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Smith–Lemli–Opitz syndrome: pathogenesis, diagnosis and management

Smith–Lemli–Opitz syndrome (SLOS) is a malformation syndrome due to a deficiency of 7-dehydrocholesterol reductase (DHCR7). DHCR7 primarily catalyzes the reduction of 7-dehydrocholesterol (7DHC) to cholesterol. In SLOS, this results in decreased cholesterol and increased 7DHC levels, both during embryonic development and after birth. The malformations found in SLOS may result from decreased cholesterol, increased 7DHC or a combination of these two factors. This review discusses the clinical aspects and diagnosis of SLOS, therapeutic interventions and the current understanding of pathophysiological processes involved in SLOS.

In brief

1. Smith–Lemli–Opitz syndrome (SLOS) is an autosomal recessive, multiple malformation syndrome due to an inborn error of cholesterol synthesis.
2. The SLOS phenotypic spectrum is very broad, ranging from a mild disorder with behavioral and learning problems to a lethal malformation syndrome.
3. Syndactyly of the second and third toes is the most common physical finding in SLOS patients. Its presence in a child with other malformations, growth failure, intellectual disability, behavioral problems or autistic characteristics should prompt testing for SLOS.
4. Mutations of 7-dehydrocholesterol reductase (*DHCR7*) result in decreased cholesterol and increased dehydrocholesterol levels.
5. A clinical diagnosis of SLOS is confirmed by finding elevated 7DHC in blood or tissues. A normal cholesterol level does not exclude SLOS.
6. The incidence of SLOS is on the order of 1/20 000–1/70 000. SLOS is more common in individuals of European heritage. Carrier frequencies of specific mutations vary widely depending on ethnic background, and for Caucasians, they are in the 1–2% range.
7. Perturbed Hedgehog signaling likely underlies some of the malformations found in SLOS.
8. Most SLOS patients are currently placed on dietary cholesterol supplementation. Anecdotal evidence suggests that dietary cholesterol supplementation is of benefit. The efficacy of simvastatin therapy is being studied.

Introduction

Smith–Lemli–Opitz syndrome (SLOS; OMIM 270400) is an autosomal recessive, malformation syndrome with intellectual disability and behavioral problems. SLOS was first delineated in 1964 by Drs Smith, Lemli and Opitz.[1] In 1993, increased levels of 7–dehydrocholesterol (7DHC) and decreased levels of cholesterol were found in SLOS patients.[2] This abnormal sterol profile was consistent with a deficiency of 7DHC reductase (DHCR7) activity (Figures 1 and 2). Subsequently, several groups identified mutations of *DHCR7* in SLOS patients.[3–5] Although present in other ethnic groups, SLOS appears to be most frequent in Caucasians of northern European heritage. The incidence of SLOS has been estimated to be on the order of 1/20 000–1/70 000.

Clinical overview

The SLOS phenotype is extremely broad (reviewed by Kelley and Hennekam[6]). Severely affected infants have multiple major congenital anomalies and typically die in

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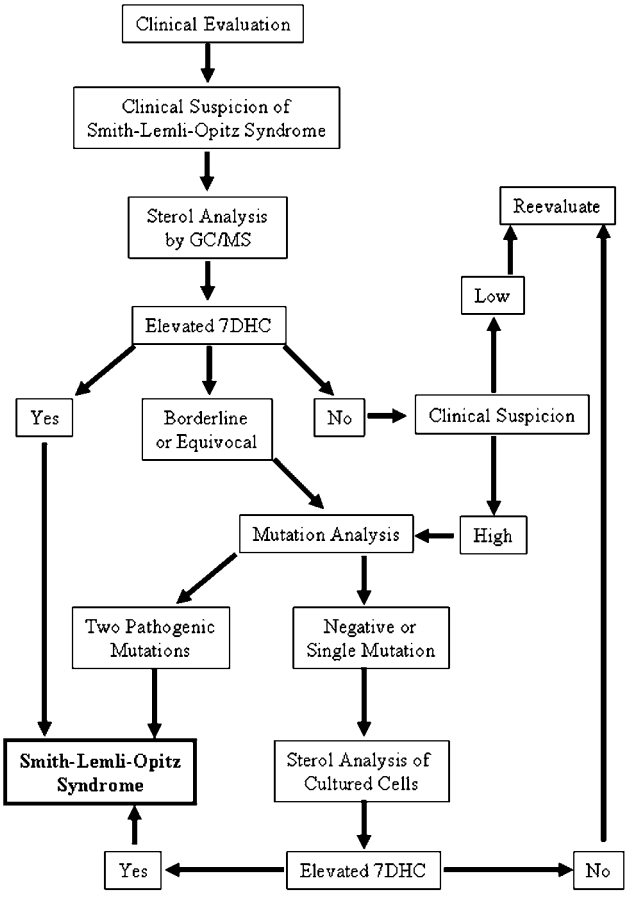

Figure 1 Diagnostic Strategy for Smith-Lemli-Opitz Syndrome. Sterol analysis by Gas Chromatography-Mass Spectrometry (GC/MS) demonstrating elevated blood or tissue 7DHC levels confirms a clinical diagnosis of SLOS. Alternatively, mutation analysis can be used to confirm a clinical suspicion of SLOS, to confirm the diagnosis if blood or tissue is not readily available, or to resolve equivocal biochemical results. In rare cases, sterol analysis of fibroblasts or lymophoblasts cultured in cholesterol-depleted medium can be used to establish or exclude the diagnosis.

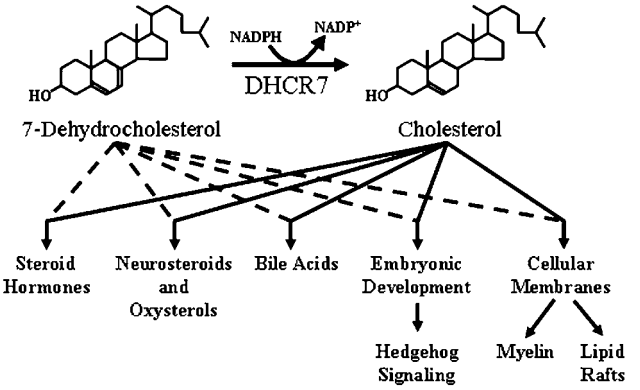

Figure 2 Biochemistry of Smith-Lemli-Opitz Syndrome. Mutations of *DHCR7* impair the reduction of 7-dehydrocholesterol to cholesterol in the final step of cholesterol biosynthesis. Cholesterol has multiple biological functions which may be disturbed due to the lack of cholesterol, toxic effects of 7DHC, or combination of these two factors.

Table 1 Clinical features and findings in Smith-Lemli-Opitz syndrome

General	*Cardiac*
Growth retardation	Atrial and ventricular septal defects
Failure to thrive	Patent ductus arteriosus
Intellectual disability	AV canal
Developmental delay	Hypertension
Craniofacial	*Pulmonary*
Microcephaly	Abnormal segmentation
Metopic prominence and bitemporal narrowing	*Gastrointestinal*
Ptosis	Poor feeding/suckling
Cataracts	Gastroesophageal reflux
Broad nasal bridge	Constipation
Anteverted nares	Pyloric stenosis
Micrognathia	Malrotation
Broad aveolar ridges	Colonic agangliosis
Arched palate	Liver disease
Cleft palate/Bifid uvula	
Extremities	*Urogenital*
Rhizomelia	Renal malformations
Postaxial polydactyly	Hypospadius
Single palmar creases	Cryptorchidism
Short, proximally placed thumbs	Ambiguous genitalia
Retention of fetal pads	*Central Nervous System*
Syndactyly of the second and third toes	Holoprosencephaly
	Agenesis or partial agenesis of the corpus callosum
Skin	Hypotonia
Photosensitivity	
Cutis marmorata	*Behavior*
Dry skin/Eczyma	Autism/Autistic features
	Hyperactivity
	Self-injurious behavior

the perinatal period. In contrast, a milder variant of SLOS combines minor physical anomalies with distinct behavioral and learning problems. Table 1 lists clinical findings and features found in SLOS patients.

Poor feeding and postnatal growth failure are frequent early manifestations of SLOS, and many infants require placement of a gastrostomy tube for adequate nutritional support. Typical craniofacial features (Figure 3a–d) include microcephaly, a small upturned nose, ptosis and micrognathia. Although cleft lip is not common, many patients have cleft palate or bifid uvula. In male patients, genital abnormalities are frequently observed. These range from small penis through various degrees of hypospadius in mild and classical cases to ambiguous genitalia or gender reversal in more severely affected infants. Limb findings are common (Figure 3e). These include short thumbs, single palmar creases, postaxial polydactyly and soft-tissue syndactyly of the second and third toes. Syndactyly of the second and third toes (Figure 3f) has been described in over 95% of SLOS patients. More severely affected patients often have major malformations of the brain

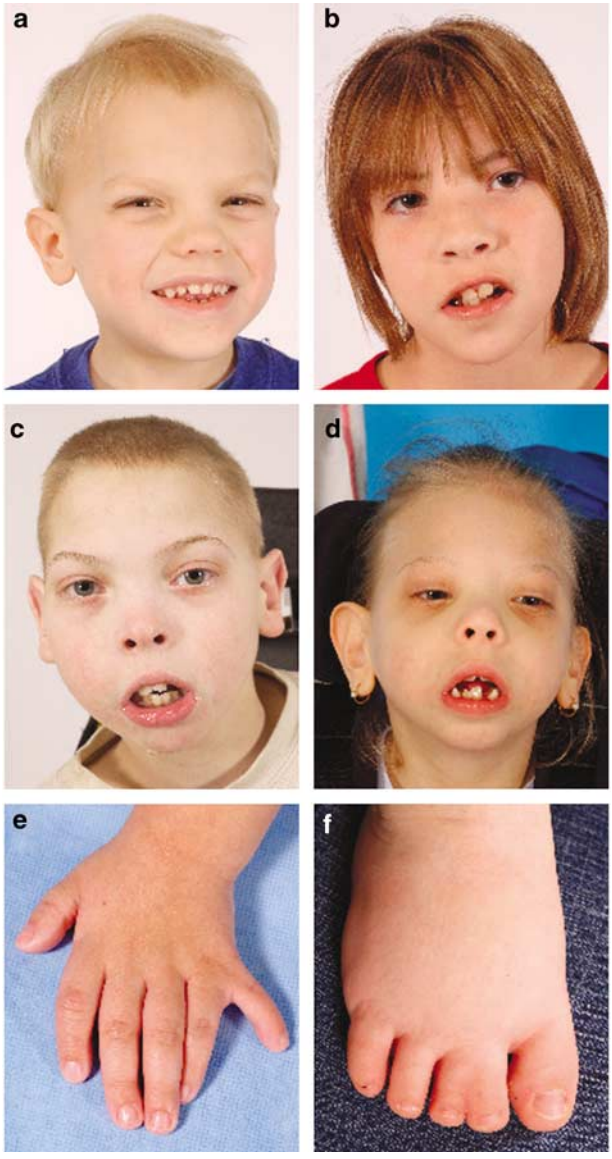

Figure 3 Typical Facial Features and Physical Findings in SLOS. (**a**–**d**) Facial photographs of a series of SLOS patients of different phenotypic severity. Facial features include microcephaly, ptosis, broad nasal bridge, upturned nose, and micrognathia. Cleft palate or bifid uvula is also common. (**e**) Limb anomalies include short proximally placed thumbs, single palmar creases, clinodactyly, and postaxial polydactyly. (**f**) Syndactyly of the second and third toes is the most frequently reported clinical finding.

(holoprosencephaly, agenesis/dysgenesis of the corpus callosum), heart (atrial and ventricular septal defects, patent ductus arteriosus and atrioventricular canal defect), lungs (abnormal segmentation) or gastrointestinal anomalies (pyloric stenosis and colonic aganglionosis).

In addition to the physical manifestations, SLOS patients have a distinct behavioral phenotype.[7] As infants, they can be irritable, lack interest in feeding and prefer not to be held. Older children demonstrate various degrees of hyperactivity, self-injurious behavior, temperament deregulation and sleep disturbances. Most SLOS children demonstrate autistic characteristics and many meet the diagnostic criteria for autism.[7,8]

Although syndactyly of the second and third toes is a normal variant, its presence in a child with other minor anomalies, failure to thrive or poor growth, developmental delay or autistic behavior should prompt consideration of SLOS.

Diagnostic approaches

A clinical suspicion of SLOS is confirmed by demonstrating elevated 7DHC in plasma or tissues (Figure 1). Although UV spectroscopy can detect 7DHC, 7DHC is best assayed using Gas Chromatography/Mass Spectroscopy (GC/MS). Sterol analysis by GC/MS allows for the identification of lathosterol and desmosterol, cholesterol precursors that accumulate in the rare 'SLOS-like' syndromes of lathosterolosis and desmosterolosis, respectively. Although frequently low, plasma cholesterol levels can be within normal limits in SLOS patients. In addition, because the standard laboratory cholesterol test does not distinguish between cholesterol and 7DHC, the measured 'cholesterol' value may fall within the normal range due to the presence of significant amounts of dehydrocholesterol (DHC). In short, a normal cholesterol level does not exclude SLOS.

Elevated 7DHC levels are relatively specific to SLOS. Occasionally, mild elevations of 7DHC are observed in patients treated with psychiatric drugs such as haloperidol or in patients with increased rates of cholesterol synthesis.[6] Mild elevations of 7DHC have also been reported in patients with cerebrotendinous xanthomatomatosis.[9] In these atypical cases, SLOS can be excluded by sterol analysis of fibroblasts or lymphoblasts grown under conditions that induce endogenous cholesterol synthesis. Similar testing can also be used in cases in which blood 7DHC levels are equivocal.

DHCR7 mutation analysis can also be performed to confirm a diagnosis of SLOS or in cases where biochemical testing is equivocal. A staged approach can be used to reduce the cost of mutation analysis. Sequencing of exons 6–9 identifies approximately 85% of *DHCR7* mutations. If both mutations are not identified, exons 3–5 can then be sequenced. Exons 1 and 2 are noncoding. In a small number of biochemically positive patients, only a single heterozygous coding mutation has been identified. In some of these cases, the second allele is not expressed (unpublished data). These nonexpressed alleles likely represent uncharacterized promoter mutations.

Molecular and genetic basis of disease

Biochemistry

SLOS is due to a deficiency of DHCR7 activity. DHCR7 catalyzes the reduction of the Δ7 bond in sterols; in

particular, it reduces 7DHC to yield cholesterol in the final step of cholesterol biosynthesis (Figure 2). Isomerization of 7DHC results in the formation of 8-dehydrocholesterol (8DHC). Both 7DHC and 8DHC, collectively referred to as DHC, accumulate in serum and tissues of SLOS patients. Although most SLOS patients have a cholesterol deficiency, cholesterol levels can be normal. In SLOS fibroblasts, residual cholesterol synthesis ranges from undetectable to over 50%, and decreased residual cholesterol synthesis correlates with increased clinical severity.[10]

Molecular biology and enzymology

DHCR7 was cloned in 1998 by multiple groups, maps to human chromosome 11q12–13, and is encoded by nine exons spanning 14 100 bp of genomic DNA.[3–5,11] Consistent with its role in cholesterol synthesis, *DHCR7* is ubiquitously expressed with highest expression levels observed in liver, adrenal and brain tissue.[11] It encodes a 425 amino-acid protein with a predicted mass of 55.5 kDa. DHCR7 is an integral membrane protein, requires NADPH as a cofactor and localizes to microsomal membranes.[12] Although computer modeling predicts a protein containing up to nine transmembrane domains,[3] the membrane topology of DHCR7 has not been experimentally verified.

Over 130 different mutations of *DHCR7* have been identified in SLOS patients.[13] The most frequently reported mutation is a splice acceptor mutation, c.964-1G>C. This mutation results in a null allele and accounts for approximately a third of the mutant alleles reported in SLOS patients. Other 'common' alleles include p.T93M (10%), p.W151X (6%), p.R404C (5%), and p.V326L (5%). Taken together, these six alleles account for approximately 60% of mutations found in SLOS patients. Different *DHCR7* mutations are associated with various European populations. The c.964-1G>C allele appears to have arisen in the British Isles, p.W151X and p.V326L appear to have arisen in Eastern Europe, p.R404C has been associated with French heritage and p.T93 M is frequently found in patients of Mediterranean descent.[14,15] The genotype–phenotype correlation in SLOS is relatively poor, and patients with the same genotype can have mild to severe phenotypes.[16] Thus, factors other than genotype significantly influence phenotype. One such factor appears to be the maternal ApoE genotype. Witsch-Baumgartner *et al*[17] reported that maternal apoε2 predisposes to a more severe phenotype in SLOS patients.

Pathogenesis

Cholesterol has multiple biological functions (Figure 2). Cholesterol is a major lipid component of cellular membranes such as myelin, and is an important structural component of lipid rafts, which play a major role in signal transduction. In addition, bile acids, steroid hormones, neuroactive steroids and oxysterols are synthesized from cholesterol. It is clear that DHC can 'substitute' for cholesterol in these various metabolic pathways; however, the effects of 7DHC on these various metabolic pathways are still being defined. In SLOS, problems may be caused by deficient cholesterol, deficient total sterols, toxic effects of either 7DHC or compounds derived from 7DHC or a combination of these factors. Given the multiple biological functions of cholesterol, it is unlikely that a single pathological mechanism underlies the varied malformations and clinical problems found in these patients.

Some of the malformations associated with SLOS are consistent with impaired sonic hedgehog (SHH) functioning.[18] SHH plays an important role in pattern formation of the central nervous system, facial structures and limbs. Mutations of *SHH* can cause holoprosencephaly, a brain malformation found in some SLOS patients.[19] SHH is cholesterol modified, secreted from a signaling cell, and binds to a receptor called Patched (PTCH). PTCH regulates transmembrane signaling in the responding cell by modulating the function of a protein called Smoothened (SMO). A number of mechanisms by which SHH signaling might be impaired in SLOS have been proposed. Cooper *et al*[20] demonstrated that reduced total sterol levels in fibroblasts derived from SLOS mutant mice impair SHH signal transduction in the responding cell due to inhibition of SMO. Work by Koide *et al*[21] suggests that the amino terminus of DHCR7 may interact directly with SMO to regulate SHH signaling and recent studies by Bijlsma *et al*[22] suggest that PTCH-mediated transport of vitamin D_3 (a metabolic product of 7DHC) modulates SMO function. Additional work is necessary to reconcile these various experimental observations.

The altered sterol composition in SLOS affects the physiochemical properties and function of cellular membranes. Lipid rafts are ordered lipid domains that function in signal transduction. Substitution of 7DHC for cholesterol alters both lipid raft stability[23] and protein composition.[24] Substitution of 7DHC for cholesterol also decreases membrane bending rigidity, a physiochemical change that may explain abnormal secretory granule formation.[25] Tulenko *et al*[26] showed that SLOS membranes had altered increased membrane fluidity and that synthetic membranes containing 7DHC studied by X-ray diffraction had an atypical membrane organization. These physical perturbations of membrane structure likely underlie functional defects in IgE receptor-mediated mast cell degranulation and cytokine production,[27] NMDA receptor function[28] and serotonin$_{1A}$ receptor ligand binding.[29]

7DHC and metabolic products of 7DHC may have toxic effects. Accumulation of 7DHC in fibroblasts impairs intracellular cholesterol transport similar to that seen in Niemann-Pick Disease, type C,[30] and 7DHC appears to increase the degradation rate of hydroxymethyl glutaryl coenzyme A reductase (HMG-CoA reductase).[31] HMG-CoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis, and increased degradation of this enzyme

may contribute to decreased sterol synthesis in SLOS patients.[32] DHC analogs of pregnenolone, pregnanetriol, DHEA and androstenediol have been identified in SLOS patients.[33] 7-dehydroallopregnanolone, a DHC analog of the neuroactive steroid allopregnanolone, has been identified in SLOS patients.[34] Wassif *et al*[35] identified a novel oxysterol, 27-hydroxy-7-DHC, in serum from SLOS patients. 27-hydroxy-7-dehydrocholesterol, in contrast to 27-hydroxycholesterol, differentially activates liver X receptors (LXR). These are nuclear receptors, which regulate lipid metabolism. It is not yet known whether these 7DHC-derived steroids and oxysterols have unique biological functions that contribute to the SLOS phenotype.

Management

Treatment

Currently, most SLOS patients are treated with dietary cholesterol supplementation. Cholesterol supplementation can be achieved by including high cholesterol foods, such as egg yolks, in the patients' diet, if tolerated, or using suspensions of pharmaceutical grade cholesterol. Observational studies report improved growth, increased socialization, decreased irritability and aggression, increased alertness, decreased tactile defensiveness, decreased photosensitivity, decreased infections, improved hearing, and improved muscle tone and strength in SLOS patients treated with cholesterol.[36,37] Anecdotal reports suggest that behavior improves within days to weeks of initiating dietary cholesterol supplementation. However, no controlled studies of this observation have been performed. In a retrospective study, Tierney *et al*[7] found that 22% (2/9) SLOS patients who were started on dietary cholesterol supplementation before 5 years of age met the Autistic Diagnostic Interview-Revised (ADI-R) criteria for autism. In contrast, 88% (7/8) SLOS patients who were started on cholesterol therapy after this age met the ADI-R criteria for autism. These data suggest that early intervention may be of benefit to SLOS patients. Although Sikora *et al*[38] found no improvement in developmental scores of SLOS children treated with a high cholesterol diet, this group did not evaluate the impact of cholesterol supplementation on behavioral or autistic aspects of SLOS.

A major limitation of dietary cholesterol supplementation is that cholesterol does not cross the blood–brain barrier. Thus, cholesterol supplementation does not treat the biochemical defect in the brain. In addition, since 7DHC levels are not normalized in SLOS patients treated with dietary cholesterol, potential detrimental effects of 7DHC may persist. To circumvent some of these limitations, treatment with simvastatin has been proposed to both reduce 7DHC levels and treat the central nervous system.[39,40] Simvastatin inhibits cholesterol synthesis at the level of HMG-CoA reductase, and simvastatin crosses the blood–brain barrier. In a two-patient trial reported by Jira *et al*,[39] simvastatin therapy improved both serum and cerebral spinal fluid DHC/cholesterol ratios, and was associated with a paradoxical increase in serum cholesterol levels. The later observation might be explained by increased expression of a *DHCR7* mutant allele with residual enzymatic function. Both *in vitro* experiments using SLOS fibroblasts[10] and *in vivo* experiments using an SLOS mouse model[41] support this explanation. Haas *et al*[42] reported improved sterol levels in a larger cohort of SLOS patients treated with combined cholesterol and simvastatin therapy. However, they did not report either a paradoxical increase in cholesterol or beneficial clinical responses. A placebo-controlled, masked, crossover trial investigating the safety and efficacy of simvastatin therapy is in progress. Until more data are available, simvastatin therapy should only be considered in the context of a clinical trial.

Genetic counseling

SLOS is a typical autosomal recessive disorder; however, for risk assessment calculations one needs to be aware that the carrier frequency is relatively high. In North American populations, the carrier frequency of the most common allele, c.964-1G>C is on the order of 1% (reviewed by Nowaczyk[43]). Witsch-Baumgartner *et al*[44] recently reported carrier frequencies for the three most common mutations (c.964-1G>C, p.W151X, and p.T93M) for various European populations. In specific European populations, the c.964-1G>C carrier frequency varied widely from 0.2 to 2.3%, and the combined carrier frequencies of c.964-1G>C and p.W151X were 1.0%, 1.4% and 2.3% in German, Polish and Czech populations, respectively. Estimating total mutation carrier frequencies based on extrapolating the observed frequency of null alleles such as c.964-1G>C and p.W151X in patients leads to major discrepancies between predicted and observed incidence of SLOS. This discrepancy is likely due to underascertainment of the c.964-1G>C allele secondary to prenatal loss of fetuses with two severe mutations. Until more information is available on carrier frequencies for other common mutations or specific information is available for a specific population, a 2% carrier frequency is a reasonable estimate for risk assessment. Plasma sterol levels cannot accurately identify *DHCR7* mutation carriers;[45] however, molecular testing can be used to evaluate carrier status.

Prenatal diagnosis

Sterol analysis can be performed on either amniotic fluid or a chorionic villus sample.[46] Molecular testing is an alternative; however, biochemical testing is faster and more economical. In a SLOS pregnancy, the fetal cholesterol synthetic defect can affect maternal steroids. The fetal cholesterol deficit can result in decreased maternal serum unconjugated estriol (uE3) levels.[47] Craig *et al*[48]have shown that an 'SLOS screening algorithm' based on maternal serum uE3, human chorionic gonadotropin and

α-fetoprotein protein levels can be used to identify fetuses with SLOS. Fetal synthesis of DHC results in the synthesis of desaturated steroids that are excreted in maternal urine.[49] Although not currently available as a clinical test, measurement of 7-dehydropregnanetriol/pregnanetriol, 8-dehydropregnanetriol/pregnanetriol, (7 + 8)-dehydropregnanetriol/pregnanetriol or dehyroestriol/estriol ratios in maternal urine appear to be a promising prenatal test.[50]

Conclusions

The recognition that SLOS is an inborn error of cholesterol synthesis gave insight into both potential pathophysiological mechanisms and potential therapeutic approaches. It will be interesting to know if the DHC-derived steroids, neuroactive steroids, bile acids and oxysterols formed in SLOS have agonistic or antagonistic properties that contribute to the pathology of SLOS. A fundamental issue regarding the treatment of SLOS patients is to determine the extent to which the behavioral and learning problems in SLOS are caused by developmental abnormalities *versus* the extent they are caused by the biochemical defect present in the central nervous system. Developmental problems are likely to be fixed deficits. However, deficits due to the biochemical disturbance may be amenable to therapy. For this reason, methods of treating the central nervous system sterol abnormality and the efficacy of such therapies need to be investigated.

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References

1 Smith DW, Lemli L, Opitz JM: A newly recognized syndrome of multiple congenital anomalies. *Journal of Pediatrics* 1964; **64**: 210–217.
2 Irons M, Elias ER, Salen G, Tint GS, Batta AK: Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. *Lancet* 1993; **341**: 1414.
3 Fitzky BU, Witsch-Baumgartner M, Erdel M *et al*: Mutations in the Delta7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome. *Proc Natl Acad Sci USA* 1998; **95**: 8181–8186.
4 Wassif CA, Maslen C, Kachilele-Linjewile S *et al*: Mutations in the human sterol delta7-reductase gene at 11q12-13 cause Smith-Lemli-Opitz syndrome. *Am J Hum Genet* 1998; **63**: 55–62.
5 Waterham HR, Wijburg FA, Hennekam RC *et al*: Smith-Lemli-Opitz syndrome is caused by mutations in the 7-dehydrocholesterol reductase gene. *Am J Hum Genet* 1998; **63**: 329–338.
6 Kelley RI, Hennekam RC: The Smith-Lemli-Opitz syndrome. *J Med Genet* 2000; **37**: 321–335.
7 Tierney E, Nwokoro NA, Porter FD, Freund LS, Ghuman JK, Kelley RI: Behavior phenotype in the RSH/Smith-Lemli-Opitz syndrome. *Am J Med Genet* 2001; **98**: 191–200.
8 Sikora DM, Pettit-Kekel K, Penfield J, Merkens LS, Steiner RD: The near universal presence of autism spectrum disorders in children with Smith-Lemli-Opitz syndrome. *Am J Med Genet A* 2006; **140**: 1511–1518.
9 Wolthers BG, Walrecht HT, van der Molen JC, Nagel GT, Van Doormaal JJ, Wijnandts PN: Use of determinations of 7-lathosterol (5 alpha-cholest-7-en-3 beta-ol) and other cholesterol precursors in serum in the study and treatment of disturbances of sterol metabolism, particularly cerebrotendinous xanthomatosis. *J Lipid Res* 1991; **32**: 603–612.
10 Wassif CA, Krakowiak PA, Wright BS *et al*: Residual cholesterol synthesis and simvastatin induction of cholesterol synthesis in Smith-Lemli-Opitz syndrome fibroblasts. *Mol Genet Metab* 2005; **85**: 96–107.
11 Moebius FF, Fitzky BU, Lee JN, Paik YK, Glossmann H: Molecular cloning and expression of the human delta7-sterol reductase. *Proc Natl Acad Sci USA* 1998; **95**: 1899–1902.
12 Nishino H, Ishibashi T: Evidence for requirement of NADPH-cytochrome P450 oxidoreductase in the microsomal NADPH-sterol Delta7-reductase system. *Arch Biochem Biophys* 2000; **374**: 293–298.
13 Correa-Cerro LS, Porter FD: 3beta-hydroxysterol Delta7-reductase and the Smith-Lemli-Opitz syndrome. *Mol Genet Metab* 2005; **84**: 112–126.
14 Witsch-Baumgartner M, Ciara E, Loffler J *et al*: Frequency gradients of DHCR7 mutations in patients with Smith-Lemli-Opitz syndrome in Europe: evidence for different origins of common mutations. *Eur J Hum Genet* 2001; **9**: 45–50.
15 Nowaczyk MJ, Martin-Garcia D, Aquino-Perna A *et al*: Founder effect for the T93M DHCR7 mutation in Smith-Lemli-Opitz syndrome. *Am J Med Genet A* 2004; **125**: 173–176.
16 Correa-Cerro LS, Wassif CA, Waye JS *et al*: DHCR7 nonsense mutations and characterisation of mRNA nonsense mediated decay in Smith-Lemli-Opitz syndrome. *J Med Genet* 2005; **42**: 350–357.
17 Witsch-Baumgartner M, Gruber M, Kraft HG *et al*: Maternal apo E genotype is a modifier of the Smith-Lemli-Opitz syndrome. *J Med Genet* 2004; **41**: 577–584.
18 Porter JA, Young KE, Beachy PA: Cholesterol modification of hedgehog signaling proteins in animal development. *Science* 1996; **274**: 255–259.
19 Kelley RL, Roessler E, Hennekam RC *et al*: Holoprosencephaly in RSH/Smith-Lemli-Opitz syndrome: does abnormal cholesterol metabolism affect the function of Sonic Hedgehog? *Am J Med Genet* 1996; **66**: 478–484.
20 Cooper MK, Wassif CA, Krakowiak PA *et al*: A defective response to Hedgehog signaling in disorders of cholesterol biosynthesis. *Nat Genet* 2003; **33**: 508–513.
21 Koide T, Hayata T, Cho KW: Negative regulation of Hedgehog signaling by the cholesterogenic enzyme 7-dehydrocholesterol reductase. *Development* 2006; **133**: 2395–2405.
22 Bijlsma MF, Spek CA, Zivkovic D, van de Water S, Rezaee F, Peppelenbosch MP: Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. *PLoS Biol* 2006; **4**: e232.
23 Megha, Bakht O, London E: Cholesterol precursors stabilize ordinary and ceramide-rich ordered lipid domains (lipid rafts) to different degrees. Implications for the Bloch hypothesis and sterol biosynthesis disorders. *J Biol Chem* 2006; **281**: 21903–21913.
24 Keller RK, Arnold TP, Fliesler SJ: Formation of 7-dehydrocholesterol-containing membrane rafts *in vitro* and *in vivo*, with relevance to the Smith-Lemli-Opitz syndrome. *J Lipid Res* 2004; **45**: 347–355.
25 Gondre-Lewis MC, Petrache HI, Wassif CA *et al*: Abnormal sterols in cholesterol-deficiency diseases cause secretory granule malformation and decreased membrane curvature. *J Cell Sci* 2006; **119**: 1876–1885.
26 Tulenko TN, Boeze-Battaglia K, Mason RP *et al*: A membrane defect in the pathogenesis of the Smith-Lemli-Opitz syndrome. *J Lipid Res* 2006; **47**: 134–143.

27 Kovarova M, Wassif CA, Odom S, Liao K, Porter FD, Rivera J: Cholesterol deficiency in a mouse model of Smith-Lemli-Opitz syndrome reveals increased mast cell responsiveness. *J Exp Med* 2006; **203**: 1161–1171.
28 Wassif CA, Zhu P, Kratz L *et al*: Biochemical, phenotypic and neurophysiological characterization of a genetic mouse model of RSH/Smith–Lemli–Opitz syndrome. *Hum Mol Genet* 2001; **10**: 555–564.
29 Singh P, Paila YD, Chattopadhyay A: Differential effects of cholesterol and 7-dehydrocholesterol on the ligand binding activity of the hippocampal serotonin(1A) receptor: implications in SLOS. *Biochem Biophys Res Commun* 2007; **358**: 495–499.
30 Wassif CA, Vied D, Tsokos M, Connor WE, Steiner RD, Porter FD: Cholesterol storage defect in RSH/Smith-Lemli-Opitz syndrome fibroblasts. *Mol Genet Metab* 2002; **75**: 325–334.
31 Fitzky BU, Moebius FF, Asaoka H *et al*: 7-Dehydrocholesterol-dependent proteolysis of HMG-CoA reductase suppresses sterol biosynthesis in a mouse model of Smith-Lemli-Opitz/RSH syndrome. *J Clin Invest* 2001; **108**: 905–915.
32 Steiner RD, Linck LM, Flavell DP, Lin DS, Connor WE: Sterol balance in the Smith-Lemli-Opitz syndrome. Reduction in whole body cholesterol synthesis and normal bile acid production. *J Lipid Res* 2000; **41**: 1437–1447.
33 Shackleton C, Roitman E, Guo LW, Wilson WK, Porter FD: Identification of 7(8) and 8(9) unsaturated adrenal steroid metabolites produced by patients with 7-dehydrosterol-delta7-reductase deficiency (Smith-Lemli-Opitz syndrome). *J Steroid Biochem Mol Biol* 2002; **82**: 225–232.
34 Marcos J, Guo LW, Wilson WK, Porter FD, Shackleton C: The implications of 7-dehydrosterol-7-reductase deficiency (Smith-Lemli-Opitz syndrome) to neurosteroid production. *Steroids* 2004; **69**: 51–60.
35 Wassif CA, Yu J, Cui J, Porter FD, Javitt NB: 27-Hydroxylation of 7- and 8-dehydrocholesterol in Smith-Lemli-Opitz syndrome: a novel metabolic pathway. *Steroids* 2003; **68**: 497–502.
36 Elias ER, Irons MB, Hurley AD, Tint GS, Salen G: Clinical effects of cholesterol supplementation in six patients with the Smith-Lemli-Opitz syndrome (SLOS). *Am J Med Genet* 1997; **68**: 305–310.
37 Irons M, Elias ER, Abuelo D *et al*: Treatment of Smith-Lemli-Opitz syndrome: results of a multicenter trial. *Am J Med Genet* 1997; **68**: 311–314.
38 Sikora DM, Ruggiero M, Petit-Kekel K, Merkens LS, Connor WE, Steiner RD: Cholesterol supplementation does not improve developmental progress in Smith-Lemli-Opitz syndrome. *J Pediatr* 2004; **144**: 783–791.
39 Jira PE, Wevers RA, de Jong J *et al*: Simvastatin. A new therapeutic approach for Smith-Lemli-Opitz syndrome. *J Lipid Res* 2000; **41**: 1339–1346.
40 Starck L, Lovgren-Sandblom A, Bjorkhem I: Simvastatin treatment in the SLO syndrome: a safe approach? *Am J Med Genet* 2002; **113**: 183–189.
41 Correa-Cerro LS, Wassif CA, Kratz L *et al*: Development and characterization of a hypomorphic Smith-Lemli-Opitz syndrome mouse model and efficacy of simvastatin therapy. *Hum Mol Genet* 2006; **15**: 839–851.
42 Haas D, Garbade SF, Vohwinkel C *et al*: Effects of cholesterol and simvastatin treatment in patients with Smith-Lemli-Opitz syndrome (SLOS). *J Inherit Metab Dis* 2007; **30**: 375–387.
43 Nowaczyk MJ, Waye JS, Douketis JD: DHCR7 mutation carrier rates and prevalence of the RSH/Smith-Lemli-Opitz syndrome: where are the patients? *Am J Med Genet A* 2006; **140**: 2057–2062.
44 Witsch-Baumgartner M, Schwentner I, Gruber M *et al*: Age and origin of major Smith-Lemli-Opitz Syndrome (SLOS) mutations in European populations. *J Med Genet* 2007; e-pub ahead of print 26 October 2007.
45 Kelley RI: Diagnosis of Smith-Lemli-Opitz syndrome by gas chromatography/mass spectrometry of 7-dehydrocholesterol in plasma, amniotic fluid and cultured skin fibroblasts. *Clin Chim Acta* 1995; **236**: 45–58.
46 Kratz LE, Kelley RI: Prenatal diagnosis of the RSH/Smith-Lemli-Opitz syndrome. *Am J Med Genet* 1999; **82**: 376–381.
47 Bradley LA, Palomaki GE, Knight GJ *et al*: Levels of unconjugated estriol and other maternal serum markers in pregnancies with Smith-Lemli-Opitz (RSH) syndrome fetuses. *Am J Med Genet* 1999; **82**: 355–358.
48 Craig WY, Haddow JE, Palomaki GE *et al*: Identifying Smith-Lemli-Opitz syndrome in conjunction with prenatal screening for Down syndrome. *Prenat Diagn* 2006; **26**: 842–849.
49 Shackleton CH, Roitman E, Kratz LE, Kelley RI: Midgestational maternal urine steroid markers of fetal Smith-Lemli-Opitz (SLO) syndrome (7-dehydrocholesterol 7-reductase deficiency). *Steroids* 1999; **64**: 446–452.
50 Shackleton CH, Marcos J, Palomaki GE *et al*: Dehydrosteroid measurements in maternal urine or serum for the prenatal diagnosis of Smith-Lemli-Opitz syndrome (SLOS). *Am J Med Genet A* 2007; **143**: 2129–2136.