Mutations in the Human Sterol Δ^7-Reductase Gene at 11q12-13 Cause Smith-Lemli-Opitz Syndrome

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Summary

The Smith-Lemli-Opitz syndrome (SLOS; also known as "RSH syndrome" [MIM 270400]) is an autosomal recessive multiple malformation syndrome due to a defect in cholesterol biosynthesis. Children with SLOS have elevated serum 7-dehydrocholesterol (7-DHC) levels and typically have low serum cholesterol levels. On the basis of this biochemical abnormality, it has been proposed that mutations in the human sterol Δ^7-reductase (7-DHC reductase; E.C.1.3.1.21) gene cause SLOS. However, one could also propose a defect in a gene that encodes a protein necessary for either the expression or normal function of sterol Δ^7-reductase. We cloned cDNA encoding a human sterol Δ^7-reductase (*DHCR7*) on the basis of its homology with the sterol Δ^7-reductase from *Arabidopsis thaliana,* and we confirmed the enzymatic function of the human gene product by expression in SLOS fibroblasts. SLOS fibroblasts transfected with human sterol Δ^7-reductase cDNA showed a significant reduction in 7-DHC levels, compared with those in SLOS fibroblasts transfected with the vector alone. Using radiation-hybrid mapping, we show that the *DHCR7* gene is encoded at chromosome 11q12-13. To establish that defects in this gene cause SLOS, we sequenced cDNA clones from SLOS patients. In three unrelated patients we have identified four different mutant alleles. Our results demonstrate both that the cDNA that we have identified encodes the human sterol Δ^7-reductase and that mutations in *DHCR7* are responsible for at least some cases of SLOS.

Introduction

The Smith-Lemli-Opitz syndrome (SLOS; also known as "RSH syndrome" [MIM 270400]) was first described in 1964 by Smith et al. (1964). Children afflicted with this syndrome have dysmorphic facial features, mental retardation, hypotonia, poor growth, and variable structural anomalies of the heart, lungs, brain, gastrointestinal tract, limbs, genitalia, and kidneys (Smith et al. 1964; Gorlin et al. 1990). Elevated levels of 7-dehydrocholesterol (7-DHC) are characteristic of this disorder (Irons et al. 1993; Natowicz and Evans 1994; Tint et al. 1994; Kelley 1995). The SLOS phenotype can be variable. Both a "mild" (type I) and a "severe" (type II) form have been described (Bialer et al. 1987; Curry et al. 1987; Gorlin et al. 1990). It is thought that both types represent a spectrum of the same disorder. Children afflicted with either the type I form or the type II form have elevated levels of 7-DHC (Tint et al. 1995; Cunniff et al. 1997). In cholesterol biosynthesis, 7-DHC is converted to cholesterol by the enzyme sterol Δ^7-reductase. This NADPH-dependent enzyme catalyzes the reduction of the Δ^7-diene bond in 7-DHC, to form cholesterol. Liver microsomes from a SLOS homozygote have been shown to have reduced activity of this enzyme (Shefer et al. 1995). On the basis of observations of increased serum 7-DHC, decreased serum cholesterol levels, and deficient sterol Δ^7-reductase activity, we and others have hypothesized that the genetic defect causing SLOS is in the gene encoding the sterol Δ^7-reductase. However, theoretically, the genetic defect could also be either in a cofactor or transporter necessary for sterol Δ^7-reductase activity or in a transcription factor necessary for efficient expression of this enzyme.

We report both the cloning of a cDNA that encodes the human sterol Δ^7-reductase and the identification of mutant cDNAs in SLOS patients. Human sterol Δ^7-reductase is homologous to a sterol Δ^7-reductase from *Arabidopsis thaliana,* to fungal sterol Δ^{14}-reductases, to fungal sterol Δ^{24}-reductases, and to the β-lamin receptor family. The gene encoding sterol Δ^7-reductase maps to

chromosome 11q12-13. We have identified four separate mutant alleles from three unrelated SLOS patients. In one patient, we have identified both mutant alleles, and another patient may be homozygous for the identified mutation. Two of the identified mutations are single-base insertions, and a third is a 134-bp insertion; these insertions introduce frameshifts. The fourth mutation is a 96-bp deletion. To our knowledge, these are the first reported mutations in SLOS, and identification of these mutant alleles establishes that mutations in the sterol Δ^7-reductase gene cause SLOS.

Subjects and Methods

Patients and Cell-Culture Methods

Institutional-review-board approval was obtained for this research, and informed consent was obtained for the skin biopsies. The A2SLO and B3SLO cell lines used in this study were obtained from patients who were clinically diagnosed with SLOS, by R.D.S. and F.D.P. Both patients had elevated serum 7-DHC levels and were severely affected (type II). Neither had a family history of consanguinity. One cell line (GM03044) was obtained from the National Institute of General Medical Sciences (NIGMS) Human Genetic Mutant Cell Repository. The available clinical description of this patient is limited; however, the presence of ambiguous genitalia and diagnosis at age 2 wk suggest a severe case of SLOS. Elevated 7-DHC levels have been found in this cell line. Control cell lines (GM05659C and GM03468A) were also obtained from the NIGMS Human Genetic Mutant Cell Repository. Skin fibroblasts were propagated in Dulbecco's minimal Eagle medium and 10% fetal bovine serum (37°C, 5% CO_2). Cholesterol-deficient conditions for growth were created with McCoy's media supplemented with 5% lipoprotein-deficient serum (35 mg protein/ml, <12 fg cholesterol/ml; PerImmune). SV40 transformation of SLOS fibroblasts was as described elsewhere (Lei et al. 1992). For expression studies of the sterol Δ^7-reductase cDNA, the open reading frame of the *DHCR7* gene was amplified by PCR (Clonetech Advantage-HF PCR kit) and was subcloned into pIRES1neo (Clonetech). SV40-transformed fibroblasts were transfected with Lipofectamine (Gibco BRL) according to the manufacturer's protocol. Initial selection of clones was done with 400 μg G418/ml. Clones were subsequently maintained in 200 μg G418/ml.

Sterol Analysis of Cell Pellets

The cell pellets were frozen and thawed twice. Lipids were then extracted as described by Folch et al. (1957). After saponification and extraction, the TMS derivatives of the cell sterols were prepared and subjected to gas-liquid–chromatography analysis (Lin et al. 1993). The gas-liquid chromatograph (Perkin-Elmer model 8500) was equipped with a 25-m × 0.32-mm (inner diameter) CP-WAX-57 CB capillary column (Chrompath). The temperatures of the column, detector, and injection port were 210°C, 250°C, and 250°C, respectively. Helium was used as the carrier gas, and cholestane was used as an internal standard. Protein content of the samples was determined by the method of Lowry et al. (1951).

Cloning of the cDNA, and Mutation Analysis

A human EST (IMAGE Consortium Clone ID704713) was labeled with $\alpha[^{32}P]$-dCTP, by means of a Boehringer Mannheim High Prime DNA Labeling Kit. This probe was then used to screen a Human Liver 5'-Stretch cDNA library (Clontech). Multiple clones were isolated. Sequencing was performed both by means of Amersham T7 Sequenase version 2.0, with gene-specific primers, and by automated sequencing on a Pharmacia ALFexpress using Cy5-labeled M13 universal and reverse primers. For mutation analysis, reverse transcriptase–PCR (RT-PCR) (Titan One Tube RT-PCR System; Boehringer Mannheim) was used to amplify overlapping fragments of the coding sequence. The mRNA encoding the sterol Δ^7-reductase was prepared from fibroblasts grown for 7 d in cholesterol-depleted media, by means of a Qiagen RNeasy Mini Kit. 5' PCR primers with *Xba*I ends (5'-AG<u>TCTAGA</u>-3') were as follows: HSP1 (5'-TCT AGA TGG CGT CAC CAA TGA C-3'), HSP3 (5'-CCC AGC TCT ATA CCT TGT GG-3'), HSP5 (5'-CTT GGC TAT GCC GTC TCC ACC-3'), HSP7 (5'-CCA TGT GAC CAA TGC CAT GG-3'), HSP9 (5'-ACC ACC AGA AGG ACC TGT TC-3'), and HSP11 (5'-TCA ACT ACG TCG GCG ACC TG-3'). 3' PCR primers with *Eco*RI ends (5'-CG<u>GAATTC</u>-3') were as follows: HSP2 (5'-ATG GCA AGA AGT CAG GGA GAG-3'), HSP4 (5'-CTG TGA AAT TGC AGT CTC TGG-3'), HSP6 (5'-CAT GGC AGA TGT CAA TGG TC-3'), HSP8 (5'-GTC ATC GAG TGC TCC TAC AC-3'), HSP10 (5'-TGT AGA AGT AGG GCA GCA GG-3'), and HSP12 (5'-CCT GGC AGG AAC ACG CTC TTG-3'). Primer pairs included HSP1/HSP2, HSP3/HSP4, HSP5/HSP6, HSP7/HSP8, HSP9/HSP10, HSP11/HSP12, HSP1/HSP8, HSP3/HSP8, and HSP7/HSP12. The reverse-transcriptase reaction was done at 50°C for 20 min, followed by 10 min at 55°C. The PCR-amplification conditions included an initial denaturation at 94°C for 2 min; denaturation between cycles, at 94°C for 30 s; annealing steps of 10 cycles at 60°C and 25 cycles at 62°C; and extension at 68°C, as recommended in the Titan RT-PCR protocol. The PCR products were then subcloned into either pCR2.1 (Invitrogen), by TA cloning, or pBlueScript SK (Stratagene). All mutations were confirmed in clones obtained from at least two independent RT-PCR reactions. Homology analysis was performed by means of BLAST

computer-based analysis (Altschul et al. 1990, 1997). Protein sequences were aligned by means of the GCG Pileup program (Wisconsin Package version 9.1-UNIX).

Radiation-Hybrid Mapping

A GeneBridge 4 radiation hybrid–mapping panel (Research Genetics), which consists of 93 radiation-hybrid clones, was screened by PCR analysis using primers specific for the *DHCR7* gene. The primer pair (5′-GCC TGG GCT CCT AAT ACAG-3′ and 5′-CAA GAG CGT GTT CTG CCA GG-3′) amplified the expected 286-bp fragment of the 3′ UTR, without cross-amplification from the hamster recipient DNA. The reactions were performed under standard buffer conditions (Perkin Elmer Cetus) and with 25 μg of hybrid DNA in a 10-μl total volume. The amplification conditions consisted of an initial denaturation at 94°C for 5 min; followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min. The final PCR products were separated and visualized on a 1% agarose/ethidium bromide gel and were scored for the presence or absence of the appropriate product. The results were confirmed in a separate set of reactions under identical conditions, with a second primer pair that amplifies a 167-bp fragment from the coding region (5′-CCC ATC GGC GGA TAT GTA GG-3′ and 5′-TGC AGC TGT CCA CCC CGC AC-3′). The results were submitted to the server at the Whitehead/MIT Center for Genome Research, for analysis. The computed results were returned and showed placement of the *DHCR7* gene relative to the framework markers on the radiation-hybrid map. The result was correlated with the chromosome genetic map, by identification of markers flanking the *DHCR7* locus that were common to both maps. The cytogenetic position of the locus was then inferred from the genetic map, given the relative physical positions of the flanking markers.

Results

On the basis of the predicted amino acid sequence of an *A. thaliana* enzyme that encodes a sterol Δ^7-reductase activity (Lecain et al. 1996), we cloned, by homology, a cDNA that encodes the human sterol Δ^7-reductase. The predicted amino acid sequence of the plant enzyme was used to identify a human expressed sequence tag (EST) (*AA282412,* tonsillar B-lymphocytes; IMAGE Consortium clone ID704713) that had significant homology. We used this human EST to isolate longer cDNA clones from a human liver library. The longest cDNA clone that we isolated was 1,852 nucleotides in length and encoded a 417-amino-acid open reading frame (fig. 1). This cDNA clone is truncated at an internal *Xba*I re-
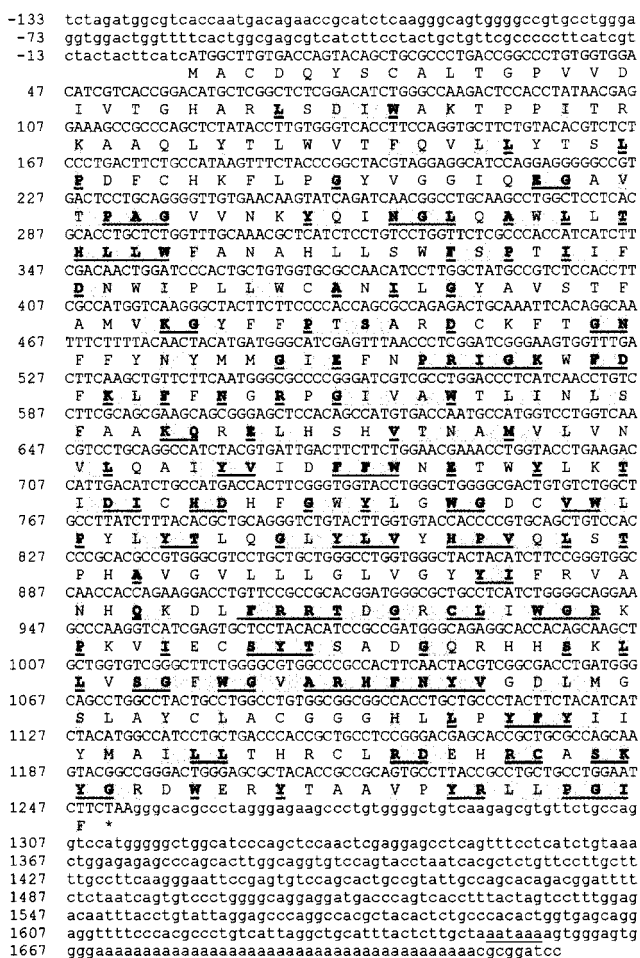

Figure 1 Nucleotide and amino acid sequence of clone pH7DHCR encoding the human sterol Δ^7-reductase. Amino acids that are identical between the human cDNA and the *A. thaliana* cDNA are underlined and are in boldface type. Amino acids that are similar are gray shaded (BLASTP 2.0.4). A putative polyadenylation signal in the 3′ UTR is underlined.

striction site. Thus, the mRNA potentially encodes a longer peptide. At the amino acid level, the predicted protein has 37% identity and 55% similarity (BLASTP 2.0.4; Altschul et al. 1997) to the *A. thaliana* enzyme. Although the highest sequence homology was found with the functionally similar *A. thaliana* sterol Δ^7-reductase, the predicted peptide also has significant homology to fungal sterol Δ^{14}-reductases (GenBank accession numbers Y10624, S30769, S44170, and X94315), to the carboxyl-terminal portions of the human and rat β-lamin receptors (GenBank accession numbers L25941 and AB002466), and to fungal sterol Δ^{24}-reductases (GenBank accession numbers S64014 and A43765) (fig. 1, and data not shown). Neither the human nor the plant sterol Δ^7-reductase has the EFGGXXG motif conserved in the sterol Δ^{14}-reductases, in the sterol Δ^{24}-reductases,

and in the β-lamin receptors (Lecain et al. 1996). Thus, on the basis of homology, this gene likely encodes the human sterol Δ^7-reductase. While the manuscript of this article was in preparation, Moebius et al. (1998) reported an independent cloning of this gene. They showed the presence of sterol Δ^7-reductase activity when this gene was expressed in *Saccharomyces cerevisiae*. Mutational analysis in SLOS was not reported. This work provides further conformation that this cDNA that we identified encodes human sterol Δ^7-reductase.

Cholesterol biosynthesis in cells is highly regulated, and transcription of cholesterol biosynthetic enzymes is down-regulated when exogenous LDL cholesterol is available. Using northern blot analysis, we compared the expression of the human sterol Δ^7-reductase in fibroblasts grown with and without an exogenous source of LDL cholesterol. Consistent with an enzyme involved in cholesterol biosynthesis, the mRNA encoding this protein is induced when fibroblasts are grown in LDL cholesterol–deficient media (fig. 2). No marked differences were observed between control lines and SLOS lines (fig. 2, and data not shown).

To prove that this cDNA encodes a sterol Δ^7-reductase, we cloned this cDNA into an expression vector, pIRES1neo, and stably transfected this construct into two SV40-transformed SLOS fibroblast lines (A2SLO-T and B3SLO-T). This vector (pIRES1neo8) constitutively expresses the putative sterol Δ^7-reductase cDNA, under control of the human cytomegalovirus major, immediate, early promotor/enhancer. Using gas chromatography, we measured sterol levels in cell lines expressing the sterol Δ^7-reductase cDNA, and we compared these levels with levels of 7-DHC in cell lines transfected with the vector alone. After 5 d of growth in lipoprotein-deficient serum, cholesterol levels increased to 166% of control levels, and 7-DHC levels decreased to 45% of control levels (table 1). The 7-DHC/cholesterol and 7-DHC/total-sterol ratios were decreased, respectively, to 26% and 38% of control levels. In a similar experiment, with the B3SLO cell line, the 7-DHC/cholesterol and 7-DHC/total-sterol ratios were decreased, respectively, to 52% and 65% of control levels ($P < .005$). The variability between cell lines is likely due to the use of pools of transfected cells. These results prove that the human cDNA isolated by homology to the *A. thaliana* Δ^7-reductase functionally encodes a sterol Δ^7-reductase.

We used radiation-hybrid mapping to learn the chromosomal location of the human sterol Δ^7-reductase gene. The data vector resulting from screening the radiation-mapping panel was 10010 00001 00000 10011 00000 00110 00000 10001 01001 10000 00100 00000 01001 00011 00110 00001 11001 00000 001. Interpretation of these data from the radiation-hybrid map placed the *DHCR7* gene 2.33 cR_{3000} from GCT16B07 on chromosome 11, linking it, at a LOD score of 19, with
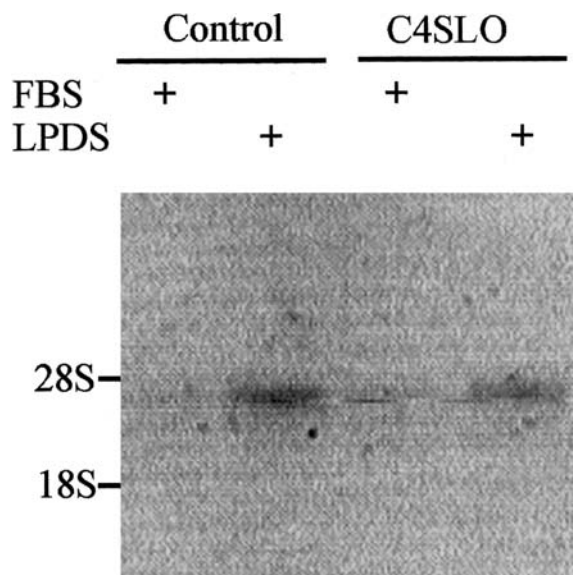

Figure 2 Northern blot analysis comparing expression of the human sterol Δ^7-reductase mRNA from fibroblasts grown with fetal bovine serum (+), compared with fibroblasts grown in lipoprotein-deficient serum (−). A 7.5-μg sample of total RNA was loaded in each lane. The *DHCR7* transcript is ~4 kb. Equivalent loading of samples was confirmed by means of a probe to human *GADPH* (data not shown).

D11S913 and D11S916. The position of *DHCR7* relative to both the radiation-hybrid map and the corresponding genetic and cytogenetic maps is shown in figure 3. Given its position between D11S913 and D11S916 on the radiation-hybrid map, *DHCR7* can be assigned a placement 70.9–80.1 cM from the top of the genetic map of chromosome 11. Cytogenetically, it is therefore likely that the *DHCR7* locus is in the 11q12-13 interval.

To determine whether mutations in the human sterol Δ^7-reductase gene cause SLOS, we used RT-PCR to subclone overlapping segments of cDNA from SLOS fibroblasts. Sequence analysis of these cDNAs allowed us to identify four mutations in three unrelated patients (table 2). A 134-bp insertion (figs. 1 [nucleotide 788/789] and 4A) was identified in the A2SLO cell line. This insertion results in a frameshift, starting at amino acid 263, that precludes translation of the highly conserved carboxyl end of the protein. This cell line may be homozygous for this insertion. Of seven cDNA clones sequenced from this region, all had the 134-bp insertion ($P < .01$). Northern blot analysis showed the presence of a slightly larger transcript in mRNA from the A2SLO cell line (fig. 4B). Finally, PCR analysis of this region demonstrated the presence of a single larger product in A2SLO samples (fig. 4C). A normal-size allele was not observed. Four separate primer pairs (HSP3/HSP8, HSP5/HSP8, HSP7/HSP8, and HSP7/HSP10) that flank this region gave sim-

ilar results (data not shown). Thus, our sequencing, northern blot, and PCR data are consistent with the presence of a homozygous mutant allele. Alternatively, a second allele could be untranscribed or could give rise to an unstable transcript that is not detectable by either RT-PCR or northern blot analysis.

Both mutant alleles were identified in a second SLOS patient (B3SLO). One mutation is a 96-bp deletion (fig. 1, nucleotides −77 to 19). This mutation potentially removes the start codon. Neither of the two ATGs present in the undeleted 5′ region are in-frame, and the next in-frame ATG encodes amino acid 138. If the initiation codon is 5′ of the XbaI site, then this mutation would result in both the removal of 32 amino acids from the amino terminus of this enzyme and introduction of a cysteine residue. Consistent with the autosomal recessive nature of this disorder, this patient has a second mutant allele. The second allele has a single cytosine insertion between nucleotides 505 and 506. This insertion results in both a frameshift starting at amino acid 170 and addition of 39 aberrant amino acids.

A fourth mutation was also identified in the C4SLO cell line. This mutation is a single thymidine insertion between nucleotides 586 and 587. The result of this insertion is both a frameshift starting at amino acid 197 and the addition of 12 aberrant amino acids. We have not yet identified the second mutation in this cell line. Both the single-base insertions in B3SLO and C4SLO would preclude normal translation of the highly conserved carboxyl-terminal half of this protein.

Discussion

The human sterol Δ^7-reductase catalyzes the reduction of the Δ^7-diene bond in 7-DHC, to form cholesterol. Children with SLOS have elevated serum levels of 7-DHC and often have low serum cholesterol levels, and cells from these patients have decreased sterol Δ^7-reductase activity. On the basis of these observations, it had been proposed that SLOS is the result of a defect in cholesterol biosynthesis. A defect in cholesterol biosynthesis can readily explain the low cholesterol levels and
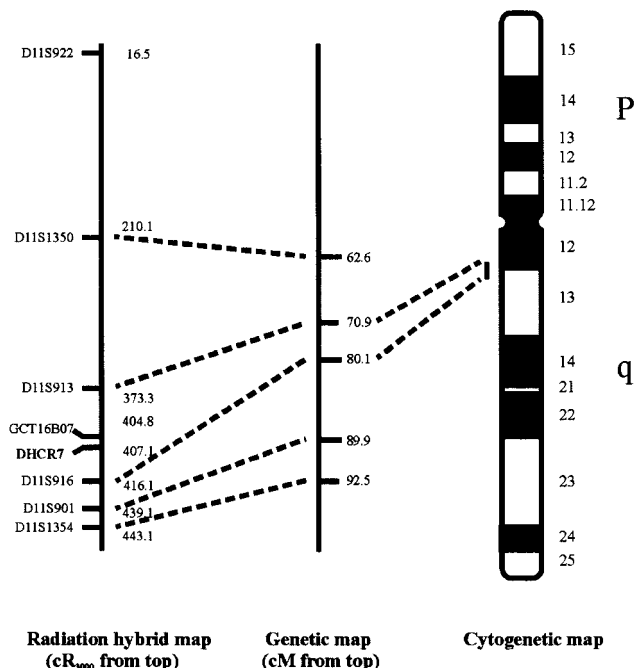

Figure 3 *DHCR7* locus integrated into the radiation-hybrid map of human chromosome 11, with correlation to the genetic and cytogenetic maps. Numbers to the right of the radiation hybrid and genetic maps indicate distances from the top of the maps, in cR_{3000} and cM, respectively. The inferred position of the *DHCR7* gene at 11q12-13 is indicated by the bar to the left of the ideogram.

high 7-DHC levels in blood and tissues, abnormal bile acid profiles, hepatic failure, growth failure, genital hypoplasia, and hypomyelination seen in this disorder. The relationship between a defect in cholesterol biosynthesis and the developmental malformations that also characterize this disorder was initially more difficult to explain. However, it is now known that sonic hedgehog (shh), a morphogen involved in patterning the CNS and limbs, is anchored to the plasma membrane via a covalent bond with cholesterol (Porter et al. 1996*a*, 1996*b*). Thus, shh function may be impaired in SLOS, because of either cholesterol deficiency or the presence

Table 1

Sterol Analysis in A2SLO Fibroblasts Expressing Human Sterol Δ^7-Reductase cDNA

	MEAN ± SD[a]			
VECTOR	Cholesterol (μg sterol/mg cellular protein)	7-DHC (μg sterol/mg cellular protein)	Total Sterol (μg sterol/mg cellular protein)	7-DHC/Cholesterol
pIRES1neo	7.03 ± 1.40	5.84 ± 1.13	14.05 ± 2.67	.83 ± .06
pIRES1neo8	11.66 ± .78[b]	2.63 ± 1.64[b]	15.32 ± 2.59	.22 ± .12[b]

NOTE.—Stably transfected cell lines were grown for 5 d in lipoprotein-deficient media. Sterol analysis of the cell pellets was done by gas chromatography.

[a] Of three samples.

[b] $P < .02$, by Student's paired *t*-test.

Table 2

Mutations of Human Sterol H⁷-Reductase, Identified in SLOS

Patient	SLO Type	Mutation	Nucleotide Position
A2SLO	II	134-bp insertion	788/789
B3SLO	II	C insertion	507/508
		96-bp deletion	−77 to 19
C4SLO	IIᵃ	T insertion	587/588

ᵃ Available clinical description is limited.

of high levels of 7-DHC. Consistent with this idea, the shh-mutant mouse has phenotypic overlap with severe SLOS (Chiang et al. 1996). In addition, pharmacological inhibitors of sterol Δ^7-reductase are teratogenic, and rat embryos exposed to these inhibitors have phenotypic overlap with both severe SLOS and the shh-mutant mouse (Roux et al. 1980; Dehart et al. 1997).

Given the observed biochemical defect of deficient sterol Δ^7-reductase activity, we and others had hypothesized that the genetic defect would be in the gene encoding this enzyme. However, it was also possible that the genetic defect could be in a cofactor of this enzyme, a transporter necessary for sterol Δ^7-reductase activity, or in a transcription factor necessary for normal expression of this enzyme. To answer this question, we have cloned a cDNA encoding a human sterol Δ^7-reductase. This cDNA encodes a 417-amino-acid open reading frame. Our cDNA is truncated at an internal *Xba*I site. Thus, it is possible that the mRNA encodes a longer peptide. A longer open reading frame, of 475 amino acids, has been reported by Moebius et al. (1998). The plant sterol Δ^7-reductase has an open reading frame of 430 amino acids; and the homology between the plant and human genes is poor in this area. However, the amino terminus predicted by the previous clone is in-frame with the peptide predicted by our cDNA. Thus, the 5′ UTR depicted in figure 1 may be translated. Functionally, our fibroblast expression studies clearly demonstrate both that our cDNA encodes a functional sterol Δ^7-reductase and that the potential initiation codon that we have identified functions in human fibroblasts. Determination of the actual initiation codon will require analysis of coupled transcription-translation products and of the native protein. The predicted protein sequence is highly homologous to other sterol reductases. The highest homology was found with the *A. thaliana* sterol Δ^7-reductase. In addition, like the plant sterol Δ^7-reductase, the human enzyme does not encode an EFGGXXG motif that is found in other members of the sterol reductase family.

Cells acquire the cholesterol that they need either from dietary cholesterol or by endogenous biosynthesis of cholesterol. This is a tightly regulated process. If exog-

enous LDL cholesterol is available, endogenous biosynthesis of cholesterol is down-regulated. Using northern blot analysis, we looked at the expression levels of the mRNA encoding the human sterol Δ^7-reductase from cells grown with and without an exogenous source of cholesterol. As we expected, the mRNA encoding this enzyme was up-regulated when fibroblasts were grown
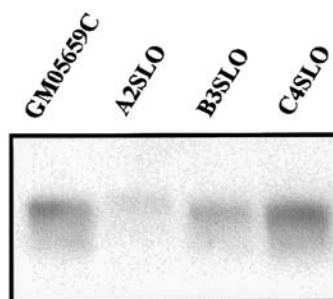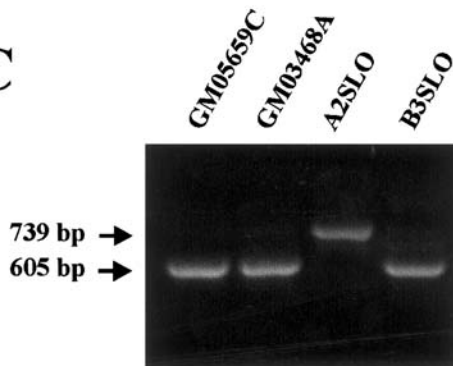

Figure 4 134-bp insertion in A2SLO. *A,* Nucleotide sequence and predicted amino acid sequence of the mutant allele. The normal nucleotide sequence is in uppercase letters, and the inserted sequence is in lowercase letters. Aberrant amino acids are in lowercase letters. *B,* Northern blot analysis of sterol Δ^7-reductase transcript from control and SLOS fibroblasts. The transcript from the A2SLO cell line is slightly larger. *C,* Ethidium bromide–stained gel of PCR products from control (lanes 1 and 2), A2SLO, and B3SLO cell lines. The expected 605-bp product was found in the control and B3SLO cell lines. A single aberrant 739-bp fragment was found in the A2SLO sample.

in cholesterol-deficient media. To prove functionally that this cDNA encodes an enzyme with sterol Δ^7-reductase activity, we expressed this cDNA in SLOS fibroblasts. Stable expression of this cDNA resulted in a significant reduction in 7-DHC levels. Thus, on the basis of both homology and function, we have identified a human sterol Δ^7-reductase.

Using radiation-hybrid mapping, we have established that the human sterol Δ^7-reductase maps to chromosome 11q12-13. Previously, it had been reported that SLOS syndrome maps to chromosome 7q32.1 (Alley et al. 1995). This was based on the finding of a similar translocation in one biochemically confirmed case of SLOS and one historical case of SLOS (Curry et al. 1987). A second historical case of SLOS had a chromosomal translocation involving chromosome 7q34 (Berry et al. 1989). Further analysis of the breakpoint region showed that the disrupted region encodes a metabotropic glutamate receptor (Scherer et al. 1997). Thus, for a chromosomal translocation in that region to cause an autosomal recessive disorder such as SLOS, one must propose both a long-range effect on the expression of one allele and an independent mutation in the second allele. We propose that these previous reports represent an unrelated coincidence of similar chromosomal translocations in SLOS patients. Alternatively, locus heterogeneity could exist for this disorder.

To establish that mutations in the human sterol Δ^7-reductase gene cause SLOS, we sequenced cDNA clones from three unrelated SLOS type II patients. We have identified four different mutant alleles from these patients (table 2). Because SLOS is an autosomal recessive disorder, the identification of mutations in both alleles is necessary for establishing that mutations in this gene cause SLOS. In B3SLO we have identified mutations in both alleles. One is a deletion of 96 nucleotides, and the second is a single cytosine insertion between nucleotides 505 and 506. This 96-nucleotide deletion has not been observed in cDNA clones either from control cells or from other SLOS cell lines. Thus, this deletion is unlikely to represent a normal splice variant. In A2SLO, we identified a 134-bp insertion. Using sequence analysis, RT-PCR, and northern blot analysis, we could not detect a second allele. Thus, A2SLO may be homozygous for this mutation. A fourth mutant allele was identified in a third patient (C4SLO). This mutant allele has a single thymidine insertion between nucleotides 586 and 587. The SLOS mutant alleles reported in this paper all come from severely affected patients and are predicted to result in a severe disruption of the sterol Δ^7-reductase. Although further genotype/phenotype correlation is necessary, this may explain the severe phenotype seen in these patients. It is possible that milder cases of SLOS are due to mutations in another gene. However, we propose that mutations that do not preclude translation of the highly conserved carboxyl half of this enzyme—mutations such as missense changes—may cause milder cases of SLOS.

The data presented here demonstrate that we have cloned a cDNA encoding the human sterol Δ^7-reductase. This conclusion is based on significant homology to an *A. thaliana* sterol Δ^7-reductase, induction of the mRNA-encoding enzyme by growth in cholesterol-deficient media, and functional correction of the biochemical defect in SLOS fibroblasts transfected with this cDNA. Furthermore, we have identified four different mutations in this gene in three unrelated SLOS patients. In one of these patients we have identified two mutant alleles, and a second patient may be homozygous for the identified mutation. Thus, we conclude that mutations in the human sterol-Δ^7-reductase gene cause at least some cases of SLOS.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

BLAST, http://www.ncbi.nlm.nih.gov/BLAST/
GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for fungal sterol Δ^{14}-reductases [Y10624, S30769, S44170, and X94315], fungal sterol Δ^{24}-reductases [S64014 and A43765], carboxyl-terminal portions of human and rat β-lamin receptors [L25941 and AB002466, respectively], and [AF062481])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for SLOS [MIM 270400])
Whitehead/MIT Center for Genome Research, http://www.genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl (for radiation-hybrid mapping)

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