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INBORN ERRORS OF STEROL BIOSYNTHESIS

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■ **Abstract** The known disorders of cholesterol biosynthesis have expanded rapidly since the discovery that Smith-Lemli-Opitz syndrome is caused by a deficiency of 7-dehydrocholesterol. Each of the six now recognized sterol disorders—mevalonic aciduria, Smith-Lemli-Opitz syndrome, desmosterolosis, Conradi-Hünermann syndrome, CHILD syndrome, and Greenberg dysplasia—has added to our knowledge of the relationship between cholesterol metabolism and embryogenesis. One of the most important lessons learned from the study of these disorders is that abnormal cholesterol metabolism impairs the function of the hedgehog class of embryonic signaling proteins, which help execute the vertebrate body plan during the earliest weeks of gestation. The study of the enzymes and genes in these several syndromes has also expanded and better delineated an important class of enzymes and proteins with diverse structural functions and metabolic actions that include sterol biosynthesis, nuclear transcriptional signaling, regulation of meiosis, and even behavioral modulation.

INTRODUCTION

For its first hundred years, following the early work of Garrod at the turn of the twentieth century, the study of inborn errors of metabolism emphasized disorders of small, water soluble metabolites, such as phenylalanine in phenylketonuria, or of macromolecule catabolism, such as mucopolysaccharide and sphingolipid storage in the lysosomal storage diseases. Of the several hundred inborn errors of metabolism discovered and characterized in the last hundred years, relatively few involved abnormal de novo synthesis of an essential small metabolite. Furthermore, most inborn errors of metabolism have featured the postnatal evolution of metabolic deficiencies or toxicities in children who are phenotypically normal at birth. However, the surprising discovery, in 1993, that Smith-Lemli-Opitz

syndrome—one of the best-known autosomal recessive malformation-mental retardation syndromes—is caused by a primary defect of cholesterol biosynthesis not only raised important questions about embryological links between abnormal sterol biosynthesis and congenital malformations but also focused attention on the special implications of a disorder involving an essential fetal metabolite that cannot be supplied in adequate amounts by the mother during gestation. Since 1993, the wider recognition that congenital malformations can result from an inborn error of cholesterol metabolism has led to the discovery of prenatal malformation syndromes caused by defects in most of the other steps in postsqualene cholesterol biosynthesis. The delineation of these new inborn errors of cholesterol biosynthesis has had obvious clinical importance, especially for diagnosis and prenatal detection of the individual syndromes. Moreover, the recognition of novel associations between aberrant cholesterol metabolism and diverse clinical problems—cerebral dysgenesis, cyclic inflammatory disease, ichthyosis, skeletal dysplasia, and the pharmacology of specific behavioral abnormalities—has opened many new avenues of biochemical and genetic investigation.

In this chapter, we present a brief overview of the essentials of human cholesterol biosynthesis and then follow with reviews of the phenotype, biochemistry, and molecular genetics of mevalonic aciduria, hyper-IgD syndrome, Smith-Lemli-Opitz syndrome (SLOS), desmosterolosis, Conradi-Hünermann syndrome, CHILD (congenital hemidysplasia with ichthyosiform erythroderma and limb defects) syndrome, and Hydrops-ectopic calcification (Greenberg) dysplasia. The larger biochemical and genetic implications of these newly discovered sterol disorders and the likely future directions of biochemical and clinical genetic research in this important biochemical pathway are also discussed.

NORMAL STEROL METABOLISM

Cholesterol Biosynthesis

Cholesterol is an essential metabolite and structural lipid in higher organisms and is synthesized by all nucleated mammalian cells. Although complex, the biosynthesis of cholesterol is only one element of the larger isoprenoid biosynthetic system, which incorporates the de novo synthesis of compounds as diverse as dolichol, ubiquinone, isopentenyladenine, and farnesyl pyrophosphate (Figure 1). Moreover, cholesterol is at once an end-product of isoprenoid metabolism and the starting substrate for the synthesis of yet another highly diverse group of metabolically active compounds, including all steroid hormones, bile acids, and signaling compounds, such as oxysterols. Cholesterol, via covalent linkage to an amino acid residue, also confers function to the hedgehog class of cell signaling proteins (discussed below in more detail).

The synthesis of sterols follows a complex series of reactions that begins with the condensation of acetyl-CoA and acetoacetyl-CoA to form 3-hydroxy-3-methylglutaryl(HMG)-CoA. The sequence reaches the halfway point with the

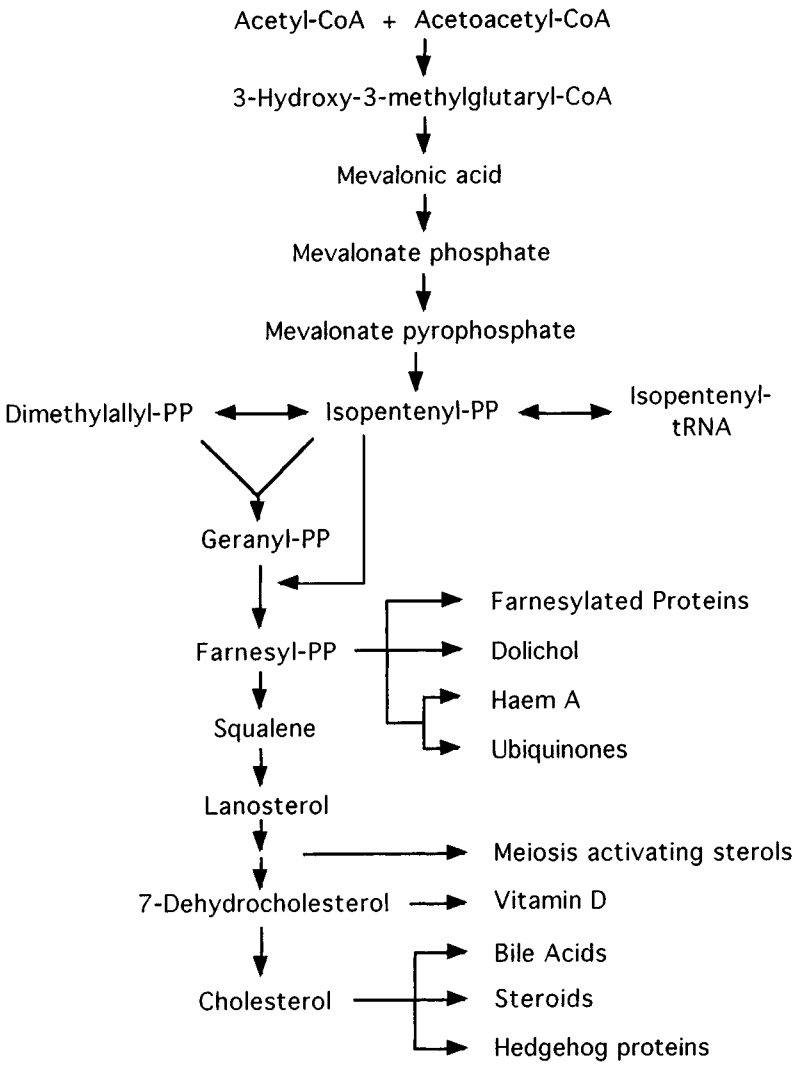

Figure 1 Outline of the pathway for the synthesis of sterols, nonsterol isoprenoids, and their derivatives.

formation of the 30-carbon precursor of all other sterols, lanosterol (4,4,14-trimethylcholesta-8(9),24-dien-3β-ol), and ending with the conversion of 7-dehydrocholesterol (7DHC) to cholesterol by the enzyme 7-dehydrocholesterol reductase (DHCR7) (Figure 2). HMG-CoA reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid, is a major rate-determining step of cholesterol biosynthesis and is subject to both transcriptional and posttranslational regulation, largely in response to the level of cholesterol in the endoplasmic

reticulum (ER) (19). The coordinated regulation of the levels of HMG-CoA reductase, acetoacetyl-CoA synthase, and the plasma membrane LDL receptor assures the maintenance of a stable cellular pool of cholesterol from both intracellular and extracellular sources (19). Cholesterol exists in several metabolically distinct pools, most importantly in the plasma membrane, lysosomal system, and ER.

Cholesterol (cholest-5-en-3β-ol), a 27-carbon, monounsaturated sterol, is synthesized from its 30-carbon sterol precursor, lanosterol, by a series of dehydrogenations, reductions, and demethylations (Figure 2). Although textbooks often indicate that desmosterol (cholesta-5,24-dien-3β-ol) is the penultimate sterol in this series of reactions, the finding, in 1993, (84) of markedly increased levels of 7DHC and almost no 7-dehydrodesmosterol in patients with SLOS indicated that reduction of the 24-25 double bond of lanosterol occurs early in the biosynthetic sequence (93). However, the relative abundance of desmosterol in neuronal tissues, the testes, and breast milk (16, 28, 116) suggests that desmosterol may have a special physiological role in these tissues.

Although all steps of cholesterol synthesis were once thought to take place in the ER, studies by Krisans and colleagues established that the second enzyme of the pathway, mevalonate kinase, is localized to the peroxisome and contains an N-terminal peroxisomal targeting amino acid sequence (PTS2) (171). Interestingly, many other enzymes of the presqualene cholesterol biosynthetic pathway also have peroxisomal targeting peptide sequences (PTS1 or PTS2) and appear to localize predominantly to the peroxisome (1). These include phosphomevalonate kinase, phosphomevalonate decarboxylase, isopentenyl diphosphate isomerase, and farnesyl pyrophosphate synthase. Sterol-carrier protein 2, a possible cofactor for DHCR7 and carrier protein for intracellular sterol transport, also appears to be targeted to and processed by peroxisomes (96). In contrast, squalene synthase resides exclusively in the ER (170). Although Appelkvist et al. published evidence that peroxisomes may harbor all the enzymes necessary for the conversion of lanosterol to cholesterol (6), the role of these putative peroxisomal activities in overall cellular cholesterol biosynthesis and homeostasis remains unknown. Nevertheless, the observations that patients with Zellweger syndrome, who have defective peroxisomal assembly, have markedly depressed serum cholesterol levels (97) and that Zellweger cells in vitro have depressed rates of cholesterol synthesis (73) strongly suggest that peroxisomes have an important role in cholesterol biosynthesis (73).

Another important, if poorly understood, aspect of cholesterol biosynthesis is the complex intracellular trafficking of cholesterol. As shown by Lange et al. (110), zymosterol (cholesta-8(9),24-dien-3β-ol), an obligatory intermediate in the synthesis of cholesterol, appears to move from the ER to the plasma membrane and then back to the ER-enriched microsomal fraction of the cell, where final conversion to cholesterol occurs. Other important pathways of intracellular cholesterol movement include transport from the plasma membrane to lysosomes and from lysosomes to the ER. A genetic disruption of the latter transport system is the cause of the abnormal lipid storage in type C Niemann-Pick disease (36, 117). Delineating intracellular trafficking of cholesterol may be important to understanding the

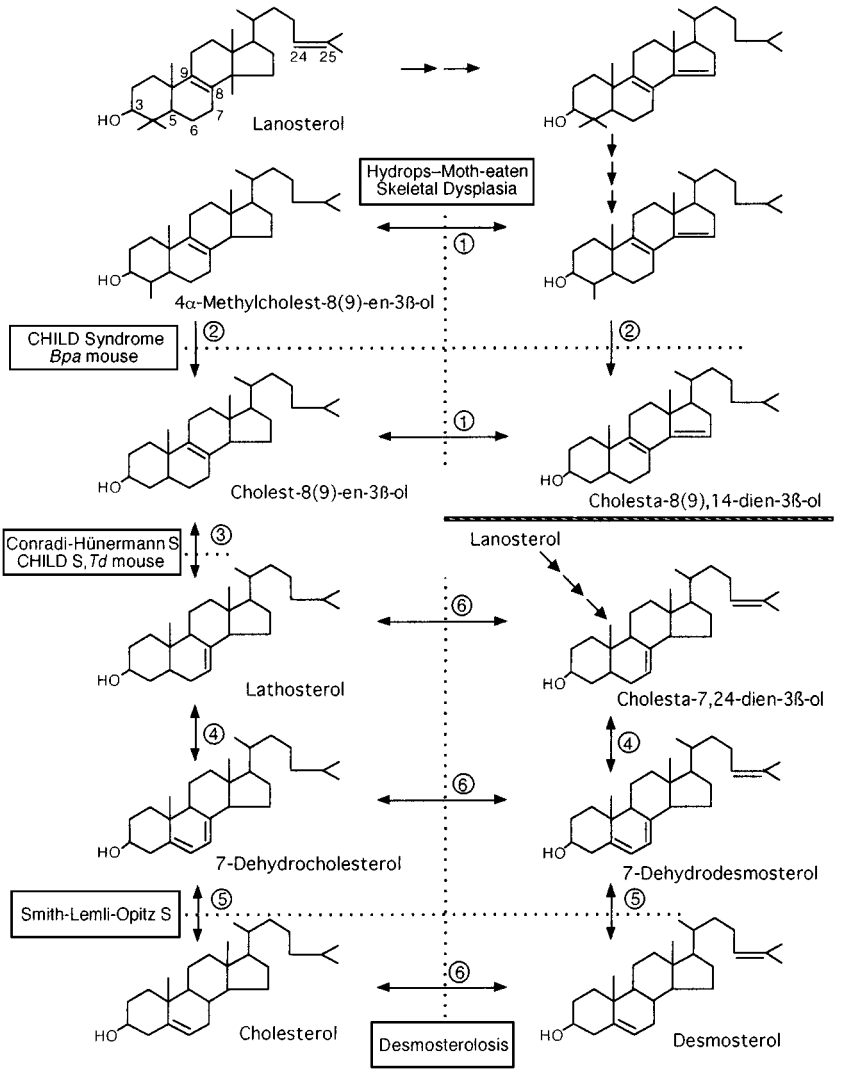

Figure 2 Enzymatic steps and principal sterol intermediates of the pathway for cholesterol biosynthesis. Clinical syndromes proven, or believed to be, caused by defects of sterol biosynthesis are indicated in the boxes. The enzymes denoted by the circled numbers are: (*1*) 3β-hydroxysteroid-Δ^{14}-reductase, (*2*) 4α-methylsterol-4-demethylase complex, (*3*) 3β-hydroxysteroid-Δ^{8},Δ^{7}-isomerase, (*4*) 3β-hydroxysteroid-Δ^{5}-desaturase (lathosterol dehydrogenase), (*5*) 3β-hydroxysteroid-Δ^{7}-reductase (7-dehydrocholesterol reductase, DHCR7), and (*6*) 3β-hydroxysteroid-Δ^{24}-reductase (desmosterol reductase).

cellular pathology of sterol disorders, in particular SLOS, because the low total sterol levels in SLOS suggest feedback inhibition of de novo sterol synthesis at one or more levels (78).

Prenatal and Postnatal Sterol Metabolism

Important to understanding the fetal pathology of disorders of cholesterol biosynthesis is evidence indicating that, in contrast to many other small metabolites, very little maternal cholesterol is transported to the fetus across the placenta (10, 22). Clinically, this is best illustrated by the finding that some SLOS newborns have plasma cholesterol levels as low as 1 mg/dl, barely 2% of the normal newborn cholesterol level (33). However, the situation may be quite different during the embryonic period, when, at least in mice, a substantial amount of LDL cholesterol reaches the developing neuroepithelium directly from the mother via a specialized, nonplacental LDL transport system mediated by megalin, a multifunctional transport protein in the embryonic neuroepithelium (46, 191). Thus, whereas delivery of cholesterol to the fetus after full development of the placenta may be minimal, critical aspects of embryonic tissue differentiation may be sensitive to maternal blood cholesterol and LDL levels.

Most cholesterol synthesized, both before and after birth, serves as a structural lipid of cell membranes. However, cholesterol also enters pathways for bile acid and steroid hormone synthesis during fetal as well as postnatal life. Cholesterol before birth is converted into a variety of fetal steroids for sexual differentiation and into estriol by combined action of the fetal adrenals and the placenta. Unconjugated estriol transferred to the mother from the fetus is believed to have a role in the maintenance of the pregnancy, but the true physiologic actions of this steroid remain unclear. Another recently discovered fate of cholesterol is its covalent linkage to hedgehog proteins, a family of embryonic signaling proteins that may be important targets of the abnormal sterol metabolism of SLOS (25, 101, 147) and possibly other sterol disorders. Less well studied than other fates of fetal cholesterol is its conversion to bile acids. However, the report of severe cholestasis and cirrhosis in one SLOS newborn (136) and the severe prenatal liver damage common to some primary defects of bile acid biosynthesis (160) suggest that abnormal species of bile acids may also have clinical consequences in defects of cholesterol biosynthesis.

MEVALONIC KINASE DEFICIENCY (MIM 251170, 260920)

History of Mevalonic Aciduria

Mevalonic aciduria (MIM 251170), caused by deficiency of mevalonate kinase (ATP:(R)-mevalonate 5-phosphotransferase; MVK), was the first reported disorder of cholesterol biosynthesis. Although elevated urinary mevalonic acid was described in 1985 by Berger et al. (11) in a one-year-old child with progressive

ataxia, the first identification of mevalonate kinase deficiency (MKD), in a second child with severe mevalonic aciduria, was reported a year later by Hoffmann et al. (74). The second patient had severe failure-to-thrive, profound psychomotor retardation, ataxia, a dysmorphic appearance, rhizomelic shortness, cataracts, hepatosplenomegaly, anemia, and recurrent crises of fever, arthralgia, edema, rash, adenopathy, and hepatomegaly. Whereas that patient also had profound physical and neurological disease, several subsequently identified patients have had milder, apparently static neurological problems, such as developmental delay, hypotonia, myopathy, or ataxia (53, 75). Although recurrent inflammatory spells occurring every 3 to 6 weeks throughout life constitute one of the most distinctive signs of MKD, not until 1999 was the less impairing disorder with similar episodic inflammatory spells, hyperimmunoglobulinemia D syndrome (HIDS; MIM 260920), shown by two different groups to be caused by missense mutations in the MVK gene (41, 80). However, in contrast to the typical 5000- to 50,000-fold elevations of urinary mevalonate in classical MKD, patients with HIDS have only 10- to 100-fold increased levels of MVA, which, during nonfebrile periods, often are detectable only by isotope-dilution gas chromatography–mass spectrometry (GC/MS). In retrospect, a number of classical MKD patients also have had increased serum IgD levels as well as chronically increased levels of the inflammatory cytokine, leukotriene E4 (125).

Clinical Characteristics of Mevalonic Kinase Deficiency

Among the more interesting biological aspects of classical mevalonic aciduria is the abnormal morphogenesis, which is evident in the dysmorphic facies and skeletal dysplasia of most affected patients (75, 122). The dysmorphic features and other structural abnormalities somewhat resemble Zellweger syndrome, which, as a disorder of peroxisomal biogenesis, might be expected to have mevalonate kinase deficiency. Although urinary mevalonic acid levels are normal in Zellweger syndrome and Zellweger syndrome fibroblasts have normal total activities of mevalonate kinase and phosphomevalonate kinase, Zellweger liver has a marked deficiency of mevalonate kinase (187). Mevalonate kinase activity also is deficient in rhizomelic chondrodysplasia punctata (RCDP), a disorder of peroxisomal biogenesis wherein a subset of peroxisomal enzymes, including MVK, is not transported into peroxisomes (18, 186). Apparently, in both Zellweger syndrome and RCDP, mevalonate kinase is synthesized, but not delivered, to the peroxisome. This may be why cholesterol synthesis is impaired in Zellweger syndrome, even though there appears to be sufficient total cellular mevalonate kinase activity to prevent the accumulation of free mevalonate.

Recurrent or cyclic fever is a well-known, if uncommon, pediatric problem that often eludes etiologic diagnosis. Although many patients with MVK deficiency have recurrent but not truly cyclic fevers, others have very regular three- to six-week cycles of fever, lymphadenopathy, leukocytosis, arthralgia, bone pain, abdominal pain, and general debilitation (75). Some patients with MVA or HIDS

have been thought to have familial Mediterranean fever or PFAPA [periodic fever, aphthous ulcers, and adenopathy syndrome (119)]. The recent recognition that MVK deficiency is the cause of the cyclic fevers of HIDS in otherwise normal individuals emphasizes that periodic fever and mevalonic aciduria are diagnostically the most important markers for MVK deficiency across the range of clinical severities (41, 80).

Pathophysiology and Treatment

Although MVK deficiency is a primary defect of cholesterol biosynthesis, plasma levels of cholesterol typically are normal or only mildly depressed (75). Considering the 10,000-fold or greater elevation of urinary mevalonic acid in classical mevalonic aciduria, HMG-CoA reductase appears to be able to upregulate mevalonate synthesis to a level sufficient to maintain adequate or nearly adequate flux through the pathway. However, cholesterol synthesis by an alternate pathway not involving mevalonate may be possible. Such a mevalonate-independent pathway for isoprenoid biosynthesis utilizing glyceraldehyde-3-phosphate and pyruvate occurs in the plant kingdom (114, 152), but no similar pathway has yet been found in higher animals.

As in any enzymatic disorder, the adverse effects of MVK deficiency may be mediated by precursor toxicity, product deficiency, or both. Unfortunately, treatment efforts directed at both metabolic consequences of MVK deficiency have met with little or no success (75). After the failure of end-product replacement therapy (cholesterol, coenzyme Q) to ameliorate the problems of mevalonic aciduria (75), an inhibitor of HMG-CoA reductase, lovastatin, was given to one child to lower the possibly toxic levels of mevalonic acid. Although lovastatin transiently lowered the level of mevalonic acid, further upregulation of HMG-CoA reductase activity seems to have occurred, followed by a life-threatening disease crisis. Pharmacological doses of corticosteroids can diminish the severity of febrile crises, but weaning MVA patients from treatment with high dose corticosteroids can be quite difficult. The finding of a direct correlation between urinary leukotriene E4 and MVA levels and the recent marketing of leukotriene receptor antagonists suggest a more direct approach to treatment of inflammation in MVA.

Genetics and Enzymology

MVA is an autosomal recessive disorder with an incidence of less than 1 in 100,000 births. Although there is no apparent ethnic predisposition for classical, severe MVA, most case reports of HIDS come from the Netherlands. The gene encoding MVK was first cloned from yeast in 1987 as *RAR1*, defined by an essential role in yeast chromosome replication (94). In 1990, the gene was independently cloned as *MVK* from yeast (ERG12) (140) and from rat (174). Two years later, a human *MVK* was cloned from a cDNA library by Schafer et al. (159). Human MVK is a homodimeric enzyme (Table 1) with a calculated monomeric weight of 42, 242 D (159). Cell localization studies by Krisans et al. (14, 171) indicate that MVK is a

TABLE 1 Molecular and enzymatic data for inborn errors of sterol biosynthesis

Disease	Mevalonic aciduria hyper IgD syndrome	Smith-Lemli-Opitz syndrome	Conradi-Hünermann, CHILD syndrome	Hydrops-ectopic calcification skeletal dysplasia	Desmosterolosis	CHILD syndrome
MIM numbers	251170 260920	268670, 270400 602858	300275 302960	215140	602938	300275 308050
Enzyme	Mevalonate kinase	7-Dehydrocholesterol reductase	Sterol-Δ7,Δ8-isomerase	Sterol Δ14-reductase	Desmosterol reductase	3β-OH-steroid dehydrogenase
EC number	2.7.1.36	1.3.1.21	5.3.3.5	1.3.1.—	1.3.—	1.1.—
Subcellular localization	Peroxisomes ?cytosol	ER	ER	ER	ER	ER
Greatest tissue abundance	Ubiquitous	Adrenal, liver, testis, brain	Liver, adrenal, gonads, uterus; low in brain & muscle		Ubiquitous	Liver, kidney, adrenal, ovary, testis
Cofactor	ATP	NADPH	NADPH	NADPH	FAD	NAD
Protein structure	Homodimer	?Homodimer	Homodimer	Homodimer (rat)		Multienzyme complex
Transmembrane domains (predicted)	0	9	4		1	1
Gene	*MVK*	*DHCR7*	*EBP*	*DHSR14*	*DHCR24/DWF1*	*NSDHL*
Yeast ortholog	ERG12	none	ERG2	ERG24		ERG26
Chromosomal localization	12q24	11q13	Xp11.22-23		1p31.1-p33	Xq28
Genomic DNA-kb	22	14	7		46.4	40
mRNA-bp	1785	2597	1073		4149	1563
Exons	11	9	5		8	8
Amino acids	396	475	230		516	373
Monomeric MW	42,424	54,516	26,335	38,000 (rat)	60,100	41,873

peroxisomal enzyme, although small amounts may normally exist in the cytosol. *MVK* has homology with ESTs from most phyla of both the plant and animal kingdoms, including organisms without the ability to make sterols (81). Genes encoding MVK in higher and lower organisms show homology in four conserved polypeptide domains (150). The second of these domains has homology with sequences in a large family of kinases for substrates as diverse as nonmevalonate isoprenoids, polypeptidyl serines, and galactose and is presumed to have a role in ATP-binding. The fourth conserved domain near the carboxy terminus of MVK likely determines mevalonate binding.

Mutation Studies

Before the *MVK* cDNA was cloned, enzymatic studies had shown a moderate correlation between the severity of clinical disease and the reduction in the MVK activity in cultured fibroblasts or peripheral lymphocytes. This phenotypic-enzymatic correlation became even more apparent in the study of HIDS. Whereas classical, severely affected MVA patients have no, or almost no, measurable MVK activity (<0.5% of control), patients with the HIDS phenotype have appreciable amounts, usually between 1% and 6% of normal in control lymphocytes (41, 80). Houten et al. (79) found that almost all patients with the Dutch-type familial periodic fever (HIDS) phenotype are heterozygous for a 1129G > A mutation (V377I). This mutation accounted for 52% of HIDS MVK alleles in their study and is presumed to be the allele responsible for most of the residual MVK activity in HIDS. To date, all MVK mutations, except one deletion mutation of exon 2, have been missense mutations distributed widely over the exons of MVK (Figure 3). The only alleles with a substantial frequency other than V377I are I268T, an allele present in both

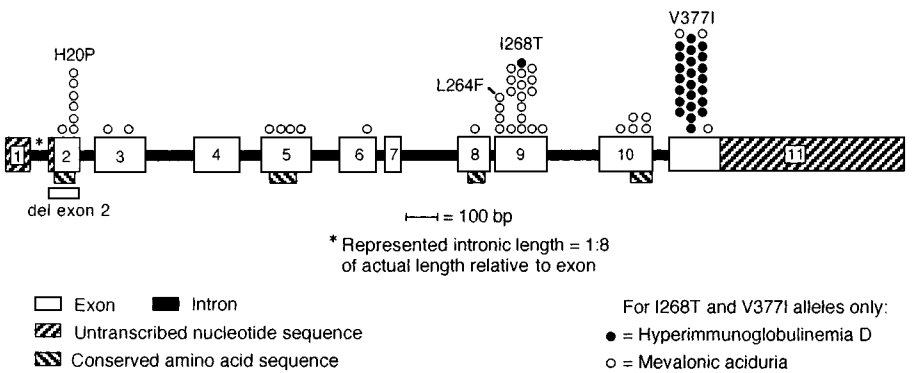

Figure 3 Distribution of *MVK* mutations in mevalonic aciduria and hyperimmunoglobulinemia D syndrome (HIDS). Approximately half of all missense mutations have been associated with HIDS. For mutations V377I and I268T only, the distrubtion of diagnoses between HIDS (closed circles) and mevalonic aciduria (open circles) is indicated.

classical MVA and HIDS patients, and H20P, which was found in one MVA patient (H20P/A334T) and five H20P/V377I HIDS compound heterozygotes. As a group, the H20P/V377I compounds had a significantly lower mean MVK activity in peripheral lymphocytes than V377I/I268T compound heterozygotes (1.8% vs. 5.0% of control). Because enzymatic assays of missense mutations in fibroblasts often overrepresent the in vivo residual activity, it is likely that I268T has intrinsically higher residual activity than H20P. That the V377I mutation is associated with high residual MVK activity also was evident in the identification of three patients heterozygous for V377I and a stop mutation (W62X, Y149X, and Y148X), two of whom were classified as HIDS and one as mild MVA (53). The inclusion of only a single homozygote for V377I among 22 HIDS patients for whom V377I represented 55% of MVK alleles suggests that homozygosity for V377I is associated with a normal phenotype in most individuals. Thus, V377I may constitute a much higher fraction of MVK alleles than indicated by its fraction of alleles among all MVA and HIDS patients. Moreover, that all four known null mutants were present in combination with only V377I suggests that truly absent MVK activity may be lethal in utero. Although one could argue that an enhanced inflammatory reaction afforded by the common V377I allele might offer a heterozygote advantage, a founder effect is also possible, especially in view of recent data concerning the apparently small number of founding Y chromosomes in Europe (153).

SMITH-LEMLI-OPITZ SYNDROME (MIM 270400; 268670)

Historical Overview

Smith-Lemli-Opitz syndrome (SLOS) is a classical, autosomal recessive multiple malformation syndrome first described in 1964 (168) and in more detail in 1969 as the RSH syndrome (139). The original SLOS patients had a distinctive phenotype consisting of a characteristic facial appearance, microcephaly, hypospadias, global developmental delay, and severe feeding problems (168). The description of new cases of SLOS in subsequent years added midline cleft palate, cataracts, postaxial polydactyly, and heart defects to the list of common abnormalities in this disorder. In 1986 and 1987, several papers described a new lethal syndrome, type II SLOS, that, in addition to the external anomalies of SLOS, had severe internal malformations, including pulmonary hypoplasia, complex congenital heart disease, renal hypoplasia or agenesis, and Hirschsprung disease (35, 40). Some 46,XY males had severe hypogenitalism or even female-appearing external genitalia.

Despite a relatively high population incidence of SLOS of about 1 in 40,000 (98), the genetic cause of the syndrome remained unsuspected until 1993 when Irons et al. (84) reported that patients with SLOS had a more than 100-fold increase in the plasma level of 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol; 7DHC), the immediate precursor of cholesterol in the Kandutsch-Russell biosynthetic pathway (93). The same biochemical abnormality has since been found not only in most patients with an accepted clinical diagnosis of SLOS but also in patients

with previously unknown or incorrect diagnoses now recognized as variant forms of SLOS. The apparent cause of the distinctive sterol abnormality, a deficiency of microsomal 7-dehydrocholesterol reductase (3β-hydroxysteroid-Δ^7-reductase; DHCR7), was supported by enzymatic assays in tissues and cultured cells (164). In 1998, Moebius et al. (130) cloned the human DHCR7 gene and localized it to chromosome 11q12-13. Shortly thereafter, the same authors reported that mutations of DHCR7 cause SLOS (49), a finding confirmed by two other groups (188, 190).

Clinical Characteristics

The clinical characteristics and phenotypic spectrum of SLOS have been described in detail in a number of clinical reviews (33, 88, 98, 99, 139, 158). The SLOS face, which combines microcephaly, bitemporal narrowing, ptosis, a short nasal root, anteverted nares, and micrognathia, is distinctive and easily recognized. Hypertelorism, epicanthal folds, cataracts, strabismus, midline cleft palate, and, more rarely, midline cleft lip (40, 101) are other important craniofacial findings in some patients. Characteristic skeletal abnormalities include postaxial polydactyly and syndactyly of the second and third toes (33, 158), limb shortness, and, more rarely, epiphyseal stippling (72, 127). Hypogenitalism in SLOS males, ranging from cryptorchidism to apparent complete sex reversal (12, 37, 91, 139), is one of the more important diagnostic characteristics of SLOS. In addition to the characteristic external anomalies of SLOS, there are important visceral and other internal malformations as well. In a cohort of 95 biochemically confirmed cases of SLOS with heart disease, Lin et al. (115) found atrioventricular canal (25%), primum atrial septal defect (20%), patent ductus arteriosus (18%), and membranous ventricular septal defect (10%) to be the most common defects. Both adrenal hyperplasia (126, 139) and hypoplasia (40) have been reported in SLOS. Pulmonary hypoplasia (24, 35, 104) and intestinal aganglionosis (35, 103, 196) are common anomalies among more severely affected patients, whereas pyloric stenosis is a prominent clinical problem for all degrees of severity of SLOS (139, 158). In addition to microcephaly, present in more than 90% of patients, common central nervous system (CNS) malformations include hypoplasia or aplasia of the corpus callosum, hypoplasia of the frontal lobes, and cerebellar hypoplasia, especially of the vermis (24, 47, 124, 158). Congenital sensorineural hearing deficits may affect as many as 10% of patients (158), and some form of the holoprosencephaly sequence—from a small midline notch of the upper lip to unilobar holoprosencephaly—occurs in about 5% of patients (101).

Natural History and Clinical Management

The neonatal period and infancy in SLOS usually are dominated by feeding problems, such as weak or abnormal suck, swallowing difficulties, vomiting, and lack of interest in feeding. As a result, more than 50% of patients require nasogastric tube feedings or prolonged or permanent gastrostomy feedings. However, failure-to-thrive is often misdiagnosed in children with SLOS, whose slow growth can

usually be explained by genetic, not nutritional, factors. Infants with SLOS typically are small for gestational age and most continue to grow substantially below the third centile despite adequate caloric intake. Moreover, because of the significant muscle hypoplasia at birth, weight for height almost always is below the third centile. During both infancy and childhood, children with SLOS appear to have an increased number of infections, and sudden overwhelming pneumonia is not rare. Because stress-related augmentation of adrenal steroid synthesis is partly dependent on circulating LDL cholesterol (21, 141), SLOS children may have inadequate adrenal function during the stress of an infection or surgery (3).

During infancy, severe hypotonia is almost universal in SLOS and stems from both CNS abnormalities and muscle hypoplasia. However, muscle mass and tone usually improve with age. Children with SLOS characteristically have global psychomotor retardation that correlates with biochemical severity (33, 158). The average SLOS child is quite sociable, has substantially better receptive than expressive language, and may be surprisingly mechanically adept for the degree of mental retardation. Gross motor development is more severely delayed than fine motor development, but most children eventually learn to walk. Approximately 10% of the children fall into the mildly retarded range (IQ 50 to 70), with a rare patient testing in the low normal or even normal range [(120, 158); R.I. Kelley, unpublished]. Behavioral abnormalities that fall within the spectrum of autistic disorder include hand flapping, abnormal obsessions, rigidity and insistence on routine, and poor visual contact (178).

Although there are no recent estimates for life expectancy in SLOS, Johnson (88) found that 27% of patients died before age 2. Because ascertainment of SLOS, even in the era of biochemical diagnosis, remains incomplete, the true percentage of cases with early or later death remains uncertain. However, life expectancy in SLOS appears to be determined largely by the severity of the internal malformations and the quality of general supportive care, not by an intrinsic toxic or degenerative process.

Although clinical management of SLOS in the past was largely symptomatic, standard care now includes treatment of the cholesterol deficiency with supplemental dietary cholesterol. The estimated daily synthetic need for cholesterol for infants is about 30 mg/kg, whereas for adults the amount decreases to approximately 10 mg/kg (31, 89). Because infants can absorb from the diet almost their entire daily cholesterol requirement (31), a child with SLOS who is given supplementary cholesterol theoretically may be able to downregulate endogenous sterol synthesis substantially and, thereby, limit the de novo synthesis of 7DHC. However, because brain cholesterol in mammals appears to be synthesized entirely in situ (133), the biochemical improvement now seen in the plasma of treated SLOS patients probably has little direct effect on brain function, except perhaps, as influenced by peripheral hormonal or other biochemical changes. Even if cholesterol could reach the brain, cognitive improvement might be limited because the microcephaly and mental retardation of SLOS probably reflect more the abnormalities of embryonic and fetal cerebral development than any ongoing effect of 7DHC or the cholesterol deficiency.

Most treatment protocols for SLOS provide between 50 and 200 mg/kg/day cholesterol, either in natural form (eggs, cream, liver, meats, and meat-based formulas) or as purified food-grade cholesterol, sometimes with supplements of bile acids (cholic acid, chenodeoxycholic acid, or ursodeoxycholic acid) (42, 83, 184). In addition to improved growth and gastrointestinal function in many SLOS patients treated with cholesterol, often there is marked improvement in behavior. The behavioral improvement, which can occur within days of treatment, is the most important clinical benefit of dietary cholesterol therapy. Treatment of several SLOS patients with simvastatin, an inhibitor of HMG-CoA reductase, has now been reported (87). Although the initial goal of treatment with simvastatin was to lower 7DHC levels to a greater degree than cholesterol (based on the speculation that 7DHC is toxic), there was paradoxical increase in the level of cholesterol at the same time that the level of 7DHC decreased (87). Most likely, treatment with an HMG-CoA reductase inhibitor, which leads to increased activity of certain enzymes with sterol response elements, caused sufficient upregulation of residual DHCR7 activity to enhance conversion of 7DHC to cholesterol. In contrast, biochemical and clinical worsening of disease has been seen in severely affected SLOS children—who have very little residual DHCR7 activity—treated with HMG-CoA reductase inhibitors (R.I. Kelley, unpublished data).

Biochemistry of Smith-Lemli-Opitz Syndrome

In almost all plasma samples from SLOS patients, the most abundant precursor sterol is 7DHC. A second diene sterol, 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol; 8DHC), which most likely derives from isomerization of 7DHC via sterol-Δ^8,Δ^7-isomerase, is normally present at about 75% of the amount of 7DHC (179). As shown in Figure 4, some SLOS patients have had cholesterol levels as low as 1 mg/dl, whereas others, between 10% and 15%, have normal cholesterol levels, even when the levels of 7DHC are substantially increased. Despite the apparently negligible transfer of cholesterol from mother to fetus (21, 22), SLOS patients with two null alleles have cholesterol levels 5 to 20 mg/dl at birth, and still higher levels in later months and years, even on cholesterol-free diets (33, 180). Thus, there may be another genetic source of DHCR7 activity, alternate splicing for certain *DHCR7* alleles, or a pathway for cholesterol synthesis not requiring DHCR7. A possible alternate source of cholesterol synthesis is the peroxisome, which, as described above, has an essential role in the early steps of cholesterol biosynthesis (13, 108, 171) and which, as shown by Appelkvist et al. (6), may have the capacity to synthesize cholesterol from lanosterol.

An interesting group of SLOS patients are those with a typical SLOS phenotype but normal plasma cholesterol levels and only mildly increased or even normal levels of 7DHC in plasma [(33); R.I. Kelley, unpublished observations]. However, if fibroblasts or lymphoblasts from these patients are grown in lipid-depleted culture medium, the level of 7DHC often rises to the same level found in cells from classical SLOS patients (2), indicating that rapidly growing cells in tissue culture

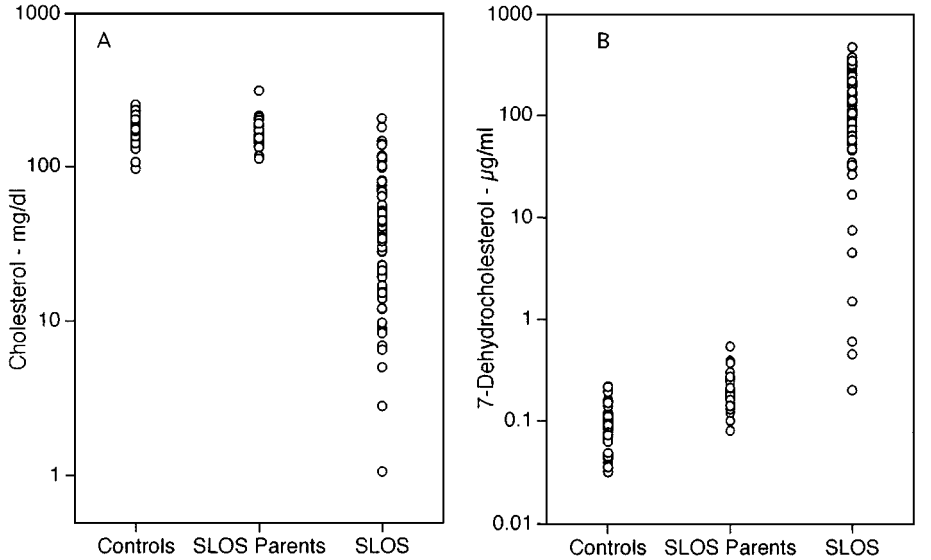

Figure 4 Distribution of plasma levels of (*A*) cholesterol and (*B*) 7-dehydrocholesterol (7DHC) in patients with Smith-Lemli-Opitz syndrome (SLOS) and their parents. Only a very rare patient with SLOS has normal plasma levels of both cholesterol and 7DHC.

may reflect better the sterol metabolism of rapidly dividing and differentiating embryonic cells.

There have been very few formal investigations of steroid hormone metabolism in SLOS. One of the earliest (23) found abnormally high levels of DHEA sulfate and low levels of testosterone in two newborns with SLOS but abnormally low levels of DHEA sulfate in an older infant and child (30 months and 5 years). More recently, Shackleton et al. (161) found 7-dehydro homologs of most of the common urinary steroid metabolites in children with SLOS. What role, if any, these abnormal 7-dehydro steroid species play in the genital malformations of SLOS in unknown. However, the production of these abnormal steroids by the fetus is sufficient to allow prenatal diagnosis of SLOS by quantification of 7-dehydro steroids in maternal urine at midgestation (162, 163). The same abnormal fetal steroid metabolism is reflected in depressed maternal unconjugated serum estriol levels at midgestation (107, 126).

Biochemical Teratology

The discovery of DHCR7 deficiency as the cause of SLOS has drawn attention to the role of cholesterol metabolism in morphogenesis. Although children with the only previously described defect of cholesterol biosynthesis, mevalonic aciduria, can have dysmorphic facies and rhizomelic shortness, that a genetic disorder of

cholesterol biosynthesis would be manifest principally as a severe multiple malformation syndrome affecting every organ system was unanticipated. However, Roux and colleagues had reported since the 1960s that, when given to pregnant rats or mice, inhibitors of enzymes of postsqualene cholesterol biosynthesis cause holoprosencephaly, microcephaly, pituitary agenesis, limb defects, and genital anomalies (154, 156). Such an apparent relationship between abnormal sterol metabolism and disordered embryologic development has been further supported by newer animal models using enzyme inhibitors and targeted genetic interruption of various steps in embryonic cholesterol metabolism (38, 112). Moreover, as noted earlier, holoprosencephaly, one of the major consequences of disrupted embryonic cholesterol metabolism, occurs in about 5% of patients with SLOS, often in patients not previously known to have SLOS (33, 101).

The mechanism by which inhibition of sterol biosynthesis disrupts normal embryologic development remained obscure until Chiang et al. (25) showed that targeted disruption of the *Sonic hedgehog* gene in mice causes not only holoprosencephaly, but also distal limb defects and other skeletal anomalies not unlike those of SLOS (25). The same group also showed that covalent addition of cholesterol to the N-terminal portion of Sonic hedgehog is an essential step in the formation of the actively signaling Shh-N hedgehog fragment (146, 147). Shortly thereafter, Roessler et al. (151) showed that haploinsufficiency for *Sonic hedgehog* in humans is one cause of sporadic as well as familial autosomal dominant holoprosencephaly. Moreover, targeted disruption of the gene for megalin (gp330), an important component of a system for delivery of maternal LDL cholesterol to the embryonic neuroepithelium, causes holoprosencephaly in homozygous deficient mice (191).

The discoveries linking holoprosencephaly, mutations in *Sonic hedgehog*, and abnormal cholesterol metabolism suggested that interference by 7DHC with cholesterol modification of Shh-N may be the cause of malformations in SLOS. However, Cooper et al. (29) published evidence that the apparent defect in Sonic hedgehog signaling instead may reside in the effect of the abnormal cellular sterol milieu on the response of the target tissue to Shh-N (29). Interestingly, the receptor for Shh, Patched, contains a sterol response element common to several proteins whose synthesis or function is regulated by the subcellular sterol environment (123). Patched, therefore, may be the element of the hedgehog signaling cascade primarily disturbed by the abnormal tissue sterol environment in SLOS tissues during embryogenesis. Because Sonic hedgehog is only one of a family of signaling proteins with a Patched receptor, similar impairment of the Desert hedgehog signaling cascade in the genital anlage (15) and of Indian hedgehog function in cartilage (85) may play a role in the abnormal morphogenesis of these structures in SLOS. Other less specific cellular disturbances, such as abnormal cell membranes and cell-cell interactions, may also contribute to the abnormal morphogenesis of SLOS and other defects of cholesterol biosynthesis (38).

Although a specific role of Sonic hedgehog in the embryological abnormalities of SLOS remains to be proven, there is now strong evidence that the malformations

of SLOS derives, at least in part, from impaired hedgehog function and the resulting downstream effects on the expression of homeobox genes. However, most genetically characterized multiple anomaly syndromes that cause disturbances in the body plan have been caused not by abnormalities of intermediary metabolism but by mutations of homeobox genes or related transcriptional factors. Even the multiple and severe metabolic abnormalities of Zellweger syndrome have little effect on the fundamental embryonic body plan. Thus, SLOS may be an exception among metabolic malformation syndromes because at least one action of its abnormal sterol biochemistry appears to disrupt embryonic signaling pathways and, thereby, mimic the effects of mutations in homeobox genes.

Enzymology of 7-dehydrocholesterol Reductase

DHCR7, the enzyme that converts 7DHC to cholesterol, is a microsomal membrane-bound enzyme with a mass of 55 kDa (Table 1). DHCR7 has been purified to near homogeneity and some of its enzymatic characteristics have been described (77, 164, 165). Important with regard to possible treatment strategies for SLOS is that DHCR7 contains a sterol regulatory element among its polypeptide domains and may undergo phosphorylation/dephosphorylation regulation (8, 166). The gene for DHCR7 was localized to 11q12-3, cloned, and sequenced in 1997 by Moebius and colleagues (130), who also showed that the human DHCR7 enzyme has strong homology with DHCR7s of both unicellular and other multicellular eukaryotes as well as homology with 3β-hydroxysteroid-Δ^{14}reductases. Indeed, there is considerable amino acid sequence homology of DHCR7 with at least other five mammalian proteins, three of which have demonstrable sterol reductase activity: sterol-Δ^{8}-isomerase, lamin B receptor (LBR), and sterol 14 reductase (Table 1). A comparison of the sequences of these enzyme proteins shows the greatest conservation of amino acid sequence in DHCR7 in exons 5 through 7, which, therefore, may be the segments that invest DHCR7 with enzymatic activity (130). Although four different sterol double bonds are acted on by the five homologous DHCR7-like proteins with known enzymatic activity, the bonds cluster in one region of the cholesterol molecule and the reaction sequence in all includes the formation of an unstable carbocation intermediate. The fifth protein with homology to the sterol reductases is LBR, a protein of the nuclear membrane. LBR has substantial homology with DHCR7 but even greater homology with TM7SF2, a protein whose gene has an intron/exon structure almost identical to the nine 3′ exons of LBR. LBR differs from both DHCR7 and TM7SF2 in having an additional three exons 5′ to the DHCR7-homologous segments that appear to encode a nuclear envelope targeting signal (169). Interestingly, LBR, which normally is tightly associated with the inner surface of the nuclear envelope, redistributes to the ER when the nuclear envelope disintegrates during mitosis (137). The complementary tissue distributions of the sigma-1 receptor and TM7SF2 (Table 1) suggest that these proteins may serve similar roles in lipid transport. That these proteins have strong homology in the putative sterol binding carboxy termini of sterol Δ^{14}-reductase

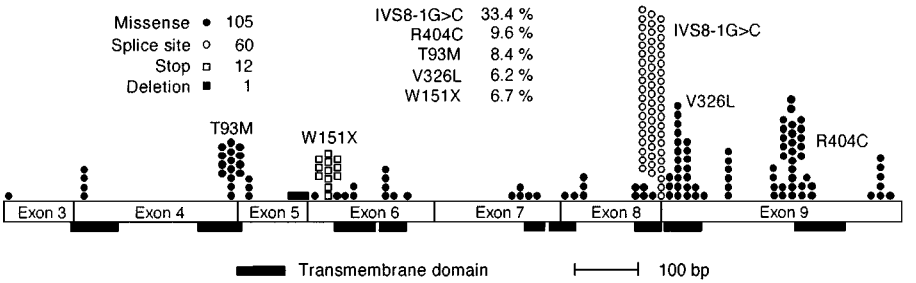

Figure 5 Structure of *DHCR7* and distribution of its mutations in Smith-Lemli-Opitz syndrome.

and sterol-Δ^8-isomerase further suggests that sterols may be preferred ligands for TM7SF2 and SIGMA1 (76).

DHCR7 Mutation Studies

Approximately 40% of known *DHCR7* mutations affect exon 9 and collectively constitute almost 75% of DHCR7 alleles in SLOS patients (Figure 5). These include four of the most common mutations, V326L, R352W, R404C, and IVS8-1G>C, which alone account for almost 50% of DHCR7 alleles. Of more than 70 known *DHCR7* mutations, almost two thirds occur in putative transmembrane regions. The most prevalent *DHCR7* mutation, IVS8-1G>C, activates a splice site 5′ to the mutation site in intron 8, leading to a 134 bp insertion in the mRNA, a premature stop codon, and a protein product shortened by 154 amino acids (7, 49). Two other common mutations are T93M in exon 4 and W151X in exon 6. Heterologous expression of c-myc cDNA constructs of the four most common missense mutations (T93M, V326L, R404C, R352W) showed that all expressed normal amounts of mRNA but reduced amounts of immunoreactive protein (<10%). The premature stop mutation, W151X, creates a truncated protein with no enzymatic activity (7, 49). Unlike the relatively common W151X mutation, all other stop or frameshift mutations have so far been unique.

Epidemiology of DHCR7 Mutations

DHCR7 alleles are notable not only for the high prevalence of a small number of severe mutations, but also for a varying geographical distribution and for discrepancies between predicted and observed frequencies of certain genotypes. All larger studies of SLOS mutations in populations in or originating from Europe find the IVS8-1G>C splice site mutation to be the most common, usually accounting for about one third of alleles. In anonymous DNA samples from different populations, Yu et al. (194) found heterozygosity for IVS8-1G>C in 1 of 90 American Caucasians, but none in similar numbers of natives of Finland, Sierra Leone, China (Han), and Japan. In a group of 1503 anonymous newborn screening blood spots collected in the U.S. state of Oregon, Battaile found 16 carriers for IVS8-1G>C,

giving an unusually high estimated carrier frequency for this single allele of 1% in a population that is between 80% and 90% of European origin. In a more detailed ethnic analysis of their previously reported DHCR7 mutation data (192), Witsch-Baumgartner et al. found evidence for an east to west European cline for IVS8-1G>C, with the highest frequency in the British Isles, and opposite west to east clines for W151X and V326L. Based on haplotype analyses, these authors speculated that the two common null mutations, IVS8-1G>C and W151X, are the most ancient among the common SLOS mutations in Europe. Interestingly, R404C and T93M, a mutation most prevalent in England and Italy, involve CpG islands and appear to have arisen on three and four different haplotypes, respectively.

Although no single DHCR7 mutation study has been large enough for a rigorous analysis of observed vs. expected frequency of *DHCR7* allele combinations, a metaanalysis of the larger studies—all of which reflect 90% or greater European ancestry—show some illuminating discrepancies. For example, among 179 SLOS patients from four major centers [(188, 192, 193); R.I. Kelley, unpublished data] there were 9 IVS8-1G>C homozygotes identified, compared to the 18 predicted by the allele fraction of 0.32 for IVS8-1G>C among all 358 alleles. In contrast, for combined heterozygosity of IVS8-1G>C with each of the four most common missense mutations (T93M, V326L, R352W, R404C), there was a twofold or greater number of patients than predicted, whereas the same relative increase was not found for IVS8-1G>C heterozygosity with the most common stop mutation, W151X (Table 2). Overall, there were 39 patients who were compound heterozygotes for IVS8-1G>C and one of the common missense alleles, compared to the 14 predicted. A similar relationship for the less common null allele, W151X, was found, with eight compound heterozygotes vs. three predicted.

Although some skewing of genotypic frequencies can be expected when combining different population samplings, the finding that all four common missense alleles show the same degree of overrepresentation in compounds with both null alleles argues that these null alleles constitute a much higher fraction of SLOS alleles in the reference populations than is evident from genotyping of identified SLOS patients. Because homozygosity for IVS8-1G>C usually leads

TABLE 2 Predicted vs. observed number of patients with specific *DHCR7* genotypes among 179 patients with Smith-Lemli-Opitz syndrome

		Mutation 2					
		Missense			Null		
Mutation 1		**T93M**	**V326L**	**R352W**	**R404C**	**W151X**	**IVS8-1 G>C**
IVS8-1 G>C	Predicted	5.6	3.3	2.2	3.3	2.9	18.2
	Observed	18	8	6	7	2	9
W151X	Predicted	0.9	1.0	0.35	0.5	0.5	—
	Observed	4	2	2	0	1	—

to a prenatal or perinatal lethal form of SLOS, the lower than expected incidence of IVS8-1G>C homozygosity could reflect substantial fetal losses of null allele homozygotes or frequently missed patients because the diagnosis of SLOS is considered less often in the severely malformed fetus or SLOS newborn who dies in the immediate perinatal period without examination by a geneticist. Furthermore, because the opportunity for DNA sampling increases with length of survival, IVS8-1G>C and W151X homozygotes and IVS8-1G>C/W151X mixed heterozygotes may be substantially underrepresented in the SLOS DHCR7 mutation studies. If DHCR7 exists as a homodimer, another, if less likely, possibility to consider is a dominant positive phenomenon, whereby one mutant protein, such as the IVS8-1G>C–encoded truncated protein, in the dimer stabilizes a more labile DHCR7 protein encoded by a *DHCR7* missense mutation. However, a dominant positive phenomenon would be unlikely to explain the increase in the observed over the predicted number of patients found for each of the four common missense mutations.

The discrepancies in the reported frequencies of specific SLOS genotypes recommend a reexamination of some of the estimates of the birth incidence of SLOS. Combining their finding of a 1% heterozygote frequency for IVS8-1G>C with the knowledge that IVS8-1G>C accounts for one third of SLOS mutations in most SLOS patient studies, Battaile et al. estimated an overall *DHCR7* mutation heterozygote frequency of 1 in 30. This gives a predicted incidence of SLOS of between 1 in 1590 and 1 in 13,500 births, which is much higher than the estimated U.S. incidence of SLOS of 1 in 40,000 births (98). However, in view of the almost threefold excess of the observed over the predicted number of null/missense mixed heterozygotes, it is possible that IVS8-1G>C and W151X together account for as many as 80% of DHCR7 mutant alleles in the U.S. population and that, consequently, the carrier frequency of *DHCR7* mutations collectively may be less than 1.3%. Whereas a 1.3% carrier frequency would predict an incidence of 1 in 24,000 births, perhaps one third of SLOS births are lethal in utero, i.e., when null alleles are involved. Moreover, as reported by Langius et al. (111) some IVS8-1G>C/missense mixed heterozygotes are developmentally normal, minimally dysmorphic (e.g., only 2/3 toe syndactyly) school-aged children, despite abnormal sterol biochemistry in plasma. Also likely is that homozygotes and mixed heterozygotes for certain missense mutations are biochemically and clinically normal, as noted above for the common V377I *MVK* mutation. Thus, although missed patients with either very mild or very severe forms of DHCR7 deficiency may remain, it is likely that the actual birth incidence of clinically significant DHCR7 deficiency in the United States is close to the reported clinical experience of 1 in 40,000 births (98).

Phenotype-Genotype Correlation

In many inborn errors of metabolism, some correlation exists between the level of the primary abnormal metabolite and clinical severity. However, as first shown in 1997 by Cunniff et al. (33), the level of 7DHC in plasma poorly correlates with

the SLOS physical severity score (33). Instead, the strongest correlation in SLOS exists between the physical severity and the dehydrosterol (7DHC + 8DHC) level expressed as a fraction of total sterols, a value that more accurately expresses the diminished cholesterol availability for both metabolic and structural fates (98). In their detailed study of phenotype-genotype correlation (192), Witsch-Baumgartner et al. divided the 40 mutations they found in 84 patients into four classes based on the structural effect or location of the mutation: missense mutations in (*a*) predicted transmembrane spanning segments and their border areas (TM), (*b*) the fourth cytoplasmic loop, (4L), (*c*) the C-terminus (C-T), and (*d*) null mutations (0), largely IVS8-1G>C and W151X. Overall, the predicted severity of the mutant allele class significantly correlated with both clinical and biochemical severity, with TM/TM compounds having the mildest biochemical and clinical phenotypes and 4L/4L and 0/0 compounds having the most severe. As would be expected, there was wide variability in the biochemical severity of 0/TM compounds. However, although 4L/4L and 0/0 were associated with the highest fractional levels of dehydrosterols, 0/4L compounds (almost all of which were IVS8-1G>C/4L compounds) had dehydrosterol fractions and clinical severities that ranged from the highest to lower values more characteristic of mild TM/TM compounds. Although the number of patients in each group was small, the unusual variability of the 0/4L compounds again raises the possibility of a dominant positive effect for some compound heterozygotes.

In addition to substantial phenotypic variability within each mutation class, much variability for single allelic combinations also exists, indicating significant contributions of other genetic or environmental factors to the clinical and biochemical phenotype of SLOS. For example, among 6 IVS8-1G>C/T93M compounds, the clinical severity score (0 = normal, 100 = most severe) ranged from 17 (mild) to 39 (moderate), whereas the pretreatment dehydrosterol fraction [(7DHC + 8DHC)/(7DHC + 8DHC + cholesterol)], ranged from 0.18 to 0.59 (mean 0.31), with no strong correlation between the clinical and biochemical measures of severity (R.I. Kelley, unpublished data). For 5 R404C homozygotes, the dehydrosterol fraction was higher and less variable—0.59 to 0.79, mean 0.67—but clinical variability was even greater, from 19 to 69. The variable dehydrosterol fraction within a single genotype and the substantial amount of cholesterol synthesized by IVS8-1G>C homozygotes suggest the existence of other DHCR7 activities or an alternate pathway of cholesterol biosynthesis, as discussed above.

DESMOSTEROLOSIS (MIM 602938)

In 1998, Fitzpatrick et al. (50) first described a third apparent defect of cholesterol biosynthesis in a 34-week gestation, 46,XX dysmorphic female infant who died 1 hour after birth from respiratory insufficiency. Anomalies included macrocephaly, thick alveolar ridges, gingival nodules, cleft palate, total anomalous pulmonary venous drainage, clitoromegaly, short limbs, and generalized osteosclerosis resembling Raine skeletal dysplasia syndrome (149). Although the infant

had macrocephaly, not microcephaly, the similarity of some of the anomalies to those of SLOS suggested a possible disorder of sterol biosynthesis as the cause of the infant's condition. Indeed, instead of 7DHC, the infant had markedly increased tissue levels of desmosterol (cholesta-5,24-dien-3β-ol), an immediate precursor of cholesterol following an alternate synthetic pathway wherein the 24-double bond is reduced last, rather than early, as in the Kandutsch-Russell pathway (Figure 2). Although fibroblasts were not available for further study, both parents had mildly increased plasma levels of desmosterol, suggesting an autosomal recessive deficiency of desmosterol reductase (3β-hydroxysteroid-Δ^{24}-reductase; DHCR24). A second infant with increased plasma and cellular levels of desmosterol was reported by Andersson et al. (4). This three-year-old male had downslanting palpebral fissures, micrognathia, submucous cleft palate, clubfoot, a persistent patent ductus arteriosus, profound microcephaly, and complete agenesis of the corpus callosum. Although the level of desmosterol in plasma was only mildly increased to 60 μg/ml (nl $<$ 1.1 μg/ml), or just 5% of the cholesterol level, the sterol fraction of desmosterol rose to 42% in lymphoblasts cultured in cholesterol-depleted medium, similar to the tissue fraction of desmosterol in the tissues of the patient of Fitzpatrick et al. (50). The parents of the second patient had two- to threefold increased levels of desmosterol in plasma (4), and desmosterol also was mildly increased in the mother's cultured lymphoblasts (R.I. Kelley, unpublished data).

Based on the description in 2000 of the human ortholog, *DWF1*, of *DIMINUTO/DWARF1*, a gene encoding a sterol-Δ^{24}-reductase–like activity in plants, Waterman identified a 169 kb human genomic clone from chromosome 1 that contained the human ortholog of *DIMINUTO/DWARF1* (189). The apparent human *DHCR24* (Table 1) mapped to 1p33.1 and spanned 46 kb of genomic DNA. Further analysis indicated a gene with eight exons that was expressed in all tissues and that, when expressed in naturally DHCR24-deficient yeast, conferred the ability to convert desmosterol to cholesterol. Testing of DNA from the two reported desmosterolosis patients and their parents revealed *DHCR24* mutations in all four alleles. Expression of the mutant alleles in yeast showed almost absent DHCR24 activity for the first patient and substantially more but still depressed activity in the second patient (19.9%). In first patient, the maternal allele carried a 1412C$>$T (Y471S) mutation, whereas the paternal allele had two mutations, 818A$>$C (N294T) and 918G$>$C (K306N). The DHCR24 activities of the identified mutations expressed in yeast paralleled the observed biochemical and phenotypic severity in the patients (189).

The embryologic abnormalities of the first desmosterolosis patient that parallel those of SLOS—cleft palate, ambiguous genitalia (although 46,XX), thick alveolar ridges with gingival nodules, and short limbs—support the conclusions of Cooper et al. (29) that the apparently impaired signaling of Sonic hedgehog in SLOS is not a specific effect of 7DHC or 8DHC but more likely reflects the intracellular cholesterol deficiency. Moreover, the studies of Roux and colleagues many years earlier had shown that Triparanol, a potent inhibitor of DHCR24, and AY-9944, an inhibitor of DHCR7, have similar malforming effects on mouse embryos

(154, 155, 157). However, other defects found in the first desmosterolosis patient, such as the marked osteosclerosis and severe limb shortness, suggest that there may be specific teratologic effects of desmosterol or, perhaps, other sterol metabolites with a 24-unsaturated bond that accumulate behind a deficiency of DHCR24. Interestingly, two other cholesterol precursors with 24-unsaturated bonds, 4,4′-dimethylcholesta-8,14,24-trien-3β-ol and 4,4′-dimethylcholesta-8,24-dien-3β-ol, have potent signaling activities in postfertilization meiosis (58, 86) and could have increased levels in desmosterolosis.

X-LINKED DOMINANT CHONDRODYSPLASIA PUNCTATA AND CHILD SYNDROME (MIM 302960, 300205, 308050)

Overview of the Chondrodysplasia Punctatas

The chondrodysplasia punctatas (CDPs) are a heterogeneous group of genetic disorders characterized by abnormal foci of calcification in the cartilaginous skeleton, termed chondrodysplasia punctata or epiphyseal stippling because of the predominant location of the lesions in the epiphyses. The severe autosomal recessive form of CDP (RCDP; MIM 215100) is associated with symmetric proximal (rhizomelic) limb shortness, ichthyosis, cataracts, growth and mental retardation, and peroxisomal abnormalities. Most patients with RCDP have mutations in the PEX7 gene, which encodes a receptor that directs proteins with a type 2 peroxisomal targeting signal (PTS2) to the peroxisomal matrix (18, 134, 148). Milder autosomal dominant, X-linked recessive (CDPX) and X-linked dominant (CDPX2) forms of CDP are also known (176). X-linked recessive CDP (MIM 302950) is often associated with terminal Xp deletions or X:Y translocations involving Xp22.32 (9). Recently, mutations in arylsulfatase E (ARSE) have been found in some CDPX patients with normal karyotypes (51). Epiphyseal stippling has also been noted in patients with a variety of other genetic and acquired disorders, including trisomy 21, congenital hypothyroidism, Zellweger syndrome, and prenatal maternal exposure to vitamin K antagonists, such as coumadin (176, 185). The occasional finding of epiphyseal stippling in Smith-Lemli-Opitz syndrome (90) and the report of a severe skeletal dysplasia in one of the two known patients with desmosterolosis (50) suggested a link between sterol metabolism and skeletal disease and led to the discovery that classic CDPX2 (102) and several other skeletal disorders (39, 56, 100, 118) are associated with a deficiency of an enzyme catalyzing one of the later steps of cholesterol biosynthesis.

Clinical Features and Genetics of X-linked Dominant CDP

CDPX2—also called Conradi-Hünermann or Happle syndrome—is a rare X-linked dominant disorder with presumed male lethality (60, 67). Affected females typically present with skeletal and skin abnormalities at birth. There is a flaky, usually erythematous, eruption (ichthyosiform erythroderma), which often resolves

completely or substantially in the first months of life, leaving linear or whorled patches of atrophic and/or pigmented skin. In addition to hyperkeratosis, acanthosis, and patchy parakeratosis in the epidermis, there are neutrophilic or lymphocytic infiltrates in both the epidermis and dermis as well as characteristic follicular plugging (30, 36, 43, 60). Electron microscopy (EM) of the epidermis shows abnormal accumulations of lipid-laden vacuoles (43, 105). The hair is coarse and lusterless, and there may be patches of cicatricial alopecia. The nails occasionally are flattened, split, and hypoplastic, whereas the teeth usually are normal. As in many X-linked genodermatoses, the skin abnormalities of CDPX2 females characteristically follow the lines of Blaschko, which reflect the functional mosaicism caused by random X inactivation (181).

Skeletal abnormalities in CDPX2 include infantile epiphyseal stippling and asymmetric rhizomelic shortness of the limbs. Scoliosis, either congenital or later onset, and kyphosis are common. Clubfoot, postaxial polydactyly, joint contractures, and vertebral anomalies also are occasional findings. The stippling in CDPX2 often involves the vertebral and tracheal cartilages and is more widespread than in most other forms of CDP. Light microscopy reveals that sections of differentiating cartilage demonstrate patchy areas of chondrocyte loss, which by EM appear to be areas of apoptosis of prechondrocytes (W. Wilcox, personal communication). Craniofacial defects include frontal bossing, a flat nasal bridge, and midface hypoplasia. Cataracts, typically congenital and asymmetric or sectorial, are present in approximately two thirds of cases (61) and may be accompanied by microphthalmia, microcornea, or optic nerve hypoplasia (173, 175). Other features reported in some patients include congenital heart disease, developmental renal anomalies, including hydronephrosis, and sensorineural as well as conductive hearing losses. CNS malformations, although rare, have been reported, and most patients have normal intelligence [(60); G. Herman, unpublished data]. Polydactyly is relatively specific for the X-linked dominant form of CDP, and the frequency of ichthyosis and cataracts is reported to be higher in CDPX2 than in RCDP.

CDPX2 affects females almost exclusively. Most cases are sporadic, presumably the result of new mutations. Four males with CDPX2, one of whom had a 47,XXY karyotype, have been reported (63, 172, 195). Presumed X linkage with male lethality has been proposed as the mechanism to explain the inheritance pattern of CDPX2 (60), and somatic mosaicism or half-sister chromatid exchange has been invoked to account for the clinical findings in CDPX2 males with apparently normal chromosome complements (63).

Clinical diagnosis of CDPX2 in infancy is based on finding epiphyseal stippling in a female with other clinical features of the disorder and is confirmed by finding a characteristic pattern of cholesterol precursors (vide infra). Prenatal diagnosis can be performed by mutation analysis of chorionic tissue or cultured amniocytes. Although not yet reported, it is likely that, as in SLOS, prenatal diagnosis by measurement of cholesterol precursors in amniotic fluid will be possible.

Treatment of CDPX2 is symptomatic. Although the ichthyotic skin lesions usually resolve within weeks to months, they may persist to varying degrees in some

patients and can be treated with a variety of emollients. Dry skin is not uncommon and alopecic patches persist throughout life. Early ophthalmologic examination to detect the presence of cataracts is essential to ensure normal visual development. Baseline head, renal, and cardiac sonographic examinations and hearing screening are also recommended. Involvement of the tracheal and laryngeal cartilages can lead to airway obstruction and difficulty with intubation. The skeletal asymmetries and, in particular, scoliosis may require surgical intervention. Also important to recognize is that congenital vertebral anomalies and underdevelopment of the foramen magnum can lead to congenital or postnatal neurological complications owing to vertebral body subluxations or spinal cord compression (34, 54).

Biochemistry and Mutation Analysis of CDPX2

In 1999, Kelley et al. reported markedly increased levels of 8DHC and 8(9)-cholestenol in five females with chondrodysplasia punctata (102). Subsequently, similar biochemical abnormalities have been detected in numerous additional females with CDPX2 (68, 82). This abnormal sterol pattern suggested a defect in 3β-hydroxysteroid-Δ^8,Δ^7-sterol isomerase, a microsomal enzyme that converts 8(9)-cholestenol to lathosterol in the terminal steps of normal cholesterol biosynthesis (Figure 2). The gene for human sterol-Δ^8,Δ^7-isomerase, also called emopamil binding protein (EBP), is X linked and maps to Xp11.2 (Table 1).

Nineteen different mutations in the human EBP gene in a total of 25 unrelated CDPX2 females have been published to date (17, 39, 68, 82) [Patients 2 and 6 in References (18) & (40), respectively, are the same.]. Biochemical studies were performed on patients in two of the reports (17, 82), and all of the females in whom mutations were found demonstrated the typical sterol pattern described above. The EBP mutations identified include 11 nonsense, 7 missense, 1 single amino acid deletion, 4 frameshift, and 2 splicing mutations. All of the missense mutations alter conserved amino acids in the predicted protein. Four of the mutations have occurred in more than one patient; two of these involve CpG islands that may be hot spots for mutations. Somatic and gonadal mosaicism has been described in one female, and gonadal mosaicism is presumed in a second family in which two sisters are affected and their mother is phenotypically normal (68). Although the possibility of gonadal mosaicism must be considered in genetic counseling for apparently sporadic CDPX2 cases, there is no conclusive evidence that this phenomenon is more common in CDPX2 than other X-linked disorders.

There are no obvious genotype-phenotype correlations among the 25 patients reported with EBP mutations, a fact that probably reflects determination of the phenotype as much by the pattern of X inactivation in affected tissues as by the nature of the mutation itself. A number of reports have documented the increasing severity of the clinical features of CDPX2 in succeeding generations (135, 138, 172, 182), a phenomenon called anticipation. In analogy with several neurological disorders (32), Traupe et al. (182) speculated that anticipation in familial CDPX2 was caused by expansion of an unstable triplet repeat. With the identification of mutations in

the EBP gene in numerous females, such anticipation now appears to result from the skewing of X inactivation, somatic and/or gonadal mosaicism, or decreased reproductive fitness of more severely affected females. However, the possibility remains that the frequently reported anticipation results from an effect of the abnormal sterol metabolism of the mother on her developing CDPX2 embryo and fetus.

Gene Structure and Enzymology

EBP, the original protein designation for human sterol-Δ^8,Δ^7-isomerase, was first identified as a high-affinity binding protein for the antiischemic drug emopamil—hence, the name. As such, EBP is a member of the sigma class of drug-binding proteins whose ligands include numerous pharmacologically active compounds, including chlorpromazine, haloperidol (129), and tamoxifen (27). EBP was later found to possess sterol isomerase activity in mammalian cells and to complement sterol isomerase deficient (*erg2*) mutants of *Saccharomyces cerevisiae* (167). Surprisingly, there is no significant amino acid homology between EBP and the yeast sterol isomerase protein encoded by the *erg2* gene. Another member of the sigma receptor family, sigma-1 receptor (Table 1), has substantial amino acid homology with the erg2 protein but has no measurable isomerase activity (95, 132)

The EBP gene spans approximately 7 kb of genomic DNA, and the genomic sequence for the entire human gene has been assembled (GenBank Accession No. NT_011609). There are five exons in the gene, with the translation start site in exon 2. The EBP protein is widely expressed, with highest levels in tissues involved in cholesterol synthesis and steroidogenesis, such as liver, adrenal gland, intestines, and gonads. It is predicted to be an integral membrane protein with four transmembrane domains and has been localized within the ER [(59); G. Herman, unpublished results]. The C-termini of the mouse and human proteins contain a lysine-rich consensus sequence for retention of proteins within the ER membrane (177). In vitro mutagenesis of the EBP protein identified six residues (His77, Glu81, Glu123, Thr126, Asn194, and Trp197) located in the cytoplasmic halves of several of the predicted transmembrane segments 2–4, which appear to be essential for enzymatic activity and, therefore, probably form part of the protein's catalytic site (131).

Mouse Models of X-linked Dominant, Male-Lethal Skeletal Dyplasias

THE TATTERED (TD) MOUSE The X-linked tattered (*Td*) mouse is a model for CDPX2. Heterozygous *Td* females are dwarfed and develop hyperkeratotic skin lesions at approximately postnatal day 4–5. The adult coat is striped, following the pattern of X inactivation in the mouse, and subtle cataracts have been detected in *Td* females. Affected male embryos die between 12.5 days post coitum (dpc) and birth, depending on the genetic background (39). Those males that survive until birth

are hydropic with a short-limbed skeletal dysplasia, craniofacial abnormalities, cleft palate, and absent mid- and hindgut. *Td* results from a missense mutation in a conserved amino acid within the murine EBP protein (G107R), and *Td* females accumulate 8DHC and 8(9)-cholestenol, similar to human CDPX2 females (39).

THE BARE PATCHES (BPA) MOUSE Interestingly, although *Td* is now the proven ortholog of human CDPX2, another X-linked dominant, male lethal mouse disorder with a similar phenotype, bare patches (*Bpa*) had long been considered the ortholog of human CDPX2 (5, 61). *Bpa* females are also dwarfed (45, 143, 144), and abnormal deposits of calcium in the tail vertebrae, consistent with epiphyseal stippling, have been reported (67). Over 50% of affected *Bpa* females have asymmetric cataracts, frequently associated with microphthalmia. On postnatal day 5–7, they develop a hyperkeratotic skin eruption, which resolves and leaves bare patches arranged in a horizontal, striped pattern, following the lines of X inactivation. Affected *Bpa* male embryos have not been recovered and die shortly after implantation [(143); B. Cattanach, personal communication]. Several *Bpa* alleles have now been identified, both X-irradiation induced and spontaneous (118). The milder alleles were originally thought to be a different locus called striated (*Str*) (145). *Str* females are normal in size, and affected *Str* male embryos die in midgestation (118). In 1999, mutations in a gene called *Nsdhl* (NADH steroid dehydrogenase-like) were identified in several *Bpa* and *Str* alleles (118). Nsdhl functions as a 3β-hydroxysteroid dehydrogenase in the oxidative decarboxylation of C-4 methyl groups in the conversion of C28, C29, and C30 precursor sterols to 8(9)-cholestenol and related 8(9)-unsaturated sterols (Figure 6). Tissues and skin fibroblasts from *Bpa* females accumulate 4-methyl, 4,4′-dimethyl, and 4-carboxy sterols, consistent with an enzymatic block at this step of the cholesterol biosynthetic pathway [(118); G.E. Herman, R.I. Kelley, unpublished data]. The removal of C-4 methyl groups immediately precedes the sterol-Δ^8,Δ^7-isomerase reaction, explaining the similarity in the phenotype between the *Bpa/Str* and *Td* mutations and the original suggestion that bare patches was orthologous to CDPX2.

NSDHL GENE STRUCTURE AND ENZYMOLOGY The NSDHL gene in the human and mouse spans approximately 40 kb and contains eight exons, with the translation start site in exon 2 (Table 1). The gene is transcribed from a dual promoter in head-to-head orientation with a gene, caltractin, involved in centrosome function, whose role appears unrelated to that of NSDHL (121). The NSDHL protein is ubiquitously expressed but with higher levels of expression in tissues with higher rates of sterol and steroid biosynthesis. The predicted protein sequence contains an N-terminal NADH cofactor binding site and a single membrane spanning region near the 3′ end of the protein (118). NSDHL also contains several conserved Tyr-X-X-X-Lys motifs, at least one of which appears to be involved in the catalytic site of other 3β-hydroxysteroid dehydrogenases (3β-HSDs) (142). NSDHL represents a new subfamily of 3β-HSDs, as it possesses more amino acid sequence identity

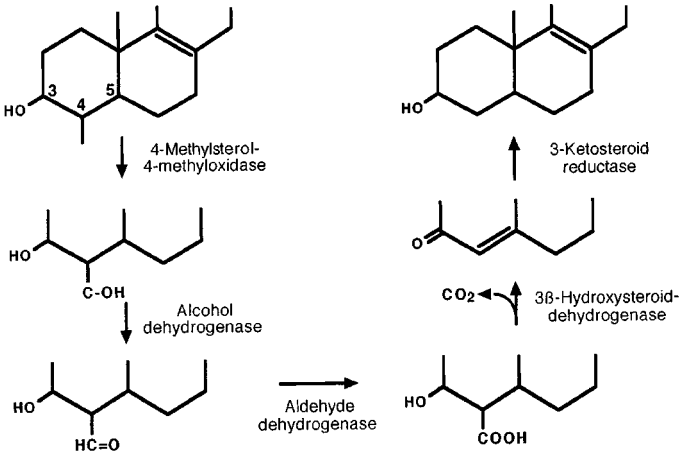

Figure 6 Enzymatic and nonenzymatic steps in the sterol-4-demethylase complex of cholesterol biosynthesis. The first three steps in the oxidative demethylation as studied in yeast appear to be carried out by a single 4-methylsterol oxidase enzyme protein. NSDHL, which is deficient in some cases of CHILD syndrome and the Bare patches mouse, is the 3β-hydroxysteroid dehydrogenase activity indicated at the fourth step.

with similar proteins from lower eukaryotes than with the other known mammalian 3β-HSDs (118).

In *S. cerevisiae*, *erg26* encodes the sterol biosynthetic 3β-HSD. In this organism, the removal of the two C-4 methyl groups from lanosterol (or related 4,4′-dimethyl intermediates) requires the sequential action of a C-4 methyloxidase (encoded by *erg25*), a 3β-HSD (*erg26*), and a keto-reductase (*erg27*) (113). A gene, *erg28*, encoding a regulatory or "scaffolding" protein that tethers the demethylase complex to the ER membrane has also recently been identified (52). Conservation of the majority of the steps in the yeast and mammalian sterol biosynthetic pathways predicts a similar C-4 demethylase complex in mammalian cells. Although a human C-4 methyloxidase gene has been identified (U60205), a mammalian 3-keto reductase gene has not as yet been isolated. A human ortholog for *erg28* has been isolated, although its function is not known (130).

CHILD Syndrome (MIM 308050)

CLINICAL FEATURES AND GENETICS CHILD syndrome is a disorder with phenotypic similarities to CDPX2 but with a striking unilateral distribution of abnormalities (65, 66, 69). Unilateral ichthyosiform skin lesions (sometimes referred to as an ichthyosiform nevus or inflammatory epidermal nevus) usually are present at birth and, in contrast to the skin lesions of CDPX2, often persist throughout life. Although the lesions may occasionally follow the lines of Blaschko, they usually involve large regions on one side of the body, with a sharp line of demarcation

at the midline. Small patches of involved skin may occur on the opposite side, although both sides of the face are spared. Alopecia may occur on the affected side and nail involvement also is common. Ipsilateral limb reduction defects are encountered with epiphyseal stippling, on X rays, during infancy. Internal malformations, including CNS, renal, and cardiac, have been reported, typically occurring on the affected side. Left-sided CHILD syndrome usually is more severe than right-sided, but it is also only half as common. The characteristic skin lesion of CHILD syndrome is a large epidermal plaque or nevus, often with a xanthomatous or even warty appearance. Histologically, there is a thick parakeratotic stratum corneum overlying a psoriasiform, acanthotic epidermis, often with inflammatory infiltrates and lipid-laden histiocytes.

Although CDPX2 and CHILD syndrome have many phenotypic similarities, there are some notable differences. For example, cataracts are not reported in CHILD syndrome, the skin lesions more often persist beyond infancy, and the skeletal anomalies—such as limb reductions or amelia—are more severe. Follicular plugging, which is characteristic of CDPX2, does not occur in classic CHILD syndrome (66, 69, 71). EM findings are similar to those reported in CDPX2 except that crystals within the lipid vacuoles, consistent with cholesterol, may be present (44, 70, 71). Despite the histologic and ultrastructural differences in the skin between CDPX2 and CHILD syndrome, the dermatological features distinguishing CDPX2 and CHILD features are not absolute, as indicated by a rare female with skin lesions typical of CHILD syndrome but with bilateral, symmetrical involvement (48) and by CDPX2 females with persistent diffuse erythroderma (92) or unilateral ichthyosis resembling the skin lesions of CHILD syndrome with only patchy involvement of the other side (30). The presence of cataracts and characteristic skin histology led the authors to classify the latter cases as CDPX2.

Like CDPX2, CHILD syndrome has been presumed to be an X-linked dominant disorder with male lethality since its delineation as a syndrome in 1980 (65). Two 46,XY males with otherwise typical right-sided CHILD syndrome (64, 195) and rare familial cases with mother to daughter transmission have been reported (64, 106). Early somatic mutations may explain the occurrence in males; however, such mutations cannot explain the unusual distribution of skin lesions in CHILD syndrome.

BIOCHEMISTRY AND GENE MUTATIONS Because of the similarities between CDPX2 and CHILD syndrome, Grange et al. (56) evaluated a four-year-old girl with CHILD syndrome and found her to have the characteristic biochemical sterol abnormalities of CDPX2 and a nonsense mutation in *EBP*. Subsequently, a second patient with CHILD syndrome and a mutation in *EBP* was identified (D.K. Grange, A. Metzenberg, G.E. Herman, & R.I. Kelley, manuscript in preparation). In contrast, following the discovery of *Nsdhl* mutations in *Bpa* mice, Konig et al. reported mutations in the human NSDHL gene in five patients with CHILD syndrome (106), including one male with a normal karyotype who was heterozygous for

wild-type and mutant alleles on the affected side. On the unaffected side, only the wild-type allele could be detected, consistent with the unilateral somatic mosaicism associated with a postzygotic mutation. In one family, a mildly affected mother had the same NSDHL mutation as her more severely affected daughter (106), excluding a postzygotic somatic mutation as the cause of CHILD syndrome, at least in that family. We have recently identified three additional CHILD patients with mutations in *NSDHL* (D.K. Grange, A. Metzenberg, G.E. Herman, & R.I. Kelley, manuscript in preparation). To date, all individuals with CHILD syndrome and mutations in the two associated X-linked genes, *EBP* and *NSDHL*, have had right-sided hemidysplasia and skin disease.

The occurrence, as in CHILD syndrome, of mutations in two or more genes in patients with similar or identical phenotypes is not uncommon. More surprising, perhaps, is the striking and unexplained unilateral distribution of lesions in CHILD syndrome. The patterning of skin lesions in CDPX2 and in *Bpa*, *Str*, and *Td* mice follows Blaschko's lines and is consistent with functional mosaicism for X-linked genes (62). However, to explain the midline demarcation and extensive uninterrupted skin lesions in CHILD syndrome, Happle proposed that a clone of midline, early embryonic organizer cells expressing the mutant NSDHL gene could affect the process of X inactivation and, thereby, alter the patterning of a large developmental field on one side of the body (56, 106). However, Happle suggested no specific molecular or biochemical mechanism. The process of X inactivation begins in the extra-embryonic cell lineages, at least in the mouse, at the blastocyst stage (3.5–4.5 dpc) [reviewed in (55)] before laterality is established. However, it is possible that genes, such as Sonic hedgehog, that play a role in laterality determination and that also are influenced by abnormalities in sterol metabolism may provide the link between the two events. Sonic hedgehog is expressed at Hensen's node, the site where asymmetry is first detected in the mammalian embryo (20). Although the expression of Shh in mammals is bilaterally symmetric, Shh-deficient mouse embryos demonstrate laterality defects (20, 25, 128, 183). Moreover, proper Shh signaling is required to prevent left-determining factors from being expressed on the right side of the embryo (128). In CHILD syndrome, Happle's organizer cells expressing the mutant NSDHL or EBP gene could exert their effect on Shh or one of its downstream effectors, such as Patched. Presumably, whether bilateral (CDPX2) or unilateral (CHILD) disease ensues from *EBP* mutations would depend on the pattern of affected vs. unaffected putative organizer cells in the laterality-determining perinodal tissues. Such a model does not explain why unilateral disease is never seen with *Td*, *Str*, or *Bpa* mutations or why unilateral involvement is so common in human *NSDHL* mutations but much less common when *EBP* is mutated. It is possible that species' differences in the timing of X inactivation or in cholesterol metabolism or transport in the developing fetus could explain the phenotypic differences. Unique species-specific features, such as the laterality defects seen in CHILD syndrome but not in the mutant mice, may require studies in in vitro human systems to understand more fully the mechanisms of disease pathogenesis.

HYDROPS–ECTOPIC CALCIFICATION–MOTH-EATEN SKELETAL DYSPLASIA (MIM 215140)

By screening tissues and cells from a skeletal dysplasia repository for evidence of abnormal sterol metabolism, a previously unreported sterol abnormality was found in several fetuses with a lethal short-limbed dwarfism known as Hydrops–ectopic calcification–moth-eaten skeletal dysplasia (HEM) (100). HEM, or Greenberg dysplasia, is a rare skeletal dysplasia characterized by fetal hydrops, short-limbed dwarfism, and severely disorganized chondro-osseous proliferation and mineralization (26, 57). Radiographic abnormalities include moth-eaten appearing, severely shortened long bones, ectopic epiphyseal calcification, laryngeal and tracheal calcification, and platyspondyly. Histologically, there is severe disorganization of cartilaginous tissues, obliteration of the marrow spaces by mesenchyme-like tissue, and extramedullary hematopoiesis. Unlike in SLOS, CDPX2, and NSDHL deficiency, HEM fetuses lack internal malformations, although two unrelated fetuses had postaxial polydactyly of the hands. The genetic characteristics of six known cases include diverse ethnic backgrounds, involvement of both sexes, affected siblings, and parental consanguinity in four of five families, thus making autosomal recessive inheritance almost certain. Possibly because of early in utero lethality—the longest surviving fetus died in utero at 30 weeks—HEM is one of the rarest skeletal dysplasias known. Although not a true chondrodysplasia punctata, HEM has enchondral bone with strikingly disordered calcification that resembles the dense punctate calcifications of severe CDPX2 and that may reflect, in part, a similar process of apoptosis or other premature death of prechondrocytes.

GC/MS of sterols extracted from the cartilage of four HEM fetuses showed increased levels of cholesta-8,14-dien-3β-ol and cholesta-8,14,24-trien-3β-ol, suggesting a deficiency of sterol-Δ^{14}-reductase (100). When grown in cholesterol-depleted culture medium, cultured skin fibroblasts or chondrocytes from two of the patients accumulated very large amounts ($>$20% of cholesterol) of the same two sterols (R.I. Kelley, unpublished observations). As shown in Figure 2, sterol-Δ^{14}-reductase just precedes the sterol-4-demethylase complex and sterol-Δ^{8},Δ^{7}-isomerase in the normal pathway for cholesterol biosynthesis. The recent discoveries that the nuclear lamin B receptor has intrinsic sterol-Δ^{14}-reductase activity (167) and that at least one 14-dehydrosterol possesses signaling activity as a postfertilization meiosis activator (58) suggest that 14-dehydrosterols may have important effects on DNA replication and nuclear signaling and may explain the especially severe phenotype in HEM dysplasia. Although the yeast sterol-Δ^{14}-reductase was cloned in 1994 (109) and the Δ^{14}-reductase activity of the nuclear lamin B receptor was demonstrated in 1998 (167), the identity of the gene encoding the primary sterol-Δ^{14}-reductase functioning in mammalian cholesterol biosynthesis has not been established (Table 1). Sequencing of all exons of the gene for TM7SF2, an ER protein with widespread tissue distribution and substantial homology with other sterol-Δ^{14}-reductases (76), failed to disclose a mutation

in HEM (G.E. Herman, unpublished data). Nevertheless, a block at the level of the sterol-Δ^{14}-reductase involved in sterol biosynthesis remains the most likely cause of HEM.

CONCLUSION

The discovery of new inborn errors of cholesterol metabolism in the past decade has provided geneticists with many new insights in several areas of biology and medicine: normal and abnormal embryogenesis, cholesterol synthesis and nutrition, biochemical genetics, the epidemiology of mutations, and even behavioral genetics. The central importance of cholesterol homeostasis in human biochemistry and development, which has been underscored by the consequences of even subtle genetic deficiencies of cholesterol biosynthesis, raises important questions about the widespread use of drugs for the reduction of blood cholesterol levels and the near elimination of cholesterol from recommended, nutritionally balanced diets.

The study of disorders of cholesterol biosynthesis has brought into focus, among many embryological phenomena, the role of cholesterol nutrition in the developing embryo. The evidence that the maternal supply of cholesterol to the developing embryo can influence the incidence and severity of certain SLOS malformations may be important with regard to the development the same malformations, such as cleft palate and holoprosencephaly, in other syndromes or even in otherwise normal individuals. There may also be important implications of the apparent interaction between cholesterol metabolism and hedgehog proteins in the abnormal morphogenesis of SLOS, if not all of the primary defects of cholesterol biosynthesis. Thus, the creation of mouse models lacking a functional 7DHC reductase gene or other, related genes of sterol biosynthesis will provide geneticists and embryologists with years of work that should bring even closer clinical dysmorphology and biochemical genetics.

Especially important among the emerging connections between sterol metabolism and cellular signaling is the evidence that sterols as well as steroids have nuclear signaling functions and that some of the most potent signaling molecules are normal, trace level intermediates in the cholesterol biosynthetic pathway. Moreover, the intriguing discovery that the lamin B receptor has sterol Δ^{14}-reductase activity—and could even be the primary Δ^{14}-reductase of cholesterol biosynthesis—suggests there may be important effects of disordered cholesterol biosynthesis and metabolism on nuclear signaling during both prenatal and postnatal life. The sequence homology of certain sterol metabolizing enzymes, like NSDHL, with other enzymes apparently dedicated to the synthesis of steroid hormones (118) raises the possibility that phylogenetically ancient sterol hormones evolved to become steroid hormones and took on new roles in interorgan signaling as the size and organizational complexity of organisms increased. Thus, there may

be a host of heretofore unsuspected sterol signaling functions at work in the nuclei or cytoplasm of both primitive and advanced eukaryotes, the elucidation of which may shed important light on less well-understood clinical consequences of inborn errors of cholesterol biosynthesis.

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