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Smith-Lemli-Opitz Syndrome Is Caused by Mutations in the 7-Dehydrocholesterol Reductase Gene

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Summary

Smith-Lemli-Opitz syndrome is a frequently occurring autosomal recessive developmental disorder characterized by facial dysmorphism, mental retardation, and multiple congenital anomalies. Biochemically, the disorder is caused by deficient activity of 7-dehydrocholesterol reductase, which catalyzes the final step in the cholesterol-biosynthesis pathway—that is, the reduction of the Δ^7 double bond of 7-dehydrocholesterol to produce cholesterol. We identified a partial transcript coding for human 7-dehydrocholesterol reductase by searching the database of expressed sequence tags with the amino acid sequence for the *Arabidopsis thaliana* sterol Δ^7 -reductase and isolated the remaining 5' sequence by the “rapid amplification of cDNA ends” method, or 5'-RACE. The cDNA has an open reading frame of 1,425 bp coding for a polypeptide of 475 amino acids with a calculated molecular weight of 54.5 kD. Heterologous expression of the cDNA in the yeast *Saccharomyces cerevisiae* confirmed that it codes for 7-dehydrocholesterol reductase. Chromosomal mapping experiments localized the gene to chromosome 11q13. Sequence analysis of fibroblast 7-dehydrocholesterol reductase cDNA from three patients with Smith-Lemli-Opitz syndrome revealed distinct mutations, including a 134-bp insertion and three different point mutations, each of which was heterozygous in cDNA from the respective parents. Our data demonstrate that Smith-Lemli-Opitz syndrome is caused by mutations in the gene coding for 7-dehydrocholesterol reductase.

Introduction

Smith-Lemli-Opitz (SLO) syndrome (MIM 270400 [Smith et al. 1964; Opitz and de la Cruz 1994; Kelley 1997]) is an autosomal recessive disorder with an estimated incidence of $\sim 1/20,000$ births (Tint et al. 1994). Patients with the disorder are characterized by a large spectrum of developmental abnormalities, including severe craniofacial malformations; multiple affected organs, including the CNS and the brain; malformations of the limbs; and incomplete development of the male genitalia (Tint et al. 1995; Cunniff et al. 1997). In addition to these morphological abnormalities, patients with SLO syndrome suffer from severe mental and growth retardation and failure to thrive. On the basis of the severity of the abnormalities, patients with SLO syndrome have been subdivided into two groups—SLO syndrome type I and SLO syndrome type II, the latter being the more severe form, which often leads to death shortly after birth (Donnai et al. 1986; Curry et al. 1987).

Biochemically, the disorder is caused by reduced/deficient activity of 7-dehydrocholesterol reductase (7-DHCR), the enzyme that catalyzes the reduction of the C_7 - C_8 (Δ^7) double bond of 7-dehydrocholesterol (cholesta-5,7-dien-3 β -ol) to produce cholesterol (cholesta-5-en-3 β -ol)—that is, the ultimate step of the cholesterol-biosynthetic pathway (Tint et al. 1994; Schefer et al. 1995). As a result, patients with SLO syndrome have low plasma cholesterol and elevated 7-dehydrocholesterol concentrations, a characteristic used for the diagnosis of the syndrome. The link between this cholesterol-biosynthesis defect and the multiple developmental anomalies typical of SLO syndrome became clear after the recent discovery that cholesterol plays an essential role in animal embryonic development, in that it determines the spatial distribution of hedgehog proteins in the developing embryo by tethering their N-terminal signaling domain to the cell surface (Porter et al. 1996; Tabin and McMahon 1997). This discovery initiated a strongly increased interest in cholesterol and its role in

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ggcatttggagcacaggtgtcta

- 102
- 79 gaaacttttaaggggcccggttcaagaagaaaagtccctctctgtgtgaaactatttggcaagaggctggaggccca

1 ATG GCT GCA AAA TTG CAA CCC AAC ATT CCC AAA GCC AAG AGT CTA GAT GGC GTC ACC AAT
M A A K L Q P N I P K A K S L D G V T N

61 GAC AGA ACC GCA TCT CAA GGG CAG TGG GGC CGT GCC TGG GAG GTG GAC TGG TTT TCA CTG
D R T A S Q G Q W G R A W E V D W F S L

121 GCG AGC GTC ATC TTC CTA CTG CTG TTC GCC CCC TTC ATC GTC TAC TAC TTC ATC ATG GCT
A S V I F L L L F A P F I V Y Y F I M A

181 TGT GAC CAA TAC AGC TGC GCC CTG ACC GGC CCT GTG GTG GAC ATC GTC ACC GGA CAT GCT
C D Q Y S C A L T G P V V D I V T G H A

241 CGG CTC TCG GAC ATC TGG GCC AAG ACT CCA CCT ATA ACG AGG AAA GCC GCC CAG CTC TAT
R L S D I W A K T P P I T R K A A Q L Y

301 ACC TTG TGG GTC ACC TTC CAG GTG CTT CTG TAC ACG TCT CTC CCT GAC TTC TGC CAT AAG
T L W V T F Q V L L Y T S L P D F C H K

361 TTT CTA CCC GGC TAC GTA GGA GGC ATC CAG GAG GGG GCC GTG ACT CCT GCA GGG GTT GTG
F L P G Y V G G I Q E G A V T P A G V V

421 AAC AAG TAT CAG ATC AAC GGC CTG CAA GCC TGG CTC CTC ACG CAC CTG CTC TGG TTT GCA
N K Y Q I N G L Q A W L L T H L L W F A

481 AAC GCT CAT CTC CTG TCC TGG TTC TCG CCC ACC ATC ATC TTC GAC AAC TGG ATC CCA CTG
N A H L L S W F S P T I I F D N W I P L

541 CTG TGG TGC GCC AAC ATC CTT GGC TAT GCC GTC TCC ACC TTC GCC ATG GTC AAG GGC TAC
L W C A N I L G Y A V S T F A M V K G Y

601 TTC TTC CCC ACC AGC GCC AGA GAC TGC AAA TTC ACA GGC AAT TTC TTT TAC AAC TAC ATG
F P P T S A R D C K F T G N F F Y N Y M

661 ATG GGC ATC GAG TTT AAC CCT CGG ATC GGG AAG TGG TTT GAC TTC AAG CTG TTC TTC AAT
M G I E F N P R I G K W F D F K L F F N

721 GGG CGC CCC GGG ATC GTC GCC TGG ACC CTC ATC AAC CTG TCC TTC GCA GCG AAG CAG CGG
G R P G I V A W T L I N L S F A A K Q R

781 GAG CTC CAC AGC CAT GTG ACC AAT GCC ATG GTC CTG GTC AAC GTC CTG CAG GCC ATC TAC
E L H S H V T N A M V L V N V L Q A I Y

841 GTG ATT GAC TTC TTC TGG AAC GAA ACC TGG TAC CTG AAG ACC ATT GAC ATC TGC CAT GAC
V I D F F W N E T W Y L K T I D I C H D

901 CAC TTC GGG TGG TAC CTG GGC TGG GGC GAC TGT GTC TGG CTG CCT TAT CTT TAC ACG CTG
H F G W Y L G W G D C V W L P Y L Y T L

961 CAG GGT CTG TAC TTG GTG TAC CAC CCC GTG CAG CTG TCC ACC CCG CAC GCC GTG GGC GTC
Q G L Y L V Y H P V Q L S T P H A V G V

1021 CTG CTG CTG GGC CTG GTG GGC TAC TAC ATC TTC CGG GTG GCC AAC CAC CAG AAG GAC CTG
L L L G L V G Y Y I F R V A N H Q K D L

1081 TTC CGC CGC ACG GAT GGG CGC TGC CTC ATC TGG GGC AGG AAG CCC AAG GTC ATC GAG TGC
F R R T D G R C L I W G R K P K V I E C

1141 TCC TAC ACA TCC GCC GAC GGG CAG AGG CAC CAC AGC AAG CTG CTG GTG TCG GGC TTC TGG
S Y T S A D G Q R H H S K L L V S G F W

1201 GGC GTG GCC CGC CAC TTC AAC TAC GTC GGC GAC CTG ATG GGC AGC CTG GCC TAC TGC CTG
G V A R H F N Y V G D L M G S L A Y C L

1261 GCC TGT GGC GGT GGC CAC CTG CTG CCC TAC TTC TAC ATC ATC TAC ATG GCC ATC CTG CTG
A C G G G H L L P Y F Y I I Y M A I L L

1321 ACC CAC CGC TGC CTC CGG GAC GAG CAC CGC TGC GCC AGC AAG TAC GGC CGG GAC TGG GAG
T H R C L R D E H R C A S K Y G R D W E

1381 CGC TAC ACC GCC GCA GTG CCT TAC CGC CTG CTG CCT GGA ATC TTC TAA ggcacgacctagga
461 R Y T A A V P Y R L L P G I F *

1444 gaacctgtggggctgtcaagagcgtgttctgcccaggtccatggggctggcatcccagctccaactcgaggagcctcag
1523 tttctcatctgtaaaactggagagcccagcacttggcaggtgtccagtagcctaatcacgctctgttcttctgttctg
1602 ccttcaaggaattccgagtgctccagcactgcccatttggccagcacagacggatttctctaatcagtgctccctggggc
1681 agggagatgaccagtcaccttactagtccttggagacaatttactgtattaggagcccaggccacgctacactct
1760 gcccacactggtagcaggaggtcttcccacgcccctgtcattaggctgcatttactcttctgtaaaataaaggggagtg
1839 gggcgtgcccgttatccatgtattgcttccagctctagatccccctccccctgctctctcagctcgtgggtggggcc
1918 cgtgcccgttctctcttggtagcgtgcacgggtgttgaactgggacactggggagaaagggcttccatgtcttctct
1997 tctctgctcctgctgcacagctgccaggagtgctctgcctgggagctgcaagacctcagagaggtcccagcactggctg
2076 tggcctttcaggtgtaggcaggtgggctctgttcccgattccctgtgagcggccaccctctcgaaagaatttctg
2155 ttgcccctgactgtgcagactctggctcgagcaaccggggaaacttccacctcaggggccctccacaccttctccagc
2234 agggagctcagtcaccagcctcgggagggcactcctttctgtgttctctccctgaggcattctctcatccctag
2313 ggtgtgtgtagaaactctttaaactctatgctccagtagagttcatctttatattaaactcccctgttcaaaaa

Figure 1 Nucleotide and deduced amino acid sequences of human 7-DHCR cDNA. 5' and 3' UTRs are denoted by lowercase letters.

Table 1
Amino Acid Sequence Homology between Different Sterol Reductases

	Human 7-DHCR	<i>A. thaliana</i> Sterol Δ 7- Reductase	Human Lamin B Receptor	<i>S. cerevisiae</i> Sterol Δ 14- Reductase	<i>S. cerevisiae</i> Sterol Δ 24(28)- Reductase
Human 7-DHCR	...	38	37	34	30
<i>A. thaliana</i> sterol Δ 7-reductase	56	...	33	31	26
Human lamin B receptor	52	47	...	40	33
<i>S. cerevisiae</i> sterol Δ 14-reductase	49	49	55	...	34
<i>S. cerevisiae</i> sterol Δ 24(28)-reductase	43	41	49	49	...

NOTE.—Data above the diagonal are percentage of identity; and data below the diagonal are percentage of similarity.

development and established SLO syndrome as the prototypical developmental disorder (Kelley et al. 1996; Lanoue et al. 1997; Farese and Herz 1998). However, it remained to be determined whether the reduced activity of 7-DHCR is due to mutations in the gene coding for 7-DHCR itself or in a gene coding for a protein that regulates the activity or expression of 7-DHCR.

We here report the identification of a full-length cDNA that codes for human 7-DHCR, as shown by heterologous expression in the yeast *Saccharomyces cerevisiae*. The identification of distinct mutations in 7-DHCR cDNA from different patients with SLO syndrome demonstrates that SLO syndrome is caused by mutations in the gene coding for 7-DHCR.

Patients and Methods

Patients

Patient 1 (SLO syndrome type I) was born at term to unrelated parents. At birth, the boy was hypotonic and showed microcephaly, micrognathia, craniofacial abnormalities, postaxial polydactyly of both hands, bilateral syndactyly of the second and third toe, inguinal testes and ambiguous genitalia that appeared to be a severe hypospadias, and micropenis. Tube feeding was started because of failure to thrive, and, at 1 year of age, the patient showed severe developmental delay. SLO syndrome was biochemically diagnosed on the basis of low plasma cholesterol and markedly-increased 7-dehydrocholesterol concentrations (0.595 and 0.559 mmol/liter, respectively, determined 2 mo after birth) and was confirmed by the finding of very reduced 7-DHCR activity in cultured skin fibroblasts, as determined on the basis of ^{14}C -mevalonate incorporation.

Patient 2 (SLO syndrome type II), the first child of unrelated parents, had severe intrauterine growth retardation and died, shortly after birth, of respiratory insufficiency. The child showed multiple dysmorphic features, including dysmorphic nose and ears, hypertrophied gingiva, retrognathia, unilateral hypoplastic fifth finger, and syndactyly of the second and third toes. The genitalia were documented as female, but chromosome analysis revealed a 46,XY karyotype. Postmortem ex-

amination showed an atrial and ventricular septum defect, unilateral abnormal pulmonary lobulation, and a horseshoe kidney. SLO syndrome was biochemically diagnosed on the basis of elevated 7-dehydrocholesterol concentrations determined in fibroblasts (7-dehydrocholesterol/cholesterol ratio 0.032 [control .005]) and was confirmed by the finding of a completely deficient 7-DHCR activity in cultured skin fibroblasts, as determined by ^{14}C -mevalonate incorporation.

Patient 3 (SLO syndrome type I), the fourth child of unrelated parents, was born at term after a pregnancy complicated by intrauterine growth retardation. At birth, the boy was microcephalic and showed multiple dysmorphic features, including a broad nasal tip with anteverted nostrils, a cleft soft palate, broad alveolar ridges, micrognathia, syndactyly of the second and third toes, and a small penis with cryptorchidism. At 3 years of age, the boy had psychomotor retardation, severe failure to thrive, and feeding difficulties that still necessitated tube feeding. SLO syndrome was biochemically diagnosed on the basis of low plasma cholesterol and high 7-dehydrocholesterol concentrations (0.30 and 0.37 mmol/liter, respectively, determined 1 mo after birth) and was confirmed by the finding of very reduced 7-DHCR activity in cultured skin fibroblasts, as determined by ^{14}C -mevalonate incorporation.

Biochemical Methods

Cholesterol and 7-dehydrocholesterol concentrations in plasma were determined as described by van Rooij et al. (1997). 7-DHCR activities in cultured skin fibroblasts were determined as described by Wanders et al. (1997).

Identification of 7-DHCR cDNA

By means of the BLAST algorithm (Altschul et al. 1990), the expressed sequence tag (EST) database of the National Center for Biotechnology Information was screened for sequences homologous to that of *Arabidopsis thaliana* sterol Δ 7-reductase. EST sequences were grouped into three sets, and contigs were composed and used to screen the GenBank protein database to identify the candidate 7-DHCR sequence. Two EST clones (IM-

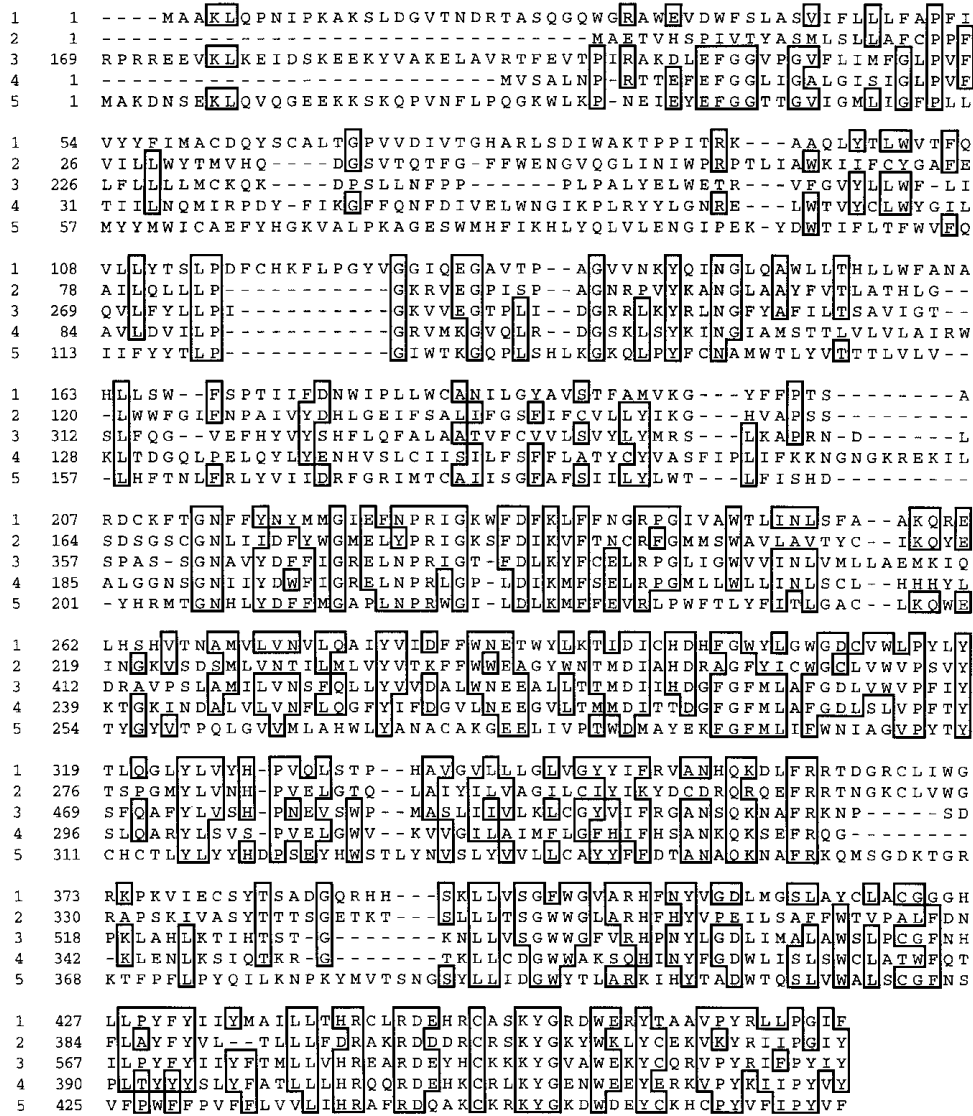


Figure 2 Sequence alignment of human 7-DHCR and different sterol reductases. Amino acids conserved in three or more sequences are boxed. Sequence designations are as follows: 1 = human 7-DHCR; 2 = *A. thaliana* sterol Δ7-reductase; 3 = human lamin B receptor (C-terminal 447 amino acids); 4 = *S. cerevisiae* sterol Δ14-reductase; and 5 = *S. cerevisiae* sterol Δ24(28)-reductase.

AGE Consortium Clone ID417125 and ID251607) for which no 5' end sequence had been deposited into the database were ordered from the UK HGMP Resource Centre in Cambridge and were entirely sequenced. The remaining 5' sequences were obtained by the "rapid amplification of cDNA ends" method (5'-RACE), by means of two nested cDNA-specific primers complementary to nucleotides 513-487 (5'-GAA CCA GGA CAG GAG ATG AGC GTT TGC-3') and 386-360 (5'-ACG TAG CCG GGT AGA AAC TTA TGG CAG-3'), and from leukocyte cDNA, according to the instructions of the manufacturer (Clontech).

7-DHCR cDNA Expression in S. cerevisiae

The coding sequence of 7-DHCR was expressed under the transcriptional control of the *GAL1* promoter, by means of the yeast expression vector pYES2 (Invitrogen). The expression construct and, as a control, pYES2 without insert were transformed separately into *S. cerevisiae* strain INVSC2 (Invitrogen), by the lithium acetate method (Invitrogen protocol). Yeast transformants were grown at 30°C in yeast nitrogen base medium (Difco) supplemented with 20 μg histidine/ml, with either 2% glucose (*GAL1*-promoter repression) or 2% galactose

Table 2**Sterol Content of Glucose- or Galactose-Grown *S. cerevisiae* Strains Transformed with Different Plasmids**

PLASMID TYPE AND CARBON SOURCE	PROPORTION OF STEROL EXAMINED ^a (%)		
	Ergosterol ^b	Brassicasterol ^c	Campesterol ^d
pYES2 + 7-DHCR:			
Glucose	100	ND	ND
Galactose	42	43	15
pYES2:			
Glucose	100	ND	ND
Galactose	100	ND	ND

^a ND = not detectable.

^b Ergosta-5,7,22-trien-3 β -ol.

^c Ergosta-5,22-dien-3 β -ol.

^d Ergosta-5-en-3 β -ol.

(*GAL1*-promoter induction) as the carbon source. In induction experiments, cells were pregrown in glucose medium, transferred by centrifugation to glucose medium and galactose medium, harvested by centrifugation after the cultures reached an optical density, at 600 nm, of ~1 (15–20 h of growth at 30°C), and then were analyzed for sterol content. In addition, glucose- and galactose-grown cells from the pYES2 + 7-DHCR transformant were resuspended in 100 mM Tris/HCl buffer, pH 7.5, and were disrupted by vigorous vortexing with glass beads (5 times for 30 s at 4°C). The resulting homogenates were incubated with 15 μ M cholesta-5,7-dien-3 β -ol (Sigma; prepared as a 0.2 mM stock in 30% 2-hydroxypropyl- β -cyclodextrin [Fluka]) and 2 mM NADPH at 37°C for 3 h and subsequently were analyzed for sterols. For sterol analysis, cells or homogenates were saponified for 2 h at 70°C in alkaline ethanol; sterols were extracted with hexane, converted to trimethylsilyl derivatives by means of bis(trimethylsilyl)trifluoroacetamide-trimethylchlorosilane (BSTFA-TMCS), and then were analyzed by gas chromatography-mass spectrometry (GC/MS).

Mutation Analysis

First-strand cDNA was synthesized from RNA isolated from cultured primary skin fibroblasts (from all three patients and the parents of patient 1) or leukocytes (from the parents of patients 2 and 3), as described elsewhere (Ijlst et al. 1994). With the first-strand cDNA used as template, the coding sequence of the 7-DHCR cDNA was amplified by PCR in two overlapping fragments, by means of two primer sets tagged with either a “-21M13” (5'-TGT AAA ACG ACG GCC AGT-3') sequence or an “M13rev” (5'-CAG GAA ACA GCT ATG ACC-3') sequence. One fragment was obtained by means of primer set DHCR_{-58 to -38} (5'-[21M13]-GGT TCA AGA AGG AAA AGT TCC C-3') and DHCR₈₂₈₋₈₁₀ (5'-[M13rev]-TGA CCA GGA CCA TGG CAT TG-3'), and

the other fragment was obtained by means of primer set DHCR₆₈₄₋₇₀₃ (5'-[21M13]-TCG GGA AGT GGT TTG ACT TC-3') and DHCR₁₅₆₃₋₁₅₄₄ (5'-[M13rev]-GGG CTC TCT CCA GTT TAC AG-3'). For patient 3 and the parents who appeared heterozygous for the 134-bp insertion, different primer sets were used, which enabled us to amplify the two cDNAs separately. The cDNA containing the insert was amplified by means of primers DHCR_{-58 to -38} and DHCR₁₅₆₃₋₁₅₄₄ in combination with primers designed on the basis of the insert sequence (5'-[M13rev]-GTC AAG CGG TGC TTT GCC C-3' and 5'-[21M13]-CGT GTG TCA GAG GCA GAG C-3', respectively). The cDNA without the insert was amplified by means of primers DHCR_{-58 to -38} and DHCR₁₅₆₃₋₁₅₄₄ in combination with primers spanning the site of insertion, which is after nucleotide 963 (primers 5'-[M13rev]-ACA CCA AGT ACA GAC CCT GC-3' and 5'-[21M13rev]-CTT TAC ACG CTG CAG GGT C-3', respectively). PCR fragments were sequenced in both directions by means of -21M13 and M13rev fluorescent primers, on an Applied Biosystems 377A automated DNA sequencer, according to the manufacturer's protocol (Perkin-Elmer).

Chromosomal Mapping of 7-DHCR cDNA

FISH mapping was performed on lymphocytes by SeeDNA Biotech, using the cDNA insert from EST clone 417125 as a probe (Heng et al. 1992; Heng and Tsui 1993). The assignment of the FISH mapping data to chromosomal bands was achieved by superimposition of FISH signals onto 6-diamidino-2-phenylindole (DAPI)-banded chromosomes (Heng and Tsui 1993). The detailed chromosomal position was determined on the basis of the data summarized from 10 photos.

Results*Identification of the Human 7-DHCR cDNA*

To identify the 7-DHCR gene, we used the BLAST algorithm (Altschul et al. 1990) to search the database of ESTs from the National Center for Biotechnology Information, for sequences homologous to that of sterol $\Delta 7$ -reductase of *Arabidopsis thaliana*. This sterol $\Delta 7$ -reductase recently has been cloned by metabolic interference in *S. cerevisiae*, based on the ability of the reductase to reduce the $\Delta 7$ double bond of ergosterol (ergosta-5,7,22-trien-3 β -ol), the final product in the sterol-biosynthesis pathway in yeast, which produces brassicasterol (ergosta-5,22-dien-3 β -ol) (Lecain et al. 1996). The BLAST search identified numerous human ESTs with significant homology to the *A. thaliana* sequence representing transcripts from three different genes. The first set of sequence codes for the human lamin B receptor (Ye and Worman 1994), a protein con-

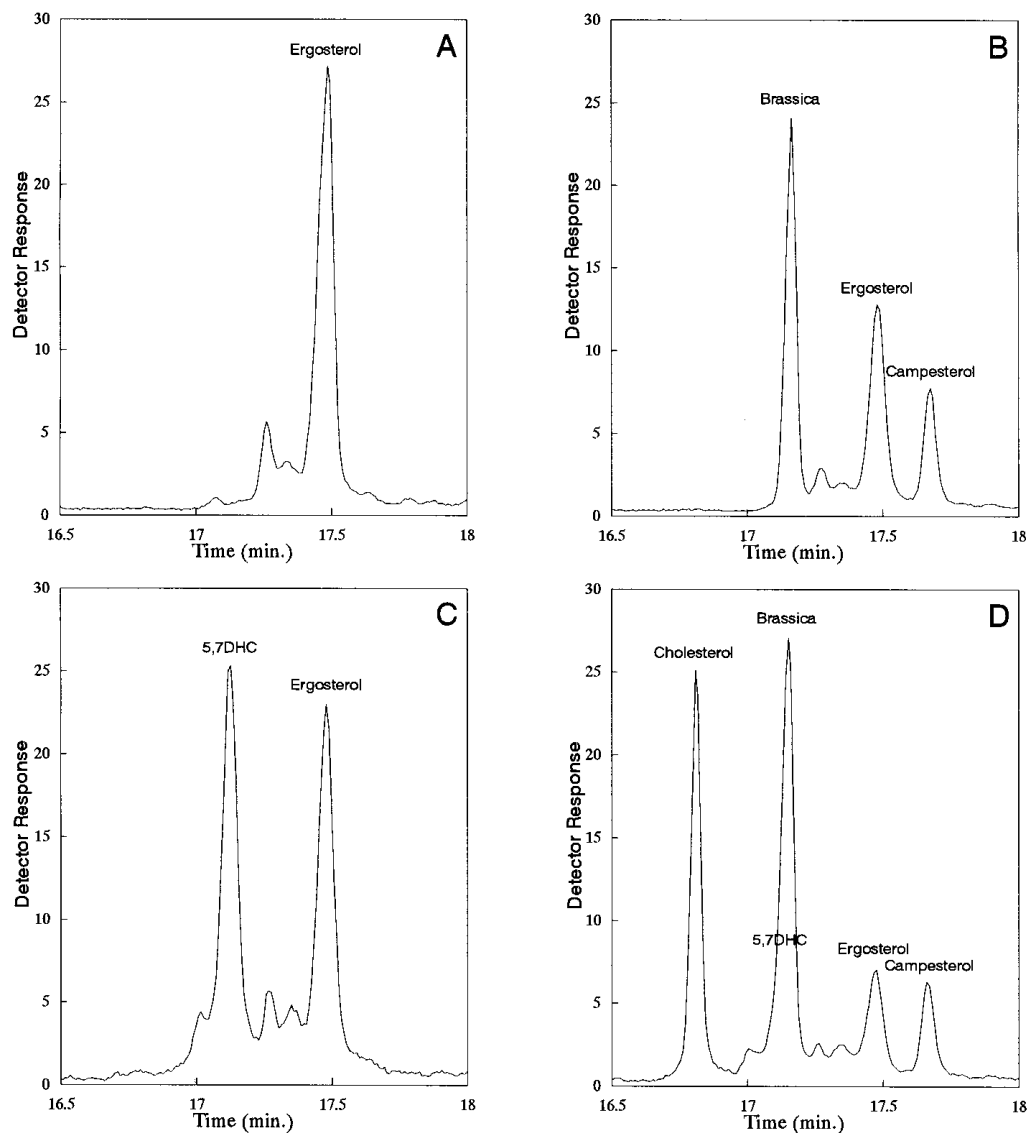


Figure 3 GC/MS analysis of total sterols of *S. cerevisiae* transformants. A, Sterol analysis of *S. cerevisiae* strain transformed with pYES2 + 7-DHCR and grown on glucose (*GAL1*-promoter repression). B, Sterol analysis of *S. cerevisiae* strain transformed with pYES2 + 7-DHCR and grown on galactose (*GAL1*-promoter induction). C, Homogenate of glucose-grown *S. cerevisiae* strain transformed with pYES2 + 7-DHCR incubated with 7-dehydrocholesterol and NADPH (*GAL1*-promoter repression). D, Homogenate of galactose-grown *S. cerevisiae* strain transformed with pYES2 + 7-DHCR incubated with 7-dehydrocholesterol and NADPH (*GAL1* promoter induction). Brassica = brassicasterol; and 5,7DHC = 7-dehydrocholesterol.

sisting of an N-terminal DNA-binding domain and a C-terminal domain of ~440 amino acids with striking homology to the amino acid sequences of various sterol reductases (Lecain et al. 1996). The two other sets of ESTs are derived from two previously unidentified genes. On the basis of the significant homology to several fungal sterol $\Delta 14$ -reductases, the second set of ESTs most likely belongs to a gene coding for human sterol $\Delta 14$ -reductase, an enzyme that catalyzes the reduction of 4,4-dimethylcholesta-8,14,24-trien- 3β -ol to produce 4,4-di-

methylcholesta-8,24-dien- 3β -ol (authors' unpublished data). The primary sequence encoded by the third set of ESTs was found to be most homologous to *A. thaliana* sterol $\Delta 7$ -reductase, suggesting that the corresponding gene codes for 7-DHCR.

Assembly of the putative 7-DHCR ESTs produced only a partial transcript, of 1,880 bp. The remaining 5' sequences were obtained by sequencing of two EST clones for which no 5' end sequences had been deposited into the database and by 5' RACE using leukocyte

Table 3**Mutation Analysis of Patients with SLO Syndrome and of Their Parents**

cDNA Source	Mutation ^a	Coding Effect
Family of patient 1:		
Patient 1	A356T (+-)	His119→Leu
	G730A (+-)	Gly244→Arg
Mother	A356T (+-)	His119→Leu
Father	G730A (+-)	Gly244→Arg
Family of patient 2:		
Patient 2	G963↓134 bp ^b (++)	Frameshift
Mother	G963↓134 bp ^b (+-)	Frameshift
Father	G963↓134 bp ^b (+-)	Frameshift
Family of patient 3:		
Patient 3	G744T (+-)	Trp248→Cys
	G963↓134 bp ^b (+-)	Frameshift
Mother	G963↓134 bp ^b (+-)	Frameshift
Father	G744T (+-)	Trp248→Cys

^a +- = Heterozygous for mutation; and ++ = homozygous for mutation.

^b The sequence of the 134-bp insert is 5'-AAG AGA ACA CGG AGG CAA GGC GTG TGT CAG AGG CAG AGC TGG GGT TTG ACC CCA GGC CGC TGG GCC CTC GAG CCC ACA CTC CTG TCC TCT CCC TGG GCA AAG CAC CGC TTG ACC CCT TCC CCC TCG CCC CCC AC-3'.

cDNA. The resulting 2,385-bp cDNA (GenBank accession number AF067127) contains an open reading frame of 1,425 bp coding for a polypeptide of 475 amino acids (fig. 1) with a calculated molecular weight of 54.5 kD and predicted to contain nine transmembrane-spanning helices. The 5' UTR and the 3' UTR are 102 and 942 bp long, respectively. Alignment of the deduced 7-DHCR amino acid sequence, the *A. thaliana* sterol $\Delta 7$ -reductase, the C-terminal 447 amino acids from human lamin B receptor, and different sterol reductases from *S. cerevisiae* reveals a high degree of homology among all reductases (also see Lecain et al. 1996), with the 7-DHCR sequence being most homologous to that of *A. thaliana* sterol $\Delta 7$ -reductase (table 1 and fig. 2).

Heterologous Expression of 7-DHCR cDNA in *S. cerevisiae*

The identified transcript was shown to code for 7-DHCR, by expression of the open reading frame under transcriptional control of the galactose-inducible *GAL1* promoter in *S. cerevisiae*, an organism devoid of endogenous sterol $\Delta 7$ -reductase activity. Sterol analysis of yeast cells transformed by the expression construct pYES2 + 7-DHCR and grown on glucose to fully repress *GAL1* promoter transcription identified ergosterol (ergosta-5,7,22-trien-3 β -ol) as the main sterol, whereas no brassicasterol (ergosta-5,22-dien-3 β -ol) was detected (table 2 and fig. 3A). However, in the same transformants grown on galactose, only 42% of the total sterol content consisted of ergosterol, whereas 43% of the total

sterol content consisted of brassicasterol, the expected product of the $\Delta 7$ -reductase reaction (table 2 and fig. 3B). In addition, campesterol (ergosta-5-en-3 β -ol) was detected (15%), as also was observed after heterologous expression of the *A. thaliana* sterol $\Delta 7$ -reductase in *S. cerevisiae* (Lecain et al. 1996), which suggests that the sterol $\Delta 7$ -reductases have some affinity for the $\Delta 22$ double bond. As a control, in yeast cells transformed with the expression vector without insert, no change in the sterol content was observed after growth on galactose, compared with that observed after growth on glucose (table 2).

Additional evidence was obtained from the in vitro conversion of 7-dehydrocholesterol (cholesta-5,7-dien-3 β -ol) to cholesterol (cholesta-5-en-3 β -ol) by a homogenate prepared from galactose-grown cells transformed with the expression construct (fig. 3D), which did not occur with a homogenate from glucose-grown cells (fig. 3C).

Sequence Analysis of 7-DHCR cDNA in Patients with SLO Syndrome

To establish that SLO syndrome is caused by mutations in the gene coding for 7-DHCR, we analyzed 7-DHCR cDNA obtained, by reverse-transcription-PCR (RT-PCR), from skin-fibroblast RNA from three patients with SLO syndrome. Sequence analysis of 7-DHCR cDNA from patient 1 showed that he is compound heterozygous for two different point mutations. The first mutation, A356T, changes the histidine at position 119 into a leucine, and the second mutation, G730A, changes

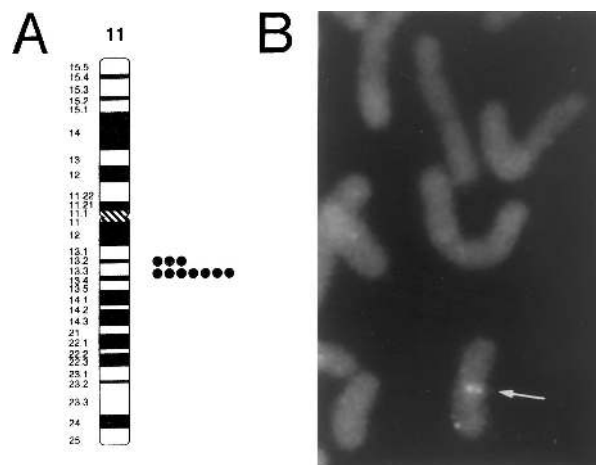


Figure 4 Chromosomal localization of human 7-DHCR gene. A, Diagram of FISH mapping results, based on a summary of 10 photos showing 11q13 as the chromosomal localization of the 7-DHCR gene. Each dot represents FISH signals detected on human chromosome 11. B, Example of FISH signal of 7-DHCR cDNA probe on chromosome 11.

the glycine at position 244, which is highly conserved among reductases (fig. 2), into an arginine (table 3). Analysis of 7-DHCR cDNA from the patient's parents revealed that the patient had inherited the A356T allele from his mother and had inherited the G730A allele from his father (table 3).

Sequence analysis of 7-DHCR cDNA from patient 2 showed a homozygous 134-bp insertion after nucleotide 963 (fig. 1), which causes a frameshift resulting in a truncated protein with a changed C-terminus (table 3). The more severely expressed phenotype of this patient (SLO syndrome type II) is in accordance with the fact that this 134-bp insertion occurs in a region strongly conserved among sterol reductases (fig. 2). The homozygosity of the 134-bp insertion in this patient was confirmed by the observation that both parents were heterozygous for the same insertion (table 3).

Sequence analysis of 7-DHCR cDNA from patient 3 showed that he is compound heterozygous for a point mutation, G744T, and for the same 134-bp insertion as was observed in patient 2 (table 3). The point mutation is inherited from the patient's father and changes the tryptophan at position 248, highly conserved among sterol reductases (fig. 2), into a cysteine. The insertion mutation is inherited from the patient's mother and introduces a frameshift, as has been discussed for patient 2 (table 3).

Chromosomal Mapping of 7-DHCR Gene

FISH mapping experiments were performed to determine the chromosomal localization of the 7-DHCR gene, with 7-DHCR cDNA used as a probe. The results showed that the 7-DHCR gene is localized on chromosome 11q13 (fig. 4).

Discussion

In this report we have shown that the gene coding for 7-DHCR is responsible for SLO syndrome. In addition to the high homology of the encoded amino acid sequence to the sterol Δ^7 -reductase of *A. thaliana*, the evidence comes from the reduction of both the C₇-C₈ (Δ^7) double bond of ergosterol in vivo and of 7-dehydrocholesterol in vitro observed after heterologous expression of the 7-DHCR cDNA in *S. cerevisiae*. The ability of human 7-DHCR to use the fungal ergosterol as a substrate is well documented, and its use to measure 7-DHCR activities has been proposed as an alternative method for the diagnosis of SLO syndrome (Honda et al. 1996). During the final stages in the preparation of the manuscript of the present article, a report appeared that describes both the molecular cloning of the human delta 7-sterol reductase cDNA and its heterologous expression in yeast and that leads to conclusions similar

to ours (Moebius et al. 1998). Comparison of the amino acid sequences confirmed that the reported delta 7-sterol reductase is identical to 7-DHCR.

The identification of distinct mutations in 7-DHCR cDNAs from different patients with SLO syndrome demonstrates that SLO syndrome is caused by mutations in the 7-DHCR gene itself, although it still may be possible that mutations in other genes lead to the same clinical phenotype. Furthermore, we have shown that both the type I (patients 1 and 3) and the more severe type II (patient 2) forms of SLO syndrome are due to mutations in the same gene. In fact, our results suggest a correlation between the severity of the disease and the type of mutation. Both patients with SLO syndrome type I are compound heterozygous for missense mutations that result in mutated 7-DHCR proteins that still may have sufficient residual activity to support life. On the other hand, the homozygous 134-bp insertion found in the patient with SLO syndrome type II produces a truncated 7-DHCR protein lacking 154 amino acids of its original C-terminal sequence. On the basis of the fact that this C terminus is highly conserved among sterol reductases (fig. 2), the resulting protein is not expected to be functional.

Interestingly, the 134-bp insertion mutation is observed in two of the three patients in the present report. Since neither these patients nor their parents are related to each other, this suggests that the 134-bp insertion may be a more frequently occurring mutation. Indeed, RT-PCR analysis of 7-DHCR cDNA from three additional unrelated patients with SLO syndrome identified two more cases of (compound) heterozygosity for the 134-bp insertion (authors' unpublished results). Analysis at the genomic level will be necessary in order to determine whether this insertion is caused by a splice-site mutation.

On the basis of a single de novo chromosomal translocation event observed in a patient diagnosed with SLO syndrome type II, chromosome 7q32 has been suggested as a candidate location for the gene responsible for SLO syndrome (Wallace et al. 1994; Alley et al. 1995, 1997). However, the results of the FISH mapping experiments reveal that the 7-DHCR gene is localized on chromosome 11q13. A possible explanation for the discrepancy may be that the chromosomal translocation event is not directly related to the clinical manifestations in the patient. Since the elevated concentration of 7-dehydrocholesterol in fibroblasts of the patient suggests that the 7-DHCR gene is affected, sequence analysis of fibroblast 7-DHCR cDNA of the patient will be the first logical step to take to resolve this matter.

Recently, we have identified a full-length cDNA coding for mouse 7-DHCR, which will be used to create an animal model for SLO syndrome. An animal SLO-syndrome model will enable us to study not only the effects of different cholesterol-enriched regimens but also why

lowered cholesterol and elevated 7-dehydrocholesterol levels cause the variable SLO phenotypes. In this respect, it will be of particular interest to study the function of embryonic signaling proteins, such as sonic hedgehog protein, that are crucial for early development and the spatial distributions of which are determined by cholesterol (Kelley et al. 1996; Porter et al. 1996; Lanoue et al. 1997; Tabin and McMahon 1997; Farese and Herz 1998).

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Electronic-Database Information

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

Genbank, <http://www.ncbi.nlm.nih.gov/Web/Genbank> (accession number AF067127)

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/omim> (for SLO syndrome [MIM 270400])

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