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Minireview

3β-Hydroxysterol Δ^7-reductase and the Smith–Lemli–Opitz syndrome

Lina S. Correa-Cerro, Forbes D. Porter*

Unit on Molecular Dysmorphology, Heritable Disorders Branch, Department of Health and Human Services, National Institute of Child Health and Human Development, National Institutes of Health, Bld. 10, Rm. 9S241, 10 Center Dr., Bethesda, MD 20892, USA

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Abstract

In the final step of cholesterol synthesis, 7-dehydrocholesterol reductase (DHCR7) reduces the double bond at C7-8 of 7-dehydrocholesterol to yield cholesterol. Mutations of *DHCR7* cause Smith–Lemli–Opitz syndrome (SLOS). Over 100 different mutations of *DHCR7* have been identified in SLOS patients. SLOS is a classical multiple malformation, mental retardation syndrome, and was the first human malformation syndrome shown to result from an inborn error of cholesterol synthesis. This paper reviews the biochemical, molecular, and mutational aspects of *DHCR7*.
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Keywords: Smith–Lemi–Opitz syndrome; 7-Dehydrocholesterol reductase; Inborn error of cholesterol synthesis

Introduction

Cholesterol is an essential lipid found in all mammalian cells, and is a major lipid component of the membrane. In addition to cholesterol's structural role in cellular membranes, cholesterol is a precursor molecule for sterol-based compounds including bile acids, oxysterols, neurosteroids, glucocorticoids, mineralocorticoids, and sex steroids such as estrogen and testosterone. Cholesterol is synthesized from acetate in a series of enzymatic reactions that can be separated into pre-squalene synthesis of isoprenoids and the post-squalene conversion of lanosterol to cholesterol. The rate limiting step for both isoprenoid and cholesterol synthesis is the conversion of 3-hydroxy-3-methylglutaryl-CoA to mevalonic acid catalyzed by 3-hydroxy-3-methylglutaryl-CoA reductase [1]. The first sterol formed in the cholesterol biosynthetic pathway is lanosterol. Lanosterol is a 30-carbon sterol formed by the cyclization of the squalene. Multiple enzymatic steps are involved in the conversion of 30-carbon lanosterol to 27-carbon cholesterol.

The principal route of cholesterol synthesis in humans is the Kandutsch–Russell pathway [2]. In this biosynthetic pathway, the immediate precursor of cholesterol is 7-dehydrocholesterol (7DHC). As shown in Fig. 1, the reduction of 7DHC to yield cholesterol is catalyzed by 3β-hydroxysterol Δ^7-reductase (DHCR7). Tissues such as brain, testes, and exocrine breast can have significant levels of desmosterol in addition to cholesterol [3–5]. Desmosterol is a 27-carbon sterol that differs from cholesterol due to the presence of a C24–25 double bond in the side chain. In the synthesis of desmosterol, DHCR7 is required for the reduction of 7-dehydrodesmosterol (Fig. 1).

To date, eight inborn errors of cholesterol synthesis have been described. Although multiple enzymatic steps are involved in the synthesis of squalene from acetyl-CoA, only one enzymatic defect of pre-squalene cholesterol synthesis has been identified. Mevalonic aciduria (MIM 251170) [6–8], and the Hyper-IgD with Periodic Fever syndrome (MIM 260920) [9–13] are both caused by mutations of the mevalonate kinase gene (*MVK*). The

* Corresponding author. Fax: +1 301 480 5791.
E-mail address: fdporter@helix.nih.gov (F.D. Porter).

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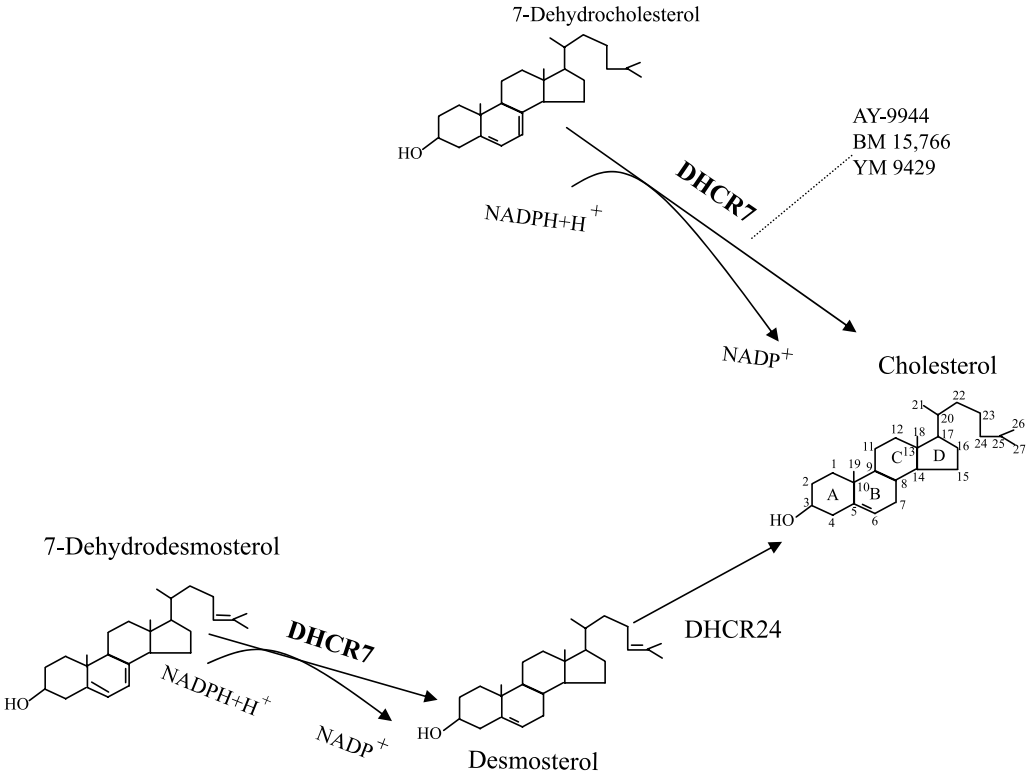

Fig. 1. Terminal enzymatic reactions in cholesterol synthesis. DHCR7 reduces the C7–8 double of bond of both 7-dehydrocholesterol and 7-dehydrodesmosterol to yield cholesterol and desmosterol, respectively. DHCR24 can reduce the C24–25 double bond in desmosterol to yield cholesterol. NADPH is a cofactor for DHCR7. AY 9944, BM,766, and YM 9429 are inhibitors of DHCR7.

post-squalene cholesterol biosynthetic defects are associated with congenital malformation syndromes. The prototypical example of this group of disorders is the Smith–Lemli–Opitz syndrome (SLOS). Other human malformation syndromes due to inborn errors of cholesterol synthesis include desmosterolosis, lathosterolosis, CHILD syndrome, autosomal dominant chondrodysplasia type 2, HEM dysplasia, and some cases of Antley–Bixler syndrome.

Desmosterolosis (MIM 602398) and lathosterolosis (MIM 607330) are rare autosomal recessive disorders of cholesterol synthesis that have clinical overlap with SLOS. To date, only two patients have been identified with each of these disorders. Desmosterolosis is due to deficiency of 3β-hydroxysterol Δ^{24}-reductase (DHCR24) activity, and desmosterolosis patients have increased desmosterol levels in plasma, tissues, and cultured cells [14]. DHCR24 reduces the unsaturated C24–25 bond in the side chain of desmosterol to yield cholesterol, and *DHCR24* maps to chromosome 1p33–p31.1 [15]. Mutations of *DHCR24* have been identified in patients with desmosterolosis [15]. Lathosterolosis patients have a deficiency of lathosterol 5-desaturase (SC5D) activity due to mutation of the *SC5D* gene [16,17]. SC5D catalyzes the conversion of lathosterol to 7DHC. The *SC5D* gene maps to chromosome 11q23.3 [18].

Autosomal dominant chondrodysplasia punctata type 2 (CDPX2, MIM 302960) and CHILD syndrome (MIM 308050) are two X-linked disorders of cholesterol synthesis. CDPX2 is due to mutation of the emopamil-binding protein gene (*EBP*) which encodes 3β-hydroxysterol-Δ^8,Δ^7-isomerase [19–21]. EBP catalyzes the conversion of cholesta-8(9)-en-3β-ol to lathosterol, and *EBP* maps to chromosome Xp11.23–p11.22 [22,23]. CHILD syndrome, or congenital hemidysplasia with ichthyosiform erythroderma and limb defects (MIM 308050) has been reported to be due to mutation of either the NAD(P)H steroid dehydrogenase-like gene (*NSDHL*) [24] or EBP [25]. The involvement of *EBP* mutations in CHILD syndrome is controversial. *NSDHL* encodes a 3β-hydroxysterol dehydrogenase that is involved in the demethylation of 4,4-dimethylcholesta-8-en-3β-ol to yield cholesta-8(9)-en-3β-ol [26]. *NSDHL* maps to Xq28 [27].

Two additional human malformation syndromes are due to genetic defects that cause a secondary abnormality in cholesterol synthesis. Kelley et al. [28] initially showed an accumulation of lanosterol and dihydrolanosterol in a patient with Antley–Bixler Syndrome (ABS, MIM 207410); however, they did not find mutations in the lanosterol-14-α-demethylase gene (*CYP51*), as would have been predicted from the sterol profile. Cytochrome P450 reductase is a cofactor for both lanosterol-14-α-demethylase and enzymes involved in steroid biogenesis. Abnormalities of steroid biogenesis have also been reported in patients with ABS [29,30]. Recently,

Fluck et al. [31] reported finding mutations of the cytochrome P450 reductase (POR) gene in some patients with ABS. This gene is localized on 7q11.2 chromosome [32]. Hydrops-ectopic calcification-moth-eaten skeletal dysplasia (HEM dysplasia, MIM 215140) was first described in 1988 by Greenberg et al. [33]. While screening cases of skeletal dysplasia for cholesterol biosynthetic defects, Kelley et al. [28] found increased levels of cholesta-8,14-dien-3β-ol and cholesta-8,14,24-trien-3β-ol in tissues from patients with HEM dysplasia. Cholesterol levels were normal. Elevated levels of cholesta-8,14-dien-3β-ol and cholesta-8,14,24-trien-3β-ol are consistent with impaired sterol Δ^{14}-reductase activity; however, HEM dysplasia is not due to mutation of the 3β-hydroxysterol Δ^{14}-reductase gene. Instead, Waterham et al. [34] found that mutations of the lamin B receptor gene (LBR) cause HEM dysplasia. The carboxyl end of the lamin B receptor is homologous to other sterol reductase enzymes, and the lamin B receptor has been shown to have sterol Δ^{14}-reductase activity [35]. The *LBR* gene is localized on 1q42.1 chromosome [36]. Hoffman et al. [37] reported that a single heterozygous mutation of *LBR* causes the Pelger–Huët anomaly (PHA). The Pelger–Huët anomaly consists of hypolobulation of nuclei in leukocytes. Based on a review of the literature, Oosterwijk et al. [38] suggest that homozygosity for LBR mutations can give rise to either a mild phenotype which includes the PHA or a severe HEM dysplasia phenotype. This wide phenotypic spectrum is presumably due to allelic heterogeneity; however, this still needs to be confirmed by molecular characterization of *LBR*.

SLOS (MIM 270400) is an autosomal recessive, multiple malformation, mental retardation syndrome first described by Smith et al. [39] in 1964. Rutledge et al. [40] later described a severe variant that became known as type II SLOS (MIM 268670). SLOS was first identified as an inborn error of cholesterol synthesis in 1993 [41,42]. The biochemical findings of increased 7DHC and decreased cholesterol levels suggested that the underlying defect was due to impaired DHCR7 function. Five years after the biochemical defect was identified, *DHCR7* was cloned [43], and mutations of *DHCR7* were identified in SLOS patients [44–46]. This review will focus on what is known about the biochemistry, molecular biology, and mutation spectrum of *DHCR7*.

DHCR7 gene structure and mRNA

The *DHCR7* cDNA (GeneID:1717, Accession: nc_000011) was cloned in 1998 by three independent groups [43–46]. *DHCR7* was mapped to chromosome 11q12–13 by radiation hybrid mapping [41], and chromosome 11q13 by FISH [45,46]. The human *DHCR7* gene spans 14,100 base pairs (bp) of genomic DNA, consisting of nine exons ranging in size from 64 bp (exon 1) to 465 bp (exon 9) [45]. The genomic structures of *DHCR7* and the corresponding rat and murine *Dhcr7* are shown in Fig. 2. The *DHCR7* mRNA is 2786 bp long, and has a 1425 bp open reading frame. The *DHCR7* open reading frame is encoded by exons three through nine. The 194 bp 5′-untranslated region of DHCR7 is encoded by exons one, two and part of three. The translation start codon is encoded by exon three. Exon nine encodes the carboxyl end of the DHCR7 protein, and a 961 bp 3′-untranslated region. The genomic structure of the rat *Dhcr7* gene is similar to the human gene [47]. Currently, only eight exons have been reported for the murine *Dhcr7* gene, and there appears to be two alternative first exons encoding the 5′-untranslated sequence [45]. Murine *Dhcr7* was mapped to chromosome 7F5 by FISH [42]. Rat *Dhcr7* was mapped to chromosome 8q2.1 by FISH [48]; however, the NCBI database indicates that rat *Dhcr7* is localized on chromosome 1q41. Consistent with the NCBI database localization, murine chromosome 7 is syntenic to rat chromosome 1.

DHCR7 expression

As expected for an enzyme involved in cholesterol synthesis, *DHCR7* is ubiquitously expressed with the highest mRNA levels detected in adrenal gland, liver, testis, and brain tissue [43]. *DHCR7* expression is induced by sterol deprivation [49]. Kim et al. [50] characterized the rat *Dhcr7* promoter. This group showed that the 179 bp 5′ of the transcription start site were necessary for sterol-regulated transcription. No TATAA box was identified in this region, which is consistent with multiple transcription start sites reported for rat *Dhcr7* [47]. The DNA sequence immediately 5′ of the rat *Dhcr7* gene encodes a number of potential regulatory elements. These include four SP1 sites, two NF-Y sites, and a single sterol responsive element-1/E-box (SRE1/E-box) binding site. DNase footprinting, electrophoretic mobility shift assays, and mutation analysis were used to demonstrate that the SP1 site at −125, the NF-Y site at −88, and the SRE1/E-box at −33/−22 regulate *Dhcr7* expression. NF-Y and SP1 are general transcription factors. SRE1 elements bind sterol regulatory binding proteins (SREBP), and regulate the expression of sterol responsive genes [51,52]. The DNA sequence corresponding to the 506 bp immediately 5′ of exon one of the human *DHCR7* gene is shown in Fig. 3. Similar to the rat gene, the human gene does not appear to have a TATAA box. This region contains three SP1 sites (−54/−59, −125/−130, and −146/−151), and an inverted NF-Y site (−84/−88). A potential partial SRE1/E-box element is located at −307/−312//−301/−306. A partial SRE1 element has been shown to function in the human squalene synthase promoter [53]. Although it needs to be experimentally demonstrated, these putative promoter regulatory sequences may function to regulate the expression of the human gene.

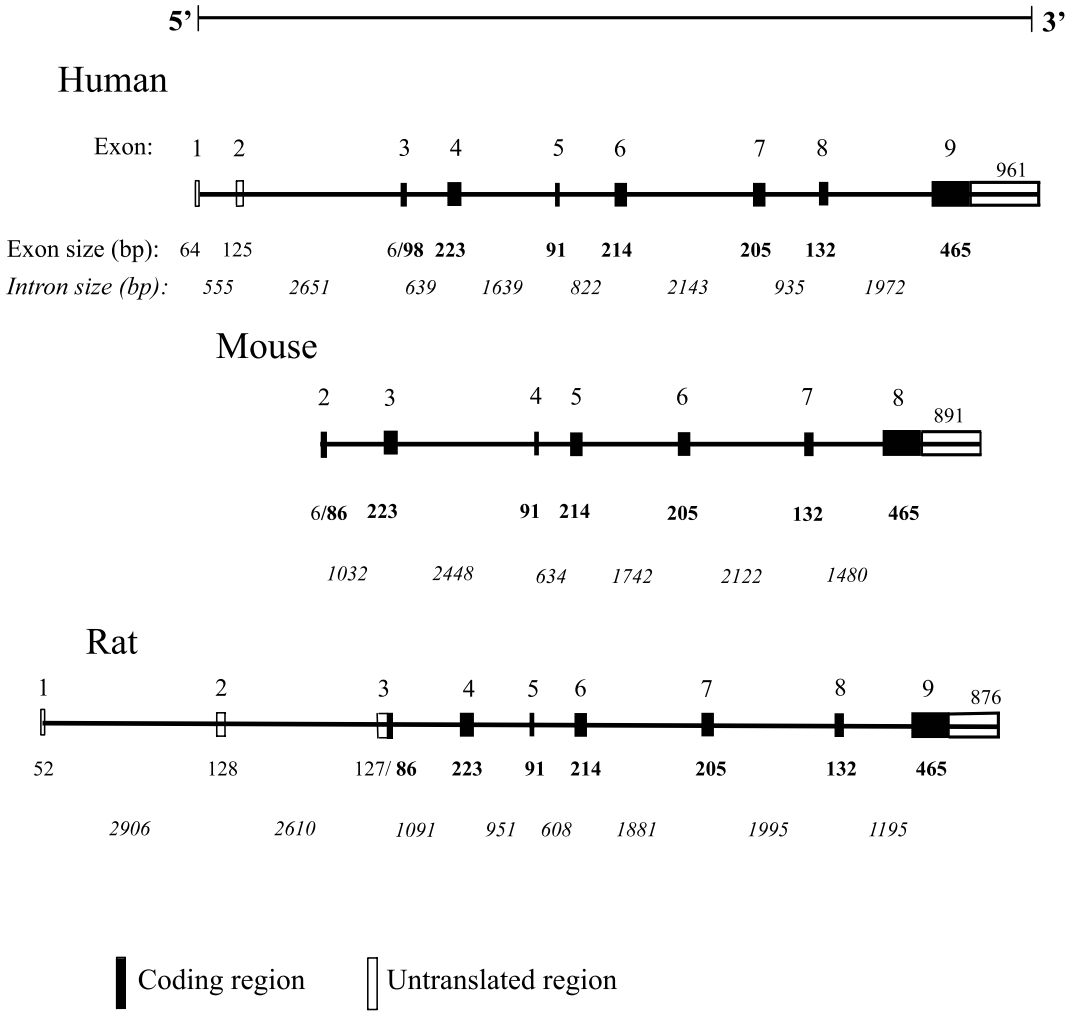

Fig. 2. Genomic structure of human, murine, and rat DHCR7. In this figure, exons are indicated by boxes. Coding regions are filled and untranslated regions are open. The size of the exons, in base pairs, is indicated by regular type and the size of introns is indicated by cursive type. The genomic structure of the 5′-untranslated region of the mouse gene is not well defined. However, two short alternatively spliced exons, 1a and 1b, have been reported approximately 6500 bp upstream of the first coding exon [45].

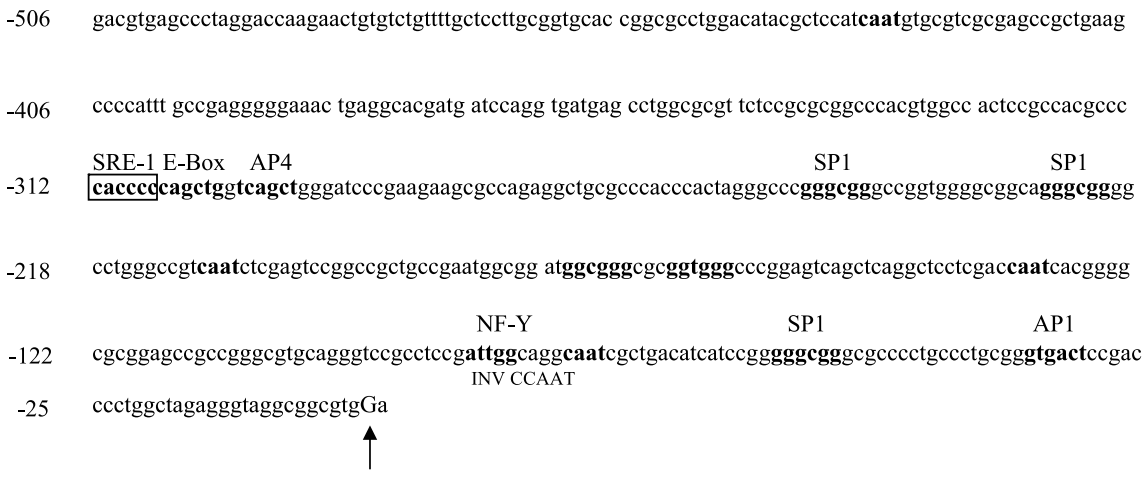

Fig. 3. Genomic DNA sequence of the potential DHCR7 promoter region. This figure shows the 506 bp of DNA immediately 5′ of the start of exon 1 (arrow). Potential regulatory binding sites are indicated.

Lee et al. [47] reported that the 5′UTR of rat *Dhcr7* undergoes alternative splicing. The different *Dhcr7* isoforms were differentially expressed in various tissues, and at different ages. Based on these observations, this group has proposed that *Dhcr7* activity may be regulated by tissue specific transcription and alternative splicing.

DHCR7 enzymology and protein structure

The terminal enzymatic steps in cholesterol synthesis are shown in Fig. 1. DHCR7 catalyzes both the reduction of 7DHC to cholesterol and the reduction of 7-dehydrodesmosterol to desmosterol. Thus, the synthesis of both cholesterol and desmosterol are impaired in SLOS. The conversion of 7DHC to cholesterol was first proposed in the early 1960s [2]. Subsequent work established 7DHC as the sterol intermediate involved in the conversion of lathosterol to cholesterol, and that reduction of 7DHC to yield cholesterol required NADPH [54]. Wilton et al. [55,56] established a direct transfer of hydrogen from NADPH to the 7α-position of cholesterol, and that the 8β-hydrogen was derived from water. Recent work by Nishino and Ishibashi [57] suggests that NADPH cytochrome-P450 oxidoreductase activity is essential for DHCR7 catalyzed reduction of 7DHC, and that DHCR7 may be an iron-containing enzyme. DHCR7 activity has been localized to microsomal fractions, and Sterol Carrier Protein 2 (SCP2) activates the conversion of 7DHC to cholesterol [58]. SCP2 can bind 7DHC and may function to increase substrate availability [59,60].

Shefer et al. [61] characterized the substrate specificity of DHCR7. Although less efficient than the reduction of 7DHC, this group demonstrated that DHCR7 can reduce the C7–8 double bond in the B-ring of ergosterol, 7-dehydrositosterol, and 7-dehydroepicholesterol. Ergosterol reduction to brassicasterol has been used as a surrogate assay to diagnose SLOS [62]. A number of synthetic compounds have been shown to inhibit DHCR7 and cause an accumulation of 7DHC (Fig. 1), including AY9944 [63,64], BM15.766 [65–67], and YM9429 [68]. AY9944 and BM15.766 are non-competitive inhibitors of DHCR7 [61]. These drugs have been used to produce teratogenic rodent models of SLOS [63,65,67,69,70].

Although the data is limited, DHCR7 activity may be regulated by phosphorylation/dephosphorylation [61]. Treatment of liver microsomes with alkaline phosphatase resulted in decreased DHCR7 activity; whereas, DHCR7 activity increased when NaF, a non-specific phosphatase inhibitor, was included in the reaction buffer. In vivo regulation of DHCR7 activity by phosphorylation/dephosphorylation has not been demonstrated, nor has a specific phosphorylation site been experimentally identified.

The human *DHCR7* cDNA predicts a protein of 475 amino acids (NP_001351). *DHCR7* homologs from *Mus musculus* (NP_031882), *Rattus norvegicus* (NP_071784), *Danio rerio* (NP_958487), and *Xenopus laevis* (AAH 54203) have been identified. Amino acid sequence identity of the predicted murine, rat, zebrafish, and frog proteins with the predicted human protein are 88, 87, 72, and 73%, respectively (Fig. 4). The human protein is predicted to have a molecular weight of 54,489 kDa, and an isoelectric point (p*I*) of 9.05 [71]. Overexpression of a FLAG epitope tagged DHCR7 protein in COS cells resulted in an apparent molecular weight of 42 kDa for FLAG-DHCR7 (Our unpublished data). Heterologous expression of DHCR7 in yeast gives a similar result [43]. The discrepancy between predicted and observed molecular weight may be due to aberrant migration in SDS–PAGE. A similar finding has been reported for the structurally related lamin B receptor [72].

The predicted amino acid sequence encodes several protein motifs. DHCR7 has both a sterol reductase 1 (amino acids 213–228, GNFFYNYMMGIEFNPR) and a sterol reductase 2 (amino acids 439–462, LLTHRCLR DEHRCASKYGRDWERY) motifs. Other proteins with sterol reductase 1 and sterol reductase 2 motifs include yeast enzymes involved in ergosterol synthesis (ERG3, ERG4, ERG24, and sts1), the 3β-hydroxysterol Δ^{14}-reductase and the lamin B receptor. DHCR24, which reduces desmosterol to cholesterol, is not a member of this protein family. DHCR24 is a FAD-dependent oxidoreductase [73]. In addition to the sterol reductase 1 and sterol reductase 2 motifs, DHCR7 also has a 12 amino acid sterol reductase signature motif (amino acids 394–405, LLVSGFWGVARH). This sterol reductase signature motif is found in sterol reductase family members [74]. The enzymatic function of these various protein motifs has not been determined.

A potential sterol-sensing domain (SSD) was identified by Bae et al. [48] in rat DHCR7. This region of rat protein has 47% amino acid similarity and 13% amino acid identity with the SSD of mouse sterol cleavage activating protein [48]. The corresponding region in the human protein spans amino acids 181–362. SSDs have been identified in a number of other proteins, including HMG-CoA reductase [75], sterol cleavage activating protein [76,77], and Niemann–Pick type C protein [78]. All three of these proteins are involved in cholesterol synthesis or homeostasis. Patched [79] and dispatched [80], two proteins involved in sonic hedgehog signaling, also have a SSD protein motif. The actual function of the SSD in these various proteins is not known. Recently, Radhakrishnan et al. [81] reported that SCAP binds cholesterol in vitro, and Ohgami et al. [82] demonstrated a direct binding between NPC1 and azocholestanol. Sterol binding required a functional SSD.

Multiple potential phosphorylation sites (S14, S25, Y64, S83, T89, T93, S113, T135, Y143, S205, Y291,

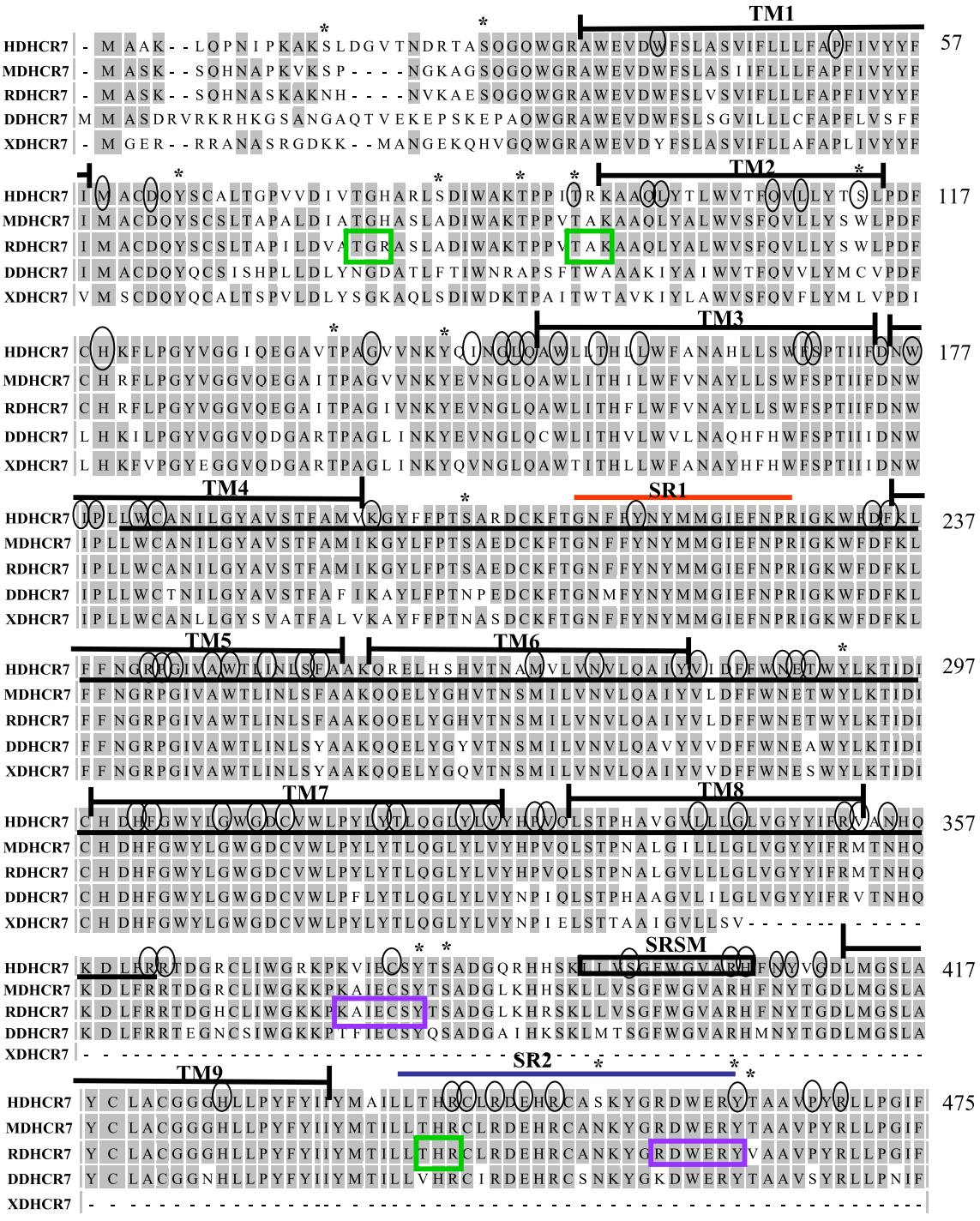

Fig. 4. Amino acid sequence alignment for DHCR7 orthologs. This figure shows the amino acid sequence for the human DHCR7 (HDHCR7), mouse (MDHCR7), rat (RDHCR7), zebrafish (DDHCR7), and *Xenopus* (XDHCR7) DHCR7 orthologs. Alignment was performed using ClustalW. Numbering is relative to the human sequence. The sterol reductase 1 motif (SR1) is indicated by a red line, the sterol reductase 2 motif (SR2) is indicated by a blue line, and amino acids corresponding to the sterol reductase signature motif (SRSM) is indicated by the black box. The putative sterol-sensing domain is indicated by a line under the human sequence. Potential phosphorylation sites predicted by NetPhos 2.0 (http://www.cbs.dtu.dk/cgi_bin/nih, [118]) are indicated by asterisks. Potential protein kinase C phosphorylation sites are boxed green, and potential tyrosine kinase phosphorylation sites are boxed in purple [48]. Hydrophilic regions that correspond to predicted intramembranous or transmembrane domains (TM) are above the human sequence. Numbering of these domains is based on the model first proposed by Fitzky et al. [45]. Amino acid residues that have corresponding mutant alleles are circled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Y382, S453, Y462, and T463) are present in DHCR7. These are indicated in Fig. 4. In the predicted amino acid sequence for rat DHCR7, Bae et al. [48] identified three potential protein kinase C phosphorylation sites (amino acids 73–75, TGR; amino acids 89–91, TAK; amino acids 437–439, THR), and two potential tyrosine kinase phosphorylation sites (amino acids 372–378, KAIECSY and amino acids 450–458, KYGRDWERY). Two of these potential sites (amino acids 437–439, THR and amino acids 450–458, KYGRDWERY) are conserved in the human, murine, rat, and zebrafish proteins (Fig. 4). The potential tyrosine phosphorylation site corresponding to amino acids 450–458 is contained within the sterol reductase 2 motif. Further work is needed to define the sites and function of DHCR7 phosphorylation.

DHCR7 is an integral membrane protein. Although the transmembrane topology of DHCR7 has not been experimentally determined, a number of models have been proposed based on the predicted amino acid sequence (Fig. 5). Fitzky et al. [45] proposed a model with nine transmembrane spanning domains. Waterham and Wanders [83] proposed a more conservative model with six transmembrane spanning domains. Both of these models assume that the amino end of the protein is on the cytosolic side of the membrane. This assumption is based on analogy with the lamin B receptor. The amino terminus of the lamin B receptor is oriented toward the nucleosol, which is contiguous with the cytoplasm. This is a reasonable assumption given that the lamin B receptor is highly homologous to 3β-hydroxysterol Δ^{14}-reductase, yeast sterol reductase enzymes, and DHCR7, and given that the lamin B receptor has sterol

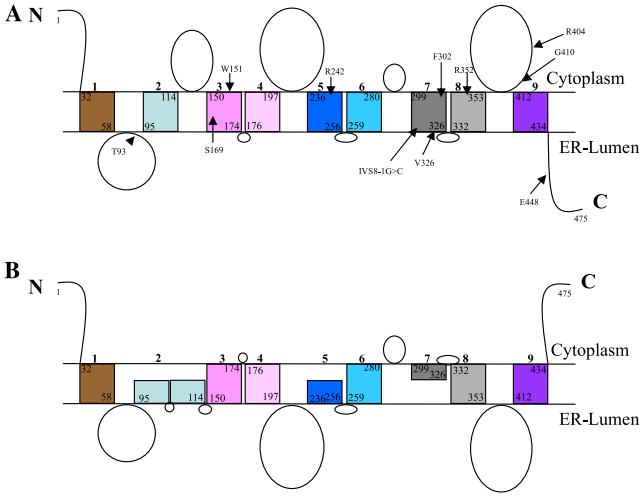

Fig. 5. Predicted membrane topologies for DHCR7. (A) The nine transmembrane domain model of DHCR7 proposed by Fitzky et al. [45]. The locations of common DHCR7 mutations are indicated by arrows. (B) The six transmembrane domain model of DHCR7 proposed by Waterham and Wanders [83]. Corresponding predicted transmembrane/intramembranous regions are indicated by color-coding. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Δ^{14}-reductase activity [35]. These two proposed models differ in orientation of the carboxy terminus relative to the cytosol, and in the localization of the extramembranous domains that Fitzky et al. [45] proposed may be involved in NADPH binding. Other models with seven transmembrane regions have also been proposed [71,84]. Until experimental data on the membrane topology of DHCR7 and functional domains becomes available, any of these models could prove accurate.

DHCR7 mutations

Mutations of DHCR7 cause SLOS. SLOS is a typical autosomal recessive disorder in which mutation of both alleles is necessary to cause the disorder. In a review of 599 published *DHCR7* mutations, we identified 105 different mutant alleles (Table 1). Missense mutations are the most common (90/105); however, nonsense mutations, splicing defects, insertions and deletions have been reported. In descending order, the twelve most frequent *DHCR7* mutant alleles are IVS8-1G > C (28.2%), T93M (10.4%), W151X (6.0%), R404C (5.2%), V326L (5.0%), R352W, and E448K (both 3.2%), G410S (2.2%), R242C (1.8%), S169L (1.7%), F302L (1.3%), and R242H (1.0%). These 12 mutations account for 69.2% of reported mutant alleles.

IVS8-1G > C is the most frequently reported *DHCR7* mutation, and accounts for almost one third of reported mutant alleles. The IVS8-1G > C mutation disrupts the splice acceptor site that is responsible for joining exons eight and nine. This results in the utilization of a cryptic splice site in intron eight, and causes the insertion of 134 nucleotides into the DHCR7 transcript. IVS8-1G > C is a null mutation; therefore, patients homozygous for the IVS8-1G > C allele are severely affected [44,46,85–88]. *DHCR7* mutations have been identified on eleven different haplotypes (Tables 2 and 3). The IVS8-1G > C mutation is most frequently found on haplotype A [89–91]. Combining mutation and haplotype analysis, Witsch-Baumgartner et al. [89] showed that the IVS8-1G > C mutation likely arose in the British Isles and is found in a distinct West to East gradient across Northern Europe.

Several groups have developed restriction fragment length polymorphism assays to identify the IVS8-1G > C mutation [90,92–94]. Using these assays, attempts have been made to determine the incidence of SLOS by ascertaining the IVS8-1G > C carrier frequency in various populations. Many of these studies then extrapolate the IVS8-1G > C allele frequency (30%) to estimate a carrier frequency for all *DHCR7* mutations. Battaile et al. [93] reported a 1.06% (16/1503) carrier frequency for the IVS8-1G > C in an Oregon population. Yu et al. [92] identified one IVS8-1G > C allele in a group of 90 American Caucasians (1.11%). Nowacyk et al. [94] reported an IVS8-1G > C carrier frequency of 1.09% (17/1559) for Caucasians of European heritage. Finally, Opitz et al.

Table 1
DHCR7 mutant alleles

Nucleotide change	Exon	Effect on coding sequence	Site[a]	Allele number (reference)[b]	%
Missense					
3G > A	3	M1(I)	Initiation codon	4 (a and b)	0.67
151C > T	4	P51S	TM1	9 (c, d, e, f, and g)	1.50
176G > T	4	M59R	loop 1–2	1 (a)	0.17
185A > T	4	D62V	Loop 1–2	1 (a)	0.17
278C > T	4	T93M	Loop 1–2	62 (c, d, e, f, g, h, i, j, q, and x)	10.35
296T > C	4	L99P	TM2	5 (c, d, and e)	0.83
326T > C	5	L109P	TM2	5 (d, h, q, and x)	0.83
356A > T	5	H119L	Loop 2–3	2 (l and f)	0.33
413G > T	6	G138V	Loop 2–3	1 (g)	0.17
433A > C	6	I145L	Loop 2–3	1 (g)	0.17
440G > A	6	G147D	Loop 2–3	6 (d, f, and h)	1.00
443T > G	6	L148R	Loop 2–3	1 (e)	0.17
461C > T	6	T154M	TM3	6 (d, g, h, and x)	1.00
461C > G	6	T154R	TM3	2 (m and n)	0.33
470T > C	6	L157P	TM3	4 (c, d, n, and q)	0.67
502T > A	6	F168I	TM3	1 (e)	0.17
506C > T	6	S169L	TM3	10 (a, d, e, f, and g)	1.67
523G > C	6	D175H	Loop 3–4	2 (e and g)	0.33
529T > C	6	W177R	TM4	1 (h)	0.17
533T > A	6	I178N	TM4	1 (m)	0.17
536C > T	6	P179L	TM4	1 (e)	0.17
545G > T	6	W182L	TM4	1 (x)	0.17
546G > C	6	W182C	TM4	2 (d and f)	0.33
548G > A	6	C183Y	TM4	1 (x)	0.17
592A > G	6	K198E	Loop 4-5	1 (x)	0.17
Not reported		D234Y	Loop 4-5	1 (k)	0.17
704T > C	7	F235S	Loop 4-5	1 (g)	0.17
724C > T	7	R242C	TM5	11 (d, g, h, p, and q)	1.84
725G > A	7	R242H	TM5	6 (f, h, m, and x)	1.00
728C > G	7	P243R	TM5	3 (e and f)	0.50
730G > A	7	G244R	TM5	4 (f, i, l, and x)	0.67
740C > T	7	A247V	TM5	3 (c, d, and h)	0.50
742T > C	7	W248R	TM5	2 (q, bb)	0.33
744G > T	7	W248C	TM5	1 (l)	0.17
753C > G	7	I251M	TM5	1 (a)	0.17
755A > G	7	N252S	TM5	2 (a)	0.33
Not reported	7	S254A	TM5	1 (f)	0.17
765C > A	7	F255L	TM5	1 (x)	0.17
808A > G	7	M270V	TM6	3 (a, m, and n)	0.50
Not reported		N274K	TM6	1 (f)	0.17
839A > G	8	Y280C	TM6	2 (q and r)	0.33
841G > A	8	V281M	Loop 6–7	2 (d and k)	0.33
852C > A	8	F284L	Loop 6–7	2 (e and z)	0.33
861C > A	8	N287K	Loop 6–7	3 (e)	0.50
862G > A	8	E288K	Loop 6-7	1 (m)	0.17
866C > T	8	T289I	Loop 6–7	4 (d, h, and q)	0.67
Not reported	8	H301Q	TM7	2 (f)	0.33
906C > G	8	F302L	TM7	8 (a, e, f, and k)	1.34
920G > A	8	G307D	TM7	1 (m)	0.17
925G > A	8	G309S	TM7	2 (m and n)	0.33
931T > G	8	C311G	TM7	1 (d)	0.17
932G > A	8	C311Y	TM7	1 (d)	0.17
952T > A	8	Y318N	TM7	2 (h and q)	0.33
956C > T	8	T319M	TM7	1 (a)	0.17
957G > A	8	T319A	TM7	2 (m and g)	0.33
970T > C	9	Y324H	TM7	4 (d and e)	0.67
976G > T	9	V326L	TM7	30 (c, d, e, f, n, q, s, and z)	5.00
986C > T	9	P329L	Loop 7–8	2 (i and t)	0.33
988G > A	9	V330M	Loop 7–8	1 (i)	0.17
1022T > C	9	L341P	TM8	1 (h)	0.17
1030G > C	9	G344R	TM8	1 (g)	0.17
1031G > A	9	G344D	TM8	1 (g)	0.17

(*continued on next page*)

Table 1 (*continued*)

Nucleotide change	Exon	Effect on coding sequence	Site[a]	Allele number (reference)[b]	%
1054C > T	9	R352W	TM8	19 (c, d, e, f, g, j, n, q, and y)	3.17
1055G > A	9	R352Q	TM8	3 (d)	0.50
1058T > C	9	V353A	TM8	1 (d)	0.17
1063A > G	9	N355D	Loop 8–9	1 (a)	0.17
1084C > T	9	R362C	Loop 8–9	1 (d)	0.17
1087C > A	9	R363C	Loop 8–9	1 (i)	0.17
1138T > A	9	C380S	Loop 8–9	2 (c and d)	0.33
1138T > C	9	C380R	Loop 8–9	4 (d and g)	0.67
1139G > A	9	C380Y	Loop 8–9	4 (d, g, and q)	0.67
1190C > T	9	S397L	Loop 8–9	1 (d)	0.17
1210C > T	9	R404C	Loop 8–9	31 (c, d, f, g, and q)	5.18
1210C > A	9	R404S	Loop 8–9	3 (d and e)	0.50
1213C > T	9	H405Y	Loop 8–9	1 (g)	0.17
1219A > T	9	N407Y	Loop 8–9	2 (f and j)	0.33
1222T > C	9	Y408H	Loop 8–9	4 (d, f, h, and q)	0.67
1228G > A	9	G410S	Loop 8–9	13 (c, d, e, f, g, and aa)	2.17
1228G > C	9	G410R	Loop 8–9	1 (d)	0.17
1277A > C	9	H426P	TM9	1 (g)	0.17
1327C > T	9	R443C	C-terminus	2 (a and d)	0.33
1328G > A	9	R443H	C-terminus	2 (g and m)	0.33
1331G > A	9	C444Y	C-terminus	1 (h)	0.17
1337G > A	9	R446Q	C-terminus	5 (d, f, and i)	0.83
1342G > C	9	E448Q	C-terminus	1 (d)	0.17
1342G > A	9	E448K	C-terminus	19 (b, d, e, g, h, i, j, q, and s)	3.17
1349G > T	9	R450L	C-terminus	3 (d, n, and p)	0.50
1384T > C	9	Y462H	C-terminus	1 (e)	0.17
1400C > T	9	P467L	C-terminus	3 (m and u)	0.50
1406G > C	9	R469P	C-terminus	2 (e)	0.33
Deletion					
99-194 del	3	W33-S65 del	TM1 del	1 (v)	0.17
384-IVS5+4del	5	Frameshift		2 (d and j)	0.33
720-735del	7	Frameshift		2 (c and d)	0.33
1057Gdel	9	Frameshift		1 (a)	0.17
1068-1070del	9	356delH	C-terminus	1 (s)	0.17
Insertion					
682Cins	7	Frameshift		1 (v)	0.17
762Tins	7	Frameshift		1 (v)	0.17
Nonsense					
99G > A	4	E37X		1 (e)	0.17
292C > T	4	Q98X		1 (n)	0.17
445C > T	6	Q149X		1 (a)	0.17
452G > A	6	W151X		36 (c, d, h, i, j, o, n, q, and aa)	6.01
651C > A	7	Y217X		1 (a)	0.17
Splice-site					
IVS8-1G > C	8	Frameshift		169 (b, c, d, e, f, h, i, k, l, n, o, q, r, s, t, v, w, x, and y)	28.21
IVS8-1G > T	8	Frameshift		1 (x)	0.17
321G > C	4	Q107H + 12aa	TM2	2 (d and h)	0.33
Total: 105 mutations				599 alleles	

References: (a) Waterham and Wanders [83], (b) Langius et al. [125] (Reported as M1(L)), (c) Fitzky et al. [45], (d) Witsch-Baumgartner et al. [85], (e) Yu et al. [110], (f) Goldenberg et al. [88], (g) our unpublished data, (h) Krakowiak et al. [119], (i) Patrono et al. [123], (j) De Brasi et al. [106], (k) Nowaczyk et al. [107], (l) Waterham et al. [46], (m) Witsch-Baumgartner et al. [121], (n) Correa-Cerro et al. [105], (o) Nowaczyk et al. [126], (p) Neklason et al. [111], (q) Waye et al. [90], (r) Prasad et al. [124], (s) Evans et al. [120], (t) Patrono et al. [128], (u) Nezarati et al. [122], (v) Wassif et al. [44], (w) Nowaczyk et al. [87], (x) Jira et al. [86], (y) De Die-Smulders et al. [129], (z) Mueller et al. [130], (aa) Bzdúch et al. [131], and (bb) Shim et al. [127].

[a] Predicted protein site is based on the topology model proposed by Fitzky et al. [45]. TM should be interpreted as indicating a potential transmembrane spanning or intramembranous location.

[b] Mutations identified in sibs have only been scored once.

[95] report an IVS8-1G > C carrier frequency of 1.13% in a Caucasian population from Utah. Based on the IVS8-1G > C allele frequency of 30% (Table 1) and a carrier frequency of 1%, one can extrapolate to estimate a total *DHCR7* mutation carrier frequency of approximately 3% for Caucasian populations. A 3% carrier frequency

Table 2
DHCR7 haplotypes

Nucleotide position	Haplotypes										
	A[a]	B[a]	C[a]	D[a]	E[a]	F[a]	G[a]	H[a]	I[a]	J[b]	K[c]
189	A	G	A	A	G	G	G	G	G	G	G
207	C	C	C	C	C	C	C	T	T	C	T
231	C	C	C	T	T	C	C	C	T	T	C
438	C	C	C	C	C	C	C	C	T	C	T
969	G	G	G	G	G	G	G	G	G	G	G
1158	C	C	C	C	C	C	C	T	T	C	T
1272	T	T	C	T	T	C	C	C	C	C	C
1350	C	C	C	C	C	C	A	C	C	C	C

[a] Witsch-Baumgartner et al. [89].
[b] Waye et al. [90].
[c] Nowaczyk et al. [107].

Table 3
DHCR7 mutations and DHCR7 haplotypes

Mutation	Haplotype[a]
IVS8-1G > C	A(32), E(1)
T93M	A(1), E(2), J(10), K(1)
W151X	C(2), F(2), G(3)
V326L	A(6)
R404C	A(1), B(1), D(1), F(8)
R352W	A(5), I(1)
E448K	A(2)
G410S	A(1)
R242C	A(1)

[a] The number of reported alleles for each haplotype is indicated inside parentheses. Data compiled from [89–91,107].

predicts a disease incidence of about 1/4500. This estimate is clearly at odds with both clinical studies that show an SLOS incidence in the range of 1/10,000 to 1/60,000 [96–102], and with diagnostic rates in US that are consistent with a clinical incidence on the order of 1/50,000 [100]. One explanation of this discrepancy is that the true frequency of the IVS8-1G > C mutation is underestimated due to underascertainment of this severe allele in viable SLOS patients [103]. Supporting this idea are the findings that very few SLOS patients of African heritage have been reported even though the carrier frequency for this population is on the order of 0.8% [91,94], and that the observed incidence of IVS8-1G > C homozygous patients is lower than expected [88]. SLOS may be a major under-recognized cause of fetal loss [95].

The missense mutation, T93M (278C > T), is the second most frequently reported *DHCR7* mutation. The threonine at amino acid position 93 is highly conserved (Fig. 4). T93M tends to be associated with a mild to typical SLOS phenotype; however, severely affected patients have been reported [104,105]. The T93M mutation has been reported in association with four different *DHCR7* haplotypes (Table 3). T93M is the most common reported mutation in Italian SLOS patients [106]. Nowaczyk et al. [107] found that T93M is the most common *DHCR7* mutation in Cuban patients, and in patients of Mediterranean heritage, the T93M mutation is found on haplotype J. In the British Isles, T93M is the third most common *DHCR7* mutation, and is found on both haplotype A and E [89]. The T93M C to T mutation is found in the context of a CpG dinucleotide. CpG dinucleotides are sensitive to mutation. In this sequence context, the cytosine can be methylated, and subsequent spontaneous deamination of the methylated cytosine results in a C > T transition [108]. The observation that T93M is found on several distinct haplotypes is consistent with multiple mutational events occurring on different DHCR7 haplotypes in different ethnic populations.

The nonsense mutation, W151X (452G > A), is the third most common *DHCR7* mutation. Nonsense mutations cause premature protein termination, and mRNA transcripts encoding nonsense mutations often undergo nonsense-mediated decay [109]. Witsch-Baumgartner et al. [89] found that the W151X mutation occurs on three related haplotypes (C, F, and G), and appears to have arisen in Southern Poland. W151X, in contrast to IVS8-1G > C, is found in an East to West gradient across Northern Europe. Other *DHCR7* nonsense mutations are E37X [110], Q98X [105], Q149X [83], and Y217X [83]. Transcripts for both Q98X and W151X undergo nonsense-mediated decay [105].

A number of *DHCR7* mutations disrupt predicted protein motifs in DHCR7, R404C, S397L, and H405Y disrupt the highly conserved sterol reductase signature motif (amino acids 394–405). R404C is the fifth most common *DHCR7* mutation. R404C is due to a C–T transition found in context with a CpG dinucleotide. R404C has been reported in association with four different haplotypes. Like T93M, it is possible that the R404C mutant allele has arisen multiple times in different populations. R404C has been reported in a number of patients of French ancestry [88,90]. This mutation, in association with a null allele, results in a classical to severe clinical phenotype [105]. Although only reported once, both S397L [88] and H405Y [unpublished data] were identified in severely affected patients. Based on this information, mutation of the sterol reductase signature motif appears to significantly impair DHCR7 function. No missense mutations of the sterol reductase 1 domain (amino acids 213–228) have been reported. Eight different missense mutations (R443C, R443H, C444Y, R446Q, E448Q, E448K, R450L, and Y462H) have been identified in the sterol reductase domain 2 (amino acids 439–462). E448K is the sixth most common *DHCR7* mutant allele. Both R443H and E448K appear to affect protein stability [88]. In contrast, the R450L mutation is associated with a mild phenotype ([111] and unpublished observation). The R450L mutant protein is stable [88] and R242C/R450L lymphoblasts appear to have a near normal apparent V_{max} for DHCR7 activity [111]. Fifty-two different mutations have been identified in the region corresponding to the potential SSD of DHCR7. Among the more common missense mutations found in this potential protein domain are V326L, R352W,

R242C, F302L, and R242H (Fig. 4 and Table 1). V326L and R352W are the fifth and sixth most common DHCR7 mutant alleles, respectively.

A number of cases of SLOS have been reported with only a single identified *DHCR7* mutation [45,88,90,106,110]. We have also identified a number of cases in which only a single heterozygous mutation can be detected in the coding regions of *DHCR7* (unpublished observations). It is possible that these cases are due to regulatory mutations that affect either transcription or stability of the *DHCR7* mRNA. To date, no *DHCR7* promoter mutations have been reported. Further work is necessary to elucidate the regulatory elements that control *DHCR7* expression, and to determine if mutations of these elements can cause SLOS.

Genotype–phenotype correlations

Establishing a genotype–phenotype correlation for *DHCR7* mutations and SLOS has been confounded by a number of factors. First, most patients are compound heterozygotes. Second, because of the large number of different mutations, except for the most common mutations, few patients with the same genotype are available to compare.

Two approaches have been used to establish a genotype–phenotype correlation for SLOS. Witsch-Baumgartner et al. [89] grouped individual mutations into four categories based on predicted protein domains. The four domains, in ascending order of mutational severity, were carboxy terminal mutations (CT), transmembrane domain mutations (TM), fourth cytoplasmic loop mutations (4L), and null mutations (0). They found a general correlation ($Rs = 0.37$) between SLOS severity scores and the following ranking of genotypes: TM/CT < TM/TM < 0/TM < 4L/TM < 4L/4L < 0/0. As would be expected, patients with a 0/0 genotype are severely affected. A limitation of this approach is that it is based on predicted protein domains that have not been experimentally determined. Clinical use of this system is limited by the large range of variability present in many of the categories.

The second approach to establishing a genotype–phenotype correlation has been to rank missense mutations found in association with one of the common null mutations (IVS8-1G > C or W151X) [104,105]. Consistent with both approaches is the observation that patients with two null mutations (IVS8-1G > C/IVS8-1G > C, W151X/W151X, and IVS8-1G > C/W151X) are typically severely affected. In these cases, one would predict that the patient's phenotype would be related to the second mutation. For common missense mutations, in general, patients with either a T93M/null or R352W/null genotype have a mild to typical phenotype, and patients with a V326L/null or R404C/null genotype have a typical to severe phenotype [105]. The clinical utility of this method is also limited by the observation that clinical phenotypes are variable even for patients with the same genotype. For example, the mean clinical severity score for patients with the T93M/null genotype is 29 ± 18 ($n = 29$) with a range of 12–75 [105].

More work clearly needs to be done to understand the high degree of phenotypic variability observed in SLOS. The phenotypic variation could be due to effects of other genes involved in cholesterol synthesis or homeostasis, variation in the conversion of 7DHC to abnormal steroids [112–114], oxysterols [115] or neuroactive steroids [116], or due to the influence of maternal factors. Recently, Witsch-Baumgartner et al. [117] found that maternal, but not patient, ApoE genotypes correlate with SLOS clinical severity scores.

Summary

Smith–Lemli–Opitz syndrome is the prototypical example of a human malformation syndrome due to an inborn error of cholesterol synthesis. Much progress has been made in identifying mutations of *DHCR7* that cause SLOS. To date, over 100 different *DHCR7* mutations have been reported. Significant work remains to determine the topology of DHCR7, identify the active sites of this enzyme, elucidate the factors underlying the phenotypic variability found in SLOS, and characterize the mechanisms regulating the activity of DHCR7 at both the transcriptional and protein level. Hopefully, such information will give insight into therapeutic approaches that will benefit these patients.

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