

## Mini Review

# The Smith–Lemli–Opitz syndrome: a novel metabolic way of understanding developmental biology, embryogenesis, and dysmorphology

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The brief history of the Smith–Lemli–Opitz syndrome (SLOS) (MIM 270400) reflects that of latter 20th century dysmorphology and biochemical and molecular genetics: from its first description as a rare but characteristic multiple malformation syndrome known only to a handful of dysmorphologists, to a relatively common Garrodian defect with a complex molecular basis that has captured the attention of researchers and basic scientists from the fields as diverse as embryology, developmental biology, sterol biochemistry, epidemiology, and teratology. The discovery of the underlying biochemical defect – deficiency of 3 $\beta$ -hydroxysteroid- $\Delta$ 7-reductase (DHCR7), an enzyme catalyzing the last step of cholesterol biosynthesis, and the resultant generalized cholesterol deficiency – has led to an explosion of knowledge of this biochemical pathway and to a paradigm shift in the recognition of metabolic deficiencies as causes of dysmorphic syndromes. Characterization of the human DHCR7 gene and the identification of mutations in patients with SLOS have revealed a complex picture of molecular heterogeneity and provided insights into the structure and function of DHCR7. SLOS is the first metabolic malformation syndrome with profound effects on the body plan, and its discovery has paved the way to the discovery of a number of other defects of the cholesterol synthetic pathway.

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In 1964, Smith et al. reported three unrelated boys with a distinctive facial appearance, microcephaly, broad alveolar ridges, hypospadias, severe feeding disorder, and global developmental delay (1). For 30 years this multiple malformation syndrome was one of many esoteric syndromes hidden away in dysmorphology journals and atlases, despite the existence of several clues pointing to an underlying defect of cholesterol metabolism. These were: a) the observation that *in utero* exposure of rat and mouse pups to chemical inhibitors of the distal cholesterol biosynthetic pathway caused holoprosencephaly, microcephaly, pituitary agenesis, limb defects, and genital anomalies (2); b) large adrenal glands with complete absence of lipid in adrenal cortex of patients

with Smith–Lemli–Opitz syndrome (SLOS) (3); c) abnormalities of the pituitary–adrenal axis (4); d) suppression of fetal estriol production in pregnancies affected with SLOS (3, 5); e) neonatal liver disease in severe SLOS cases in association with low serum cholesterol level (6); and f) male pseudohermaphroditism not because of testosterone–dihydrotestosterone receptor defects (7). Finally, in 1994 two patients with SLOS were found to have abnormally low plasma cholesterol and elevated levels of 7-dehydrocholesterol (7DHC), the immediate precursor of cholesterol in the Kandutsch–Russell biosynthetic pathway, suggesting that this multiple malformation syndrome was caused by a simple Garrodian enzymatic defect (8).

Deficient cholesterol synthesis in SLOS is caused by the abnormally low activity of 3 $\beta$ -hydroxy-

steroid-  $\Delta^7$ -reductase (7-dehydrosterol reductase, DHCR7), the enzyme responsible for conversion of 7DHC to cholesterol and, in a parallel step, of 7-dehydrodesmosterol to desmosterol (Fig. 1). In 1998, three teams characterized the human DHCR7 gene and identified mutations in SLOS patients (9–12). To date, 67 DHCR7 mutations have been reported in almost 200 patients with SLOS representing a continuum of clinical severity (9–23). A mouse model has been developed which will allow further study of the embryonic effects of cholesterol deficiency, and the effects of cholesterol therapy (24).

### Incidence

SLOS is most prevalent among populations of northern and central European origin, and appears to be extremely rare among Asian and African

populations (25). Estimates of the incidence of SLOS vary considerably depending on the diagnostic criteria and the reference population. The incidence of severe, clinically diagnosed SLOS in a completely ascertained population of newborns in the Czech Republic is estimated to be greater than 1 in 10 000 (26), and the incidence of severe, biochemically confirmed SLOS in Slovakia is estimated to be 1 in 15 000 to 1 in 20 000 (27). These observations indicate that the carrier frequency in some European populations may be as high as 2.0% (1 in 50).

As one might predict, the incidence of SLOS is lower for the more heterogeneous populations of the United Kingdom and North America. The incidence of SLOS is estimated at 1 in 30 000 for New England (28), 1 in 20 000 for British Columbia (29), and 1 in 26 500 for Ontario (30). In contrast, the rate of biochemical diagnosis of SLOS in two American referral laboratories suggests an incidence of 1 in 50 000 (31). A review of all diagnosed SLOS cases

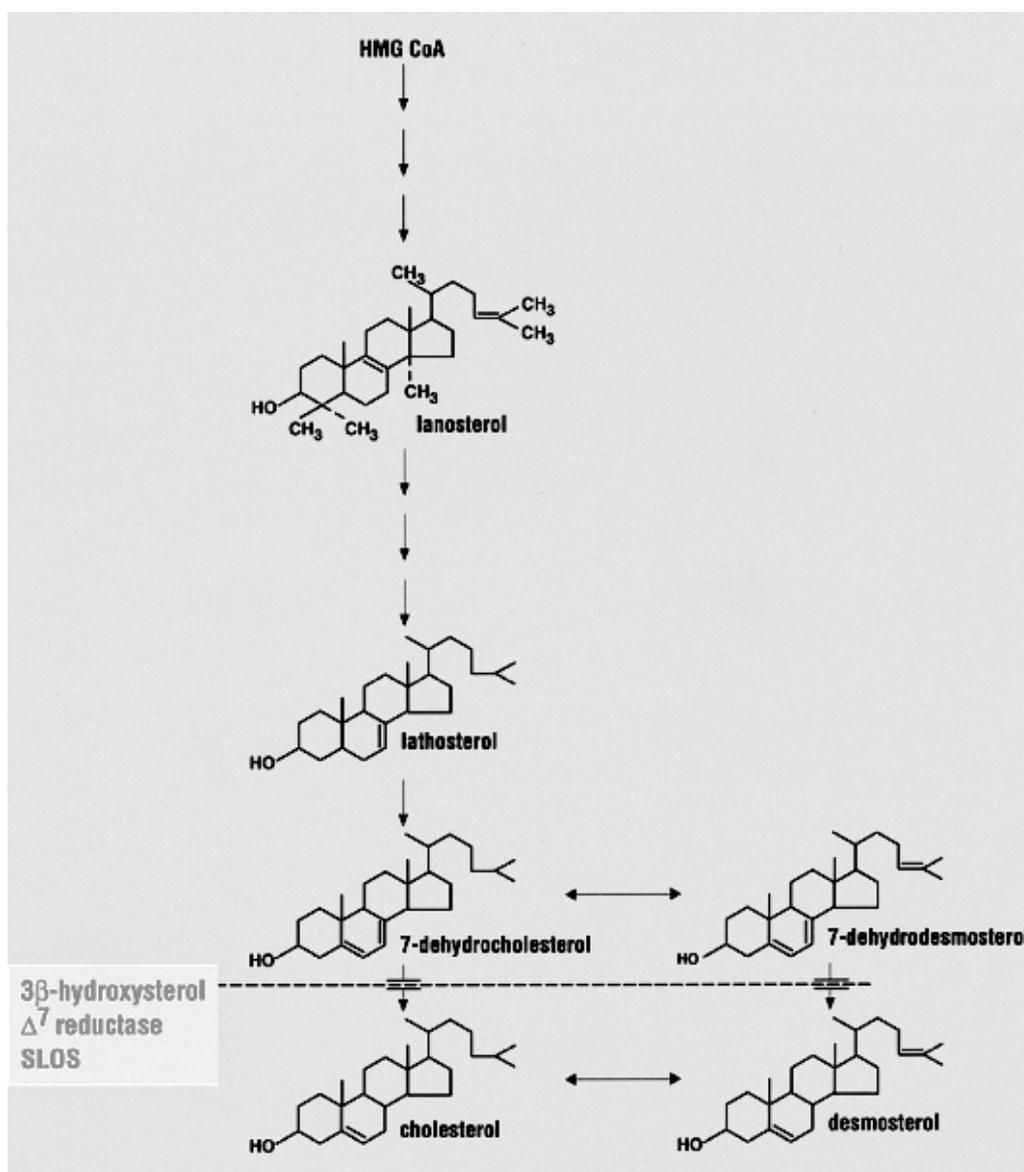


Fig. 1. The Kandutsch-Russell pathway of cholesterol biosynthesis with the defect at the level of 3 $\beta$ -hydroxysteroid- $\Delta^7$ -reductase that results in SLOS.

in the United Kingdom from 1984 to 1998 indicates a minimum prevalence of 1 in 60 000 (32). With the exception of the latter two studies, the above estimates are based on small numbers of affected individuals identified over short periods of time. These estimates may be too low because of ascertainment bias favoring diagnosis of cases with the classical SLOS phenotype and underestimating the incidence of both severe cases resulting in fetal demise and mild cases with subtle phenotypes (33, 34).

Several lines of circumstantial evidence indicate that the carrier frequency and overall prevalence of SLOS may be higher than currently estimated. In contrast to most rare recessive disorders, consanguinity of parents of patients with SLOS is infrequent. Of the almost 200 patients for whom molecular data are available, only two sib pairs were from consanguineous matings (14, 20) and of those, two brothers were compound heterozygotes (14). In addition, there have been several accounts of secondary affected cases with SLOS families (26, 33). Lastly, population surveys have indicated that the carrier frequency of the most common SLOS mutation (IVS8-1G . C) is approximately 1% (35, 36, our unpublished data). Given that this mutation represents less than 30% of the alleles in surveys of affected individuals, the carrier rate for SLOS mutations may be as high as 1 in 30 (35).

### Phenotype

SLOS has protean manifestations of variable severity (1, 6, 29, 37–47). The use of the biochemical diagnostic test has led to the expansion of the SLOS phenotype to include more severely affected patients with holoprosencephaly (48) and renal agenesis, as well as patients with mental retardation, behavioral abnormalities, and minimal clinical findings, who had been diagnosed as having idiopathic mental retardation, attention deficit-hyperactivity disorder, or autism (33, 34, 45).

The comparison of the series of patients reported before and after the discovery of the biochemical defect shows similar frequencies of the major clinical features (31, 45–47). Before the discovery of the underlying biochemical defect, SLOS was divided into two groups based on clinical severity: classic form (SLOS type I) and severe form (SLOS type II) (5, 6, 49). However, a scoring system designed to evaluate the clinical severity of SLOS showed a unimodal frequency distribution (44, 46). A revised scoring system was devised to weigh distinct embryological organ systems equally and reduce the influence of visceral anomalies (Table 1) (31). Based on the severity score, the SLOS phenotype can be

Table 1. Clinical criteria for the revised severity score

Organ	Score	Criteria
Brain	1	Seizures; qualitative MRI abnormality
	2	Major CNS malformations; gyral defects
Oral	1	Bifid uvula or submucous cleft
	2	Cleft hard palate or median cleft lip
Acral	0	Non-Y-shaped minimal toe syndactyly;
	1	Y-shaped 2–3 toe syndactyly; club foot;
	2	upper or lower polydactyly; other syndactyly Any two of the above
Eye	2	Cataract; frank microphthalmia
Heart	0	Functional defects
	1	Single chamber or vessel defects
	2	Complex cardiac malformation
Kidney	0	Functional defect
	1	Simple cystic kidney disease
	2	Renal agenesis; clinically important cystic disease
Liver	0	Induced hepatic abnormality
	1	Simple structural abnormality
	2	Progressive liver disease
Lung	0	Functional pulmonary disease
	1	Abnormal lobation; pulmonary hypoplasia
	2	Pulmonary cysts; other major malformations
Bowel	0	Functional GI disease
	1	Pyloric stenosis
	2	Hirschsprung disease
Genitalia	1	Simple hypospadias
	2	Ambiguous or female genitalia in a 46,XY; frank genital malformation in a 46,XX

The severity score is obtained as follows: categories of cerebral, ocular, oral, skeletal, and genital defects are identified, the scores for the 10 categories are summed, and the scale normalized by dividing the maximum score of by 20 and multiplying by 100 (31).

divided into three categories: mild ( $\leq 20$ ), classical (20–50), and severe ( $> 50$ ) (50). A correlation of the severity score was found with biochemical parameters (44, 46, 50), and the severity score has provided a basis for establishing a genotype–phenotype correlation (15, 16, 19).

### Physical features

The characteristic facial features of SLOS are microcephaly, bitemporal narrowing, ptosis, short nasal root, short nose with anteverted nares and micrognathia, epicanthal folds, and capillary hemangioma over the nasal root extending onto the glabella (1, 45) (Figs. 2A and 3A). The ears appear low-set, and are posteriorly rotated, but are otherwise normal. The classic, characteristic ‘SLOS face’ may be very subtle in some patients (33, 45, 46) (Fig. 3). Oral findings include a high-arched and narrow hard palate, broad and ridged alveolar ridges

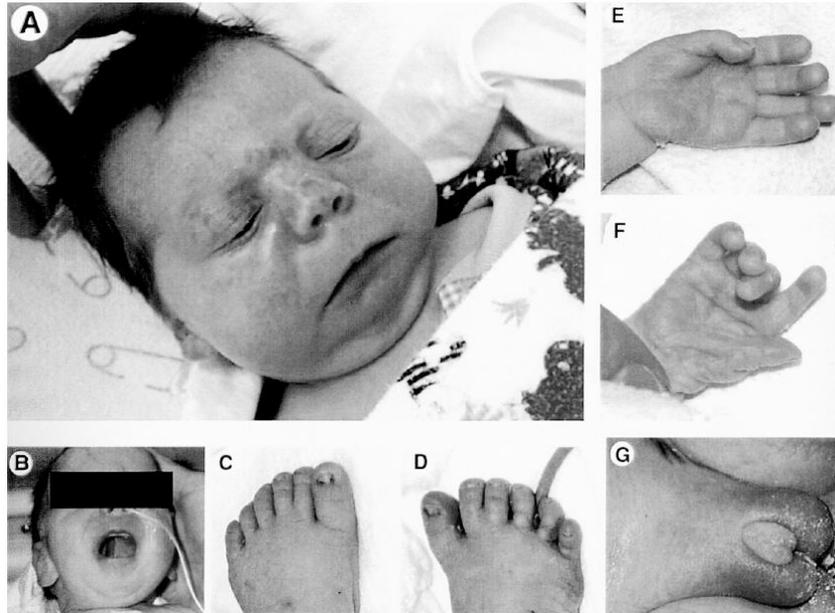


Fig. 2. Clinical features of severe SLOS in a newborn. A: Facial appearance with bitemporal narrowing, micrognathia, short nose with anteverted nares, and a capillary hemangioma of the forehead; B: midline cleft palate; C and D: polydactyly of the feet with 2–3 cutaneous syndactyly; E: short, proximally placed thumb and hypoplasia of the thenar eminence; F: 'Zigzag' index finger; G: ambiguous genitalia in a 46,XY infant.

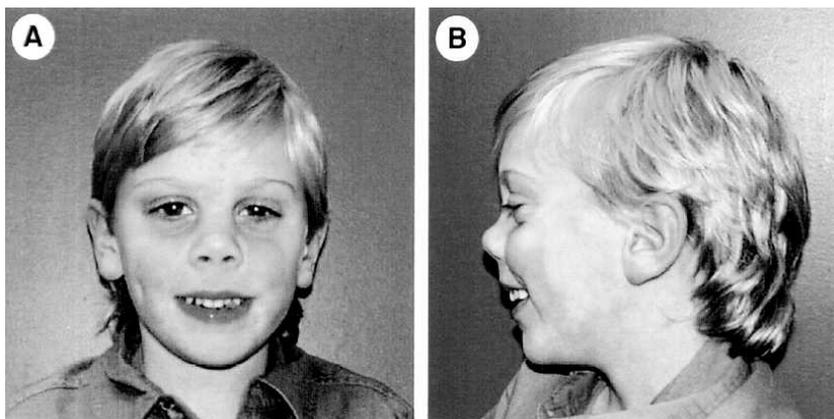


Fig. 3. Facial features of a 5-year-old boy with mild SLOS and minimal facial changes of SLOS. A and B: Capillary hemangioma visible on anterior view of the face, short nose with anteverted nares on profile view.

(Fig. 4B), and redundancy of sublingual tissues (5, 6, 48). In cases with cleft lip or palate, or with holoprosencephaly, the facial features of cleft lip or palate sequence or of holoprosencephaly sequence may overshadow those of SLOS (6, 48, 51) (Fig. 5).

CNS anomalies include agenesis or hypoplasia of corpus callosum, cerebellar hypoplasia, increased ventricular size, and decreased size of frontal lobes. About 5% of patients have various forms of the holoprosencephaly sequence (3, 46, 48, 52). Bilateral or unilateral postaxial polydactyly can be present in the hands or feet, or both (Fig. 2C,D). The thumb is short and proximally placed, the first metacarpals

are short, and the thenar eminences are hypoplastic (Fig. 2E,F). The index finger often has a subtle 'zigzag' alignment of the phalanges (Fig. 2E,F). The most characteristic finding for patients with SLOS is the Y-shaped cutaneous syndactyly of second and third toes (Fig. 2C,D) (6, 45–47); however, syndactyly may be minimal and appreciable only from the plantar aspect (33).

The external genitalia in the male range from normal to female-appearing or ambiguous (Fig. 2). Internal genitalia of 46,XY patients with sex-reversal may include blind vaginal pouch and rudimentary uterus; testes are usually palpable in the

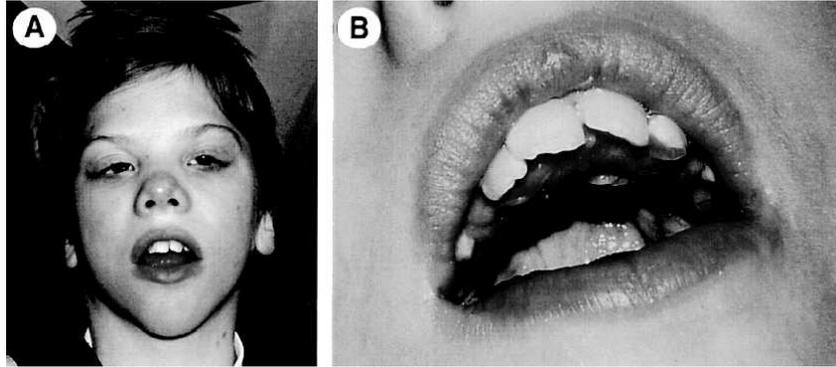


Fig. 4. A: Facial appearance of a 10-year-old girl with classical SLOS. B: Gingival hypertrophy and high-arched and narrow palate.

inguinal region (5, 6, 37–39, 43, 44, 49). Female patients with SLOS usually have normal genitalia, or there may be a characteristic hypoplasia of labia minora and majora.

Congenital heart defects are seen in about 50% of patients with SLOS (53). Endocardial cushion defects and hypoplastic left heart syndrome are most common. About one-quarter of patients have renal anomalies, such as renal hypoplasia or agenesis, renal cortical cysts, hydronephrosis, and structural abnormalities of the collecting system (5, 45, 50). Laryngomalacia and tracheomalacia may result in sleep apnea (54). In more severely affected newborns, abnormal pulmonary lobation, pulmonary hypoplasia, and Hirschsprung disease, involving variable lengths of the gut, are common (5, 6, 44). Cholestatic liver disease is seen infrequently in severely affected newborns (5, 44, 55). Eye abnormalities include congenital cataracts and optic nerve hypoplasia (40, 56).

*Behavioral and developmental phenotype.* With the availability of biochemical testing, the mental phenotype of SLOS has expanded to include patients with low normal intelligence (18, 30, 34). Patients with SLOS have a characteristic behavioral phenotype with sensory hypersensitivity with tactile defensiveness (oral, hands, and feet), and an unusual hyperresponsivity to certain auditory and visual stimuli (57). Many patients, even with mild clinical disease, manifest significant aggressiveness and selfinjury. A severe sleep disturbance characterized by markedly reduced sleep duration, fragmented sleep, and difficulty falling asleep, and autistic behaviors are observed frequently (57).

*Natural history.* Newborns and infants with SLOS have feeding problems, requiring gavage feeding in many cases. Abnormalities of intestinal motility (pyloric stenosis, vomiting, and gastroesophageal

reflux), feeding intolerance, gastrointestinal irritability, and allergies are frequent and contribute significantly to the morbidity (31). A hypermetabolic state requiring caloric intake significantly in excess of the calculated need based on weight and level of activity is seen in many patients (31). Hypotonia, central as well as secondary to muscle hypoplasia, is universal in infancy, but improves with age often leading to hypertonia, contractures, and orthopedic complications in non-ambulatory children. Recurrent infections (otitis media, pneumonia) are common, as is significant photosensitivity (45–47). Clinically significant adrenal insufficiency is uncommon; however, it may become manifest during periods of physiologic stress (58, 59). Anesthesia may be difficult because of micrognathia, but no other significant anesthetic complications have been observed (31, 60).

Life expectancy of infants and children with SLOS depends on the number and severity of congenital malformations, which in turn are inversely correlated with plasma cholesterol level (61)

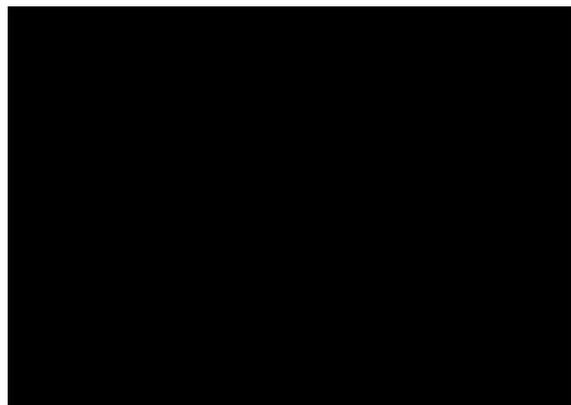


Fig. 5. Facial features of a 24-week-gestation fetus with severe SLOS, holoprosencephaly, and the holoprosencephaly facial sequence (midline cleft palate, midline cleft lip, single nostril, and hypotelorism).

and the ratio of cholesterol to total sterols (16). Currently, the severity of clinical disease is being related to the underlying molecular change in DHCR7 and the enzyme activity level (12, 15, 16).

### **Sterol biosynthesis**

Cholesterol is a 27-carbon, mono-unsaturated sterol, synthesized from lanosterol by a series of oxidations, reductions, and demethylations. The Kandutsch–Russell pathway of cholesterol synthesis (Fig. 1) is the principal route of cholesterol synthesis in man (62). However, recent studies of DHCR7 mutations suggest that an alternate pathway of cholesterol synthesis may exist (12, 15), or that the transplacental transfer of cholesterol is higher than thought previously (31).

Cholesterol serves as a major structural lipid of cell and mitochondrial membranes and of myelin. Cholesterol is the immediate precursor for the synthesis of all known steroid hormones and of bile acids. Intermediates proximal to squalene in the cholesterol biosynthetic pathway serve as precursors for the synthesis of isoprenoids (dolichol, ubiquinone) and of RNA via isopentenyl adenine (62).

### **Biochemical phenotype**

The clinical diagnosis of SLOS requires confirmation by demonstrating elevated serum levels of 7DHC (8, 46, 63–66). Approximately 10% of patients with DHCR7 deficiency have normal levels of cholesterol. Both 7DHC and 8DHC cross-react as cholesterol in the cholesterol oxidase assay methods, leading to a false elevation of ‘cholesterol’ level (31, 55). Retrospective or post-mortem biochemical diagnosis can be performed using frozen or formalin preserved tissue samples, neonatal blood spots, stored amniotic fluid (AF), or plasma (64, 67, 68). Elevated levels of 7DHC, but with normal 7DHC/ total sterol ratio are observed in forms of hypercholesterolemia, as well as in conditions with increased enteric circulation of bile acids (31). Mild to moderately elevated levels of 7DHC have been observed in 3 patients treated with haloperidol; 7DHC levels were directly proportional to the dose of haloperidol, and decreased to normal upon discontinuation of haloperidol therapy (our unpublished data).

The biochemical profile of SLOS reveals a continuum of both 7DHC and cholesterol levels. Serum 7DHC levels range from 10-to over 1000-fold greater than normal (8, 64). There is an inverse correlation between severity of the SLOS phenotype and either the absolute level of cholesterol in plasma, or the level of cholesterol as a fraction of total sterols

(16, 45, 64). However, even the most severely affected newborns have detectable levels of cholesterol at birth. Prenatally, there is a direct correlation between severity of phenotype and the level of 7DHC in amniotic fluid, while the level of cholesterol remains relatively constant between affected pregnancies and heterozygous and homozygous normal pregnancies (50).

### **Prenatal diagnosis and pregnancy screening**

The biochemical abnormalities of SLOS allow an accurate and rapid prenatal diagnosis. Demonstration of elevated 7DHC/total sterol ratio in fetal tissues or of increased levels of 7DHC in AF is diagnostic of SLOS (50, 69–72). The measurement of the 7DHC/total sterol ratio in fetal tissues can be performed at 11–12 weeks of gestation using material obtained by chorionic villus sampling (71). Elevated 7DHC in AF can be demonstrated as early as 13 weeks of gestation (72). With knowledge of the parental mutations, direct DNA testing of amniocytes or CVS can also be used for prenatal diagnosis (73).

Abnormally low maternal serum level of unconjugated estriol in pregnancies affected with SLOS may allow prenatal screening for SLOS as part of the routine triple marker screen for Down syndrome and neural tube defects (74). Non-invasive prenatal diagnosis based on the demonstration of abnormal equine-type estriols in maternal urine is currently being investigated (75).

### **Treatment**

Cholesterol therapy is associated with accelerated somatic growth, and with a decrease in the frequency of infections (76, 77). Although cholesterol supplementation does not appear to alter the developmental outcome (78), treatment with cholesterol decreases irritability, hyperactivity, and self-injury, renders patients with SLOS more alert, sociable, and affectionate, and significantly reduces autistic behaviors (57). No side effects of cholesterol therapy have been reported to date.

Exogenous cholesterol supplements begin at a dose of 40–50 mg/kg/day, increasing as needed for somatic growth requirements; occasional patients require doses of cholesterol of up to 300 mg/kg/day. Cholesterol is supplied either in a natural form (eggs, cream, liver) or as purified food grade cholesterol. Because of the feeding difficulties in infants and younger children and because the diet is unpalatable, tube feeding is often required. Clinical and animal model studies have shown little added

benefit of bile acid supplementation (31, 55). Fresh frozen plasma is used as a source of cholesterol for acute management of infections or surgical procedures. During intercurrent illness, patients with SLOS might develop overt adrenal insufficiency requiring treatment (58, 59). There is limited but encouraging evidence that inhibitors of HMG-CoA reductase may augment residual DHCR7 activity leading to increase in the plasma cholesterol levels in some patients (79).

### Mutational spectrum

In 1998, the human DHCR7 gene was cloned and mapped to 11q12 (9–12), and the complete genomic and cDNA sequences became available (GenBank accession numbers AF110060, NM-001360) (Fig. 6). The 2957 bp cDNA has an open reading frame of 1425 bp, which codes for a protein with 475 amino acid residues and a calculated mass of 54.5 kDa. DHCR7 is a microsomal, membrane-bound protein encoded by exons 3–9 of DHCR7. By hydropathy plot, there are nine  $\alpha$ -helix transmembrane domains. The large fourth cytoplasmic loop likely contains the active site of the enzyme and the binding site for NADPH. The C-terminus (CT) is predicted to be located within the lumen of the endoplasmic reticulum

Sixty-seven DHCR7 mutations were found in the almost 200 SLOS patients who have been genotyped (10, 11, 13–23, our unpublished results). In all but a few patients, both mutant alleles were identified. Missense mutations comprise the largest class of

mutant alleles (85%), followed by a small number of nonsense mutations, small deletions or insertions, and mutations that affect splicing or translation initiation. Extensive gene deletions or rearrangements are yet to be encountered.

The DHCR7 mutation spectrum is strikingly different from that of most other autosomal recessive diseases. Missense mutations generally account for only 30–50% of the mutational spectrum, with the majority of mutations having more a deleterious effects on gene function (e.g. frameshifts, nonsense mutations, and splice-site mutations). This difference may reflect the fact that the current spectrum of SLOS mutations is derived largely from surviving SLOS patients, and that the most severe cases (and their underlying mutations) may be under-represented because of prenatal or perinatal lethality. Moreover, there might be other DHCR7 mutations that give rise to milder forms of SLOS (e.g. isolated behavioral problems, isolated mental retardation, isolated subtle malformations) which are currently not recognized as part of the clinical spectrum of SLOS.

### Missense mutations

Fifty-seven missense mutations have been described, the vast majority of which are clustered within and adjacent to the transmembrane domains, or within the fourth cytoplasmic loop and CT domains (Fig. 6). There is a disproportionate number of missense mutations in the latter two domains, which constitute approximately 21% of the enzyme but contain

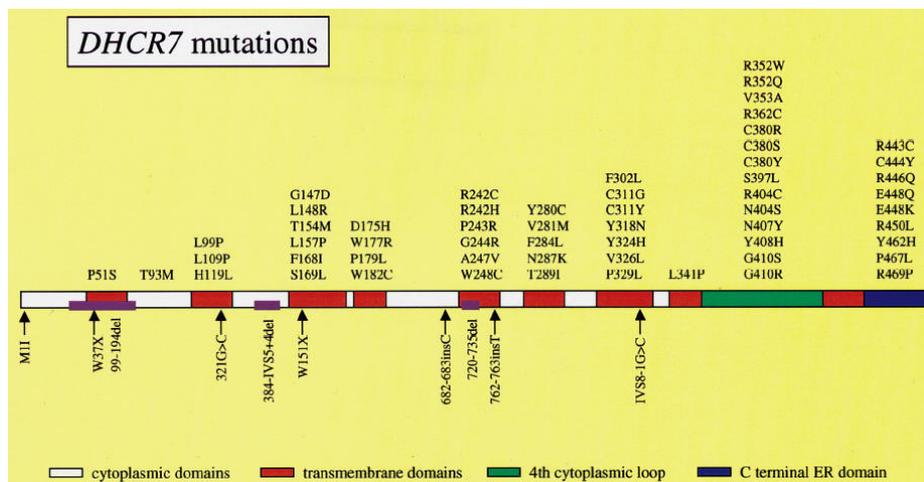


Fig. 6. DHCR7 gene and the 67 known mutations that result in SLOS.

40% of the missense mutations. Most of the missense mutations target amino acid residues that are conserved between plant and vertebrate sterol reductase enzymes, and are associated with reduced levels of protein expression (12, 19, 22).

#### *Nonsense mutations*

Two nonsense mutations have been reported, W151X and W37X. Both mutations would result in non-functional truncated products. W151X is the only common nonsense mutation, and the most common SLOS allele in Slovakia and the Czech Republic (21).

#### *Splice-site mutations*

The most frequent SLOS allele, IVS8-1G . C, alters the canonical AG6 sequence of the IVS8 splice acceptor site. Alternative splicing from a cryptic acceptor site within IVS8 results in a transcript containing 134 bp of intron sequences at the end of exon 8. This in turn leads to a frameshift and premature termination of translation prior to the highly conserved CT domains.

A second mutation, Q107H or c.321G . C, causes an amino acid substitution and alters the splice donor site of IVS4 at the - 1 position. Alternative splicing from a downstream donor sequence results in a 36 bp insertion of intron sequences at the end of exon 4, resulting in an insertion of 12 amino acid within the second transmembrane domain (19).

A third splice-site mutation results from a 33 bp deletion that extends from position c.384 (codon 128, exon 5) to position + 4 of IVS5 (13, 15). This mutation deletes the donor splice sequence of IVS5. Novel mRNA species as a result of alternative splicing were not identified, suggesting that the transcript is highly unstable (13).

#### *Frameshift mutations*

Two single base insertions have been reported, c.682-683insC and c.762-763insT (10). Both mutations cause frameshifts within exon 6. A third frameshift mutation is caused by a 16 bp insertion within exon 7, c.720delTGCGCCCCGGGATC (15). All three frameshifts result in premature termination of translation before the highly conserved CT domains.

A 96 bp deletion beginning at c.98 or c.99 was detected from cDNA, but the underlying mutation in the genomic DNA was not determined (10). This mutation may actually be a deletion of the first 96 bp of exon 2 (c.99-194del). Alternatively, the cDNA

deletion could be the result from a point mutation that alters the splice acceptor sequence of IVS3 (e.g. IVS3-1G \ C). If splicing of IVS3 utilized a cryptic splice acceptor within exon 4, specifically the AG dinucleotide at the extreme 3% end of the 'deletion', the resulting mRNA would contain the observed in-frame 96-bp deletion. Regardless of the mechanism, the mutant mRNA would code for a protein that is missing 32 amino acid residues that encompass the second transmembrane domain.

#### *Translation initiation mutation*

A novel mutation affecting the translation initiation codon (M1I) was recently reported in 3 very mildly affected patients (two IVS8-1G→C/M1I, one E448K/M1I) (18). A downstream in-frame initiation codon in exon 4 may be utilized for translation initiation. The sequences immediately flanking this putative alternative initiation codon conform to the so-called Kozak consensus sequence required for efficient initiation of mRNA translation in eukaryotes (YNNAUGG) (80). Translation initiation from this codon would result in a slightly truncated product, missing the cytoplasmic amino terminal 58 residues and first transmembrane domain. This initiation codon is functional in fibroblasts and the resulting product has DHCR7 activity (10).

#### *Common SLOS alleles*

Six mutations account for approximately two-thirds of the mutant alleles identified in SLOS patients. These are the splice acceptor site mutation IVS8-1G . C (29.6%), nonsense mutation W151X (8.1%), and four missense mutations: T93M (10.8%), R404C (6.5%), V326L (6.3%), and R352W (3.3%). At least two haplotypes have been identified with the most common allele, IVS81G→C, suggesting that it is an old mutation and that it arose independently in different populations (15, 81).

The relative frequencies of DHCR7 mutations differ significantly between populations (Table 2). Although the IVS8-1G→C mutation is panethnic (German, Dutch, Belgian, Spanish, North American Caucasian, Italian, Czech, Slovak, Croatian, and Polish), it is the most frequent mutation in patients of Western European ancestry and least common in Czech and Slovak (21), and in Polish patients (G. Utermann, personal communication). Conversely, W151X is the most frequent mutation of patients from Slovakia and the Czech Republic (21). The R404C mutation is frequent in the Acadian population in Louisiana (15); 3 heterozygotes and 1 homozygote for this mutation in our Centre have French-Canadian parents who are carriers.

Table 2. Relative *DHCR7* mutation frequencies in different populations (%)

Mutation	Countries				
	US	UK	Germany	Czech Republic	Poland
IVS8-1G→C	29	28	24	5	3
R404C	11	11			
V326L			16	25	23
W151X			11	50	33
R352W					13

T93M mutation is the most frequent mutation in Italian patients (13).

A selective heterozygote advantage for northern Europeans has been suggested based on the increased synthesis of vitamin D from increased levels of 7DHC observed in heterozygotes (26, 31). Although high frequencies of *DHCR7* mutations may have evolved because of special heterozygote advantage afforded by a particular mutation, it is more likely that a combination of founder effect and heterozygote advantage encouraged the persistence of these several mutations and contributed to the selection of these mutations.

### Genotype–phenotype correlation

The continuous spectrum of severe to mild clinical and biochemical phenotypes in SLOS results mainly from the large number of *DHCR7* mutations and is related to the level of residual *DHCR7* activity. While affected siblings with the same genotype have virtually identical phenotypes (12, 14, 16, 21, 33), markedly different phenotypes can occur in unrelated patients with the same mutations. This indicates that the SLOS phenotype may be influenced by: a) additional modifiers of *DHCR7* expression, b) other genes, or c) modulation by other factors affecting the activity of cholesterol synthetic pathway. Maternal genetic or environmental factors, either affecting the transplacental cholesterol transport, or the availability of cholesterol to the developing embryo and fetus, may also influence the phenotypic severity.

The correlation between genotype and phenotype has been difficult to establish because of the relatively small number of patients for whom complete mutational and clinical data have been reported, and ascertainment bias toward patients with the classical SLOS phenotype. This situation is further complicated by the broad spectrum of SLOS mutations and the fact that most patients are compound heterozygotes. As a consequence, the vast majority of genotypes have only been described in 1 or 2 patients.

In an effort to facilitate the genotype–phenotype correlation, a classification of *DHCR7* mutations based on their severity and location within the 3D-structure of the protein was proposed (15). The mutation classes are: a) null alleles as a result of small insertions or deletions resulting in frameshifts, nonsense mutations, or splice-site mutations (class 0); b) missense mutations within or immediately adjacent to the transmembrane domains (TM); c) missense mutations in the fourth cytoplasmic loop, the NADPH-binding region and possibly the catalytic site of the enzyme (4L); and d) missense mutations in the highly conserved CT domain. All but one (4L/CT) of the possible combinations of mutation classes has been reported in the literature.

In general, the most severely affected patients are either homozygotes or compound heterozygotes for mutations that result in little or no *DHCR7* activity (0/0, 0/4L). Most classical SLOS patients are compound heterozygotes for a severe, null mutation and a missense mutation associated with residual enzyme activity (0/TM, 0/4L, 0/CT). Milder phenotypes have been reported for missense mutations affecting TM domains (TM/TM) or the CT domain (CT/CT), or a combination of these (TM/CT). The phenotype of 0/TM compound heterozygotes varies depending on the missense mutation, ranging from mild to severe (Table 3).

The most frequent SLOS mutations are null alleles (e.g. IVS8-1G→C and W151X), yet the observed incidence of 0/0 genotypes is significantly lower than expected. Of the 164 reported genotypes in the literature, there are only 12 cases of homozygosity or compound heterozygosity for null alleles (15, 21, 23, 82). Given that the IVS8-1G→C and W151X mutations account for approximately 37% of the SLOS alleles, one would expect a significantly higher incidence of 0/0 genotypes. The under-representation of 0/0 genotypes provides indirect evidence that a proportion of the severe cases remains undiagnosed as a result of fetal or neonatal death.

Table 3. Genotypes reported in more than 2 patients and their corresponding phenotypes

Genotype (number of cases)	Phenotype (severity score)	References
IVS8-1G→C/ IVS8-1G→C (8)	Severe	(11, 15, 22, 81)
IVS8-1G→C/ R404C (4)	Severe (78, 65,68); moderate (35)	(15)
W151X/W151X (3)	Severe (> 65)	(15, 20)
R404C/R404C (4)	Severe	(15)
W151X/V326L (7)	Moderate	(12, 20)
IVS8-1G→C/ R352W (6)	Mild (<25), moderate, severe (100)	(14 – 16)
IVS8-1G→C/ T93M (13)	Mild-severe	(12, 15, 16, 19)
IVS8-1G→C/ V326L (4)	Moderate (40, 45), severe	(15, 16, 20) (75)

## Morphogenesis

How does a simple Garrodian enzymatic deficiency result in such a plethora of seemingly unrelated malformations? SLOS is the first bona fide multiple malformation syndrome, with profound effects on the body plan, found to be caused by a discrete biochemical deficiency. This discovery will lead to expanded understanding of the embryological mechanism underlying such diverse processes as forebrain development, palatine shelf fusion, neuronal migration, and limb and digit formation. The discovery that the covalent addition of cholesterol to Sonic hedgehog (SHH), an embryonic signaling protein, is an essential part of SHH autoprocessing was the first step in this process (27, 83, 84). SHH participates in patterning of development in the vertebrate ventral forebrain and limb buds, among other structures. A similar process may be responsible for abnormal responses to Desert hedgehog (DHH) and Indian hedgehog (IHH), signaling proteins involved in the development of the genital tract and skeleton, respectively. Targeted disruption of SHH results in holoprosencephaly, distal limb defects, and skeletal anomalies (85), while a homozygous mutation in DHH was demonstrated in a child with partial gonadal dysgenesis and minifascicular neuropathy (86). Other levels at which the sterol defect in SLOS may cause impaired signalling of Shh, Dhh, and Ihh could involve the sterol-sensing domain of receptors PATCHED-1 and PATCHED-2 (87). The lamin B receptor, a nuclear protein involved in cell-cycle signaling, has a sterol-binding domain similar to DHCR7 and a sterol-14-reductase activity; the abnormal 7DHC/total sterol ratio in SLOS may interfere with its functions either in the regulation of the cell-cycle or, possibly, sterol synthesis in the nuclear compartment (88). Cholesterol deficiency in tissues and the abnormal 7DHC/total sterol ratio in cell membranes of patients with SLOS may affect the developmental processes that involve cell-cell interaction (89), and lead to other disturbances of embryonic development such as generalized receptor dysfunction.

## Future directions

Future research directions include the determination of carrier frequency of SLOS in the general population, including severe and mild DHCR7 mutations. These data will provide a precise estimate of the population incidence of SLOS, including cases at the severe and mild extremes of the clinical spectrum. The actual incidence of SLOS in Canada is currently investigated by the Canadian Paediatric Surveillance Program using a 3-year prospective study, which reports proven SLOS cases, and investigates probable cases. Comparison of the observed incidence of SLOS with the theoretical incidence calculated from the carrier frequencies will allow the determination of the prenatal and neonatal loss rate. A multi-center prospective study of the efficacy of prenatal screening for SLOS using maternal serum markers will begin in 2001.

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