American Journal of Medical Genetics 138A:56–60 (2005)

Clinical Report

Recognition of Smith–Lemli–Opitz Syndrome (RSH) in the Fetus: Utility of Ultrasonography and Biochemical Analysis in Pregnancies With Low Maternal Serum Estriol

Marwan Shinawi,[1] Sara Szabo,[2] Edwina Popek,[2] Christopher A. Wassif,[3] Forbes D. Porter,[3] and Lorraine Potocki[1]*

[1]*Department of Molecular and Human Genetics, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas*
[2]*Department of Pediatric Pathology, Texas Children's Hospital, Houston, Texas*
[3]*Unit on Molecular Dysmorphology, Heritable Disorders Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland*

Smith–Lemli–Opitz syndrome (SLOS), or RSH, is an autosomal recessive disorder caused by mutations of the gene encoding 7-dehydrocholesterol reductase (*DHCR7*). The utility of maternal serum screens and ultrasound as prenatal screening methods for SLOS is presently undetermined. We report the clinical, cytogenetic, biochemical, and molecular findings of a stillborn with SLOS. The diagnosis was made postnatally on the basis of physical findings and confirmed by biochemical and DNA analyses of fetal tissue. Although abnormalities were detected by maternal serum triple screen and prenatal ultrasonography, a diagnosis of SLOS was not suspected before delivery. This study demonstrates that patients with SLOS may escape prenatal diagnosis despite the presence of multiple anomalies and abnormal maternal serum screen results, and lends support for consideration of prenatal biochemical testing for SLOS in pregnancies with these findings. As SLOS is a severe autosomal recessive disorder with a recurrence risk of 25%, ultrasonographic, cytogenetic, and biochemical analyses in the second trimester should be considered if abnormal maternal serum screening results, specifically low levels of unconjugated estriol, are detected.

KEY WORDS: Smith–Lemli–Opitz syndrome; unconjugated estriol; 7-dehydrocholesterol; fetal demise

INTRODUCTION

Smith–Lemli–Opitz syndrome (SLOS), or RSH [OMIM #268670] is an autosomal recessive Multiple Congenital Anomalies and Mental Retardation (MCA/MR) syndrome affecting 1 in 20,000–1 in 40,000 newborn infants and caused by a deficiency of DHCR7, an essential enzyme in the biosynthesis of cholesterol [Tint et al., 1994]. The clinical severity of SLOS ranges from cutaneous syndactyly of the second and third toes or minor anomalies to a severe MCA/MR syndrome, to prenatal lethality [Cunniff et al., 1997].

The best biochemical predictor of clinical severity of SLOS is the plasma cholesterol to DHC ratio which decreases with increasing clinical severity [Tint et al., 1995]. It has been demonstrated also that cultured fibroblasts from typical and atypical cases of SLOS accumulate 7-DHC when they are grown in lipoprotein deficient medium [Honda et al., 1997].

Maternal serum unconjugated estriol (uE3) levels in combination with abnormal sonography may provide useful diagnostic information in the absence of a family history of SLOS [Canick et al., 1997; Bradley et al., 1999; Kratz and Kelley, 1999]. Kratz and Kelley found an inverse relationship between clinical severity and amniotic fluid 7-DHC and maternal serum uE3 levels: i.e., the lower the uE3 level, the higher the 7-DHC level and the severity score [Kratz and Kelley, 1999]. However at the present time, the utility of interpreting maternal serum screen results in conjunction with prenatal ultrasonography as screening methods for SLOS is undetermined.

Our case emphasizes the need for awareness to the severe prenatal presentation of SLOS especially if associated with abnormal maternal serum screen results. It also suggests that it would be reasonable to consider simultaneously measuring 7-DHC levels in the amniotic fluid in pregnancies where SLOS is suspected and in all pregnancies undergoing amniocentesis for chromosome analysis where the maternal serum screen indicates either a low uE3 or an increased risk for trisomy 18.

CLINICAL REPORT

The propositus is a chromosomally male fetus who died at 38 weeks of gestation and was delivered to a 33-year-old gravida 2, para 0, healthy mother and 41-year-old healthy father, both of Caucasian ancestry. The couple's first pregnancy was aborted spontaneously at 10 weeks of gestation but no genetic testing was performed. The family history is otherwise noncontributory. Prenatal history documents vaginal bleeding at 6 weeks of gestation, decreased fetal movements, and no exposure to medications, smoking, alcohol or illicit substances.

Specific analyte analysis documented a maternal serum alpha-fetoprotein (AFP), 0.7 MoM; uE3, 0.53 MoM; and total human chorionic gonadotropin (hCG), 0.43 MoM. This maternal serum triple screen indicated a 1:4,400 risk for Down syndrome. Although the three markers were low, they did not reach the laboratory cut off risk of 1:100 for trisomy 18. Prenatal ultrasonography at 20 weeks of gestation identified intrauterine growth retardation, apparently female genitalia, a cystic mass adjacent to the 5th digit of the left hand, bilateral club feet, and lower limb polydactyly. Chromosome analysis of

*Correspondence to: Lorraine Potocki, M.D., Department of Molecular and Human Genetics, Baylor College of Medicine, Texas Children's Hospital, Clinical Care Center, 6621 Fannin, Suite 1560, Mail Code CC-1560, Houston. Texas 77030.
E-mail: lpotocki@bcm.tmc.edu

Received 22 February 2005; Accepted 24 May 2005
DOI 10.1002/ajmg.a.30898

cultured amniocytes showed 46,XY in 19 of 20 colonies and 47,XY,+6 in one colony (interpreted as culture artifact). Six weeks prior to delivery, thickened cardiac ventricles with calcium deposits in the left ventricle were noted by ultrasonography; however, fetal echocardiography did not detect a major congenital heart defect.

Physical examination of this stillborn fetus documented multiple congenital anomalies and dysmorphic features (Fig. 1). Head circumference was 31 cm (<5th centile), weight was 2,493 g (10–25th centile), and length 42.5 cm (<10th centile). Craniofacial abnormalities included hypertelorism, broad nasal root, bilateral epicanthal folds, flat and broad nasal root and flat nasal bridge, a short and upturned nose with hypoplastic alae nasi, a long smooth upper lip with downturned mouth and micrognathia. There was a midline cleft of the hard and soft palate, broad alveolar ridges, and multiple, white lingual nodules. The neck was short and webbed with thickened and redundant posterior skin folds. The ears were low-set, posteriorly angulated, with thick and simplified helices. There was tetramelic rhizomelic shortness with contractures of the large joints, axillary ptyergia (mild), bilateral postaxial polydactyly of the hands and the right foot, bilateral 5th finger clinodactyly, an infarcted pedunculated 2 cm mass connected by a thread-like segment to the remnant of left sixth finger, cutaneous syndactyly of the 2nd and 3rd toes bilaterally, smooth palms and soles with absence of creases on the right thumb, and nail hypoplasia (Fig. 2). There was a shallow sacral dimple and indistinct gluteal folds. The fetus had a microphallus, vaginal pouch, labial scrotal folds, and non-palpable testes. Autopsy examination documented microcephaly (brain weight 246 g; expected weight 400 g) with small frontal lobes and abnormal orientation of the temporal gyri. There were no major intercranial malformations and the corpus callosum was present. Examination of the heart and great vessels revealed tetralogy of Fallot with anomalous, retroesophageal right subclavian artery. The fetus also had pulmonary hypoplasia with unilobed lungs, small accessory spleen, and hypoplastic left kidney with reduced number of medullo-calyceal units and focal dysplasia. Postmortem radiographs showed fusion of L5-S1 and short bones in all four limbs.

MATERIALS AND METHODS

Sterol concentrations in cultured fibroblasts were measured by gas chromatography-mass spectrophotometry as described previously [Kelley, 1995]. After growth in cholesterol deficient medium for 5 days, 51% of total sterols were 7-DHC. Cholesterol, 8-DHC, and lathosterol accounted for 28%, 2%, and 19% of total sterols, respectively. These results are consistent with sterol profiles obtained from other previously confirmed cases of severe SLOS (personal communication, FDP).

In order to establish a molecular diagnosis, DNA was isolated from parental blood and fetal fibroblasts according to standard methods. The common splice acceptor site mutation, IVS8-1G > C, was identified using a fluorogenic probe based allelic discrimination assay in DNA obtained from the affected fibroblasts. DNA sequencing identified a c.452G > A (W151X) mutation and confirmed the IVS8-1G > C mutation. The W151X mutation was confirmed using a PCR-RFLP assay in which the mutant allele is specifically digested using *AluI*. Sequencing of the parental DNA showed that the father was heterozygous for the nonsense mutation W151X, and the mother was a carrier for the splice-site mutation IVS8-1G > C.

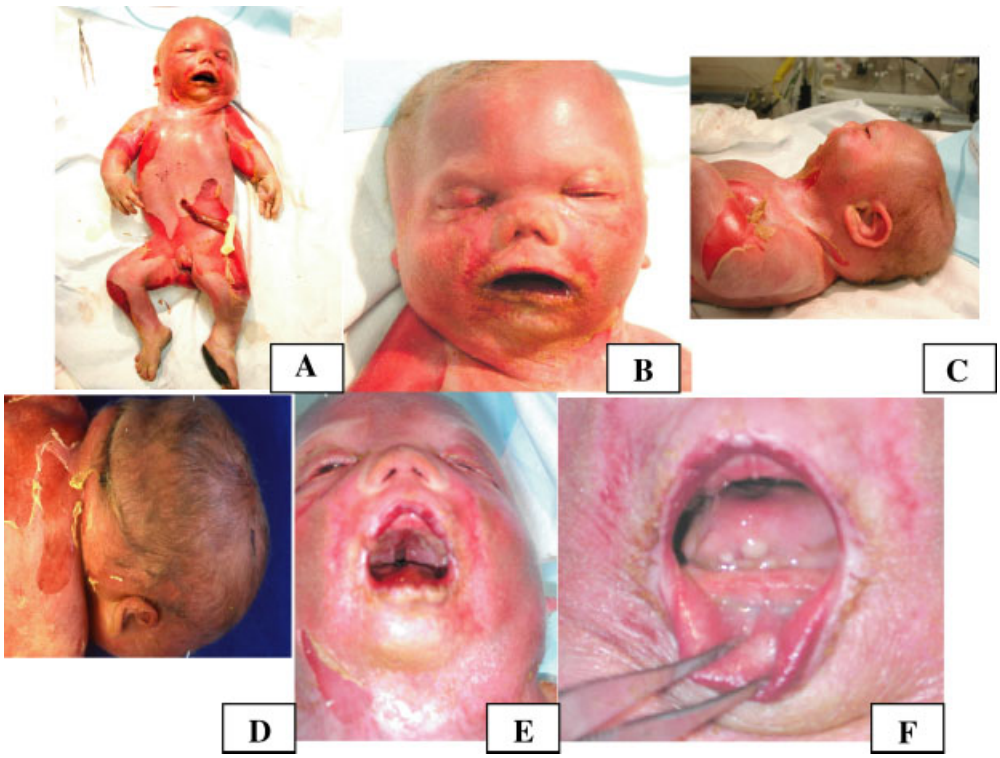

Fig. 1. Stillborn fetus with Smith–Lemli–Opitz syndrome (SLOS). **A**: Note rhizomelic shortness of limbs. **B**: Facial appearance: hypertelorism, flat nasal bridge, very short and upturned nose with hypoplastic alae nasi, long and smooth philtrum with downtenting of mouth. **C**: Note micrognathia and low-set ears. **D**: Posterior thickening of webbed neck. **E**: Midline cleft palate. **F**: Thick, broad alveolar ridges with several lingual nodules which were noted to be adipose tissue on histology.

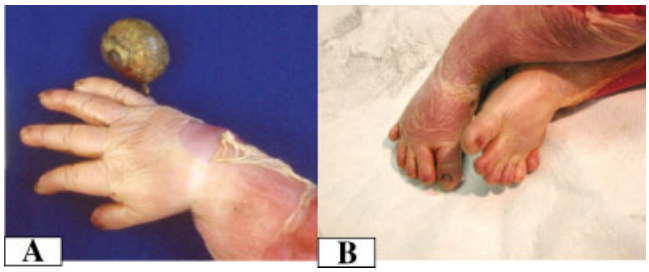

Fig. 2. **A**: Nodular mass connected to the remnant of left 6th finger. Histological examination showed near complete devitalization with islands of cartilage in the center and epidermal surface suggestive of torsion and subsequent infarction of the digit (not shown). **B**: Bilateral club feet and polydactyly with Y-shaped cutaneous syndactyly of the 2nd and 3rd toes.

DISCUSSION

Mutations in the gene *DHCR7* cause SLOS by blocking the conversion of 7-DHC to cholesterol and subsequently leading to low tissue content of cholesterol and increased content of 7-DHC.

Cholesterol is a key regulator of eukaryotic membranes and a precursor in the synthesis of steroids and other sterols. It also has an important role in the activation of the Hedgehog proteins which are cell signaling molecules critical for embryonic growth, patterning and morphogenesis [Ingham and McMahon, 2001]. Interestingly, the disorder known as Pallister–Hall syndrome shares many manifestations with SLOS and is due to mutations in the *GLI3* gene which encodes a signaling factor downstream of the sonic hedgehog pathway [Kang et al., 1997]. Low levels of cholesterol in the fetus also results in steroid precursor deficiencies which presumably contribute to decreased uE3 in maternal circulation [Shackleton et al., 1999]. There are several case-reports and series of pregnancies in which, in the presence of an affected fetus, undetectable to low levels of uE3 in maternal serum were found [McKeever and Young, 1990; Blitzer et al., 1994; Canick et al., 1997; Angle et al., 1998; Kratz and Kelley, 1999; Shackleton et al., 1999] and occasionally, a characteristic pattern of low uE3, low hCG, and low AFP in these pregnancies indicating an increased risk for trisomy 18 [Palomaki et al., 1995] was seen [Bradley et al., 1999] (Table I). Bradley et al. [1999] showed results for 26 pregnancies resulting in a child with SLOS. The median uE3 in this study was 0.23 MOM (range 0.1–0.52), corresponding to <1% of the level in normal pregnancies. Kratz and Kelley [1999] diagnosed SLOS postnatally in three of four pregnancies with a low uE3 and an SLOS-type fetal abnormality (e.g., polydactyly, ambiguous genitalia) however, none of the pregnancies were tested for SLOS prenatally.

In another series, approximately 30% pregnancies affected with SLOS screened positive for trisomy 18 based on low levels of all analytes [Bradley et al., 1999], thus lending further credibility to specific prenatal testing for SLOS in the setting of low uE3 marker analysis. Therefore, the combination of ambiguous genitalia in a chromosomally male fetus, multiple congenital anomalies with low maternal serum markers were highly indicative for SLOS in the case presented. Although the frequency of SLOS in pregnancies with low maternal estriol levels or multiple congenital anomalies is unknown, the diagnosis of SLOS should, nevertheless, be considered in both clinical settings. Fetal disorders that are associated with low maternal serum estriol and multiple congenital anomalies include chromosomal abnormalities, steroid/multiple sulphatase deficiency and anencephaly [Glass et al., 1998]. Bick et al. [1999] reported on 26 pregnancies that had low maternal estriol, 9 of which ended in spontaneous miscarriage but none of live born children was clinically diagnosed with SLOS. Several other studies found a high fetal loss rate associated with low maternal serum level of estriol, with and without low levels of AFP and beta hCG but none of these studies evaluated the frequency of SLOS among pregnancies with low estriol [Schleifer et al., 1995; Santolaya-Forgas et al., 1996]. In fact, one study has debated the utility and the cost-effectiveness of maternal serum screen as a screening tool for SLOS [Schoen et al., 2003]. In this study, of the total of 103 women with unexplained low uE3, only two SLOS cases were detected: one infant who died soon after birth and a fetus with SLOS, diagnosed after therapeutic abortion for severe oligohydramnios. Interestingly, intrauterine fetal death occurred in 39 pregnancies [Schoen et al., 2003]. It is important to note, however, the authors of the study did not perform sterol analysis in the amniotic fluid or fetal tissues to rule out the possibility of SLOS.

The carrier frequency (2pq) of the most common mutation (IVS-1G > C), which accounts for approximately one third of the ascertained SLOS mutant alleles is 1.1%, thus predicting a

TABLE I. Smith–Lemli–Opitz Syndrome (SLOS) Cases Associated With Abnormal Maternal Serum Screen

Reference	Number of cases	Comments
McKeever and Young [1990]	2	Undetectable uE3 during the late stages of pregnancy
Blitzer et al. [1994]	2	Maternal serum uE3, AFP, and hCG levels were low
Hyett et al. [1995]	1	Low uE3, 46,XY karyotype, female genitalia with nuchal fluid accumulation by fetal ultrasonography
Rossiter et al. [1995]	1	Maternal serum uE3, AFP, and hCG levels were low
Canick et al. [1997]	2	Maternal serum uE3 (MOM): 0.44; AFP and hCG levels were unremarkable
Angle et al. [1998]	1	uE3, 0.44 MoM; AFP, 0.77 MoM; hCG, 2.11 MoM, antenatal ultrasound was abnormal, amniocentesis demonstrated a normal 46,XY karyotype
Bick et al. [1999]	1	One out of 26 pregnancies with estriol level 0.25 MoM had SLOS, 46,XY karyotype, female genitalia and mid-trimester intrauterine growth retardation by ultrasound examination
Bradley et al. [1999]	26	The median uE3 measurement (0.23 MoM) is lower than the 1st centile of control pregnancies ($P < 0.001$); 24 of the 26 uE3 measurements are below the 5th centile
Kratz and Kelley [1999]	3	Of the four pregnancies with low uE3 and SLOS-type fetal abnormalities, three were confirmed to have SLOS, of the seven pregnancies with low uE3 and normal sonographic testing, none were affected with SLOS
Shackleton et al. [1999]	1	Undetectable uE3, abnormal sonography, amniotic fluid confirmed SLOS, termination at 18 weeks
Schoen et al. [2003]	2	Two of 103 pregnancies with unexplained low uE3 were diagnosed with SLOS

3%–4% carrier frequency for all mutant alleles and a disease incidence of 1/2,500 to 1/4,400 [Battaile et al., 2001; Nowaczyk et al., 2001]. This discrepancy between calculated and observed frequencies most likely represents undiagnosed mild cases, misdiagnosed severe cases, death prior to diagnosis, and intrauterine fetal demise and lack of diagnosis. Underascertainment of severely affected cases likely accounts for at least part of the estimated and the observed birth incidence of SLOS. In fact, in approximately 25% of reports of biochemically confirmed SLOS, the initial diagnosis was in error [Cunniff et al., 1997]. Fortunately, biochemical analysis of 7-DHC is a highly sensitive and specific method for the prenatal and postnatal diagnosis of SLOS [Rossiter et al., 1995], and mutation analysis of *DHCR7* is clinically available and positive in more than 80% of SLOS patients. We speculate that a prudent utilization of these diagnostic methods would correct the ascertainment bias that is significantly contributing to the discrepancy between the calculated and observed frequencies.

Both W151X and IVS8-1G > C are null alleles. It has been shown in genotype–phenotype correlation studies that patients with two functional null *DHCR7* alleles have the most severe phenotypes that results in intrauterine or perinatal lethality [Witsch-Baumgartner et al., 2000]. IVS8-1G > C disrupts a splice acceptor sequence. Using of a cryptic splice acceptor site results in the insertion of 134 intronic nucleotides into the *DHCR7* mRNA. Thus, at the protein level, this mutation results in a frame shift and a truncated product. W151X is a nonsense mutation that predicts truncation of the DHCR7 protein. However, a recent study has demonstrated that the *DHCR7* W151X transcript undergoes nonsense mediated decay [Correa-Cerro et al., 2005]. It is notable that these two null mutations (IVS81G > C and W151X) were among the five most frequent SLOS mutations identified and accounting for over one-third of all SLOS mutations [Correa-Cerro and Porter, 2005].

Currently, many pregnant women in the United States have multiple marker serum screening for chromosomal syndromes and neural tube defects. As the prenatal diagnosis of SLOS is clinically available and highly sensitive and specific, testing for 7DHC in addition to chromosome analysis should be standard of care when the uE3 is low even if multiple anomalies are not detected on prenatal US. In the future, a maternal urine test for 7DHC derived steroids may provide a non-invasive method of prenatal diagnosis [Shackleton et al., 2001]. The accurate postnatal diagnosis in this stillborn infant not only provided an explanation for the IUFD and physical abnormalities, but was essential for giving an accurate recurrence risk and offering prenatal diagnosis in future pregnancies for this couple. While prenatal diagnosis can be made biochemically following amniocentesis, DNA analysis can allow for preimplantation diagnosis for carrier couples.

ACKNOWLEDGMENTS

We thank Beth K. Dupper, M.P.H., B.S.N, R.N., for her professional assistance. We thank the parents for their consent to publish the photographs in this article.

REFERENCES

Angle B, Tint GS, Yacoub OA, Clark AL. 1998. Atypical case of Smith–Lemli–Opitz syndrome: Implications for diagnosis. Am J Med Genet 80:322–326.

Battaile KP, Battaile BC, Merkens LS, Maslen CL, Steiner RD. 2001. Carrier frequency of the common mutation IVS8-1G > C in DHCR7 and estimate of the expected incidence of Smith–Lemli–Opitz syndrome. Mol Genet Metab 72:67–71.

Bick DP, McCorkle D, Stanley WS, Stern HJ, Staszak P, Berkovitz GD, Meyers CM, Kelley RI. 1999. Prenatal diagnosis of Smith–Lemli–Opitz syndrome in a pregnancy with low maternal serum oestriol and a sex-reversed fetus. Prenat Diagn 19:68–71.

Blitzer MG, Kelley RI, Schwartz MF. 1994. Abnormal maternal serum marker pattern associated with Smith–Lemli–Opitz (SLO) syndrome. Am J Hum Genet 55:A277.

Bradley LA, Palomaki GE, Knight GJ, Haddow JE, Opitz JM, Irons M, Kelley RI, Tint GS. 1999. Levels of unconjugated estriol and other maternal serum markers in pregnancies with Smith–Lemli–Opitz (RSH) syndrome fetuses. Am J Med Genet 82:355–358.

Canick JA, Abuelo DN, Bradley LA, Tint GS. 1997. Maternal serum marker levels in two pregnancies affected with Smith–Lemli–Opitz syndrome. Prenat Diagn 17:187–189.

Correa-Cerro LS, Porter FD. 2005. 3β-hydroxysterol Δ7-reductase and the Smith–Lemli–Opitz Syndrome. Mol Genet Metab 84:112–126.

Correa-Cerro LS, Wassif CA, Waye JS, Krakowiak PA, Cozma D, Dobson NR, Levin SW, Anadiotis G, Steiner RD, Krajewska-Walasek M, Nowaczyk MJ, Porter FD. 2005. DHCR7 nonsense mutations and characterization of mRNA nonsense mediated decay in Smith–Lemli–Opitz syndrome. J Med Genet 42:350–357.

Cunniff C, Kratz LE, Moser A, Natowicz MR, Kelley RI. 1997. Clinical and biochemical spectrum of patients with RSH/Smith–Lemli–Opitz syndrome and abnormal cholesterol metabolism. Am J Med Genet 68: 263–269.

Glass IA, Lam RC, Chang T, Roitman E, Shapiro LJ, Shackleton CH. 1998. Steroid sulphatase deficiency is the major cause of extremely low oestriol production at mid-pregnancy: A urinary steroid assay for the discrimination of steroid sulphatase deficiency from other causes. Prenat Diagn 18:789–800.

Honda A, Tint GS, Salen G, Kelley RI, Honda M, Batta AK, Chen TS, Shefer S. 1997. Sterol concentrations in cultured Smith–Lemli–Opitz syndrome skin fibroblasts: Diagnosis of a biochemically atypical case of the syndrome. Am J Med Genet 68:282–287.

Hyett JA, Clayton PT, Moscoso G, Nicolaides KH. 1995. Increased first trimester nuchal translucency as a prenatal manifestation of Smith–Lemli–Opitz syndrome. Am J Med Genet 58:374–376.

Ingham PW, McMahon AP. 2001. Hedgehog signaling in animal development: Paradigms and principles. Genes Dev 15:3059–3087.

Kang S, Graham JM, Olney AH, Biesecker LG. 1997. GLI3 frameshift mutations cause autosomal dominant Pallister–Hall syndrome. Nat Genet 15:266–268.

Kelley RI. 1995. 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 236:45–58.

Kratz LE, Kelley RI. 1999. Prenatal diagnosis of the RSH/Smith–Lemli–Opitz syndrome. Am J Med Genet 82:376–381.

McKeever PA, Young ID. 1990. Smith–Lemli–Opitz syndrome. II: A disorder of the fetal adrenals? J Med Genet 27:465–466.

Nowaczyk MJ, Nakamura LM, Eng B, Porter FD, Waye JS. 2001. Frequency and ethnic distribution of the common DHCR7 mutation in Smith–Lemli–Opitz syndrome. Am J Med Genet 102:383–386.

Palomaki GE, Haddow JE, Knight GJ, Wald NJ, Kennard A, Canick JA, Saller DN Jr, Blitzer MG, Dickerman LH, Fisher R. 1995. Risk-based prenatal screening for trisomy 18 using alpha-fetoprotein, unconjugated oestriol and human chorionic gonadotropin. Prenat Diagn 15:713–723.

Rossiter JP, Hofman KJ, Kelley RI. 1995. Smith–Lemli–Opitz syndrome: Prenatal diagnosis by quantification of cholesterol precursors in amniotic fluid. Am J Med Genet 56:272–275.

Santolaya-Forgas J, Jessup J, Burd LI, Prins GS, Burton BK. 1996. Pregnancy outcome in women with low midtrimester maternal serum unconjugated estriol. J Reprod Med 41:87–90.

Schleifer RA, Bradley LA, Richards DS, Ponting NR. 1995. Pregnancy outcome for women with very low levels of maternal serum unconjugated estriol on second trimester screening. Am J Obstet Gynecol 173:1152–1156.

Schoen E, Norem C, O'Keefe J, Krieger R, Walton D, To TT. 2003. Maternal serum unconjugated estriol as a predictor for Smith–Lemli–Opitz syndrome and other fetal conditions. Obstet Gynecol 102: 167–172.

Shackleton CH, Roitman E, Kratz LE, Kelley RI. 1999. Equine type estrogens produced by a pregnant woman carrying a Smith–Lemli–Opitz syndrome fetus. J Clin Endocrinol Metab 84:1157–1159.

Shackleton CH, Roitman E, Kratz L, Kelley R. 2001. Dehydro-oestriol and dehydropregnanetriol are candidate analytes for prenatal diagnosis of Smith–Lemli–Opitz syndrome. Prenat Diagn 21:207–212.

Tint GS, Irons M, Elias ER, Batta AK, Frieden R, Chen TS, Salen G. 1994. Defective cholesterol biosynthesis associated with the Smith–Lemli–Opitz syndrome. N Engl J Med 330:107–113.

Tint GS, Salen G, Batta AK, Shefer S, Irons M, Elias ER, Abuelo DN, Johnson VP, Lambert M, Lutz R, et al. 1995. Correlation of severity and outcome with plasma sterol levels in variants of the Smith–Lemli–Opitz syndrome. J Pediatr 127:82–87.

Witsch-Baumgartner M, Fitzky BU, Ogorelkova M, Kraft HG, Moebius FF, Glossmann H, Seedorf U, Gillessen-Kaesbach G, Hoffmann GF, Clayton P, Kelley RI, Utermann G. 2000. Mutational spectrum in the delta7-sterol reductase gene and genotype-phenotype correlation in 84 patients with Smith–Lemli–Opitz syndrome. Am J Hum Genet 66: 402–412.