

INVITED EDITORIAL

RSH/Smith-Lemli-Opitz Syndrome: Mutations and Metabolic Morphogenesis

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For almost 30 years following its description in 1964, the RSH syndrome—now more often called the “Smith-Lemli-Opitz syndrome” (SLOS)—was just one of many autosomal recessive, multiple anomaly syndromes that filled the pages of dysmorphology atlases (MIM 270400; Smith et al. 1964). Its specific combination of craniofacial anomalies, polydactyly, cleft palate, and genital malformations in males was readily diagnosed by dysmorphologists but otherwise caused little interest. In 1993, however, SLOS was abruptly lifted from relative obscurity by the discovery that affected patients have as much as 2,000-fold-increased blood levels of 7 α -dehydrocholesterol (7DHC), the immediate precursor of cholesterol in the Kandutsch-Russell pathway of cholesterol biosynthesis (Irons et al. 1993; Tint et al. 1994). The now apparent high specificity of increased 7DHC levels for diagnosis of SLOS (Cunniff et al. 1997) has made possible not only definitive diagnosis of SLOS but also prenatal diagnosis by measurement of 7DHC in amniotic fluid or chorionic villus (Abuelo et al. 1995; Rossiter et al. 1995; Mills et al. 1996). Not unexpectedly, the availability of a laboratory test for SLOS also has led to an expansion of its clinical phenotype to include near-normal children with no discrete malformations, as well as severely malformed fetuses that die in utero. Studies of blood sterol levels of SLOS patients show that clinical severity correlates best not with the absolute level of 7DHC but inversely either with the level of cholesterol or with the level of cholesterol as a fraction of total sterols (Cunniff et al. 1997). The most severely affected, “type II” SLOS patients (Curry et al. 1987) typically have blood cholesterol levels of 1–10 mg/dl (0.03–0.3 mmol/liter) and die in the newborn period because of multiple internal anomalies (Tint et al. 1995). At the other extreme, ~10% of SLOS patients have minimal

disease and normal or low-normal cholesterol levels at diagnosis (Cunniff et al. 1997). Although, historically, a clinical distinction often was made between classic (“type I”) SLOS and the more severe, type II patients, there is, in reality, a clinical and biochemical continuum from mild to severe SLOS (Cunniff et al. 1997).

Cholesterol Biosynthesis and 3 β -Hydroxysterol- Δ 7-Reductase

The biosynthesis of cholesterol and its related family of “isoprenoid” compounds, including coenzyme Q and dolichols, begins with the conversion of 3-hydroxy-3-methylglutaric acid to mevalonic acid and progresses to the synthesis of polyisoprenoids, such as geraniol-PP and farnesol-PP. After the formation of the first sterol, lanosterol, from squalene, there follows a succession of demethylations and double-bond rearrangements, the last of which is the reduction of the 7-8 double bond of 7DHC to form cholesterol, mediated by the microsomal enzyme, 3 β -hydroxysterol- Δ 7-reductase (DHCR7; E.C.1.3.1.21). The finding of marked elevations of 7DHC in SLOS immediately implicated *DHCR7* as the site of the genetic defect (Tint et al. 1994). Although the chromosomal location of *DHCR7* was unknown in 1993, attention soon turned to 7q32.1 because of two unrelated SLOS patients, one of whom was biochemically confirmed, who were found to have translocations at that position (Alley et al. 1995). However, earlier this year, Fabian Moebius and colleagues reported the cloning of a human microsomal *DHCR7* gene and mapped it to chromosomal position 11q12-13 (Moebius et al. 1998). The same laboratory has now reported that all 13 patients with classical SLOS whom they studied had mutations in *DHCR7* (Fitzky et al. 1998). In this and the July issue of the *Journal*, two other groups working independently, one at the National Institutes of Health and headed by Forbes Porter and another that is a consortium of Dutch centers, report mutations of the same *DHCR7* gene in six additional patients with SLOS (Was-siff et al. 1998; Waterham et al. 1998), confirming that, for most patients with a clinical diagnosis of SLOS and increased 7DHC levels, the gene for *DHCR7* harbors

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the responsible mutations. However, because one pair of siblings with phenotypic SLOS and moderately increased levels of 7DHC has been found to have an apparent defect of sterol transport (Anderson et al. 1998), mutations in genes other than *DHCR7* are possible.

The three articles describing *DHCR7* mutations reveal interesting aspects of the biochemistry and population genetics of SLOS. The historically relatively high incidence of SLOS—~1/20,000–30,000 births among those of northern- and central-European background (Opitz 1994; Cunniff et al. 1997)—suggests either heterozygote advantage or one or more founder mutations. Possibly, both mechanisms are involved. Of 19 different *DHCR7* mutations found in, coincidentally, 19 patients, there were 13 missense mutations, 1 nonsense mutation, and 5 frameshift mutations. Of these mutations, three occurred in four or more patients, the most interesting being a 134-bp insertion found in 7 of 38 alleles. This particular mutation is predicted to abolish all *DHCR7* activity and was homozygous in 2 of the 19 patients studied, both of whom had a diagnosis of type II SLOS. Because the most severely affected patients have measurable cholesterol levels at birth, typically 5–10 mg/dl (Tint et al. 1995; Cunniff et al. 1997), there may be another genetic source of *DHCR7* activity. Alternatively, there could be either a pathway of cholesterol synthesis not requiring *DHCR7* or more maternal-to-fetal transfer of cholesterol than currently is thought. The finding of many different *DHCR7* mutations in this relatively common malformation syndrome suggests a heterozygote advantage, such as increased production of vitamin D from the mildly increased plasma levels of 7DHC in SLOS parents (Opitz and de la Cruz 1994; Kelley 1995). The diversity of *DHCR7* mutations also means that measurement of plasma and tissue sterol levels will remain the primary method for diagnosis of SLOS. However, mutational analysis may be useful in the evaluation of suspected SLOS patients with normal or equivocally increased 7DHC levels.

SLOS, Holoprosencephaly (HPE), and the Metabolism of Morphogenesis

As work progressed on the molecular and biochemical characterization of SLOS, major advances also were being made in the study of HPE and the “hedgehog” class of embryonic signaling proteins, two areas of genetics at first seemingly unrelated to SLOS. However, these three areas of research now have converged in a remarkable way that provides new insights into human morphogenesis. This part of the SLOS story actually begins before the first clinical description of SLOS when, in the early 1960s, researchers discovered that inhibitors of enzymes of the distal cholesterol biosynthetic pathway, including *DHCR7*, caused HPE, pituitary agenesis,

and, less frequently, limb and genital anomalies in the pups of pregnant rats or mice (Roux and Aubry 1966; Roux et al. 1980). An even more convincing SLOS phenotype recently has been produced by treatment of pregnant, cholesterol-deficient mice with an inhibitor of *DHCR7* (Lanoue et al. 1997). HPE, a failure of normal bilobar development of the forebrain and a prominent characteristic of all animal models of fetal exposure to inhibitors of *DHCR7*, was reported in only one SLOS patient prior to 1993 (McKeever and Young 1990). However, by use of an increased 7DHC level as a diagnostic biochemical marker, SLOS now has been identified in at least seven patients with HPE (Kelley et al. 1996; author’s unpublished data). Although all of these HPE patients had other physical characteristics of SLOS, in most of them the diagnosis of SLOS could not have been made without measurement of plasma sterols, because the facial anomalies associated with HPE, such as midline cleft lip and hypotelorism, obscured the diagnostically important facial dysmorphism of SLOS.

At the same time that dysmorphologists were dusting off their biochemistry books to study cholesterol metabolism, Jeffery Porter, Phil Beachy, and their colleagues at Johns Hopkins made the startling discovery that covalent addition of cholesterol to Sonic hedgehog (Shh), an embryonic signaling protein, was an essential part of Shh’s “autoprocessing” reaction (Porter et al. 1995, 1996b). In this reaction, precursor Shh protein in the presence of cholesterol cleaves itself to form a non-signaling COOH-terminal half and a mature, cholesterol-substituted, N-terminal half, “Shh-N.” Shh-N appears to possess all Shh signaling activity, which in vertebrates includes patterning of development in the ventral forebrain and limb buds, among other structures. Although the covalently attached cholesterol moiety is not essential for intrinsic signaling activity of Shh-N, it appears to have a role in the attachment and localization of Shh-N to cell membranes (Porter et al. 1996a).

The first link between Shh and HPE was made when Chiang et al. (1996) reported that homozygous Shh knockout mice developed HPE. This discovery soon was followed by the report that mutations in *SHH*, the 7q36-linked gene encoding SHH in humans, caused autosomal dominant HPE (Roessler et al. 1996). The possibility that cholesterol might play an important role in embryonic forebrain development had been suggested somewhat earlier by the discovery that transgenic mice deficient in the synthesis of megalin (Willnow et al. 1996), an important component of a system for delivery of maternal LDL cholesterol to the embryonic neuroepithelium, also develop HPE. Possibly related CNS malformations also were found in transgenic mice lacking apolipoprotein B, another component of the embryonic cholesterol-delivery system (Farese et al. 1995). This unexpected convergence of HPE, SLOS, Shh, and chole-

terol metabolism immediately focused attention on the possibility that the covalent attachment of cholesterol to Shh-N and related hedgehog proteins is the link between the abnormal cholesterol metabolism of SLOS and abnormal morphogenesis of SLOS.

Like many other wonderful unitary theories that preceded it, the proposal that abnormalities of cholesterol-mediated Hedgehog autoprocessing causes HPE and other malformations is probably wrong. The story is indeed much more complex. Addressing the relationship between Shh function and abnormal cholesterol metabolism, Michael Cooper, Phil Beachy, and coworkers (Cooper et al. 1998) have shown that autoprocessing of Hedgehog is not impaired when cholesterol in the reaction medium is replaced by 7DHC or any of many other 27-carbon sterols tested. Although the possibility that 7DHC-modified Shh-N might not have normal signaling function was not assessed directly, additional *in vitro* studies with AY-9944 (which produces SLOS-like sterol abnormalities) and teratogens that cause SLOS-like malformations in rats strongly suggested that the defect in Shh signaling resided in the target tissue and not in the Shh-N signal itself. For example, treatment of target tissue (chick neural-plate ectoderm) for 48 h with AY-9944, Triparanol, or jervine at concentrations that produce HPE in whole chick embryos completely blocked signaling by recombinant Shh-N (Cooper et al. 1998). Moreover, studies in my laboratory suggest that it is not just impaired cholesterol biosynthesis in the target tissue that limits Shh signaling. For example, whereas both AY-9944 and the plant alkaloid tomatidine cause severe impairment of cholesterol biosynthesis and marked increases in 7DHC in cultured cells and chick embryos, only AY-9944 is teratogenic and blocks Shh signaling (author's unpublished observations). However, as shown by Cooper et al. (1998), all of the known synthetic and plant teratogens that cause HPE also impair intracellular cholesterol transport, as measured by uptake and esterification of extracellular cholesterol. One possible target of teratogen-induced abnormal intracellular cholesterol metabolism is "Patched," a putative hedgehog receptor (Ingham et al. 1991; Stone et al. 1996; Goodrich et al. 1997) that contains a sterol-sensing domain (Stone et al. 1996).

Shh is just one of several similarly functioning hedgehog proteins, all of which may interact with Patched in a sterol-sensitive manner. Thus, it is possible that impaired signaling activities of other hedgehog proteins, such as Desert hedgehog (Bitgood et al. 1996) and Indian hedgehog (Iwasaki et al. 1997), also play a role in the abnormal morphogenesis of SLOS tissues that are not targets of Shh signaling. For example, although steroid abnormalities often are presumed to be the cause of the hypogenitalism of SLOS, the persistence of Müllerian remnants in some severely affected SLOS males (Bialer

et al. 1987) could be explained by inadequate signaling of Desert Hedgehog, which is prominently expressed in Sertoli cells, the source of Müllerian inhibitory factor (Bitgood et al. 1996). Despite the attractiveness of and experimental support for the role of impaired Hedgehog signaling in SLOS morphogenesis, other, less-specific disturbances also could be involved. In several 10–20-wk SLOS fetuses tested in my laboratory, cholesterol is severely deficient in all tissues, with 7DHC and related cholesterol precursors typically constituting $\geq 75\%$ of fetal sterols. Such a severe disturbance in membrane sterol composition is likely to affect many critical developmental processes that involve cell-cell interactions, as suggested by Sulik and colleagues in their recent studies with the SLOS-mimicking teratogen, BM 15,776 (Dehart et al. 1997). Even in tissue culture, SLOS fibroblasts interact abnormally with themselves and with the growth surface (author's unpublished observations).

Future Directions

Where do all these remarkable findings leave our SLOS patients and their families? What clinical benefit can be derived from these discoveries? What other syndromes are likely to involve abnormalities of cholesterol metabolism or Hedgehog proteins?

Although most geneticists interested in SLOS are learning more about cholesterol metabolism and Sonic hedgehog than they ever imagined, the most important changes are in the homes of the SLOS children. A reading of any issue of the newsletter of the RSH/Smith-Lemli-Opitz Advocacy and Exchange, the family support group for SLOS, will show how profoundly some SLOS children and adults are affected when their cholesterol-deficiency syndrome finally is treated. Growth improves, older children learn to walk, and adults speak for the first time in years. Equally important is how much better the children feel. Sometimes after just days or weeks of cholesterol treatment, head banging stops, agitation passes to calm, and older children and adults verbalize how much better they feel. As we become more involved in the treatment of SLOS patients, it is also important to note that *DHCR7* first was cloned not as the SLOS gene but as the gene for a promiscuous drug-binding protein (Moebius et al. 1997, 1998). We should remember this fact not only in evaluating the behavioral pharmacology of SLOS but also in considering *DHCR7* and cholesterol biosynthesis as potentially significant ancillary sites of action of various drugs in any patient.

The discovery of mutations of *DHCR7* as the cause of SLOS will lead to the inevitable *DHCR7* knockout mouse and, we hope, to animal models of different therapy modalities. Mutational analysis will allow more-accurate carrier testing as well as preimplantation testing. In the meantime, work on various aspects of sterol

metabolism in SLOS continues. An earlier observation now under active reinvestigation is that mothers carrying SLOS fetuses have much lower than normal levels of serum unconjugated estriol (McKeever and Young 1990). If confirmed as a consistent marker for SLOS, the finding of a low maternal serum estriol level may allow detection of this relatively common disorder in families without a known risk for SLOS. Another important clinical question to be answered is whether differences in human maternal cholesterol levels influence the delivery of cholesterol to the developing embryo, as some studies of AY-9944 in rats have suggested (Barbu et al. 1984).

Finally, the new molecular embryology of SLOS and its connection with HPE and, possibly, with Shh already has spawned a small industry of sterol teratology. Dysmorphologists naturally will look at other biochemically undiagnosed malformation syndromes and wonder whether therein lurk new disorders of cholesterol biosynthesis. Indeed, an apparent defect in desmosterol (24-dehydrocholesterol) metabolism already has been described in a single stillborn infant with some characteristics of SLOS (FitzPatrick et al. 1998). However, SLOS as a "metabolic malformation" syndrome may be the exception rather than the rule. In this era of weekly discoveries of new genes for old syndromes, most mutations that cause multiple congenital malformation syndromes—that is, disturbances of the body plan—have not been disorders of intermediary metabolism but, instead, mutations of homeobox genes and other transcriptional regulators and signaling systems. Even the widespread and severe biochemical disturbances of a classic prenatal metabolic disorder such as Zellweger syndrome have few effects on the body plan. Thus, SLOS and possibly a few other sterol defects may be exceptional, because their abnormal sterol biochemistry, which cannot be corrected by maternal sources, appears to disrupt the action of Hedgehog proteins, important embryonic proteins with diverse effects on the activity of transcriptional regulators. Nevertheless, because clinical screening for abnormal cholesterol biosynthesis was unknown before the discovery of increased 7DHC levels in SLOS, we should remind ourselves as we see our patients with unexplained syndromes that there are many other categories of obligate fetal metabolite biosynthesis for which no systematic metabolic screening exists and for which no genetic defects are known.

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