Review

Smith-Lemli-Opitz Syndrome and the *DHCR7* Gene

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Summary

Smith-Lemli-Opitz syndrome, a severe developmental disorder associated with multiple congenital anomalies, is caused by a defect of cholesterol biosynthesis. Low cholesterol and high concentrations of its direct precursor, 7-dehydrocholesterol, in plasma and tissues are the diagnostic biochemical hallmarks of the syndrome. The plasma sterol concentrations correlate with severity and disease outcome. Mutations in the *DHCR7* gene lead to deficient activity of 7-dehydrocholesterol reductase (DHCR7), the final enzyme of the cholesterol biosynthetic pathway. The human *DHCR7* gene is localised on chromosome 11q13 and its structure has been characterized. Ninety-one different mutations in the *DHCR7* gene have been published to date. This paper is a review of the clinical, biochemical and molecular genetic aspects.

Keywords: Smith-Lemli-Opitz Syndrome, 7-dehydrocholesterol reductase, mutations, cholesterol

Introduction

Smith-Lemli-Opitz syndrome (SLOS MIM 270400), a severe developmental disorder associated with multiple congenital anomalies, is caused by a defect of cholesterol biosynthesis, i.e. a deficiency of the enzyme 7-dehydrocholesterol reductase (DHCR7; EC 1.3.1.21), the final enzyme of the cholesterol biosynthetic pathway. Low cholesterol and high concentrations of its direct precursor, 7-dehydrocholesterol (7DHC), and its isomer 8-dehydrocholesterol (8DHC), in blood and tissues are the biochemical hallmarks of the syndrome (Smith *et al.* 1964; Irons *et al.* 1993; Tint *et al.* 1995). The plasma sterol concentration generally correlates with severity and outcome (Tint *et al.* 1995; Witsch-Baumgartner *et al.* 2000). That a single metabolic (enzymatic) defect in humans could lead to a multiple malformation syndrome was a novel and unexpected discovery. Cholesterol, an important constituent of the cell membrane of most eukaryotic cells, has important interactions with proteins which control embryonic development. In addition, cholesterol acts as the precursor for steroid hormones, bile acids and myelin formation in the brain, spinal cord and peripheral nervous system (Figure 1). Recently, several other reviewers have discussed various aspects of SLOS and other defects of cholesterol biosynthesis (Opitz, 1999; Fitzky *et al.* 1999; Kelley & Hennekam, 2000; Moebius *et al.* 2000; Waterham & Wanders, 2000; Haas *et al.* 2001; Battaille & Steiner, 2000; Nwokoro *et al.* 2001; Kelley & Herman, 2001). Here we discuss the clinical, biochemical and molecular genetic aspects of SLOS.

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Clinical Aspects of SLOS

Since the first description of SLOS as a clinical entity in 1964 (Smith *et al.* 1964) many papers have appeared describing a variety of common and less common clinical findings in patients with this multiple malformation/retardation syndrome (e.g. Opitz, 1999; Kelley & Hennekam, 2000; Cunniff *et al.* 1997; Ryan

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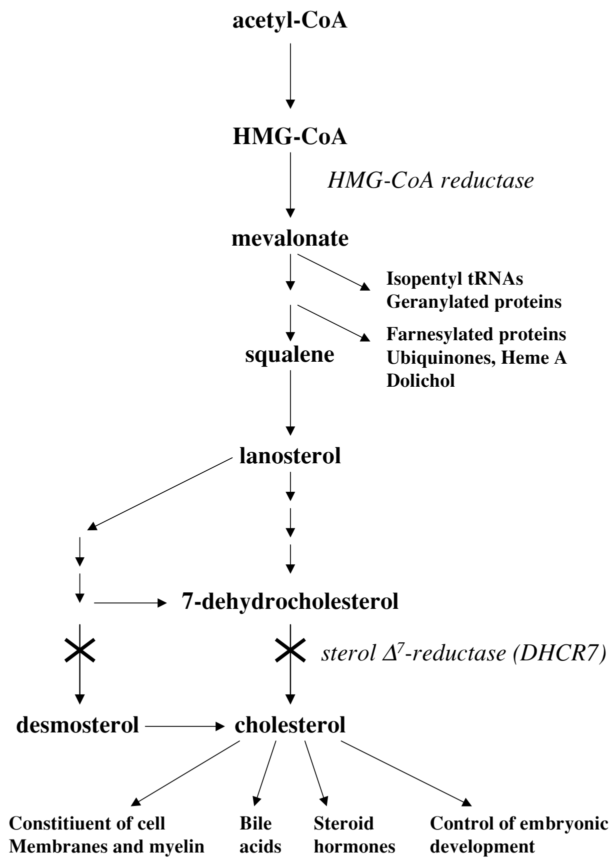

Figure 1 Cholesterol biosynthetic pathway.

et al. 1998). The clinical spectrum includes different morphogenic abnormalities such as craniofacial, internal organ, limb/skeletal and urogenital anomalies, as well as (intrauterine) growth and mental retardation, failure to thrive and behavioural problems. The frequently occurring clinical symptoms apparent in 164 biochemically confirmed SLOS cases (Kelley & Hennekam, 2000) are summarized in Table 1. While many of the signs alone are not disease-specific for SLOS, the combination of several may point to this disorder. Indeed the occurrence of second/third toe syndactyly, polydactyly, microcephaly, ptosis, long philtrum, (congenital) cataract, photosensitivity, Hirschprungs' disease (colonic aganglionosis), pyloric stenosis, or genital developmental anomalies (ranging from hypospadia to a complete sex reversal) in combination with neurodevelopmental delay, should raise clinical suspicion of SLOS (Figure 2). Through the years attempts have been made to distinguish the severe (lethal) type II form from the relatively mild type I form. The identification of the

Table 1 Clinical symptoms in SLOS. Findings in 164 biochemically confirmed cases (Kelly & Hennekam, 2000)

Finding	(%)
2/3 toe syndactyly	97
Mental retardation	95
Microcephaly	84
Postnatal growth retardation	82
Anteverted nares	78
Ptosis	70
Genital anomalies	65
Congenital heart defects	54
Polydactyly[†]	48
Cleft palate[*]	47
Abnormal lung lobation	45
Renal anomalies	43
Structural brain anomalies	37
Cataract	22
Colonic aganglionosis	16
Pyloric stenosis	14

[*] Includes cleft soft palate, submucous cleft, and cleft uvula.
[†] Includes postaxial polydactyly of hand(s)/foot.

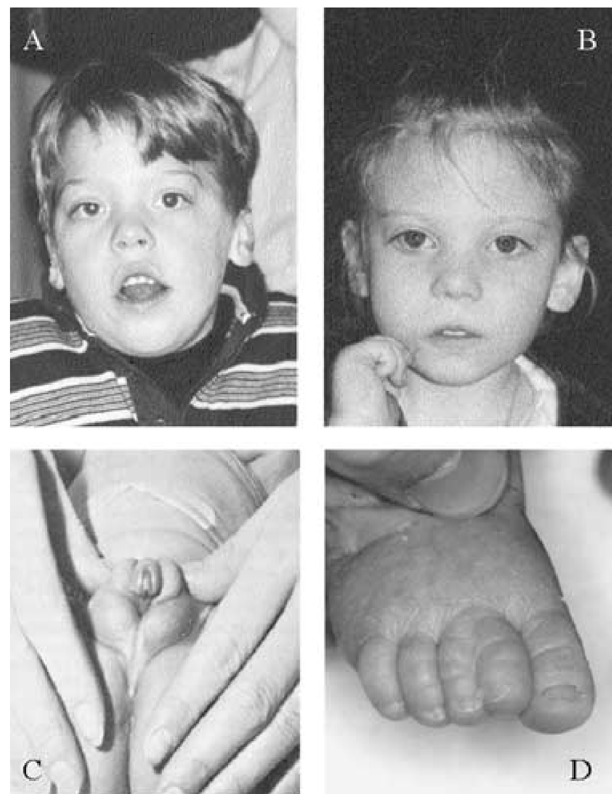

Figure 2 Clinical characteristics in SLOS. Facial dysmorphias including: mild ptosis, a long philtrum anteverted nares in a 5 year old boy (A) and a 3 year old girl (B). Genital anomalies in boys (C) and 2/3 toe syndactyly (D) present in respectively 65% and 97% of SLOS cases.

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biochemical and molecular basis of SLOS, however, has made clear that SLOS forms a clinical and biochemical continuum ranging from hardly recognizably mild diseases to severe lethal forms (Kelley, 1998, 2000; Waterham *et al.* 1998; Cunniff *et al.* 1997; Tint *et al.* 1995; Jira *et al.* 2001). The clinical description in the literature on SLOS is probably biased towards those more severely affected, and thus readily recognizable, patients. Reviewing published SLOS families, the incidence of fetal death and spontaneous abortions is twice as high as in the general population, suggesting the occurrence of severe cases leading to early death. With the introduction of selective screening and the availability of biochemical and genetic testing following the recent elucidation of the underlying cause of SLOS, mildly affected cases or even patients without clinical suspicion of SLOS can be readily diagnosed.

Therapeutic trials using dietary supplementation of cholesterol with or without bile acids have shown that plasma cholesterol levels can be increased in some patients. Concentrations of the precursors 7DHC and 8DHC, however, were only marginally altered, and clinical effects of the treatment so far have been rather disappointing (Irons *et al.* 1995; Ullrich *et al.* 1996; Elias *et al.* 1997). An alternative therapeutic strategy has been developed, treating 5 SLOS patients with Simvastatin (an oral HMG-CoA reductase inhibitor) for a median period of 2 years. The overall biochemical effect was impressive, with a decrease of 7DHC and 8DHC, and increase of cholesterol, in plasma to respectively 28% and 162% of the initial concentration with promising clinical improvement (Jira *et al.* 1997; Jira *et al.* 2000; and unpublished data). This therapeutic statin approach is currently being tested in a multicentre European Trial.

Biochemical Aspects

SLOS is caused by a deficiency of the enzyme 7-dehydrocholesterol reductase (DHCR7; EC 1.3.1.21), the final enzyme of the cholesterol biosynthetic pathway. Molecular cloning of the cDNA showed that the human enzyme is a protein with a calculated molecular weight of 54.5 kDa and nine putative/predicted transmembrane segments. It is microsomal membrane-bound and in humans the mRNA is expressed ubiquitously, with the highest expression in the adrenal gland, liver, testis and brain (Moebius *et al.* 1998).

Cholesterol is the main endproduct derived from the isoprenoid biosynthetic pathway (Goldstein & Brown, 1990). This pathway supplies cells with a variety of compounds, collectively called isoprenoids, which function in diverse cellular processes. Among these are *ubiquinone-10* and *heme A*, involved in electron transport, *dolichol*, a mediator of protein glycosylation, *isopentyl tRNAs*, and *farnesyl and geranyl groups* for prenylation of proteins that are involved in cell signalling and differentiation (Goldstein & Brown, 1990). Acetyl-CoA (C2) is converted into the isoprene unit, isopentenyl-PP (C5) by six different serial enzymatic reactions. After adding C5 to geranyl-PP (C10) through farnesyl-PP (C15), squalene (C30) is transformed to lanosterol (4,4,14-trimethylcholesta-8(9), 24-dien-3-ol) by cyclization. A series of enzymatic reactions is required to eventually produce cholesterol (Figure 1). Not only humans but the majority of organisms including animals, plants, fungi and micro-organisms are equipped with this important *de novo* (chole)sterol biosynthetic pathway, supplying sterols and steroids to cells, membranes and tissues. Cholesterol has a pivotal role during embryogenesis where it functions as a transporter-molecule for the Sonic hedgehog (Shh) signalling protein, which is essential for normal morphogenesis. Without cholesterol, Shh-transport and/or function is impaired (Hall *et al.* 1995; Porter *et al.* 1996; Hall *et al.* 1997; Lanoue *et al.* 1997; Cooper *et al.* 1998). The sterol derangement in SLOS (accumulation of 7DHC/8DHC and shortage of available cholesterol) undoubtedly influences the proper activation of the Shh-receptor. These findings may explain the phenotypic consequences of DHCR7 deficiency as observed in this syndrome: microcephaly, distinctive facies, cataract, syn/polydactyly, and a variety of organ malformations including genital abnormalities ranging from intersex to complete sex reversal in boys. Although the mechanism of the Shh-induced signalling pathway in vertebrates is not completely defined, it is known to regulate dorso-ventral patterning within the neural tube, limb, lung, genital, ocular, and retinal development as well as craniofacial morphogenesis (Krishnan *et al.* 1997; Hayes *et al.* 1998; Imokawa *et al.* 1997; Levine *et al.* 1997; Helms *et al.* 1997; Marigo *et al.* 1995; Hall

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et al. 1995; Kumar *et al.* 1996). In a recent null mutation mouse model without DHCR7 activity, accumulated 7-dehydrocholesterol was found to suppress sterol biosynthesis posttranslationally, in line with earlier observations in skin fibroblasts from SLOS patients (Shefer *et al.* 1997). This effect might exacerbate abnormal development in SLOS by increasing fetal cholesterol deficiency (Fitzky *et al.* 2001). The most predictive biochemical value in SLOS is the 7DHC/cholesterol ratio in plasma (Tint *et al.* 1995; Witsch-Baumgartner *et al.* 2000). In general, patients with a plasma 7DHC/cholesterol ratio between 0.5 and 1.0 have moderate SLOS. Patients with a plasma ratio <0.5 have a mild presentation and course, while plasma 7DHC/cholesterol ratio >1.0 is associated with severe SLOS (Krakowiak *et al.* 2000; Jira *et al.* 2001). This biochemical ratio could be a useful tool for prognosis and treatment in SLOS but cannot predict severity accurately.

To date, seven distinct inherited disorders have been linked to different defects in cholesterol biosynthesis. Two disorders are known to result from an enzyme defect in the pre-squalene segment of the pathway: the classical form of mevalonic aciduria (MIM 251170: Hoffmann *et al.* 1986; Houten *et al.* 2000; Haas *et al.* 2001), and the hyperimmunoglobulinemia D and periodic fever syndrome also known as Dutch-type periodic fever (MIM 260920: Houten *et al.* 1999; Drenth *et al.* 1999; Houten *et al.* 2000). Of the remaining five disorders, all due to different enzyme defects in the post-squalene segment of the pathway, four have been resolved at the molecular level recently by the demonstration of disease-causing mutations in the encoding genes. These include SLOS and the two X-linked dominant inherited and male-lethal disorders, Conradi-Hünermann-Happle syndrome (*CDPX2*; MIM 302960: Happle, 1979) and CHILD syndrome (MIM 308050: Happle *et al.* 1980) caused by deficiencies of sterol 8-7 isomerase (*EBP* gene at Xp11.22–23) or sterol C-4 demethylase (*NSDHL* gene at Xq28) respectively. In patients with desmosterolosis, a rare autosomal recessive disorder, characterized by multiple congenital anomalies, elevated levels of the cholesterol precursor desmosterol in plasma, tissue, and cultured cells suggested a deficiency of the enzyme 3beta-hydroxysterol Δ24-reductase (MIM 602398: FitzPatrick *et al.* 1998). Four mutations in two patients only recently gave the molecular confirmation of this defect (*DHCR24* gene at 1p31.1-p33: Waterham *et al.* 2001). The last, extremely rare and probably autosomal recessively inherited, disorder of sterol synthesis, namely Greenberg skeletal dysplasia (MIM 215140: Greenberg *et al.* 1988), presumably due to a deficiency of sterol 14-reductase, is detected by elevated levels of specific sterol intermediates in conjunction with decreased levels of cholesterol in tissues and cells of affected patients. Confirmation at the molecular level of this disorder awaits the identification of the corresponding gene and/or molecular analysis in affected patients.

Molecular Genetics of The *Dhcr7* Gene

Organisation and Conservation

In 1998, the human 7-dehydrocholesterol reductase gene (*DHCR7*, GenBank accession number: AF034544) was identified and assigned to chromosomal region 11q13 (Fitzky *et al.* 1998; Wassif *et al.* 1998; Waterham *et al.* 1998). Ninety-one different mutations in the *DHCR7* gene of patients with Smith-Lemli-Opitz syndrome have been described to date (Fitzky *et al.* 1998; Wassif *et al.* 1998; Waterham *et al.* 1998; Yu *et al.* 2000; De Brasi *et al.* 1999; Witsch-Baumgartner *et al.* 2000; Patrone *et al.* 2000; Waterham & Wanders, 2000; Krakowiak *et al.* 2000; Jira *et al.* 2001; Witsch-Baumgartner *et al.* 2001a; Witsch-Baumgartner *et al.* 2001b; Evans *et al.* 2001; Prasad *et al.* 2002). The cDNA has an open reading frame of 1425 base-pairs, coding for a polypeptide of 475 amino acids (Figure 3); alternative splicing has not been described. Structurally, the protein is strongly related to plant and yeast sterol reductases (Rahier *et al.* 1996; Waterham *et al.* 1998). The degree of identity of human *DHCR7* with *A. thaliana* sterol Δ7-reductase and *S. cerevisiae* sterol Δ14-reductase is 38% and 34% respectively (Waterham *et al.* 1998).

Frequency and Ethnic Aspects

SLOS is the most frequently occurring defect of cholesterol biosynthesis known to date. Estimated incidences of SLOS have historically been based on clinical diagnosis and initially ranged from 1:40,000–50,000 births (Opitz *et al.* 1994; Cunniff *et al.* 1997). Now that biochemical and genetic testing for this trait has become available the incidence turns out to be

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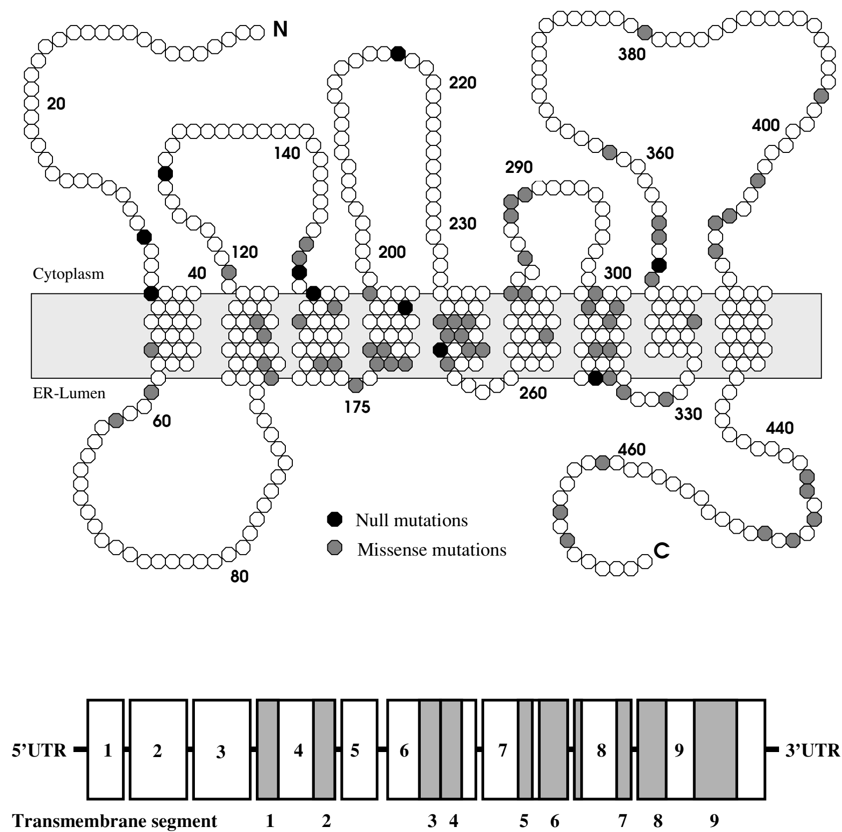

Figure 3 Predicted membrane topology (upper part) and intron-exon organisation (lower part) of the 7-dehydrocholesterol reductase, based on the data of Fitzky *et al.* (1998). Transmembrane segments 4 to 8 represent the, highly conserved, sterol-sensing domain. The coding sequences of the transmembrane segments are given as grey areas in the lower part of the figure. Position of null mutations (black) and missense mutations (grey) are indicated.

significantly lower due to formerly incorrect inclusions of cases with SLOS-like phenotypes. Based on biochemically confirmed cases an incidence of approximately 1:60,000 births has now been reported in the UK and the USA (Ryan *et al.* 1998; Kelley & Hennekam, 2000). From the number of biochemically and genetically confirmed Dutch SLOS cases we have estimated an incidence of approximately 1:80,000 births in The Netherlands (Jira *et al.* 2001; Waterham & Wanders, 2000). On the other hand, an incidence of 1: 20,000–26,500 of biochemically confirmed SLOS cases has been reported for Slovakia and Canada, respectively (Bzduch *et al.* 2000; Nowaczyk *et al.* 2001a).

The reason for the rather high incidence of SLOS is unclear but may be a consequence of both founder effects and heterozygote advantage. A founder effect has been suggested to explain the fact that SLOS is most common in Caucasian populations of east-European descent. A possible advantage for heterozygotes is a relative protection from atherosclerosis due to lowered blood cholesterol levels; also a lower risk for children to acquire rickets due to increased vitamin D production from elevated 7-DHC levels has been suggested (Kelley, 1998; Kelley & Hennekam, 2000).

Pathogenic Mutations and Polymorphisms

We performed a survey of the literature on mutations described in patients diagnosed with SLOS. Molecular data are presented in Table 2. The position of the mutations related to the position of the transmembrane segments and loops of the DHCR7 protein is illustrated in Figure 3.

Table 2 summarizes mutations described in SLOS patients by: nucleotide change, effect on coding sequence (for numbering of nucleotides and amino acids see: Waterham *et al.* 1998), affected exon,

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Table 2 91 Mutations in the DHCR7 gene causing SLOS (A) and 15 polymorphic mutations found in SLOS patients and controls (B).

A

Nucleotide Change	Effect on coding sequence	Exon	Localisation in DHCR7	Percentage of published mutations	Reference	Overall Incidence
3G>A	M1	3	N-terminus	2/64	2	
IVS3-1-95	Frameshift	4	-	1/6	5	
99G>A	W37X	4	-	1/60	3	
151C>T	P51S	4	MAH1	2/168, 2/60	1,3	
176G>T	M59R	4	MAH1 (loop1-2)	1/64	2	
185A>T	D62V	4	Loop1-2	1/64	2	
278C>T	T93M	4	Loop1-2	14/168, 6/64, 7/60, 7/18, 4/30	1,2,3,4,7	11.2%
296T>C	L99P	4	MAH2 (loop1-2)	2/168, 1/60	1,3	
321G>C	Q107H	4	MAH2	1/68, 1/30	1,7	
326T>C	L109P	5	MAH2	1/168, 1/64, 1/30	1,2,7	
356A>T	H119L	5	MAH2	1/64	2	
384-IVS5+4del	Frameshift		-	1/18	4	
385-IVS5+5del	Frameshift		-	1/168	1	
440G>A	G147D	6	Loop2-3	1/168, 1/30	1,7	
443T>G	L148R	6	Loop2-3	1/60	3	
445C>T	Q149X	6	-	1/64	2	
452G>A	W151X	6	-	14/168, 2/64, 1/18, 1/30	1,2,4,7	6.4%
461C>T	T154M	6	MAH3	3/168, 2/64, 1/30	1,2,7	
461C>G	T154R	6	MAH3		8	
470T>C	L157P	6	MAH3	1/168	1	
502T>A	F168I	6	MAH3	1/60	3	
506C>T	S169L	6	MAH3	4/168, 1/64, 1/60	1,2,3	
523G>C	D175H	6	Loop3-4	1/60	3	
529T>C	W177R	6	MAH4	1/30	7	
533T>A	I178N	6	MAH4		8	
536C>T	P179L	6	MAH4	1/60	3	
545G>T	W182L	6	MAH4	1/64	2	
546G>C	W182C	6	MAH4	1/168	1	
548G>A	C183Y	6	MAH4	1/64	2	
592A>G	K198E	6	MAH4	1/64	2	
651C>A	Y217X	7	-	1/64	2	
682Cins	Frameshift	7	-	1/6	5	
720-735del	Frameshift	7	-	1/168	1	
724C>T	R242C	7	MAH5	3/168, 1/30	1,7	
725G>A	R242H	7	MAH5	1/64, 1/30	2,7	
728C>G	P243R	7	MAH5	2/60	3	
730G>A	G244R	7	MAH5	2/64	2	
740C>T	A247V	7	MAH5	1/168, 1/30	1,7	
744G>T	W248C	7	MAH5	1/64	2	
753C>G	I251M	7	MAH5	1/64	2	
755A>G	N252S	7	MAH5	2/64	2	
762Tins	Frameshift	7	-	1/6	5	
765C>A	F255L	7	MAH5	1/64	2	
808A>G	M270V	7	MAH6	1/64	2	
839A>G	Y280C	8	MAH6	1/2	10	
841G>A	V281A	8	MAH6	1/168	1	
852C>A	F284L	8	MAH6	2/64, 1/60	1,3	
861C>A	N287K	8	Loop6-7	3/60	3	
862G>A	E288K	8	Loop6-7		8	
866C>T	T289I	8	Loop6-7	2/168, 2/30	1,7	
906C>G	F302L	8	Loop6-7	1/64, 1/60	1,3	

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Table 2 (*continued*)

A

Nucleotide Change	Effect on coding sequence	Exon	Localisation in DHCR7	Percentage of published mutations	Reference	Overall Incidence
920G>A	G307D	8	MAH7		8	
925G>A	G309S	8	MAH7		8	
931T>G	C311G	8	MAH7		1	
932G>A	C311Y	8	MAH7		1	
952T>C	Y318N	8	MAH7	1/30	7	
956C>T	T319M	8	MAH7	1/64	2	
957G>A	T319A	8	MAH7		8	
IVS8-1G>C	Frameshift		-	49/168,19/64,20/60, 1/6,1/2,11/30,5/8,1/2	1,2,3,5,6,7,9,10	31.5%
IVS8-1G>T	Frameshift		-	1/64	2	
970T>C	Y324H	9	MAH7	3/168, 1/60	1,3	
976G>T	V326L	9	MAH7	12/168, 1/64, 5/60, 1/8	1,2,3,9	6.3%
986C>T	P329L	9	Loop7-8	1/2	6	
1022T>C	L341P	9	MAH8	1/30	7	
1054C>T	R352W	9	Loop8-9	5/168, 1/64, 2/60, 2/18	1,2,3,4	3.2%
1055G>A	R352Q	9	Loop8-9	3/168	3	
1057Gdel	Frameshift	9	-	1/64	2	
1058T>C	V353A	9	Loop8-9	1/168	1	
1063A>G	N355D	9	Loop8-9	1/64	2	
1068-1070del	356delH	9	Loop8-9	1/8	9	
1084C>T	R362C	9	Loop8-9	1/168	1	
1138T>A	C380S	9	Loop8-9	1/168	1	
1138T>C	C380R	9	Loop8-9	1/168	1	
1139G>A	C380Y	9	Loop8-9	2/168, 1/64	1,2	
1190C>T	S397L	9	Loop8-9	1/168	1	
1210C>T	R404C	9	Loop8-9	18/168	1	10.7%
1210C>A	R404S	9	Loop8-9	2/168, 1/60	1,2	
1219A>T	N407Y	9	Loop8-9	1/18	4	
1222T>C	Y408H	9	Loop8-9	1/168, 1/30	1,7	
1228G>A	G410S	9	Loop8-9	2/168, 1/60	1,3	
1228G>C	G410R	9	Loop8-9	1/168	1	
1327C>T	R443C	9	C-terminus	1/168, 1/64	1,2	
1328G>A	R443H	9	C-terminus		8	
1331G>A	C444Y	9	C-terminus	1/30	7	
1337G>A	R446Q	9	C-terminus	2/168	1	
1342G>C	E448Q	9	C-terminus	1/168	1	
1342G>A	E448K	9	C-terminus	3/168,2/64,2/60, 2/18, 1/30,1/8	1,2,3,4,7	2.9%
1349G>T	R450L	9	C-terminus	1/168	1	
1384T>C	Y462H	9	C-terminus	1/60	3	
1400C>T	P467L	9	C-terminus		8	
1406G>C	R469P	9	C-terminus	2/60	3	

localisation in DHCR7 protein, incidence in study cohorts, their references and overall incidence. DNA sequencing of almost 400 SLOS-alleles identified 91 different mutations. The *DHCR7* gene mutations identified in SLOS patients were shown to have deleterious effects on the function of the DHCR7 protein (Fitzky *et al.* 1998; Wassif *et al.* 1998; Waterham *et al.* 1998).

Apart from the majority of missense mutations, four nonsense mutations (W37X, Q149X, W151X, Y217X), eight frameshift mutations leading to a premature stop: four deletions (IVS3-1-95del, 384-IVS5+4del, 720-735del, and 1057Gdel); two splice site mutations (IVS8-1G>C and IVS8-1G>T); and two insertions (682insC and 762insT), have been reported. Recently a 3 bp deletion has been detected resulting in

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Table 2 (*continued*)

B
15 Polymorphisms (silent mutations)

Nucleotide Change	Effect on coding sequence	Exon	Localisation in DHCR7	Percentage of published mutations	Reference	Overall Incidence
-223T>C	Non coding	1	Polymorphism		1	
-23T>C	Non coding	2	Polymorphism		8	
139C>T	L47	4	Polymorphism		8	
189A>G	Q63Q	4	Polymorphism		1,2,3,4	
207C>T	T69T	4	Polymorphism		2,3	
231C>T	T77T	4	Polymorphism		1,2,3,4	
285A>G	K95K	4	Polymorphism		1	
IVS4-60T>G	Non coding	-	Polymorphism		8	
438C>T	N146N	6	Polymorphism		1,2,3	
969G>T	L323L	9	Polymorphism		1	
1020C>T	V340V	9	Polymorphism		9	
1158C>T	D386D	9	Polymorphism		1,2,3	
1272T>C	G424G	9	Polymorphism		1,2,3,4	
1341C>T	D447D	9	Polymorphism		2	
1350C>G	R450R	9	Polymorphism		1	

*) MAH = Membrane associated helix (see figure 3). Total of 358 SLOS-alleles.
1) Fitzky *et al.* 1998; Witsch-Baumgartner *et al.* 2000.
2) Waterham *et al.* 1998; de Die-Smulders *et al.* 1999; Waterham & Wanders 2000; Jira *et al.* 2001.
3) Yu *et al.* 2000.
4) De Brasi *et al.* 1999.
5) Wassif *et al.* 1998.
6) Patrono *et al.* 2000.
7) Krakowiak *et al.* 2000.
8) Witsch-Baumgartner *et al.* 2001a and 2001b. (these studies are not included in the calculation of the overall incidence of mutations).
9) Evans *et al.* 2001.
10) Prasad *et al.* 2002.

the in-frame removal of a single histidine residue from position 356 of the DHCR7 protein. This deletion of one codon does not lead to a premature truncation of the protein and some residual activity may be expected, although expression has not been determined.

The seven most frequent mutations described to date are: IVS8-1G>C, T93M, R404C, W151X, V326L, R352W and E448K with a frequency of: 31.5, 11.2, 10.7, 6.4, 6.3, 3.2 and 3.2%, respectively, representing two thirds of the mutations in analysed alleles. Eighty-one mutations detected in SLOS alleles are single amino acid substitutions.

Two splice site mutations have been described at the same position (the frequent IVS8-1G>C mutation and a rare variant, namely IVS8-1G>T; Jira *et al.* 2001). The G>C mutation causes aberrant splicing producing an mRNA with 134 base-pairs of retained intron 8 sequence at nucleotide position 963, which upon translation will lead to a frame shift and a stopcodon at nucleotide 1235 (TGA), predicted to produce an inactive, truncated protein lacking 154 amino acids of its original C-terminal sequence (Waterham *et al.* 1998; Waterham *et al.* 2000). This was confirmed by enzyme activity measurements in patients homozygous for this mutation (Moebius *et al.* 1998; Waterham *et al.* 1998; Witsch-Baumgartner *et al.* 2000; Waterham & Wanders, 2000; Yu *et al.* 2000; Bataille *et al.* 1999) which revealed no activity. Severely affected patients with extremely short life span have been identified who were homozygous for this mutation (Waterham *et al.* 1998; Witsch-Baumgartner *et al.* 2000) with plasma cholesterol levels as low as 20 μmol/l (Jira *et al.* 2001). The number of homozygotes for this mutation, however, is lower than expected on the basis of the Hardy Weinberg equation. Hence, homozygosity for this *null* allele has been predicted to lead to spontaneous termination of pregnancy in many cases, and consequently to an underestimation of the true

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incidence of SLOS (Kelley & Hennekam, 2000; Waterham & Wanders, 2000; Nowaczyk *et al.* 2001a).

So far fifteen silent polymorphic mutations have been found in the *DHCR7* gene (Table 2).

Localization of Mutations in the Protein

Mutations occur throughout the whole gene/protein without evident hotspots. A substantial number of mutations, however, are found in the so called "sterol-sensing domain" encompassing five transmembrane segments 4–8, as proposed by Fitzky *et al.* (1999) and Bae *et al.* (1999) (Figure 3). The specific DHCR7 membrane-spanning segments show strong homology to segments found in five other human proteins. In nature these proteins all have crucial interaction with sterols: 1] HMG-CoA reductase (Olender *et al.* 1992), 2] Niemann-Pick C1 gene product (Loftus *et al.* 1997), 3] Sterol regulatory element-binding protein-SCAP (Nohturfft *et al.* 1998), 4] the morphogene receptor PATCHED (Loftus *et al.* 1997) and 5] the DISPATCHED protein (Burke *et al.* 1999). According to hydrophobicity in the DHCR7 protein, membrane-spanning segments 4 to 8 are closely spaced and connected by relatively short hydrophilic amino acid loops. In contrast to DHCR7, SCAP and HMG-CoA reductase have a long COOH-terminal domain that projects into the cytosol (Nohturfft *et al.* 1998).

The majority of SLOS patients are compound heterozygotes for two different mutations in *DHCR7*. Sibs with the same *DHCR7*-mutations or genotypically identical patients, reported by different groups, may display similar or rather different plasma sterol values and/or different phenotypes (Krakowiak *et al.* 2000; Jira *et al.* 2001). The mutational site and effect on the coding sequence seemed to be only partially predictive for clinical and biochemical severity.

Expression Studies and Genotype-Phenotype

Enzymatic activities of DHCR7 can be determined by assaying the conversion of either isotope-labelled 7DHC, or its precursors such acetate or lathosterol (cholesta-7-en-3β-ol), to cholesterol using thin layer chromatography or HPLC (Lund *et al.* 1996; Shefer *et al.* 1997; Necklason *et al.* 1999). An alternative is the use of non-radiolabelled ergosterol (ergo-5,7,22-trien-3β-ol) as a substrate for DHCR7. Incubation of microsomal preparations or cell homogenates from rat and human with ergosterol results in the reduction of the $\Delta 7$ double bond, which produces brassicasterol (ergo-sta-5,22-dien-3β-ol). The ergosterol conversion followed by GC-MS detection of produced sterols, although less sensitive than the isotope-based assays, avoids the problems caused by the instability and availability of the radiolabelled precursors (Honda *et al.* 1996; Shefer *et al.* 1998). Functional analysis, by expression of DHCR7 mutants in mammalian cells, demonstrated that all but one of the missense (R450L; C-terminus) mutations result in unstable protein (Waterham *et al.* 1998; Witsch-Baumgartner *et al.* 2000; Jira *et al.* 2001). Residual enzyme activity may explain some of the phenotypic variability seen in SLOS. Enzyme kinetic studies support this theory (Neklason *et al.* 1999).

Mutations that alter, interfere with, or truncate the sterol-sensing domain of the DHCR7 protein are likely to cause a more severe clinical and biochemical SLOS-phenotype. Making predictions of SLOS phenotype from genotype, however, continues to be difficult since there is significant clinical and biochemical variability among genetically identical infants. A higher incidence of severe null mutations (IVS8-1C>G insertion and W151X) in a population could explain a lower incidence of SLOS. The 50% incidence of the IVS8-1G>C mutations observed in our European SLOS-study (Jira *et al.* 2001) exceeds the findings of others who identified the IVS8-1G>C mutation in 21 of 66 (32%) and 18 of 52 (35%) SLOS-alleles, respectively (Yu *et al.* 2000; Bataille *et al.* 2000). The development of a simple PCR-RFLP can be used as a screening-method for detecting this frequent SLOS-mutation. Data on population screening for the IVS8-1G>C mutation indicated the high carrier frequency of about 1 in 100 and 1 in 30 for U.S. Caucasians and European Caucasians, respectively (Yu *et al.* 2000; Nowaczyk *et al.* 2001b). A study of *DHCR7* mutations from three European areas (Poland, Germany/Austria and Great Britain) revealed extreme frequency gradients for this mutation. The frequency gradient of the W151X mutation is in a direction opposite to the gradient of the IVS8-1G>C mutation (Witsch-Baumgartner *et al.* 2001b).

In conclusion, SLOS is a multiple malformation/ retardation syndrome with a clinical and biochemical

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spectrum ranging from lethal to difficult to diagnose with very mild presentation. Among the 91 different mutations observed and reviewed, the majority are missense mutations (81). There is a clustering in three domains of the DHCR7 protein; 1] in the transmembrane domain; 2] in the fourth cytoplasmatic loop; 3] and at the C-terminus.

Twelve *null* mutations (4 nonsense, 4 deletions, 2 splice site mutations and 2 single nucleotide insertions) lead to absent enzyme activity. As far as studied, missense mutations, on the other hand, have demonstrated only decreased protein stability in expression studies.

Severe clinical phenotype in SLOS patients is due to *null* allele and fourth loop mutations, whereas C-terminal and transmembrane mutations cause a mild to moderate clinical phenotype. Further molecular genetic studies will enable insight into carrier frequency of specific *DHCR7* mutations in various populations. Additional clues for prenatal, maternal, environmental, other genetic and compensatory biochemical determinants that can modify the phenotypical consequences of the functional DHCR7 deficiency in SLOS are needed.

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