franco et al., 1991; Bick et al., 1992; Hardelin et al., 1992; Boulox et al., 1993; Hardelin et al., 1993a; Hardelin et al., 1993b; Meindl et al., 1993; Klink et al., 1994; Paige et al., 1994; Martul et al., 1995; Pareti et al., 1995). Although different inheritance patterns have been described in KS, indicating the involvement of several genes, the 5 to 7 fold excess of affected males versus females suggested that the X-linked inheritance pattern was the most frequent (Pawlowitzki et al., 1987). However, recent studies have demonstrated that the X-linked form of the disease accounts for the minority of patients and that most cases are presumably due to mutations in autosomal genes, thus indicating the genetic heterogeneity of the disease (Waldstreicher et al., 1996; Georgopoulos et al., 1997).

XLI is caused by STS deficiency (Cuevas et al., 1995). It is caracterized by an early onset with dark, dry and irregular scales affecting limbs and trunk. The enzymatic defect can be recognized by decreased STS activity in various cell lines (Shapiro, 1983).

Here we report the molecular characterization of a deletion which involved the entire STS gene and only the first 3 exons of the KAL gene, in a patient who presented Kallmann syndrome and ichthyosis.

SUBJECT AND METHODS

The patient was admitted to the hospital at the age of 20 years. He was the product of an uneventful pregnancy and had seven brothers and one sister. Since early childhood, generalized ichthyosis and anosmia were present. Psychomotor development was considered normal. During adolescence there was no pubertal development. Physical Examination showed short stature (139 cm) generalized ichthyosis, absence of secondary sexual characteristics, bilateral cryptorchidism and a small penis (length 3.0 cm). Olfactory tests (Rosen et al., 1979) revealed the presence of anosmia. No ocular albinism or skeletal abnormalities were found. The karyotype was 46, XY. An intravenous pyelogram showed no renal malformations and computed tomography of the hypothalamic-pituitary region was normal. Apparently, one brother who lives in the United States, 25 years old, presents anosmia, hypogonadism and ichthyosis; however, he has a normal stature. Both parents and all the other sibs are normal, their stature is above the third centile for Mexican population; neither ichthyosis nor hypogonadisin was detected in any of them.

METHODS

Endocrinological Studies

Baseline plasma levels of LH, FSH and GH were measured by specific double-antibody radioimmunoassays. Reagents for analysis of LH, FSH, testosterone (T) and estradiol (E,) were kindly provided by the Special Program on Research, Development, and Research Training in Human Reproduction, WHO (Geneva, Switzerland). Reagents for GH analysis were purchased in New England Nuclear (Boston, MA, USA) and from NIH (Bethesda, MD, USA). Plasma concentrations of LH and FSH are expressed as IU/1 according to the First International Reference Preparation. Cortisol was measured using reagents purchased in Amersham International (Buckinghamshire, England) and in Radioassay System Laboratories (Carson, CA, USA).

GH Secretion Test: Clonidine (0.15 mg/ml body surface area) was administered and GH was quantitated every 15 to 30 minutes for 3 hours.

GnRH Stimulation Test: The LH and FSH response to GnRH (100 ug i.v.) was determined every 15 minutes for 2 hours, after four days of GnRH priming (100 ug daily).

hCG Stimulation Test: The patient received 2500 units of hCG (Gonadotropil C, Roussel, México) intramuscularly every 24h for 4 consecutive days. Serum T was measured before, during and after gonadal stimulation.

High Resolution Banding

High resolution banding was performed by conventional methods.

Specific Enzymatic Investigation

Leukocyte concentrate was obtained from the patient and controls to determine steroid sulphatase activity. The assay was performed as previously described (Cuevas et al., 1995). Briefly, I0 ml of blood were drawn with a heparinized syringe. The leukocyte fraction was obtained through

centrifugation and washed three times with 150mM NaCl. The STS assay was performed in a leukocyte pellet that was homogenized with a polytron in two cycles of 20 and 10 seconds respectively. 7-[3-H]-dehydroepiandrosterone sulphate (New England Nuclear, Boston, MA, USA) was used as enzymatic substrate and the product of the hydrolisis was recovered with bencene and read in a scintillation spectrometer.

DNA Extraction and PCR Analyses

Genomic DNA was prepared from peripheral blood leukocyte by standard techniques (Sambrook et al., 1989). For each RNA amplification, genomic DNA (0.5-1.0 pg) in the presence of 0.1 mM DNTP, 2U Taq DNA polymerase (Ampli Taq, Perkin Elmer Corp., NJ, USA) and 250 nM of each specific set of KAL primers was used. The sequences of the KAL primers and splice site junctions, the sizes of the amplified products, as well as the PCR conditions were previously described by Hardelin et al. (1993a); concentrations were slightly modified. Thirty cycles of PCR amplifications were performed in a Thermal Cycler with denaturation at 94°C for 1 minute, annealing at 55-63°C for 1 minute and extension at 72°C for 1 minute. 5' and 3' extremes of the STS gene were amplified with a PCR amplification kit (Perkin Elmer Corp., NJ, USA). Thirty cycles were carried out with denaturation at 94'C for 1 minute, annealing at 60°C for 30 seconds and extension at 72°C for 2 minutes. The primers used and the PCR conditions are detailed elsewhere (Ballabio et al., 1990). The amplification of a fragment of the Duchenne muscular dystrophy gene was used as an internal control (Ballabio et al., 1990). After amplification, PCR products were electrophoresed on 1% agarose gels stained with ethidium bromide in order to verify the correct size of the expected fragments.

RESULTS

Endocrinological Investigations: After GnRH priming, LH responded from 1.2 IU/l to 7.8 IU/l, whereas FSH only augmented from 0.2 IU/l to 2.8 IU/l. Serum T showed a slight response (considering the low basal values) from 1.28 nmol/1 to 5.78 nmol/1 after the hCG challenge. GH showed normal results to clonidine stimulation demonstrating a peak of 57 ug/l. Basal concentrations of cortisol and E2, were within normal ranges.

High Resolution G Banding: 100 metaphases were analyzed. No numerical, nor structural abnormalities were observed.

STS Activity: Steroid sulphatase activity was absent in the patient.

DNA Analyses: A complete deletion of the STS gene was observed in the patient due to no amplification product of the 5' and 3' extremes of the gene (Figure 1). The KAL gene presented a deletion which comprises from exon 1 to exon 3; the remaining exons of the gene amplified in a normal fashion (Figure 2).

DISCUSSION

In this study we present a patient with a contiguous syndrome due to a partial deletion of the KAL gene and a complete deletion of the STS gene.

Clinical features of KS (hypogonadotropic hypogonadism + anosmia) and XLI were evident during physical examination. The low basal levels of gonadotropins and T, as well as the LH and FSH response to GnH demonstrated the hypothalamic origin of the hypogonadism (Spratt et al., 1988).

The poor response, in terms of T, to the hCG challenge should be attributed to the long history of cryptorchidism causing a primary testicular failure (Griffin & Wilson, 1992). The diagnosis of ichthyosis corroborated by the absence of STS activity (Cuevas et al., 1995).

The distal short arm of the X-chromosome undergoes chromosomal rearrengements at a high rate, resulting most commonly in interstitial deletions involving the STS gene, causing isolated XLI (Ballabio & Andria, 1992). In some instances deletions or unbalanced translocations of the Xp22.3 region may span other adjacent genes originating the so-called contiguous gene syndromes. A contiguous gene syndrome involving the STS and KAL genes is not a frequent finding. A complete deletion of both genes has been reported previously in six different families (Ballabio et al., 1989; Boulox et al., 1993; Meindl et al., 1993; Klink et al., 1994; Paige et al., 1994; Martul et al., 1995); whereas Parenti et al. (1995) , described 3 sibs in whom a complete deletion of the STS gene and a partial deletion of the KAL gene was the cause of the disorder. In our patient, we found a complete deletion of the STS gene and a partial deletion of the KAL gene involving exons 1 to 3. This is an unexpected finding since both genes (STS and KAL) run in opposite directions, KALL gene is proximal to the STS gene with its 3' end oriented to the telomere (Parenti et al., 1995). We consider that this rearrengement could be due to an uneven recombination. It is noteworthy that this is the first report in which a deletion (or a point mutation) has been observed exclusively in this region of the KAL gene. All other mutations previously described (Ballabio et al., 1989; Franco et al., 1991; Bick et al., 1992; Hardelin et al., 1992; Boulox et al., 1993; Hardelin et al., 1993a; Hardelin et al., 1993b; Meindl et al., 1993; Klink et al. 1994; Paige et al., 1994; Martul et al., 1995; Parenti et al., 1995; Georgopoulos et al., 1997) have been located from exon 14 to exon 4, excepting Parenti's where the deletion comprised from exon 14 to exon 2. All genetic defects identified to date, occur within the region encoding the four fibronectin type III repeats of the KAL protein; whereas our patient had the mutation in the conserved cysteine-rich N-terminal region which corresponds the whey acidic protein motif (Franco et al., 1991;

Georgopoulos et al., 1997). Our finding demonstrates that a deletion in this region is sufficient to cause the complete loss of function of the KAL gene.

The absence of mental retardation and chondrodysplasia punctata, as well as the normality of the X-chromosome by high resolution G banding, showed that the contiguous gene syndrome in this patient was limited to the KAL and STS genes. The origin of the short stature observed in the patient can't be ascertained by this study, due to the excellent response of GH to clonidine and the absence of chromosomal abnormalities.

In conclusion, this is the first case in which a contiguous gene syndrome includes the STS gene and only the first 3 exons of the KAL gene, genetic region in which no mutations had been previously observed, thus demonstrating the importance of this region for the normal function of the KAL gene.

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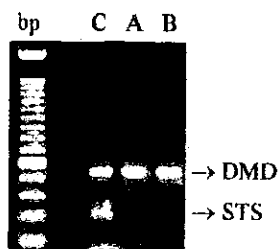
FIGURE LEGENDS

Figure 1- PCR amplification of the 5' and 3' extremes of the STS gene in DNA from a normal control (C), an individual with X-linked ichthyosis (A) and the patient with the contiguous gene syndrome (B)   Amplification of a fragment of the Duchenne Muscular dystrophy gene (DMD) was used as an internal control Deletion of the STS gene is observed in the individual with X-linked ichthyosis as well as in the patient.
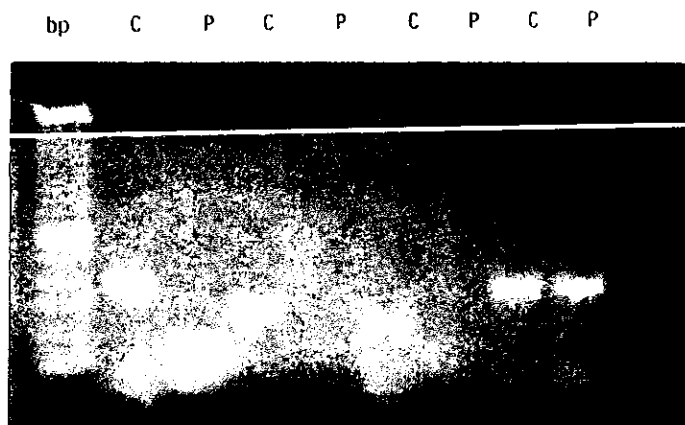

Figure 2- PCR amplification of exons 1-4 of the KAL gene in DNA from the patient (P) and a normal control (C) Deletion of the first 3 exons are observed in the patient's DNA.