thematic review

Thematic Review Series: Genetics of Human Lipid Diseases

Malformation syndromes caused by disorders of cholesterol synthesis

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Abstract Cholesterol homeostasis is critical for normal growth and development. In addition to being a major membrane lipid, cholesterol has multiple biological functions. These roles include being a precursor molecule for the synthesis of steroid hormones, neuroactive steroids, oxysterols, and bile acids. Cholesterol is also essential for the proper maturation and signaling of hedgehog proteins, and thus cholesterol is critical for embryonic development. After birth, most tissues can obtain cholesterol from either endogenous synthesis or exogenous dietary sources, but prior to birth, the human fetal tissues are dependent on endogenous synthesis. Due to the blood-brain barrier, brain tissue cannot utilize dietary or peripherally produced cholesterol. Generally, inborn errors of cholesterol synthesis lead to both a deficiency of cholesterol and increased levels of potentially bioactive or toxic precursor sterols. Over the past couple of decades, a number of human malformation syndromes have been shown to be due to inborn errors of cholesterol synthesis. Herein, we will review clinical and basic science aspects of Smith-Lemli-Opitz syndrome, desmosterolosis, lathosterolosis, HEM dysplasia, X-linked dominant chondrodysplasia punctata, Congenital Hemidysplasia with Ichthyosiform erythroderma and Limb Defects Syndrome, sterol-C-4 methyloxidase-like deficiency, and Antley-Bixler syndrome.—Porter, F. D., and G. E. Herman. **Malformation syndromes caused by disorders of cholesterol synthesis. *J. Lipid Res.* 2011. 52: 6–34.**

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Altered cholesterol homeostasis contributes to multiple human diseases. These range from common disorders such as atherosclerotic cardiovascular disease and stroke to rare genetic syndromes. Cholesterol can be endogenously synthesized from acetate in a series of enzymatic steps (**Figs. 1** and **2**). In addition to cholesterol, mevalonate metabolism also is involved in the synthesis of isoprenoids such as farnesylpyrophosphate, geranylgeranylpyrophosphate, heme, ubiquinones, and vitamin D. Cholesterol synthesis can be divided into two major components, presqualene cholesterol synthesis and postsqualene cholesterol synthesis. Presqualene cholesterol synthesis (Fig. 1) contributes to both sterol and isoprenoid synthesis, whereas postsqualene cholesterol synthesis (Fig. 2) represents a commitment to sterol and vitamin D synthesis. This review will focus on malformation syndromes due to inborn errors of postsqualene cholesterol synthesis. Mevalonate kinase deficiency, an inborn error of presqualene cholesterol synthesis that can result in mevalonic aciduria or hyperimmunoglobulinemia D syndrome, has previously been reviewed by Hass and Hoffmann (1). The first committed step of sterol synthesis involves the synthesis of lanosterol from squalene-2,3-epoxide, a reaction catalyzed by lanosterol synthase (2). Synthesis of cholesterol, a C27 sterol, from lanosterol, a C30 sterol, involves multiple enzymatic reactions, including reduction of Δ7, Δ14, and Δ24 double bonds; removal of methyl groups at positions C4α, C4β,

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Abbreviations: ABS, Antley-Bixler Syndrome; Bpa, bare patche; CDPX2, X-linked dominant chondrodysplasia punctata, Conradi-Hunermann Syndrome; CHILD Syndrome, Congenital Hemidysplasia with Ichthyosiform erythroderma and Limb Defects Syndrome; 8(9)chl, cholesta-8(9)-en 3β-ol; CNS, central nervous system; CYP, cytochrome P450; CYP17A1, sterol 17α-hydroxylase; CYP21A2, sterol 21-hydroxylase; DHC, dehydrocholesterol; 7DHC, 7-dehydrocholesterol; 8DHC, 8-dehydrocholesterol; DHCR7, 3β-hydroxysterol Δ7-reductase; DHCR14, 3β-hydroxysterol Δ14-reductase; DHCR24, 3β-hydroxysterol Δ24-reductase; DSD, disordered sexual development; EBP, emopamil binding protein; ERG, ergosterol; FGFR2, fibroblast growth receptor 2; HEM dysplasia, Hydrops-Ectopic Calcification-Moth-Eaten Skeletal Dysplasia; 3β-HSD, 3β-hydroxysteroid dehydrogenase; HSD17B7, 3β-ketosterol reductase; LBR, Lamin B Receptor; LXR, liver X receptor; MAS, meiosis-activating sterol; PHA, Pelger-Huët anomaly; POR, Cytochrome P450 oxidoreductase; PTCH, patched; SC4MOL, sterol-C-4 methyloxidase-like; SC5D, 3β-hydroxysteroid-Δ5-desaturase; SHH, sonic hedgehog; SLOS, Smith-Lemli-Opitz Syndrome; SMO, Smoothened; SREBP2, sterol regulatory element binding protein; Str, striated; Td, tattered.

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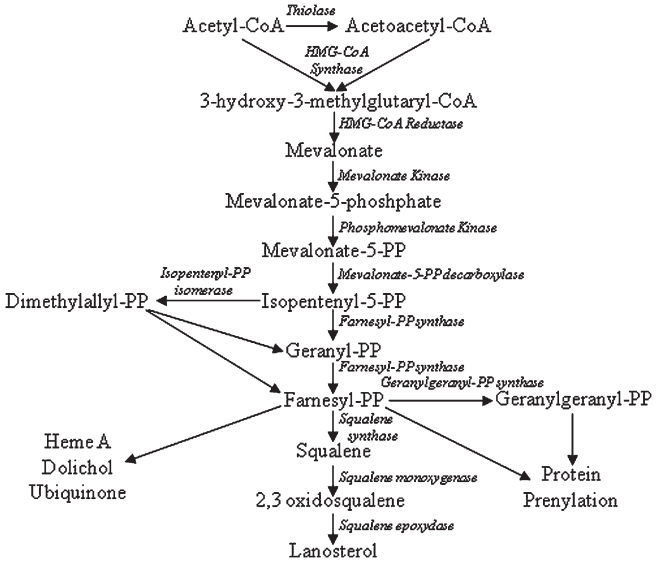

Fig. 1. Presqualene cholesterol synthetic pathway. Cholesterol is synthesized from acetate in a series of enzymatic reactions. The biosynthetic pathway can be divided into two components. The presqualene cholesterol synthetic pathway is depicted in this figure. In addition to cholesterol, isoprenoid precursors are used to synthesize heme A, dolichol, and ubiquinone. Protein prenylation is a post-translational modification that involves the addition of either farnesyl or geranylgeranyl. Protein prenylation plays a role in localizing proteins to cellular membranes. Formation of squalene represents a commitment to sterol synthesis. Squalene undergoes cyclization to form lanosterol, the first sterol in the cholesterol synthetic pathway. PP: pyrophosphate.

and C14 to reduce the C30 precursor lanosterol to a C27 sterol; isomerization of the Δ8(9) double bond to Δ7(8); and a desaturation reaction to introduce the Δ5 double bond found in cholesterol.

The majority of inborn errors of metabolism are catabolic defects involving small organic acids. In these disorders, ready transport across the placenta and maternal metabolism of accumulating intermediates protect the developing fetus prior to birth. Although minor dysmorphic features can occasionally be found in some of these disorders, developmental malformations are not typically associated with inborn errors of metabolism. The inborn errors of cholesterol synthesis are anabolic defects that result in deficiency of cholesterol and accumulation of precursor sterols. In contrast to most small molecule inborn errors of metabolism, the blood-brain and placental barriers limit the ability of maternal metabolism to compensate for the metabolic defect found in the inborn errors of cholesterol synthesis. Unlike in the adult where cholesterol is in steady state, there is net accrual in the fetus [reviewed in (3)]. Studies by both Belknap and Dietschy (4) and Jurevics et al. (5) concluded that the developing rat fetus is able to synthesize sufficient cholesterol and receives little to no cholesterol from the dam. In contrast, Woollett (6) concluded that only ~40% of the mass of fetal cholesterol in the Golden Syrian hamster could be accounted for by fetal synthesis; however, essentially all of the cholesterol could be accounted for if cholesterol synthesized by the uterine membrane and decidua were included. Using the *Dhcr7* mutant mouse model, Tint et al. (7) concluded that early in gestation (prior to a gestational age of 12 d), the dam is the major source of cholesterol and that fetal sterol synthesis becomes the primary source of cholesterol in mid to late gestation. A number of studies, in various species, have evaluated cholesterol transfer from maternal circulation to the fetus (4–12) and Lin et al. (13) showed transfer of ^{14}C-labeled cholesterol from maternal circulation to human fetal tissues. However, it is not clear to what degree maternal cholesterol contributes to a normally developing fetus and if it is necessary for human fetal development. Complicating the interpretation of these results with respect to the impact that maternal cholesterol could have on human fetal development is the fact that in contrast to rodents, the human yolk sac involutes early in development. The blood-brain barrier also contributes to the fetal dependence on endogenous cholesterol synthesis for normal development. A number of studies have shown that both the developing and mature central nervous system (CNS) are dependent on endogenous cholesterol synthesis (5, 7, 14–16). The dependence of fetal development on endogenous synthesis of cholesterol explains why the inborn errors of cholesterol synthesis, in contrast to most inborn errors of metabolism, are associated with significant disruptions of embryonic development.

Over the past couple of decades, a number of human malformation syndromes have been associated with defects in sterol synthesis (Fig. 2; **Table 1**). These include autosomal recessive disorders such as Smith-Lemli-Opitz Syndrome (SLOS), lathosterolosis, desmosterolosis, and sterol-C-4 methyloxidase-like (SC4MOL) deficiency, as well as X-linked dominant disorders such as X-linked dominant chondrodysplasia punctata (CDPX2) and Congenital Hemidysplasia with Ichthyosiform erythroderma and Limb Defects (CHILD) syndrome. Furthermore, impaired cholesterol synthesis has been proposed to contribute to some cases of Antley-Bixler syndrome and Hydrops-Ectopic Calcification-Moth-Eaten Skeletal Dysplasia (HEM dysplasia). Prior reviews on SLOS and malformation syndromes due to inborn errors of cholesterol synthesis include those by Anderson (17), Herman (18, 19), Kelley (20), Kelley and Herman (21), Porter (22–24), and Yu and Patel (25).

To understand the pathological processes underlying the developmental defects found in this group of human malformation syndromes, one needs to consider both the consequences of cholesterol deficiency and the potential consequence of accumulation of bioactive precursor sterols. Whereas cholesterol deficiency is common to these disorders, it is the accumulation of specific precursor sterols that likely contributes to the unique aspects of this series of malformation syndromes. In this paper, we will review the human malformation syndromes due to inborn errors of cholesterol synthesis, what is known about the pathological processes underlying these disorders, and how this understanding may provide insight into pathological mechanisms contributing to more common human diseases.

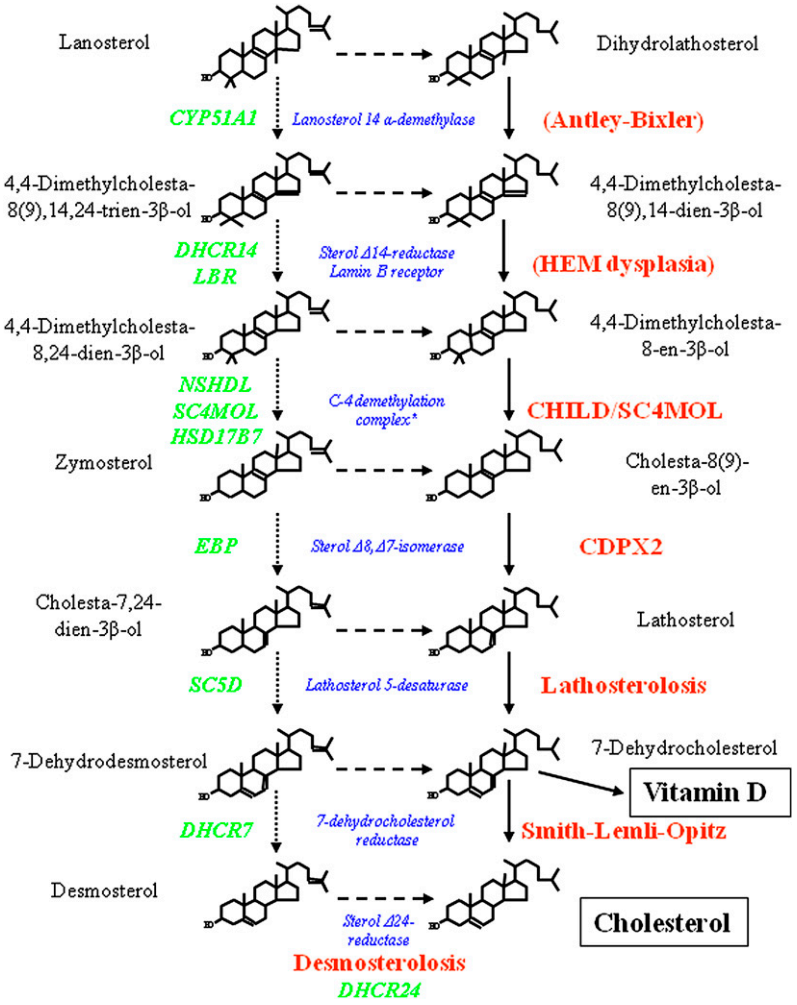

Fig. 2. Postsqualene cholesterol synthetic pathway. This figure depicts the postsqualene cholesterol synthetic pathway. Lanosterol is the first sterol formed after cyclization of squalene. Lanosterol is converted to cholesterol in a series of enzymatic reactions. Two major synthetic pathways exist, primarily distinguished by the timing of the reduction of the Δ24-bond in the aliphatic side chain by sterol Δ24-reductase. If reduction of the Δ24-bond occurs early, cholesterol is synthesized via the Kandutsch-Russel pathway (right side of the figure). This pathway appears to be favored in most tissues. In the Bloch pathway (left side of the figure) reduction of the Δ24-bond occurs as the last enzymatic step that converts desmosterol to cholesterol. Significant levels of desmosterol are found in the developing brain. The enzyme names and the corresponding genes are in italics (small and large type, respectively). *The C-4 demethylation complex consists of three proteins (NSHDL, sterol C-4 methyloxidase, and 3β-ketosterol reductase). Syndromes associated with defects at specific steps are indicated in red, bold type, genes in green, and enzymes in blue. HEM dysplasia and Antley-Bixler syndrome are in parentheses to indicate that they are not simply due to disruption of the corresponding enzymatic reaction. 7DHC is a precursor for both cholesterol and Vitamin D. In skin tissue photolysis of 7DHC in the skin leads to the formation of previtamin D3.

SLOS

SLOS phenotype

SLOS (MIM no. 270400) was the first human syndrome discovered to be due to an inborn error of sterol synthesis (26, 27). "The discovery of the deficiency of 7-dehydrocholesterol (7DHC) reductase as a causative factor of the SLO syndrome made this syndrome the first true metabolic syndrome of multiple congenital malformations" (28). SLOS was first described in 1964 by Drs. Smith, Lemli, and Opitz (29). These physicians described three male patients with distinctive facial features, mental retardation, microcephaly, developmental delay, and hypospadias (type I SLOS). A SLOS variant with a severe phenotype (type II SLOS) was later initially delineated in case reports by Rutledge et al. (30), Donnai et al. (31), and Curry et al. (32). After identification of a common biochemical defect, it was recognized that both type I and type II SLOS phenotypes represent the clinical spectrum of one disorder (33).

The SLOS phenotypic spectrum is extremely broad [reviewed in (20, 34, 35)]. Severely affected cases often die in utero or soon after birth due to major developmental malformations, whereas mild cases combine minor physical findings with learning and behavioral problems. **Figure 3** illustrates typical SLOS phenotypic findings. Most SLOS patients have a distinctive facial appearance (Fig. 3A–C). Although submucosal and U-shaped cleft palates are common, cleft lip is uncommon. A mild manifestation of cleft palate that is frequently observed in SLOS is a bifid uvula (Fig. 3D). The classical facial features become less recognizable in older patients (35, 36). Prenatal cataracts have been described in 12–18% of cases (35, 37), and, although rare, vision-impairing postnatal cataracts can develop rapidly (38). Limb anomalies are common in SLOS. Limb malformations that are frequently present include short proximally placed thumbs, single palmar creases, postaxial polydactyly, and syndactyly of the second and third toes (Figs. 3E, F). Syndactyly of the second and third toes is the most commonly reported physical finding in SLOS patients. Notably, syndactyly of the second and third toes is one of the few malformations observed in a hypomorphic mouse model (39). Although considered a normal variant, given the wide phenotypic spectrum for SLOS, syndactyly of the second and third toes in combination with other malformations, behavioral disturbances, or cognitive issues should prompt consideration of SLOS. Congenital heart defects, including atrioventricular canal, hypoplastic left heart sequence, and septal defects, are common in classical and severe cases (40), and hypertension can be a

TABLE 1. Malformation syndromes associated with inborn errors of cholesterol synthesis

Disorder	MIM number	Inheritance pattern	Gene	Human chromosome	Enzyme
SLOS	270400	Autosomal recessive	*DHCR7*	11q12-13	7DHC reductase
Lathosterolosis	607330	Autosomal recessive	*SC5D*	11q23.3	SC5D
Desmosterolosis	602398	Autosomal recessive	*DHCR24*	1p31.1-p33	DHCR24
CDPX2	302960	X-Linked dominant	*EBP*	Xp11.22-11.23	3β-Hydroxysteroid Δ8,Δ7-sterol isomerase
CHILD Syndrome	308050	X-Linked dominant	*NSDHL*	Xq28	3β-Hydroxysteroid dehydrogenase
SC4MOL	607545	Autosomal recessive	*SC4MOL*	4q32-q34	Sterol C-4 methyloxidase
Antley-Bixler	207410	Autosomal recessive	*POR*	1q11.2	Cytochrome P450 oxidoreductase
HEM dysplasia[a]	215140	Autosomal recessive	*LBR*	1q42.1	Lamin B receptor
			DHCR14 (TM7SF2)	11q13	Sterol Δ14-reductase

[a] As discussed in the text, the HEM dysplasia phenotype is likely due to a laminopathy rather than an inborn error of cholesterol synthesis.

clinical problem (20, 41). Physical gastrointestinal anomalies associated with SLOS, including colonic aganglionosis, pyloric stenosis, and malrotation occur in some patients; however, functional gastrointestinal problems, including gastroesophageal reflux, formula intolerance, and constipation, are frequent problems. Slow growth and poor weight gain are typical. The majority of SLOS infants are poor feeders, and gastrostomy tube placement is required in many cases. Genital malformations are common in male patients, and these can range from various degrees of hypospadias to ambiguous genitalia. Structural brain malformations including holoprosencephaly and abnormalities of the corpus callosum (35, 42–44) can be observed in more severely affected subjects. Phenotypic severity, based on the degree and number of physical malformations, can be quantified using a severity score initially developed by Bialer et al. (45) and subsequently modified by Kelley et al. (20).

Photosensitivity is a common finding in SLOS (35, 46, 47). Both anecdotally (47) and objectively (48), cholesterol supplementation reduces photosensitivity. Anstey et al. (49) characterized the photosensitivity found in SLOS and demonstrated that it was UVA mediated. This group argued that the sensitivity was unlikely simply due to photolysis of accumulating 7DHC, because 7DHC preferentially absorbs shorter wavelength UV than UVA and the lack of correlation between degree of photosensitivity and serum 7DHC levels. They postulated that the UVA sensitivity could be secondary to the photosensitivity of other sterol accumulation in SLOS or lysosomal membrane destabilization due to low cholesterol levels. Based on subsequent work by other groups, it is likely that 7DHC or 7DHC metabolites underlie the UVA sensitivity. Chignell et al. (50) showed that the UVA photosensitivity in SLOS might be a result of oxidative stress induced by photolysis of cholesta-5,7,9(11)-trien-3β-ol or 9-DDHC, and Valencia et al. (51) showed that 7DHC enhances UVA-induced reactive oxidative stress by enhancing free radical-mediated membrane lipid oxidation.

In addition to the physical manifestations, SLOS subjects have a distinct cognitive and behavioral phenotype (52, 53).

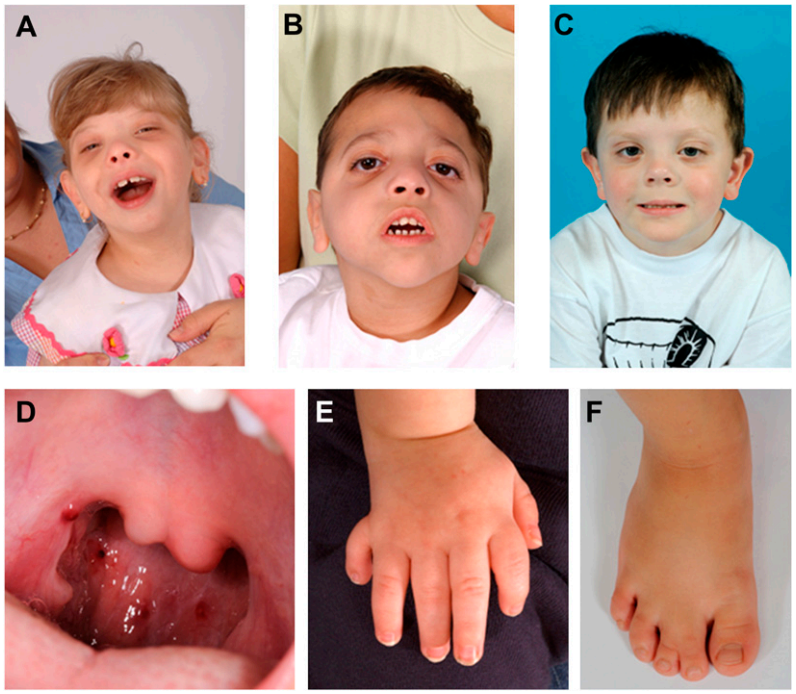

Fig. 3. Common physical findings in SLOS. Facial appearance in severe (A), classical (B), and mild (C) cases of SLOS. Typical facial features include microcephaly, ptosis, midface hypoplasia, small upturned nose, and micrognathia. Cleft palate and submucosal clefts are frequently observed. Bifid uvula (D) are an observable manifestation of a submucosal cleft. Limb anomalies can include short proximally placed thumbs (E), postaxial polydactyly (E), or syndactyly of the second and third toes (F). Permission was obtained from guardians for the publication of these photographs.

Although near normal intelligence is possible (54), moderate to severely impaired cognitive function is typical. SLOS infants are frequently described as irritable, inconsolable, and hypersensitive to stimulation (35, 36, 55). Ryan et al. (35) reported a disturbed sleeping pattern. Self injurious behavior, including head banging and biting, is observed in many patients (52). Autistic and ritualistic behaviors have been noted in SLOS (35, 55). Tierney et al. (53) found that 53% (9/17) of SLOS patients met the Autism Diagnostic Interview-Revised criteria for autism, and Sikora et al. (56) confirmed a very high frequency of Autism Spectrum Disorder in SLOS patients (71–86%), and proposed that the incidence of Autistic Spectrum Disorder may be higher in SLOS than in any other single gene disorder.

Biochemical and molecular aspects

SLOS was first shown to be due to an inborn error of cholesterol synthesis in 1993 by Tint and Irons (26, 27). This group found decreased levels of cholesterol and elevated levels of 7DHC in two SLOS patients. This sterol profile suggested a deficiency of 3β-hydroxysterol Δ7-reductase (DHCR7) activity. DHCR7 catalyzes the reduction of the Δ^7 bond in 7DHC to form cholesterol in the last step of the Kandutsch-Russel cholesterol synthetic pathway (57) (**Figs.** 2 and **4**). In addition to accumulation of 7DHC, elevated levels of 8-dehydrocholesterol (8DHC) can also be detected in serum and tissue from SLOS patients (58). 8DHC is likely formed by the action of 3β-hydroxysteroid- Δ^8-Δ^7-sterol isomerase on 7DHC (58, 59). Data on the enzymology and structure of DHCR7 are limited but have been reviewed by Correa-Cerro and Porter (60). DHCR7 is an NADPH-dependent (61) integral membrane protein. The membrane topology of DHCR7 has not been experimentally determined; however, using computer modeling and analogy to the lamin B receptor, Fitzky et al. (62) proposed that DHCR7 has nine membrane spanning domains, with the N terminus oriented toward the cytoplasm. The human *DHCR7* cDNA sequence predicts a protein of 475 amino acids that has significant homology with 3β-hydroxysterol Δ14-reductase (DHCR14), the C terminus of the lamin B receptor, and several enzymes involved in ergosterol synthesis (ERG3, ERG4, and ERG24).

DHCR7 is encoded by nine exons (62–65). In 1998, three groups independently established that mutations of *DHCR7* were present in SLOS subjects (62, 64, 65).

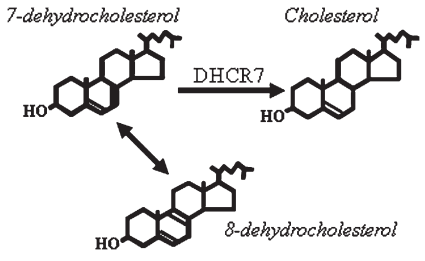

Fig. 4. Reduction of 7DHC. DHCR7 catalyzes the reduction of Δ7-bond in 7DHC to yield cholesterol. In SLOS there is a deficiency of DHCR7 activity that leads to the accumulation of 7DHC and its isomer 8DHC.

Subsequently, over 100 *DHCR7* mutations have been identified in SLOS subjects [reviewed in (25, 60)]. The most common mutation, c.964-1G>C (IVS8-1G>C), is a splice acceptor mutation that accounts for approximately one-third of all reported mutant alleles. Inappropriate splicing of the IVS8-1G>C allele leads to the insertion of 134 bp of intronic sequence into the mRNA transcript (62) and is a null allele (66). Other common (5–10% allele frequency) mutations are p.T93M, p.W151X, p.V326L, and p.R404C. Genotype-phenotype correlations in SLOS are poor (67). Many of the missense mutations result in residual enzymatic activity, and, in general, residual DHCR7 activity is associated with less severe SLOS phenotypes (66). However, factors other than genotype and residual activity appear to significantly influence subject phenotype (67). These could include endogenous factors affecting the function of other genes involved in cholesterol homeostasis and embryonic development or maternal factors. Witsch-Baumgartner et al. (68) reported that the maternal apolipoprotein E genotype is a modifier of the clinical severity of SLOS. Specifically, maternal ε2 genotypes were associated with a more severe phenotype. This finding is supported by work in a SLOS mouse model showing that lack of functional apolipoprotein E potentiates the phenotypic severity (69). Given that ApoE is a major component of lipoproteins in the central nervous system, it is possible that apolipoprotein E genotype could influence cognitive development in SLOS. Further work is necessary to define the environmental, maternal, and genetic factors that contribute to the SLOS phenotype.

Many of the common *DHCR7* mutations can be traced to specific European populations and demonstrate frequency gradients across Europe (70). IVS8-1G>C appears to have arisen in the British Isles and decreases in frequency as one progresses eastward across Europe. In contrast, p.W151X and p.V326L are higher in Eastern Europe and demonstrate a westward gradient across Northern Europe. IVS8-1G>C and p.W151X are estimated to have arisen approximately 3,000 years ago in northwest and northeast Europe, respectively (71). The most common missense mutation, p.T93M, is frequently observed in individuals of Mediterranean heritage (70, 72–74) and is estimated to have arisen approximately 6,000 years ago (71).

Incidence and carrier frequency

SLOS is a relatively frequent autosomal recessive disorder with a high carrier frequency in Caucasians. The incidence of SLOS is reported to be 1:10,000 to 1:70,000 in populations of Northern and Central European heritage (35, 75–78). Based on biochemical testing, Kelley (76) estimated an incidence of 1:50,000 in the United States. In North American populations, the carrier frequency of c.964-1G>C (IVS8-1G>C) is 1% [reviewed in (79)]. In Poland, the combined carrier frequency of p.W151X and p.V326L was found to be 2.4% (80), and in European populations, the combined carrier frequency of IVS8-1G>C and p.W151X ranges from 1.0 to 2.3% (71). Extrapolation of carrier frequency of common *DHCR7* mutations to esti-

mate clinical incidence frequently results in a case incidence much higher than clinically appreciated (79, 81).

The discrepancy between the predicted incidence based on carrier frequency and clinical incidence is likely due to several factors. Underascertainment of mild cases (82, 83) could explain some of this discrepancy. However, the full SLOS phenotypic spectrum and the frequency of mild cases are not likely to be determined until a newborn screening program is implemented. In a meta-analysis of *DHCR7* mutation frequencies, Kelley and Herman (21) found overrepresentation of four common missense alleles and underrepresentation of two null alleles, IVS8-1G>C and p.W151X. They concluded that incomplete ascertainment of null alleles due to prenatal or neonatal death likely leads to skewing of allele frequency in genotyped patients. Nowaczyk et al. (79) and Opitz (84) also concluded that a high frequency of fetal loss occurs. Based on results of prenatal screening for SLOS, Craig et al. (85) estimated a second trimester prevalence of 1 in 101,000, a finding consistent with early prenatal pregnancy loss. Similarly, although the IVS8-1G>C carrier frequency is 0.7% in African Americans (86), few African American cases have been reported.

High carrier frequencies for *DHCR7* mutations suggest the possibility of a heterozygous advantage. Although no direct data is available to support this hypothesis, a number of plausible mechanisms bear further consideration and study. Although cholesterol has been implicated as a major factor in atherosclerotic cardiovascular disease, both the late onset of health problems related to atherosclerosis and the relatively recent dietary changes that contribute to hypercholesterolemia make it unlikely that this is a plausible mechanism to explain a heterozygous advantage that would influence carrier frequency. A more plausible mechanism is that increased vitamin D levels in the skin of DHCR7 heterozygotes could protect against vitamin D-deficient rickets. In addition to being the precursor for cholesterol, 7DHC is the precursor for vitamin D (Fig. 2). Photolysis of the B-ring of 7DHC in the skin leads to the formation of previtamin D_3. Given the association of *DHCR7* mutations with Northern European populations, it is plausible that heterozygosity for a null *DHCR7* mutation may have provided some protection against development of vitamin D-deficient rickets (20). Decreased vitamin D levels have been implicated as a contributing factor for a number of autoimmune disorders (87, 88). Like atherosclerotic cardiovascular disease, the late age of onset for many autoimmune disorders would argue against a significant gain in fitness for a DHCR7 heterozygote. However, multiple sclerosis frequently affects young women, and vitamin D intake is inversely associated with risk of developing multiple sclerosis (89). Another hypothesis is that small, but significant, increases of 7DHC in tissues from heterozygotes might increase resistance to infectious diseases. Many aspects of viral replication and infectivity involve cholesterol (90). Although the functional significance has yet to be defined, an example of modified cholesterol homeostasis in response to viral infection is a selective increase in 7DHC associated with human hepatitis B infection (91). Mycoplasm infectivity, including tuberculosis, is also dependent upon host cholesterol (92). Thus, it is plausible that alterations in cholesterol homeostasis or sterol composition of cellular membranes could influence infectivity by pathogens. Although both potentially attractive and plausible, the concept of a heterozygous advantage for carriers of *DHCR7* mutations and potential mechanisms are hypothetical at this time and require experimental support.

Diagnosis and treatment

A biochemical diagnosis of SLOS is based on demonstration of increased 7DHC in serum or tissues relative to control levels. Serum cholesterol levels can range from <10 mg/dl to normal for age. Because serum cholesterol levels can be within the normal range in mild cases of SLOS, an age-appropriate serum cholesterol level cannot be used to exclude a diagnosis of SLOS. Control reference values for serum 7DHC are 0.1 ± 0.05 μg/ml for children < 10 years old and 0.13 ± 0.06 μg/ml for subjects 10 years and older. Although there are equivocal cases of SLOS with serum 7DHC levels just above control levels, 7DHC levels are typically more than 50-fold elevated. Prenatal diagnosis of SLOS can be accomplished by GC-MS or liquid chromatography-tandem MS sterol analysis of amniotic fluid or chorionic villus (93–95). Alternatively, prenatal diagnosis can be made noninvasively by measuring maternal urine or serum dehydrosteroids (96). Molecular analysis of *DHCR7* is an alternative to biochemical testing; however, it is more expensive. Sequencing of coding exons and adjacent splice domains is estimated to have sensitivity on the order of 96% (http://www.genedx.com/pdf_files/info_sheet_slo.pdf).

In equivocal cases with 7DHC levels just above control levels, sterol analysis of either fibroblasts or lymphoblasts cultured in cholesterol-depleted medium is often useful. This is especially true if two pathogenic *DHCR7* mutations have not been identified. When cultured in cholesterol-depleted medium, endogenous cholesterol synthesis is induced in growing cell cultures. Under these conditions, it is frequently easier to detect accumulation of sterol precursors in comparison to control cultures. A diagnostic algorithm combining biochemical and molecular testing for SLOS has recently been published (20).

After the biochemical defect in SLOS was defined, dietary cholesterol supplementation became standard therapy. The rationale was to both provide an exogenous source of cholesterol and to decrease the endogenous synthesis of 7DHC via cholesterol-mediated downregulation of HMG-CoA reductase. A series of observational case reports showed that dietary cholesterol supplementation both improved the serum cholesterol-total sterol ratio and decreased serum 7DHC levels (36, 47, 97, 98). These studies also reported improved nutritional status, growth, muscle tone, and strength. Dietary cholesterol therapy also appears to improve photosensitivity (48). No long-term, placebo-controlled trials have been performed. In a masked comparison of therapy with 50 mg/kg/day versus 150 mg/kg/day of cholesterol, no differences were noted;

however, the low dose of 50 mg/kg/day exceeds daily cholesterol needs in children, and thus the potential effect of dietary cholesterol supplementation may have already been maximized (20). Dietary cholesterol supplementation has been reported to impact the behavioral manifestations observed in SLOS. Anecdotal reports (20, 36, 47, 97, 99) describe rapid improvement in irritability, tactile defensiveness, sociability, self-injurious behavior, and hyperactivity. Parents of SLOS children frequently relate rapid changes in behavior related to dietary cholesterol supplementation to physicians managing the supplementation. However, these anecdotal reports are difficult to explain mechanistically. A direct effect of cholesterol supplementation on the CNS is difficult to postulate, because dietary cholesterol does not cross the blood-brain barrier (100). However, an indirect mechanism mediated through sterol metabolites, such as neuroactive steroids (101) or oxysterols (102, 103) that are able to cross the blood-brain barrier, is plausible. Another possible indirect mechanism would be an effect of dietary cholesterol on endothelial cell function in brain capillaries and thereby secondary modulation of CNS function. Unfortunately, to date, the anecdotal reports of improved behavior and learning have not been quantified in subsequent studies. Sikora et al. (56) were not able to document improvement in developmental quotients, and Tierney et al. (104) did not detect rapid behavioral changes in a placebo-controlled trial. Although dietary cholesterol supplementation appears to improve the health and well-being of SLOS patients, an impact on behavior and learning has not been demonstrated in a controlled trial.

Simvastatin therapy has also been investigated in SLOS; however, therapeutic efficacy of combined simvastatin and dietary cholesterol supplementation therapy has not yet been substantiated. The initial rationale for simvastatin therapy was to decrease endogenous synthesis of 7DHC, because dietary cholesterol decreases but does not normalize 7DHC levels. Initial case reports provided conflicting data. Jira et al. (105) found that simvastatin therapy paradoxically increased serum cholesterol level in two patients, whereas Starck et al. (106) encountered clinical problems in treating SLOS patients with simvastatin. A potential mechanism that could explain the paradoxical increase in serum cholesterol levels would be increased SREBP2 mediated expression of a mutant *DHCR7* allele with residual enzymatic function. If increased expression of a hypomorphic *DHCR7* allele increases cholesterol synthesis, then, because it crosses the blood-brain barrier, simvastatin might be effective in increasing brain cholesterol synthesis. This hypothesis is supported by both in vitro (66) and in vivo experiments utilizing a hypomorphic mouse model (39). However, to date, a paradoxical increase in serum cholesterol levels in response to simvastatin therapy has not been confirmed. In a retrospective study that included 14 SLOS patients treated with cholesterol and simvastatin, Haas et al. (107) reported a decrease in dehydrocholesterol levels and improvement of the dehydrocholesterol-cholesterol ratio but did not observe an increase in cholesterol levels. Fractional cholesterol synthesis rate has been measured in three SLOS patients comparing a high cholesterol and high cholesterol plus simvastatin treatment regimen (108). Although difficult to interpret due to low power, no significant difference was observed. Reports are also mixed on perceived clinical benefit of combined dietary cholesterol supplementation and simvastatin therapy (105–107, 109). However, to date, a placebo-controlled trial necessary to interpret changes in subjective behavioral symptoms has not been reported.

SLOS animal models

To assist in studying the pathological processes underlying SLOS, both genetic mouse and pharmacological rat models have been developed. Pharmacological inhibitors of DHCR7 that have been used to model SLOS include YM9429 (110, 111), BM15.766 (112–115), and AY9944 (116–119). All three of these compounds appear to be noncompetitive inhibitors of DHCR7 (110, 120). AY9944 crosses the blood-brain barrier and can be used to inhibit cholesterol synthesis in the brain (121). Fliesler et al. (122–126) have extensively used AY9944 to functionally characterize the effect of 7DHC accumulation in rat retinae. Several mouse models of SLOS have been developed. These include two independent null mutations, *Dhcr7*$^{\Delta 3\text{-}5}$ and *Dhcr7*$^{\text{delEx8}}$ developed by Wassif et al. (127) and Fitzky et al. (128), respectively, and a p.T93M knockin hypomorphic mouse (39). The *Dhcr7*$^{\Delta 3\text{-}5}$ and *Dhcr7*$^{\text{delEx8}}$ alleles are null alleles, whereas the *Dhcr7*$^{\text{T93M}}$ allele encodes a protein with residual enzymatic activity and thus is a hypomorphic mutation. Mice homozygous for the null mutation have decreased cholesterol (>5-fold) and markedly increased 7DHC levels (250- to 2000-fold) in serum and tissues (127). In brain tissue, 7-dehydrodesmosterol substitutes for desmosterol. The cholesterol found in *Dhcr7* mutant embryos is likely of maternal origin (7, 9). Phenotypic overlap between the null mouse models and SLOS patients includes: *1*) intrauterine growth retardation; *2*) cleft palate; *3*) poor feeding and an abnormal suck; and *4*) neurological abnormalities, including hypotonia. In comparison to human patients homozygous for null mutations, the mutant mice have very few malformations. This is likely due to differences in the availability of maternal cholesterol in mice and humans during embryogenesis (7, 9). Both *Dhcr7*$^{\text{T93M/T93M}}$ and *Dhcr7*$^{\text{T93M}/\Delta 3\text{-}5}$ mice are viable, fertile, and physical malformations are limited to syndactyly of the second and third digits (39). The toe syndactyly is an interesting finding given that it is the most penetrant physical finding reported in SLOS patients and involves homologous digits. Sterol analyses of tissues from 1-day-old hypomorphic mice show markedly decreased levels of cholesterol and increased levels of 7DHC consistent with the genotypic spectrum (39). However, although sterol levels do not completely normalize, they do correct spontaneously with age in mutant mice with a *Dhcr7*$^{\text{T93M}}$ allele (39, 129). This phenomenon has not been observed in human patients with hypomorphic missense mutations. It is likely a consequence of a combination of higher transcription levels of the hypomorphic mutant allele in mice compared with humans and decreased postnatal need for endogenous

cholesterol synthesis (39). Although the spontaneous improvement in sterol levels makes the hypomorphic mouse model difficult to work with, it does suggest that therapeutic strategies designed to increase the expression of *DHCR7* mutant alleles with residual function may be efficacious.

SLOS pathogenesis

Although the primary biochemical defect underlying SLOS is well defined, the pathophysiological processes that give rise to the physical, cognitive, and behavioral problems found in SLOS are still under investigation. It is unlikely that one single pathophysiological mechanism explains the myriad of symptoms seen in SLOS. Multiple pathological mechanisms are likely due to two primary factors. First, cholesterol has multiple biological functions. Second, normal biological processes could be impaired by a deficiency of cholesterol, a direct toxic effect of DHC, or a toxic effect of DHC-derived metabolites.

Cholesterol is a major lipid component of plasma membranes and, specifically, a structural component of lipid rafts. Lipid rafts are liquid-ordered subdomains composed of cholesterol, sphingolipids, and proteins that play a major role in signal transduction and membrane trafficking (130). Although it substitutes for cholesterol reasonably well in raft formation (131) and behaves similarly to cholesterol in phosphatidylcholine-sterol monolayer films (132, 133), substitution of 7DHC for cholesterol may alter the physiochemical properties and function of cellular membranes. In comparing artificial vesicles formed from admixtures of either cholesterol or 7DHC and egg sphingomyelin, liquid ordered domains formed with 7DHC appeared to be smaller and had more diffuse boundaries than those formed with cholesterol (134). Data published by both Megha et al. (135) and Xu et al. (136) showed that, relative to cholesterol, 7DHC stabilizes lipid rafts in model membranes. Both Tulenko (137) and Staneva et al. (134), using X-ray diffraction techniques, showed that 7DHC results in an atypical membrane organization. In addition to studies with artificial membranes, membranes from SLOS cells have been shown to have altered fluidity (137), and Boesze-Battaglia (124) showed reduced membrane fluidity in rod outer segments derived from AY9944-treated rats due to decreased content of docosahexaenoic acid. These physiochemical alterations have functional consequences on raft protein composition, signal transduction, and membrane trafficking. Keller et al. (131) have shown that the protein composition of lipid rafts purified from AY9944-treated rat brain tissue is altered. Analyses of specific receptor systems have shown dysfunction in SLOS model systems. *Dhcr7* mutant mast cells demonstrate constitutive cytokine release and hyper-degranulation after stimulation of the high affinity IgE receptor (138). These defects appear to result from displacement of Fyn kinase from lipid rafts containing 7DHC and a resulting increase in Fyn kinase activity and Akt phosphorylation (138). Neurophysiological studies have demonstrated that frontal cortex neurons from *Dhcr7* mutant embryos have an impaired N-methyl-D-aspartic acid receptor response to glutamate stimulation (127). 7DHC cannot substitute for cholesterol in restoring ligand binding to solubilized hippocampal serotonin1A receptors (139), and serotonin1A receptor signaling is impaired in AY9944-treated cells (140). These in vitro findings may be mechanistically related to the observation by Waage-Baudet et al. (141) of abnormal serotonergic development in *Dhcr7* mutant embryos and are especially intriguing given the high frequency of autistic symptoms in SLOS patients (53, 56). Alteration of the sterol composition also appears to alter other physiochemical properties of membranes. Gondrè-Lewis et al. (142) found, in comparison to cholesterol, that 7DHC decreases the bending rigidity and intrinsic curvature of artificial membranes. This observation may explain abnormal pancreatic secretory granule formation (142). In addition to altered sterol composition, Pappu et al. (143) demonstrated increased levels of dolichol and ubiquinone synthesis in SLOS and postulated that these nonsterol isoprenoids could alter membrane fluidity, permeability, and function.

In addition to its structural role in cellular membranes, cholesterol is a precursor for the synthesis of steroids, neuroactive steroids, oxysterols, and bile acids. Therefore, in SLOS, there may be a deficiency of the normal cholesterol-derived metabolites or formation of DHC-derived analogs. Both 7DHC and 8DHC can enter the cholesterol biosynthetic pathway, and dehydrocholesterol analogs of pregnenolone, pregnanetriol, dehydroepiandrosterone, and androstenediol have been identified in SLOS patients (144–146). As noted above (SLOS diagnosis and treatment section), these DHC-derived steroids can be used for the prenatal diagnosis of SLOS. The identified dehydrosteroids and dehydrosteroid metabolites suggest that dehydrocholesterol can be transported into the mitochondria and participate in the following enzymatic reactions: p450 side chain cleavage, 17-hydroxylase/17,20-lyase, 3β-hydroxysteroid dehydrogenase, 3α-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, 20α-hydroxysteroid dehydrogenase, and 5β-reductase (144). A 7DHC derived analog of allopregnanolone, 7-dehydroallopregnanolone, has also been identified in urine from postpubertal, female SLOS patients (101). Allopregnanolone is a neuroactive steroid. Neuroactive steroids are modulatory ligands for neurotransmitter and nuclear steroid hormone receptors and have functional roles in neurogenesis, neuroprotection, and myelination (147). It is not known to what degree the steroids synthesized from dehydrocholesterol have biological activity. It is plausible that these DHC-derived steroid analogs have either antagonist or agonistic activity and may functionally contribute to the SLOS cognitive or behavioral phenotype. Unlike cholesterol, neuroactive steroids can cross the blood-brain barrier, thus providing a hypothetical mechanism that could explain anecdotal reports of rapid behavioral effects reported in association with dietary cholesterol supplementation.

Cholesterol is also a precursor for bile acid synthesis. Bile acid deficiency has been reported in severe cases of SLOS (27, 148), and bile acid supplementation therapy has been tried in SLOS (36, 97). However, in a milder cohort of

patients, Steiner et al. (149) observed a normal rate of bile acid synthesis, and bile acid supplementation is not currently a standard therapeutic intervention. Dehydrocholesterol-derived bile acids have been reported. Honda et al. (113) reported 27-hydroxylation of both 7DHC and 8DHC and partial metabolism to 3β-hydroxycholestadienoic acids in BM15.766-treated rats. BM15.766 is a pharmacological inhibitor of DHCR7. Natowicz and Evans (148) reported abnormal bile acids in urine from four SLOS patients. These initial studies have not been pursued in more detail, and it is not known if the dehydrocholesterol-derived bile acids have clinical importance.

Cholesterol is also the precursor for oxysterols. Oxysterols can be derived from cholesterol by either chemical or enzymatic mechanisms and are biologically active [reviewed by Schroepfer (150)]. Sterol 27-hydroxylase catalyzes the formation of 27-hydroxycholesterol, which is the first step in the alternative bile acid synthetic pathway (151). Absence of sterol 27-hydroxylase results in cerebrotendinous xanthomatosis (152). 24S-hydroxycholesterol is synthesized in neurons from cholesterol by CYP46 (153). Oxysterols are biologically active, cross the blood-brain-barrier, and may contribute to disease processes (154, 155). Oxysterols, including 24(S)-hydroxycholesterol, 24(S),25 epoxycholesterol, and, to a lesser extent, 27-hydroxycholesterol, appear to be endogenous ligands for liver X receptors (LXRs), which are critical regulators of cholesterol homeostasis (156–158). Oxysterols can be cytotoxic, induce apoptosis, or modulate the immune response [reviewed by Vejux and Lizard (159)]. Altered oxysterol homeostasis may contribute to or be a marker of various neurodegenerative diseases such as Alzheimer disease, Parkinson disease, multiple sclerosis, and macular degeneration (155, 159–162).

It is plausible that oxysterols may contribute to the pathology of SLOS. Björkhem et al. (102) found decreased levels of 24(S)-hydroxycholesterol and increased levels of 27-hydroxycholesterol in serum from SLOS patients. Decreased 24(S)-hydroxycholesterol is consistent with decreased brain cholesterol turnover. However, increased 27-hydroxycholesterol levels are more puzzling. Although increased production was not excluded, decreased levels of 3β,7α-dihydroxy-5-cholestenoic acid suggested that the increased 27-hydroxycholesterol levels were due to decreased metabolism (102). Wassif et al. (103) identified markedly elevated levels of both 27-hydroxy-7DHC and 27-hydroxy-8DHC in serum from SLOS patients compared with control levels (**Fig. 5**). It is plausible that these novel oxysterols have biological activity and thus may have a functional role in the development of the SLOS phenotype. Consistent with this hypothesis, increased levels of 27-hydroxy-7DHC in the SLOS mouse model are associated with a more severe phenotype (163). Chemically produced oxysterols may also play a role in SLOS pathogenesis. 7DHC is highly reactive. Xu et al. (164) have recently identified a series of 7DHC-derived oxysterols, and Korade et al. (165) have shown that oxysterols formed from 7DHC peroxidation were cytotoxic to neuroblastoma cells in micromolar concentrations (165). Oxidative stress may also

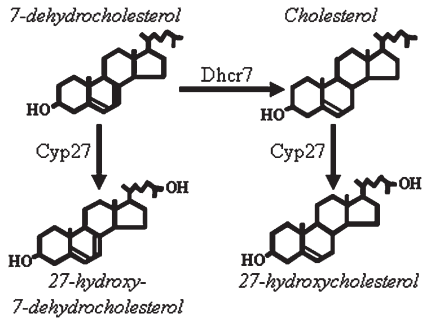

Fig. 5. Oxysterol synthesis in SLOS. DHCR7 catalyzes the reduction of the Δ-7-bond in 7DHC to yield cholesterol in the final step of cholesterol synthesis. CYP27 normally functions to synthesize 27-hydroxycholesterol from cholesterol. 27-Hydroxy-7DHC and 27-hydroxy-8DHC have been identified in serum from SLOS patients. They likely arise due to CYP27 hydroxylation of 7DHC or 8DHC, respectively. 8DHC and the corresponding oxysterol are not depicted in this figure.

result in oxidation of lipids and proteins in addition to cholesterol. This occurs in the AY9944 rat model and has been proposed to contribute to the pathology of SLOS (126, 166). Additional work is necessary to establish the extent to which either oxidative stress or oxysterols functionally underlie or contribute to the pathology of SLOS.

Many of the malformations found in SLOS are consistent with impaired sonic hedgehog (SHH) function. SHH is a signaling factor that functions in the patterning and growth of embryonic structures, including the central nervous system, facial structures, and limbs. SHH is post-translationally modified by cholesterol (167–169) and palmitic acid (170). Lipid-modified SHH is secreted by the signaling cell, and cholesterol modification of SHH limits diffusion and is important for proper gradient formation (171). Secreted SHH binds to the receptor Patched (PTCH) in the responding cell. PTCH then regulates transmembrane signaling in the responding cell by relieving inhibition of Smoothened (SMO). PTCH and SMO are localized to the primary cilium, and activation of SMO initiates a regulatory cascade that involves activation of glioma-associated oncogene homolog family transcription factors [reviewed in (172)]. A number of mechanisms by which SHH signaling might be impaired in SLOS have been postulated. Cooper et al. (173) demonstrated that dehydrocholesterol does not appear to directly inhibit the procession of SHH, but the reduced total sterol levels in *Dhcr7* mutant mouse embryonic fibroblasts impaired SHH signaling in the responding cell by inhibiting SMO. Another potential mechanism that has been proposed includes a direct interaction between the N terminus of DHCR7 and SMO that could be impaired due to mutation of DHCR7 (174). Metabolites of 7DHC could modulate signaling by this pathway. Studies by Bijlsma et al. (175) suggest that PTCH-mediated transport of vitamin D3 (a metabolic product of 7DHC) modulates SMO function, and oxysterols stimulate hedgehog signaling (176, 177). Thus, it is plausible that 7DHC or oxysterols derived from 7DHC could impair SHH signaling.

A number of de novo discovery studies have used pharmacological or genetic animal models CNS SLOS to

identify biological pathways that contribute to the SLOS phenotype. Lipidomic analysis of retinal tissue from AY9944-treated rats demonstrated a significant decrease in docosahexaenoic acid containing phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine species (125). Although these findings need to be confirmed in SLOS subjects, studies such as these demonstrate alteration of metabolic pathways in addition to the primary defect in cholesterol biosynthesis that could contribute to a pathological cascade resulting in the SLOS phenotype. These other metabolic pathways may be amendable to therapeutic intervention. Waage-Baudet et al. (178) reported microarray expression profiling of hindbrain tissue from *Dhcr7* mutant, heterozygous, and control E14 embryos. Hierarchical clustering analysis identified, in addition to genes involved in cholesterol homeostasis, altered expression of genes involved in apoptosis and cell cycle control, development and morphogenesis, vesicular transport, neurodegeneration, neural cytoskeleton, and axonal guidance. Functional analysis confirmed a defect in the netrin/deleted in colorectal cancer axonal guidance pathway that may contribute to defects in corpus callosum formation. Proteomic analysis of E18.5 embryonic *Dhcr7* mutant brain tissue demonstrated altered expression of proteins involved in apoptosis, intracellular trafficking, glycolysis, cytoskeleton, and mevalonate metabolism (179). Based on the identification of increased cofilin-1 phosphorylation in *Dhcr7* mutant brain tissue, Jiang et al. (180) demonstrated aberrant activation of the Rho-Rock-Limk-Cofilin-1 and Rac/Cdc42-Pak-Limk-Cofilin-1 pathways. Consistent with the functional role these Rho GTPase-regulated pathways are known to play in dendrite and axonal formation (181), axon and dendrite growth was demonstrated to be abnormal in *Dhcr7* mutant hippocampal neurons (180). Altered Rho/Rac signaling underlies other human syndromes with cognitive impairment (182), and it is plausible that aberrant axonal/dendritic growth combined with defects of axonal guidance contribute to the cognitive deficiencies found in SLOS patients.

Smith-Lemli-Opitz-like syndromes: desmosterolosis and lathosterolosis

Two very rare autosomal recessive malformation syndromes, desmosterolosis and lathosterolosis, have been referred to as SLOS-like syndromes due to phenotypic overlap with SLOS. Given that the initial cases of desmosterolosis and lathosterolosis were ascertained while testing for SLOS, phenotypic overlap is to be expected. Due to the rarity, the aforementioned ascertainment bias and the broad phenotypic range observed to date, the phenotypic spectrums of desmosterolosis and lathosterolosis are not fully delineated.

Desmosterolosis

Desmosterolosis (MIM no. 602398) is an inborn error of cholesterol synthesis due to impaired reduction of the Δ^{24} bond in the aliphatic side chain of cholesterol. Reduction of the Δ^{24} bond is catalyzed by 3β-hydroxysterol Δ24-reductase (DHCR24), and this reduction can occur at different times in the cholesterol synthetic pathway. Reduction of the Δ^{24} bond occurs early in the Kandutsch-Russel cholesterol synthetic pathway (57) but is the penultimate step in the Bloch pathway of cholesterol synthesis (183). In the Bloch pathway of cholesterol synthesis, DHCR24 reduces desmosterol to yield cholesterol (Fig. 2). Although its functional role has not been defined, desmosterol accumulates in the developing CNS just prior to the onset of myelination (184–186). Desmosterol is also present in the testes (187) and spermatozoa (188, 189). Its concentration in the flagella suggests a role in sperm motility. Thus, in addition to being a sterol precursor of cholesterol, desmosterol likely has independent functions. The diagnosis of desmosterolosis is made by demonstrating elevated levels of desmosterol by GC-MS analysis of serum or tissue sterols. In the case reported by Anderson et al. (190), serum cholesterol levels were normal but desmosterol was increased 120-fold above control levels (60 μg/ml vs. control values of 0.5 ± 0.3 μg/ml). In the case reported by Fitzpatrick et al. (191), the tissue desmosterol-cholesterol ratio was increased at least 6-, 107-, and 11-fold above control values for brain, liver, and kidney tissue, respectively.

DHCR24 was initially identified as a transcript with markedly reduced expression in the inferior temporal lobes of patients with Alzheimers disease and was called seladin-1 (192). In addition to its role in cholesterol synthesis, DHCR24 protects cells against oxidative stress-induced apoptosis (193–195). Subsequently, Waterham et al. (196) recognized that the corresponding gene encoded *DHCR24* based upon its homology with an analogous sterol reductase from *Arabidopsis thaliana* (DWF1/DIM). *DHCR24* encodes a predicted 516 amino acid polypeptide with one transmembrane domain. Based on sequence homology, DHCR24 belongs to a family of FAD-dependent oxidoreductases. Enzymatic activity is stimulated by FAD and is dependent on NADPH (196). Similar to other enzymes involved in postsqualene cholesterol synthesis, DHCR24 is present in the ER; however, in response to oxidative stress, DHCR24 translocates to the nucleus (195). Activity reducing missense mutations of *DHCR24* have been reported in two patients with desmosterolosis (196).

Phenotypic descriptions have been published for only two cases of desmosterolosis (191, 197). The case reported by FitzPatrick (191) involved a 34 week estimated gestational age premature infant with SLOS-like features of thick alveolar ridges, gingival nodules, cleft palate, short limbs, severe congenital heart defect, and ambiguous genitalia. In addition to the SLOS-like phenotypic findings, this infant had microcephaly and generalized osteosclerosis. Three *DHCR24* missense mutations (p.Y471S/p.N294T and p.K306N) were identified in this child, and all three missense mutations independently decreased DHCR24 activity (196). The case reported by Andersson (197) resembled SLOS in that this child had agenesis of the corpus callosum, micrognathia, submucosal cleft palate, club foot, and congenital heart disease. In contrast to the first case, this child had severe microcephaly (−7 SD at 3 years of age). Waterham et al. (196) demonstrated that this child was homozygous for a deleterious p.E191K mutation of

DHCR24. Three additional cases of desmosterolosis have been identified but not published (Richard Kelley, personal communication). Two of the cases were noted to have severe microcephaly and agenesis of the corpus callosum in utero.

Pharmacological and genetic models of desmosterolosis have been developed. The developmental effects of Dhcr24 inhibition using either triparanol (198) or U18666A (199) have been studied in rats. Cenedella (200) has reviewed the use of U18666A in studies of sterol metabolism and trafficking. Wechsler et al. (201) reported the generation of a viable mouse model of desmosterolosis. *Dhcr24* mutants are growth retarded and infertile but can survive to adulthood. At 3 months of age, sterol analysis of plasma and liver tissue showed that desmosterol accounted for 99% of total sterols. The lack of malformations in the genetic mouse model, in comparison to both human patients and pharmacological models of desmosterolosis (202), is likely due to availability of maternal cholesterol during embryogenesis in the mouse. The viability of *Dhcr24* mutant mice is variable. Mirza et al. (203) reported defects in skin development that resulted in a lethal dermopathy. This dermopathy results in increased transepidermal water loss and may be due to inappropriate expression of aquaporin-3 (204). Consistent with the hypothesis that accumulating sterol intermediates are biologically active and thus may contribute to the pathology of these disorders, accumulation of desmosterol in *Dhcr24* mutant mice stimulates expression of LXR-target genes (205).

Lathosterolosis

Lathosterolosis (OMIM no. 607330) results from impaired 3β-hydroxysteroid-Δ5-desaturase (SC5D) activity. In the Kandutsch-Russel synthetic pathway, SC5D catalyzes the conversion of lathosterol to 7DHC in the enzymatic step immediately preceding the defect in SLOS, whereas in the Bloch pathway of cholesterol synthesis, SC5D catalyzes the conversion of cholesta-7,24-dienol to 7-dehydrodesmosterol (Fig. 2). Arthington et al. (206) initially cloned the *ERG3* gene from *Sacchromyces cerevisiae*. *ERG3* encodes a C-5 sterol desaturase essential for ergosterol biosynthesis that is homologous to SC5D. Ergosterol is the major sterol synthesized by yeast. Matsushima et al. (207) cloned the human *SC5D* gene based on its homology to ERG3 and mapped it to chromosome 11q23.3. To date, deleterious mutations of SC5D have been reported in only two families (208, 209).

The initial case of lathosterolosis was reported in 2002 by Burnetti-Pierri et al. (208). Updated descriptions along with a description of an aborted sibling have subsequently been published (210, 211). The proband had multiple malformations typically observed in SLOS, including microcephaly, bitemporal narrowing, ptosis, cataracts, anteverted nares, micrognathia, and postaxial polydactyly. Syndactyly was present but involved the second through fourth toes. Two *SC5D* missense mutations, p.R29Q and pG211D, were identified in this family. The second reported case of lathosterolosis was identified by Krakowiak et al. (209). This case was initially reported by Parnes et al (212) as a case of SLOS associated with nonneuronal mucolipidosis. Phenotypic findings in this case included microcephaly, ptosis, congenital cataracts, micrognathia, broad alveolar ridges, postaxial polydactyly, second to third cutaneous toe syndactyly, and ambiguous genitalia. Sequence analysis demonstrated that this child was homozygous for p.Y46S mutations of *SC5D* (209). Parents were not consanguineous; however, both were of French Canadian ancestry. The mucolipidosis observed in the case reported by Parnes was not observed in the two Italian siblings; however, lamellar lysosomal inclusions could be induced in cultured fibroblasts from both families (209, 210).

Krakowiak et al. (209) disrupted *Sc5d* to produce a lathosterolosis mouse model. *Sc5d* mutant pups were stillborn and had craniofacial malformations, including cleft palate and limb defects such as postaxial polydactyly. Comparison of abnormalities in both the SLOS and lathosterolosis mouse models can be used to help separate problems that are caused by decreased cholesterol/sterol from those that are specifically due to increased 7DHC or lathosterol. Cholesterol levels are decreased to a similar extent in both *Dhcr7* and *Sc5d* mutant embryos, but the accumulating intermediates are 7DHC and lathosterol, respectively. Jiang et al. (179) reported proteomic analysis of both *Dhcr7* and *Sc5d* mutant brain tissue. Consistent with the defect being due to decreased cholesterol rather than a toxic effect of increased 7DHC, cofilin 1 phosphorylation is similarly altered in both the SLOS and lathosterolosis mouse models (180).

Hydrops-Ectopic Calcification-Moth-eaten skeletal dysplasia

Hydrops-Ectopic Calcification-Moth-eaten skeletal dysplasia (HEM dysplasia, Greenberg Dysplasia, MIM no. 215140) is an autosomal recessive, lethal skeletal dysplasia first described by Greenberg et al. (213). The phenotypic, radiological, and histological aspects of HEM dysplasia have been described by several groups (213–215). The HEM dysplasia phenotype includes hydrops fetalis, cystic hygroma, rhizomelic and mesomelic shortening of the limbs, postaxial polydactyly, incomplete lung lobation and pulmonary hypoplasia, intestinal malrotation, and extramedulary hematopoiesis. Radiographic findings are diagnostic with a characteristic "moth-eaten" or mottled appearance of the long bones, platyspondyly, ectopic ossification of both the ribs and pelvis, absent distal phalanges, and deficient ossification of the skull. Histological analysis is notable for disorganization of both cartilage and bone with absence of cartilage column formation, nodular calcifications in cartilage, and islands of cartilage surrounded by lamellar-like bone. The possibility that HEM dysplasia was an inborn error of cholesterol synthesis was first raised by Kelley (21) due to the finding that sterol analysis of tissue from HEM dysplasia fetuses had minor (<1% total sterols), but clearly abnormal, elevations of cholesta-8(9), 14-diene-3β-ol, and cholesta-8(9),14,24-trien-3β-ol (Fig. 2). Accumulation of these two cholesterol precursors would be consistent with impaired sterol Δ^{14}-reductase activity. Because cholesterol levels were normal, to explain the severe

HEM dysplasia phenotype, it was postulated that the malformations were a result of hormonal-like activity of the Δ^{14}-sterols rather than a sterol deficiency (21). Unique among the postsqualene cholesterol biosynthetic steps, two different proteins can catalyze the reduction of Δ^{14}-sterols. These are DHCR14 (TM7SF2, SR-1) and the lamin B receptor (LBR). DHCR14 localizes to the ER, catalyzes the reduction of C14-C15 unsaturated sterol intermediates, and has a high degree of sequence similarity with DHCR7 (216, 217). LBR localizes to the inner nuclear membrane (218), has a carboxyl-terminal domain homologous to both DHCR14 and DHCR7 (216), and has sterol Δ^{14}-reductase activity (219). Based on the observation of increased Δ^{14}-sterols in HEM dysplasia tissue (21), Waterham et al. (220) performed sequence analysis on both *DHCR14* and *LBR* and identified a homozygous mutation of *LBR* in a fetus with HEM dysplasia. Based on this finding, they concluded that HEM dysplasia is due to sterol Δ^{14}-reductase deficiency and that LBR functions as the primary sterol Δ^{14}-reductase in human cholesterol biosynthesis (220). Interestingly, heterozygous mutations of LBR result in hypolobulated neutrophil nuclei, a finding known as Pelger-Huët anomaly (PHA) (221). Homozygosity for PHA has previously been described and appears to result in a spectrum of phenotypes (222, 223). Oosterwijk et al. (222) reviewed 11 cases of homozygous PHA and 8 cases of HEM dysplasia. Homozygous PHA appeared to be phenotypically distinct from HEM dysplasia, with only mild skeletal manifestations (short stature, short metacarpals, and polydactyly) being observed in 5 of 11 (45%) cases. The mild phenotype observed for homozygous PHA is also in contrast to the severe phenotype found in a rabbit model (224). Oosterwijk et al. (222) concluded that the difference is likely related to allelic heterogeneity and suggested that the two phenotypes correlate to the two functional domains of LBR. Specifically, the mild phenotype is related to a defect in nuclear membrane function, and the more severe HEM dysplasia phenotype is an inborn error of cholesterol synthesis. To date, data have not been published on specific mutations or sterol analysis in homozygous PHA patients and only in a few HEM dysplasia cases. However, it is unlikely that LBR is the primary sterol Δ^{14}-reductase in cholesterol biosynthesis and that HEM dysplasia is due to sterol Δ^{14}-reductase deficiency. First, the sterol defect reported in HEM dysplasia is minimal (21), and second, subsequent mouse work does not support this hypothesis. Mutations of *Lbr* are found in the ichthyosis mouse (225). Wassif et al. (226) disrupted the murine *Dhcr14* gene and measured tissue sterol levels in *Dhcr14*, *Lbr*, and compound *Dhcr14/Lbr* mutant mice. Their data strongly support the conclusion that Dhcr14 and Lbr provide significant redundancy with respect to sterol Δ^{14}-reductase activity and that accumulation of Δ^{14}-sterols does not underlie the mouse ichthyosis phenotype. Sterol Δ^{14}-reductase enzymatic redundancy is also supported by the work of Bennati et al. (227), which confirmed that disruption of *Dhcr14* does not significantly impair cholesterol biosynthesis. The combined available data support the hypothesis that HEM dysplasia and ichthyosis due to *LBR/Lbr* mutations are laminopathies rather than inborn errors of cholesterol synthesis.

CDPX2

CDPX2 (Conradi-Hunermann syndrome; MIM no. 302960) is a rare, X-linked, often male-lethal disorder associated with skin, skeletal, and ophthalmologic anomalies [for a recent review, see (18)]. Asymmetry in the skeletal findings and patterned skin lesions result from random X-inactivation in affected tissues in heterozygous females. The defective gene is called *EBP*, emopamil binding protein, and encodes a Δ^{8}-Δ^{7}-sterol isomerase that converts cholest-8(9)-en-3β-ol or zymosterol to lathosterol or cholesta-7,24-dien-3β-ol, respectively (Fig. 2) (228, 229). Recently, several males with hypomorphic *EBP* mutations and a phenotype distinct from that of CDPX2 have been described (230, 231).

Clinical features of CDPX2

The major clinical findings in heterozygous females with CDPX2 are typically present at birth and involve the skin and skeleton (**Fig. 6**A–D). There is rhizomelic shortening of the limbs that is often asymmetric. Radiographs in infancy demonstrate epiphyseal stippling (chondrodysplasia punctata) due to abnormal calcium deposition (Fig. 6B). Epiphyseal stippling can be found in numerous genetic and acquired disorders; however, the stippling found in CDPX2 is often more widespread, with involvement of the vertebrae and tracheal cartilage, in addition to the long bones (232). Stippling cannot be detected after normal epiphyseal ossification occurs. Short stature and scoliosis are common and may be congenital. Clubfoot, joint contractures, and vertebral anomalies have also been reported. The latter may contribute to scoliosis. There is a characteristic craniofacial appearance with frontal bossing, midface hypoplasia, and a flat nasal bridge. Postaxial polydactyly occurs in about 10% of reported cases and appears to be most common in this form of inherited CDP (18, 233, 234). Adult height is often reduced, averaging 60–63 inches in mildly affected females (235).

Skin findings at birth typically include a scaling, erythematous eruption with a linear or patchy distribution that follows lines of X-inactivation, the so-called lines of Blaschko (236, 237) (Fig. 6A). Occasionally, a diffuse erythroderma may be present (238). The initial eruption usually fades over the first several months of life and may be replaced with linear or whorled pigmentary abnormalities (hyper- or hypopigmentation) and/or atrophic patches that may involve hair follicles (follicular atrophoderma) (Fig. 6C). The nails may occasionally be involved, but the teeth are normal. The hair has been described as coarse and lusterless and patches of scarring alopecia are common (Fig. 6D).

Histologic examination of involved regions of ichthyosis in infancy demonstrates hyperkeratosis, acanthosis, and parakeratosis, with the presence of calcium in the stratum corneum and follicular plugging. The latter features are not found in involved skin in CHILD Syndrome (see below) or in other types of inherited ichthyoses (239, 240).

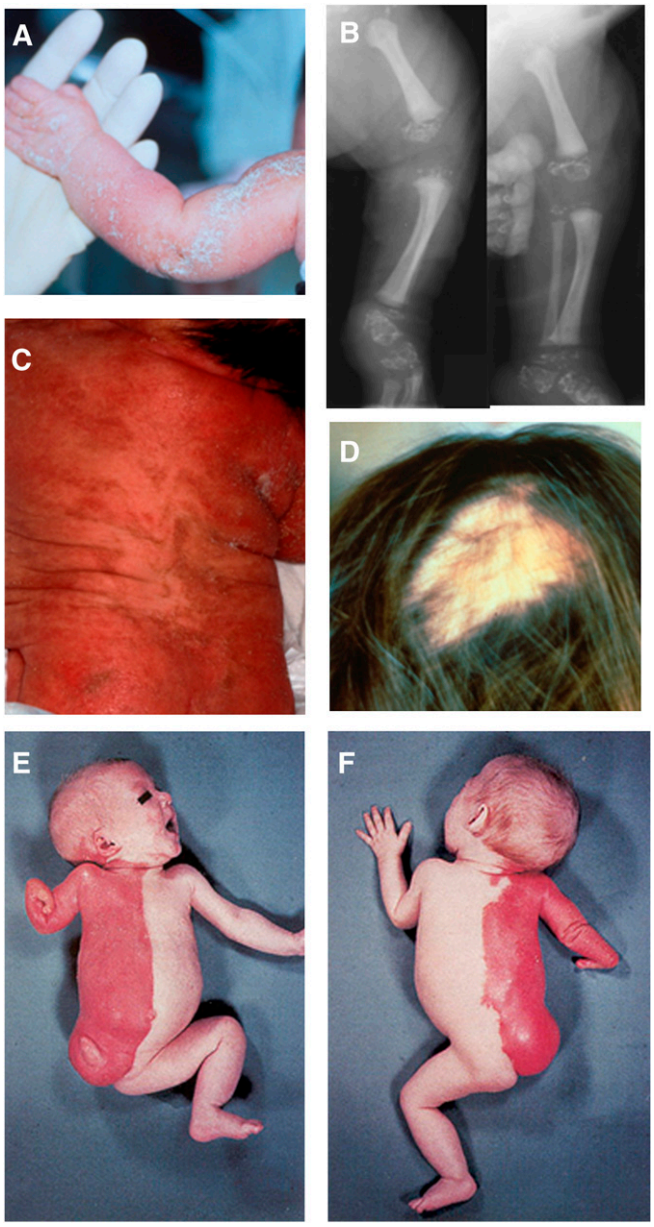

Fig. 6. Phenotypic findings in CDPX2 and CHILD syndrome. Typical skin findings observed at birth in patients with CDPX2 include scaling and erythematous eruption in a linear or patchy distribution that follows the lines of Blaschko. This distinctive patterning is due to X-inactivation. B: Radiographs demonstrating epiphyseal stippling or chondrodysplasia punctata in a patient with CDPX2. Later hyperpigmentation at age 2 months following lines of Blaschko on the back (C) and scarring alopecia (D) in CDPX2. Unilateral, sharply demarcated, erythematous, ichthyosiform nevus, and ipsilateral limb reduction are characteristic findings in CHILD Syndrome (E and F). The photographs in this figure were originally published in reference (18) and reproduced with permission from Elsevier Ltd.

Inflammatory infiltrates of neutrophils or lymphocytes may be found in areas of involved skin.

Cataracts are found in approximately 65% of affected females. They are often congenital and may be bilateral, unilateral, or sectorial (234, 241). Microphthalmia and microcornea have also been reported. In one series of females with CDPX2 and sterol isomerase mutations, 10 of 12 females for whom information was available had cataracts (83%) (233). Sensorineural hearing loss has been identified in about 10% of patients, and there are single reports of affected females with a tethered cord, Dandy-Walker malformation, and cervical myelopathy. Intelligence is usually normal. Other reported visceral anomalies include congenital heart disease, hydronephrosis, and other developmental renal anomalies. Newly diagnosed patients should receive a renal ultrasound and an echocardiogram if there is a murmur present.

The phenotype of affected females is extremely variable. At the severe end, one can see early fetal loss and stillbirths with severe skeletal and internal anomalies (242). In these cases, a skeletal dysplasia, with asymmetric shortening of the long bones detected on prenatal ultrasound may suggest CDPX2, among other diagnoses (243). The ability to screen for mutations in the X-linked *EBP* gene in patients with suspected CDPX2 has resulted in the identification of much more mildly affected individuals. Some gene carriers may demonstrate only short stature, and completely asymptomatic carrier mothers of infants with classic CDPX2 have been identified (233, 235). Gonadal mosaicism has also been described, which is important for genetic counseling regarding recurrence risks.

Several males with typical features of CDPX2 have been reported with a 47,XXY karyotype or somatic mosaicism as a mechanism (235, 244–246). However, five males with 46, XY karyotypes, a neurodevelopmental phenotype, and mutations in the Δ^8-Δ^7-sterol isomerase gene have been reported (230, 231). Although there is some phenotypic overlap with features seen in females with CDPX2, all three surviving males had moderate to severe mental retardation. Central nervous system (CNS) malformations (agenesis or hypoplasia of the corpus callosum, Dandy-Walker malformation) were seen in three of the four patients for whom clinical information was available. Other reported malformations included cleft lip and palate (one patient), hypospadias or hypoplastic genitalia (three), cataract (one), renal anomalies (two), and postaxial polydactyly (one). Two of the males had stippled epiphyses, and one demonstrated ichthyosis. The facies, where reported, were dysmorphic with hypertelorism, prominent nasal bridge, and micrognathia. All of the males had the typical abnormal sterol profiles found in females with CDPX2 (see below), although molecular analysis with an *EBP* mutation was reported in a single case (231). In this patient, hypotonia, seizures, ptosis, and patchy hypopigmentation were also noted. Some of the features in these males demonstrate overlap with SLOS-like disorders involving more distal enzymes in the pathway. It is presumed that the sterol isomerase mutations in these males would function as hypomorphs, with some residual enzymatic activity accounting for their postnatal survival (age 1 day–4 years at time of biochemical diagnosis; L. Kratz, personal communication).

Sterol biochemistry and molecular biology

In 1999, Kelley et al. (247) found abnormal elevations of sterol metabolites in tissue samples from several females

with CDPX2. Specifically, they noted increased levels of 8DHC and cholesta-8(9)-en 3β-ol [8(9)chl], a unique pattern of sterols not previously found in any genetic disorder or following treatment of cells with pharmacologic inhibitors. Elevation of 8(9)chl suggested a block at the level of 3β-hydroxysteroid- Δ^8-Δ^7-sterol isomerase (Fig. 2). The accumulation of 8DHC is presumed to result from the action of lathosterol 5-desaturase on the increased levels of 8(9)chl. We (233) and others (228, 248, 249) have reported elevations of these compounds in plasma from most, but not all, females diagnosed with CDPX2 subsequently determined to have mutations in the *EBP* gene. In our series (233), the mean plasma 8DHC in affected females was 3.8 μg/ml ± SD 3.5 and ranged from 0 to 14.9 (normal < 0.1). Similarly, the mean plasma 8(9)chl was 9.8 μg/ml ± SD 10.3 and ranged from 0.14 to 41.3 (normal < 0.1). In a series of 105 females with presumed CDPX2 studied in their laboratory, Kratz and Kelley (personal communication) found plasma levels of 8(9)chl of 0.18–186 μg/ml and 8DHC of <0.01–138 μg/ml (normal < 0.01). Plasma total cholesterol levels did not differ from those of the general population. It should be noted that the levels of these sterol intermediates are typically 1–10% of total plasma sterols, much lower than the levels of intermediates found in SLOS. However, the detection of specific elevations of 8DHC and 8(9)chl in plasma in a female with clinical features of CDPX2 has a high correlation with detection of an *EBP* mutation. Heterozygous *EBP* mutations were identified in 20 of 22 females (91%) with the characteristic abnormal sterol profile in the series of Herman et al. (233). Similar results have been found by others (248, 249). Thus, plasma sterol analysis can be a useful biochemical screening test for CDPX2, particularly in atypical cases.

The gene defect underlying CDPX2 was identified in 1999 by two groups based on the pattern of sterol abnormalities (228) and by homology with the X-linked mouse mutant tattered [(229) and see below]. The gene called EBP was originally identified as a sigma type receptor for a variety of drugs, including tamoxifen (250, 251). It was subsequently shown to have Δ^8-Δ^7-sterol isomerase activity and can complement *S. cerevisiae* that lack the yeast ortholog (ERG2) (228).

The *EBP* gene maps to Xp11.22, spans ~7.0 kb, and has five exons, four of which are coding. More than 50 different mutations have been reported in the gene in females with CDPX2 (252). Missense, nonsense, frameshift, and splicing mutations have been found throughout the gene as well as small insertions and deletions. Whittock et al. (249) provide a nice summary and schematic of the 49 different mutations identified by 2003. There are several recurrent mutations, primarily at cytosine phosphate guanine dinucleotides representing mutation "hot spots" (233). There are no genotype/phenotype correlations, and wide phenotypic variation within a single family has been noted (235). These findings are likely due to the fact that random X-inactivation is the major determinant of clinical severity in affected tissues in individual female patients. As noted above, survival of males with a CDPX2-like phenotype has been associated with 47,XXY karyotypes or somatic mosaicism, whereas survival of hemizygous males with a neurologic phenotype is likely secondary to residual enzyme activity and a hypomorphic mutation.

Finally, several females with focal dermal hypoplasia caused by mutations in the X-linked *PORCN* gene and submicroscopic deletions that include the adjacent *EBP* locus have been described (253, 254). Interestingly, none of the females exhibited any features of CDPX2. All such patients had extreme skewing of X-inactivation (>95%) in favor of the normal X chromosome, likely accounting for their survival and lack of additional phenotypes.

Pathogenesis and mouse models

The X-linked male lethal Tattered mouse mutant (*Td*) is a model for human CDPX2. Two alleles are known: Td^{1H}/+ results from the *Ebp* missense mutation p.G107R (229), and Td^{Ho}/+ contains the adjacent "*cis*" missense mutations p.L132P and p.S133C (255). Affected heterozygous *Td* females are dwarfed, may have cataracts, and exhibit a hyperkeratotic eruption on postnatal day 4–5 that resolves and results in striping of the adult coat. As with human CDPX2 patients, cholest-8(9)-en-3β-ol and 8DHC accumulate in plasma from *Td*/+ females (229, 255). Affected hemizygous male embryos die between E12.5 and birth, depending on their genetic background, and exhibit nonimmune hydrops with a short-limbed skeletal dysplasia, cleft palate, and absent intestines (229). In Td^{Ho} male embryos, diminished expression of embryonic globin genes was detected at E12.5, as well as increased apoptosis of fetal, yolk-sac derived erythrocytes (256). The authors speculate that defective erythropoiesis may contribute to the male embryonic lethality. No further characterizations of these mouse models or possible mechanisms of pathogenesis in human CDPX2 have been reported, including possible roles of the EBP protein as a drug receptor.

CHILD SYNDROME AND DISORDERS OF THE C-4 STEROL DEMETHYLASE COMPLEX

CHILD Syndrome

CHILD Syndrome (MIM no. 308050) is a rare, X-linked, male-lethal disorder, first described under this acronym by Happle et al. (257) in 1980. Fewer than 100 cases have been reported in the medical literature worldwide. The syndrome is characterized by skin and skeletal abnormalities that typically demonstrate a striking unilateral predominance or distribution (Fig. 6E,F). The majority of cases have been sporadic, although rare mother to daughter transmission has been described. CHILD Syndrome results primarily from mutations in the X-linked *NSDHL* (NADH steroid dehydrogenase-like) gene that is involved in the demethylation of C4-methyl groups from the sterol intermediate lanosterol (**Figs.** 2 and **7**) (258, 259).

Clinical features of CHILD Syndrome

The hallmarks of CHILD Syndrome are the presence of unilateral ichthyosiform skin lesions and ipsilateral limb

reduction defects (245, 257, 258) (Fig. 6E,F). The skin lesion(s) typically affect one side of the body, with a sharp line of demarcation at the midline, and they may be extensive. Happle et al. (245) have argued that the characteristic lesion of CHILD Syndrome is a distinct type of inflammatory nevus with an erythematous base and yellow, waxy scales, although some regions may have a more "warty" or verrucous appearance. The lesions are present at birth or within the first few months of life. Some lesions may be present on the contralateral side, and bilateral, more symmetric involvement has been described (260, 261). Some of the lesions may follow lines of X-inactivation of the underlying dermatomes (lines of Blaschko) (237), although most do not. Unlike CDPX2, the lesions often persist throughout life, although there may be some resolution over time, and new lesions may continue to appear. Lesions are often observed in skin folds, a finding called ptychotropism (262). The face is usually spared, but the scalp may be involved. Alopecia may occur, usually on the more involved side, and nails are often dystrophic. Involvement of the right side of the body is more common than the left, although visceral involvement occurs more frequently in left-sided cases (258, 263).

All of the disorders of postsqualene cholesterol biosynthesis are associated with skeletal defects. However, aplasia of an entire limb, severe phocomelia, or significant limb hypoplasia on the side of skin involvement, is unique to human CHILD Syndrome. Oligo- or hypodactyly have also been reported in some cases. X-rays in infancy may demonstrate epiphyseal stippling of the involved limb(s), similar to that found in CDPX2. Milder defects, such as distal digit shortening, as well as occasional syndactyly or polydactyly, have been reported (258, 264–266). Hypoplastic or hemivertebrae and scoliosis have also been described. With the discovery of the *NSDHL* gene and the ability to perform molecular diagnosis, milder cases with minimal to no skeletal and/or skin involvement have been identified (264, 265). Visceral involvement is fairly common and frequently present in cases with extensive skin and skeletal involvement or in those with left-sided predominance. Deafness was reported in 3 of 22 cases (14%; 2 sensorineural and 1 unilateral, type unspecified) by Bornholdt et al. (258). Mild cognitive problems have been reported in a few surviving females, although intelligence is usually normal. Documented CNS malformations are present in only a small number of cases (≤10%) (258, 267). The most common abnormalities are hypoplasia of the involved side of the brain and/or cranial nerve involvement. Recently, Schmidt-Sidor et al. (268) reported severe left cerebral hypoplasia with cortical polymicrogyria and ventriculomegaly, absent corpus callosum, and dysplastic left cerebellar hemisphere in a premature newborn female with left-sided mutation-positive CHILD Syndrome who succumbed shortly after birth. Right-sided structures appeared normal, and the authors concluded that the morphologic changes on the involved side resulted from altered proliferation and neuronal migration.

A variety of congenital heart defects were noted in 10–20% of cases prior to gene identification. However, in the cases reported since 2000 with *NSDHL* mutations (258, 268–270), a single instance of hypoplastic left heart syndrome was noted in the newborn with CNS malformations who died (268). Three of 23 (13%) patients reported by Bornholdt et al. (258) had unilateral renal agenesis. Lung hypoplasia, with a small chest wall leading to respiratory distress, has also been reported (263, 268).

Gene identification and molecular biology

In 2000, Konig et al. (259) identified mutations in the X-linked candidate *NSDHL* gene in four unrelated females and one male with clinical features of CHILD Syndrome. They chose to examine *NSDHL* based on some similarities in the phenotypes of CHILD Syndrome and CDPX2. None of the CHILD Syndrome patients of Konig et al. (259) had *EBP* mutations.

The first mutations in the *Nsdhl* gene were reported in mice with the X-linked, male-lethal bare patches (*Bpa*) and striated (*Str*) mutations (271). *Nsdhl* encodes a 3β-sterol dehydrogenase that is involved in the removal of C-4 methyl groups in postsqualene cholesterol biosynthesis. The function of NSDHL as a C-4 sterol dehydrogenase was substantiated by the accumulation of 4-methyl and 4,4-dimethyl sterol intermediates in tissue samples and cultured skin fibroblasts from heterozygous *Bpa/Str* mutant females (271). In addition, the mouse NSDHL protein can rescue the conditional lethality of *S. cerevisiae* that lack the orthol-

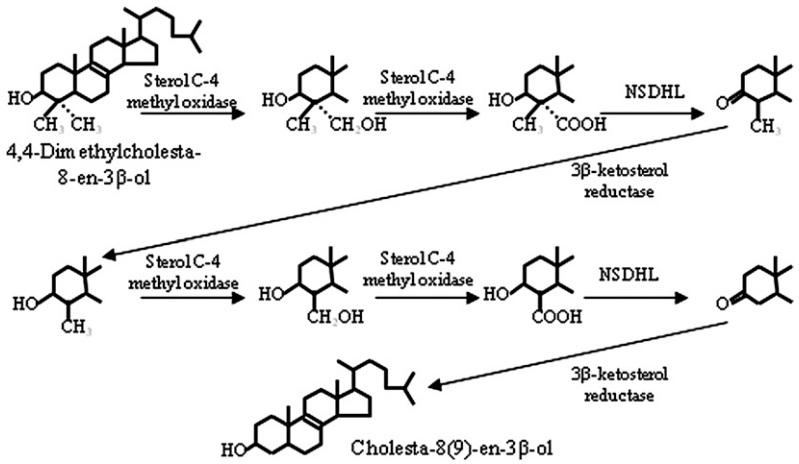

Fig. 7. Sterol C-4 demethylation. The sterol C-4 demethylation complex consists of three enzymes that act in concert in the demethylation of 4,4-dimethylcholesta-8-en-3β-ol and 4,4-dimethylcholesta-8,24-dien-3β-ol (not shown). These enzymes include sterol C-4 methyl oxidase, NSDHL, and 3β-ketosterol reductase.

ogous ERG26 protein involved in the synthesis of ergosterol (272).

The predicted NSDHL protein includes an N-terminal NADH cofactor binding domain with a conserved $GX_2GX_2GX_{17}D$ motif, as well as the YX_3K motif that is found at the active site of all 3β-hydroxysteroid dehydrogenases (HSDs) (273, 274). The mouse protein exhibits 82.9% amino acid identity with human NSDHL and 34.7% amino acid identity with the yeast ERG26 protein (271, 275). NSDHL represents a new HSD subfamily, because it demonstrates higher homology to proteins from yeast and plants than to known mammalian 3β-HSDs that function in steroidogenesis. The NSDHL protein is localized to ER membranes, the site of postsqualene cholesterol synthesis (276), as well as the surface of lipid droplets (277, 278). Based on in vitro trafficking studies, it has been proposed that the protein buds from the surface of ER membranes with lipid droplets as they form (277). Several additional cholesterol biosynthetic enzymes have been detected in lipid droplets (279), and it has been postulated that the compartmentalization of cholesterol synthetic enzymes may serve as another mechanism for cholesterol homeostasis in the cell (277, 279).

Based on homology with yeast, it was predicted that NSDHL would function in a complex with a sterol C-4 methyloxidase [encoded by the *SC4MOL* gene in humans (280)] and a 3β-ketosterol reductase [encoded by the *HSD17B7* gene (281)] (Fig. 7). HSD17B7 also participates in the conversion of estrone to estradiol in steroidogenesis (282, 283). In *S. cerevisiae*, the *ERG28* gene encodes a regulatory protein for the complex that may function as a scaffold and tether multiple cholesterologenic enzymes to ER membranes (284–286). Mammalian orthologs of *ERG28* have been identified (284–286), including the anonymous murine gene *Orf11* (NM_021446). The *Orf11* gene is coordinately upregulated with *Sc4mol* and *Hsd17b7* and other genes involved in cholesterol biosynthesis in *Nsdhl* deficient cultured skin fibroblasts (287), providing circumstantial evidence for its role in mammalian sterol metabolism.

A total of 19 distinct *NSDHL* mutations have now been reported in 24 unrelated CHILD Syndrome patients (252, 258, 259, 269, 270). This total includes one complete and one partial gene deletion, one splicing mutation, a 4 bp insertion producing a frameshift and premature termination, six nonsense mutations, and nine different missense mutations spaced throughout the gene. The missense mutation p.A105V has been reported in five unrelated cases and involves a cytosine phosphate guanine dinucleotide that can be a "hotspot" for mutations. Four of the reported cases were familial, including one family with five mildly affected females in three generations (264).

All of the mutation-positive affected cases are female with the exception of one male with the nonsense mutation R88×, a 46,XY blood karyotype, and classic CHILD Syndrome with extensive unilateral skin involvement and lower limb hypoplasia (288). The authors postulated that an early somatic mutation accounted for postnatal survival as has been found in 46,XY males with selected other X-linked male lethal disorders. Indeed, in DNA prepared from cultured skin fibroblasts from the unaffected side, only the normal allele was detected (258). As with CDPX2, there are no clear genotype/phenotype correlations in affected females, probably because the pattern of X-inactivation in affected tissues influences the phenotype as much or more than the mutation itself.

It should be noted that a female with clinical features of CHILD Syndrome has been reported with the nonsense mutation p.R110× in the *EBP* Δ^8-Δ^7-sterol isomerase gene, typically associated with CDPX2 (289). She exhibited primarily unilateral ichthyosiform skin lesions with ipsilateral limb hypoplasia and patchy alopecia. Subsequently, a second female with clinical features of typical CHILD Syndrome and an *EBP* mutation was identified [(21) and D.K. Grange, A. Metzenberg, G.E. Herman, and R.I. Kelley, unpublished results]. There has been some disagreement about the clinical diagnosis of the first case (290, 291) and whether only those females with *NSDHL* mutations should be designated as CHILD Syndrome. However, given the recent description of males with hypomorphic *NSDHL* mutations and distinct neurologic phenotypes (see below), it seems reasonable to use the designation of CHILD Syndrome based on clinical features, as suggested by Grange et al. (290).

Sterol biochemistry

Although CHILD Syndrome results from a cholesterol biosynthetic enzyme deficiency, cholesterol levels in plasma from affected females are normal, and abnormal metabolites may be difficult to detect (263). Indeed, in *Nsdhl* deficient female mice, plasma sterol profiles are normal. This finding likely results from the presence of cells expressing a normal *NSDHL* allele in affected heterozygous females. Indeed, Cunningham et al. (292) noted approximately 50% wild-type and mutant expressing cells in Bpa^{1H} /+ females shortly after birth. However, by 1 year of age, mutant NSDHL negative cells were significantly reduced in both brain and liver. The authors hypothesize that there is selection against NSDHL deficient cells over time, although their presence in appropriate numbers at birth suggests that they are able to survive and differentiate in the developing embryo.

Biochemical evidence for a defect in sterol metabolism often requires culturing skin fibroblasts in lipid-depleted media where elevations in 4-methyl and 4,4-dimethylsterols, as well as more modest increases in lathosterol and desmosterol, can be detected (18, 271). The latter accumulate whenever there is a block in cholesterol synthesis and the entire pathway is upregulated, as would occur in a heterozygous female with populations of cells expressing either an abnormal or normal *NSDHL* allele (247). However, few of the reported CHILD patients with *NSDHL* mutations have had sterol analysis performed. Due to the high likelihood of a normal sterol profile in plasma, molecular diagnosis should be employed in any patient suspected of CHILD Syndrome.

Pathogenesis and mouse models of NSDHL deficiency

The unilateral distribution of anomalies and skin lesions in CHILD Syndrome does not follow the pattern of

X-inactivation and remains unexplained. The existence of familial cases with an *NSDHL* mutation excludes somatic mosaicism as a unifying mechanism (258, 264). Happle (237, 259) has proposed that disruption of a clone of early midline "organizer" cells expressing a mutant *NSDHL* allele could affect X-inactivation and the lateralization process itself. Because such a process appears not to be active in the mouse (see below), it will be extremely difficult to investigate experimentally.

What can be more easily studied is the pathogenesis of anomalies and lethality in *Nsdhl* deficient mice. Heterozygous *Bpa* females have a skeletal dysplasia and are dwarfed compared with normal littermates. They develop a hyperkeratotic skin eruption on postnatal day 5–7 that resolves, producing a striping of the adult coat consistent with random X-inactivation [reviewed in (18)]. Milder alleles, called Striated (*Str*), appear normal in size and cannot be distinguished from their wild-type littermates until postnatal day 12–14, when striping of their coat becomes apparent. While some asymmetry may be noted in affected mice, limb reduction anomalies and diffuse unilateral skin lesions have never been observed [(292–294); G.E. Herman, unpublished results].

Seven distinct *Nsdhl* mutations have been identified in the mouse that provide an allelic series (271, 272). The original Bpa^{1H} allele has the most severe phenotype and results from a nonsense mutation, K103×, which is predicted to be a null. All of the known *Nsdhl* alleles are prenatally lethal in affected male embryos (295). Male lethality for moderate (Bpa^{8H}) and mild (Str^{1H}, Str^{1Or}) alleles occurs at midgestation (E10.5-12.5) and is associated with a thin and poorly vascularized fetal placental labyrinth and yolk sac (295). Decreased proliferation was noted in the labyrinth in affected versus control males with increased, but relatively low levels (<1%), of apoptosis. Defective hedgehog signaling in placental allantoic mesoderm was subsequently found in affected Bpa^{8H} male embryos following chorioallantoic fusion at ~E8.5 (296). No consistent abnormalities were observed in the embryos themselves. In addition, cholesterol and total sterol levels in extracts prepared from affected E10.5 male Bpa^{8H} embryos were normal, compared with those of wild-type male littermates (295), almost certainly secondary to maternal transport (3, 11). The majority of affected male embryos for the most severe Bpa^{1H} null allele die prior to E9.5. At E7.75, expected Mendelian ratios were found, although the affected Bpa^{1H} males were small, pale, disorganized, and typically demonstrated defective gastrulation (D. Cunningham and G.E. Herman, unpublished results).

Given the normal sterol levels in affected *Nsdhl* deficient male embryos at the time of their death, we and others have suggested that the accumulation of potentially toxic methylsterols above the enzymatic block, rather than cholesterol deficiency per se, may be responsible. Methylsterols dramatically alter the fluidity of cell membranes compared with lathosterol, 7DHC, or cholesterol itself (297). Whereas 7DHC and lathosterol are incorporated into artificial rafts generated in vitro as well as cholesterol itself, sterols containing C-4 methyl groups are not (136, 298). In addition, C4 methylsterols are members of the class of meiosis-activating sterols (MASs) that includes 4,4-dimethyl-5α-8,14,24-triene-3β-*ol* (follicular fluid MAS) and 4,4-dimethyl-5α-8,24-diene-3β-*ol* (testis MAS) but not lanosterol itself (299). MASs normally accumulate in gonads, induce cell division during meiosis, and are ligands for LXRs (156). In support of the hypothesis that methylsterol accumulation is toxic, forebrains of Bpa^{8H} male embryos are smaller than those of wild-type littermates. On histology, at E10.5, the cortex is thinned and disorganized with increased apoptosis despite increased cellular proliferation (300). Unfortunately, studies directly examining the toxicity in vitro or in vivo of the C4 methylsterols that accumulate in *Nsdhl* deficient cells have not been performed, partly due to the lack of specific inhibitors of the enzyme and the lack of appropriate, stable, synthetic sterol intermediates.

Additional phenotypes associated with genes of the C-4 sterol demethylase complex in human and mouse

Recently, two families with males with X-linked syndromic mental retardation and mutations in *NSDHL* have been described (300, 301). Affected males demonstrate mild to severe mental retardation, microcephaly, CNS malformations, seizures, dysmorphic facies, and no features of CHILD Syndrome. The disorder has been given the eponym of CK syndrome (302). The first family of two brothers was reported among 208 males with X-linked mental retardation; they had an insertion and frameshift mutation near the C terminus of *NSDHL* that disrupted the ER localization signal of the protein (301). In the second family, affected individuals had a 3 bp deletion of a single amino acid in exon 7 (p.K232del) of NSDHL (300). Interestingly, in the second family, carrier females exhibited significant psychopathology, including callousness and conduct problems (C. Boerkoel, personal communication). Functional studies in vitro demonstrated that both mutations act as hypomorphs. The authors propose that the neurodevelopmental phenotypes and psychopathology in affected males and carrier females, respectively, result from accumulation of methylsterols in the brain rather than from cholesterol deficiency, as in SLOS. They further state that these families provide a link between cholesterol metabolism and behavior.

A single human patient has been identified with autosomal recessive mutations in the *SC4MOL* sterol C4 methyloxidase gene. The phenotype is characterized by psoriasiform dermatitis, arthralgias, immune dysfunction, congenital cataracts, microcephaly, and developmental delay (303). Total serum cholesterol was low at 85 mg/dl (normal 140–176) with elevated 4,4'-dimethyl and 4α-monomethylsterols by GC-MS. The authors found increased proliferation of affected skin fibroblasts in lipid-depleted media that they attributed to the meiosis-activating effects of the sterol metabolites that accumulate. They postulate that the hyperproliferative skin phenotype in disorders of the C-4 demethylase complex results from elevations of these intermediates.

Finally, although human patients have not as yet been identified with deficiency of HSD17B7 sterol reductase,

two groups have generated targeted mutations in the mouse (304, 305). Similar to the moderate *Nsdhl* deficient *Bpa*^{8H} allele, homozygous null *Hsd17b7* embryos for both targeted alleles die at ~E10.5 with very similar phenotypes, including pericardial effusion, vascular defects in the embryo and yolk sac, and malformations and delayed development of the brain. In particular, the forebrains were very small and demonstrated increased apoptosis, similar to that seen in *Nsdhl* deficiency (300). For one of the two *Hsd17b7* mutants (304), tetraploid aggregation did not rescue the prenatal lethality, and the authors comment that this excluded defects of the placental labyrinth as a cause. However, as shown for *Nsdhl* alleles, lethality due to defects in allantoic mesoderm can produce a thin, poorly vascularized labyrinth (295), and primary defects in this mesodermal lineage are not rescued in tetraploid chimeras (306, 307).

Antley-Bixler Syndrome

Antley-Bixler Syndrome (ABS; MIM no. 207410) is a rare, congenital multiple malformation syndrome with cardinal features that include craniosynostosis and other craniofacial anomalies, as well skeletal defects (308). Many patients demonstrate defects in steroidogenesis and disturbances of sexual development. However, ABS is genetically heterogeneous; a subset of patients with an ABS phenotype without ambiguous genitalia have heterozygous, autosomal dominant gain-of-function mutations in fibroblast growth receptor 2 (*FGFR2*) (309, 310). This group can be considered part of the *FGFR*-related craniosynostosis syndromes and will not be discussed further. Confusion regarding the genetics and spectrum of clinical and biochemical phenotypes associated with the remainder of cases of ABS, including the presence of occasionally abnormal sterol profiles, was resolved with the discovery that mutations in a gene encoding a cytochrome P450 oxidoreductase (*POR*) were responsible for them (311–313). Specifically, the POR protein acts as an electron donor to many cytoplasmic P450 enzymes, including the cholesterogenic C14 lanosterol demethylase encoded by the *CYP51* gene and several enzymes involved in steroid hormone synthesis (**Figs.** 2 and **8**). Although POR deficiency is now considered primarily a disorder of steroidogenesis, it is included here because more severely affected individuals do demonstrate abnormal sterol profiles. Further, some of the malformations associated with the syndrome likely relate to altered cholesterol metabolism (see below).

Based on the occasional occurrence of ABS in siblings and in infants from consanguineous matings, inheritance has been presumed to be autosomal recessive, a fact confirmed following identification of mutations in the *POR* gene. The incidence and prevalence of ABS caused by POR deficiency is not known. However, prior to the *POR* gene discovery, approximately 50 cases were reported in the medical literature, and ABS was considered extremely rare. Since gene identification in 2004, at least 50 more cases have been detected, including much milder variants, suggesting that POR deficiency is more common than originally thought. ABS is now considered as the most severe presentation of a broad spectrum of phenotypes, as described below.

Clinical features

ABS was first described by Antley and Bixler in 1975 in an infant with "trapezoidocephaly" and skeletal anomalies, including radiohumeral synostosis, bowing of the femurs, and fractures of the long bones (314). Typical cases present at birth with severe craniofacial anomalies, skeletal defects, and ambiguous genitalia (308, 315). Craniofacial anomalies include severe craniosynostosis, usually involving the coronal and lambdoid sutures. There is brachycephaly with a high, broad forehead and severe midface hypoplasia. Choanal stenosis or atresia may be present, requiring prompt neonatal resuscitation. There may be a depressed nasal bridge, with a very short upturned nose and small mouth. The ears may be dysplastic with stenotic external auditory canals. Hydrocephalus and Arnold-Chiari malformations can also occur.

Bottero et al. (316), in a report of 2 new cases and review of the literature of 20 cases from 15 unrelated fami-

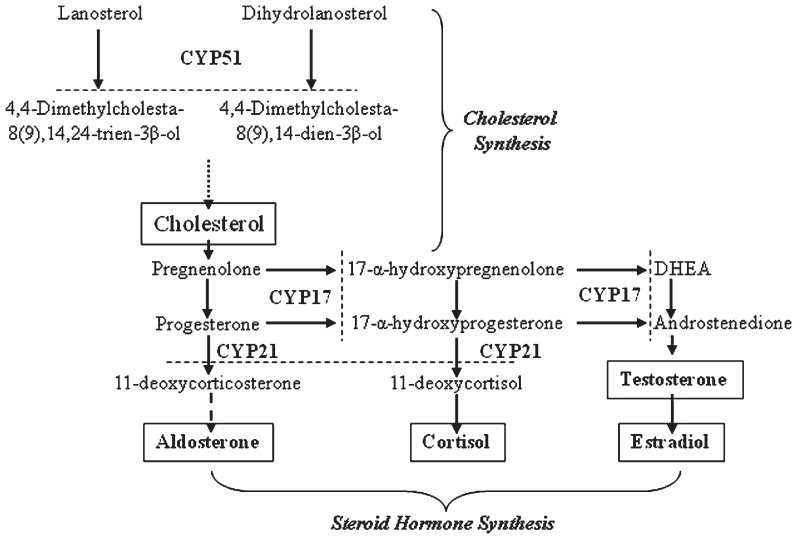

Fig. 8. Sterol and steroid biochemical abnormalities in cytochrome P450 oxidoreductase deficiency. Cytochrome P450 oxidoreductase is an essential electron donor for P450 cytochrome containing enzymes involved in cholesterol and steroid synthesis. In cholesterol synthesis, deficient CYP51 activity leads to accumulation of lanosterol and dihydrolanosterol. In steroid hormone synthesis, impaired CYP17 and CYP21 activity leads to elevated pregnenolone, 17-OH progesterone, and other progesterone metabolites in combination with low androgen levels.

lies ascertained clinically, found an incidence of coronal synostosis of 95%; additional sutures were involved in 65%. Choanal stenosis or atresia was present in 55%, and external auditory canal stenosis was reported in 27%. In a more recent series of 35 Japanese patients with POR deficiency (317), overt craniosynostosis was present in 57% (20 of 35) and choanal stenosis in 17% (6 of 35), reflecting the broader spectrum of phenotypes with the ability to perform molecular diagnosis of milder cases.

Per the reviews of Bottero (316) for clinically ascertained cases and Fukami (317) for cases with POR mutations, additional skeletal features include radiohumeral or other forearm synostoses (95 and 31%) causing fixed flexion at the elbow. Bowing of the femurs, multiple contractures (91 and 80%), and neonatal fractures can occur, and clubfoot and arachnodactyly (77 and 74%) are common. Vertebral and rib anomalies and scoliosis have also been reported.

Urogenital anomalies, reported in 64% of the patients of Bottero et al. (316) and by others (308, 317), include absent, dysplastic, ectopic, or horseshoe kidneys; abnormal ureters with or without reflux; and abnormalities of the external genitalia, including cryptorchidism. A variety of congenital heart defects occur in 10–20% of patients, and GI malformations, such as malrotation and imperforate anus, have been reported.

There is increased mortality in the neonatal period and during the first year of life due primarily to the severe midface hypoplasia, choanal stenosis, and associated infections and respiratory failure (234, 318). Aggressive and careful management of the airway, with tracheostomy if needed, can improve the overall prognosis. Developmental delay and mental retardation have been reported in >50% in a small series of long-term survivors (316). Although some of the cognitive impairment may be due to respiratory insufficiency after birth or complications from severe craniostenosis, mental retardation can occur in patients without these problems.

Individuals with so-called moderate POR deficiency (308) demonstrate milder craniofacial and skeletal malformations. Cognitive function is usually normal. At the mildest end of the phenotypic spectrum are individuals with only subtle defects of steroidogenesis, such as amenorrhea, polycystic ovarian syndrome, and infertility in both sexes (308, 319, 320).

Abnormalities of steroid metabolism

It was recognized early on that many newborns with ABS had ambiguous genitalia [now referred to as disordered sexual development (DSD)]. ABS is unique among the variant forms of congenital adrenal hyperplasia in that the DSD affects both sexes, with underdeveloped genitalia and cryptorchidism in affected 46,XY males and external virilization, with clitoromegaly and fused labia, in 46,XX females (316, 317, 319, 320). In contrast to classic congenital adrenal hyperplasia, there is no postnatal progression of the virilization in untreated females. Prenatal androgen excess is occasionally manifested as maternal virilization during pregnancy, with acne, hirsutism, and deepening of the voice. This is also reversed following delivery. In addition, very low or undetectable unconjugated estriol has been described in maternal serum screening samples obtained in midgestation in pregnancies affected with a fetus with ABS (321).

Although more mildly affected POR deficient patients may have no clinical manifestations of defective steroidogenesis, they all demonstrate biochemical evidence of partial blocks at multiple steps in the conversion of cholesterol to cortisol, estrogens, and androgens (Fig. 8). Definitive biochemical diagnosis of POR deficiency can be made by GC-MS of urinary steroids, which reveals a characteristic profile of elevated pregnenolone and 17-OH progesterone and other progesterone metabolites, in the presence of low androgens (313, 322). Mineralocorticoid synthesis and metabolism is normal. Some cases have been identified on newborn screening for other forms of congenital adrenal hyperplasia (308, 323). Mild abnormalities of serum steroids are often, but not always, present, and serum analysis should not be employed as a definitive diagnostic test. The steroid metabolites that accumulate in POR deficiency are consistent with partial deficiencies of 21-hydroxylase (CYP21A2) and 17α-hydroxylase (CYP17A1). The biochemical findings are explained by the fact that the POR enzyme serves as an electron donor for all cytoplasmic (Type II) P450 (CYP) enzymes which includes CYP17A1 and CYP21A2 (313) (Fig. 8).

Partial deficiencies of 21- and 17α-hydroxylase explain impaired glucocorticoid production in ABS and POR deficiency (Fig. 8). Prenatal virilization of affected females, and, occasionally, even pregnant females, provides evidence of androgen excess. However, postnatally, there are often decreased or low levels of androgens in both males and females (313, 320). Although the mechanism for this finding is not known, it has been proposed that an alternative pathway for androgen production, operating only in the fetus, may be responsible (320, 324).

The defects in steroidogenesis associated with POR deficiency are partial, and, hence, baseline glucocorticoid production may be sufficient. However, response to stress is usually impaired, and all individuals identified with POR deficiency require prompt endocrinologic evaluation and close surveillance. As mentioned above, more mildly affected individuals may present at puberty or to fertility clinics as adults. For additional information concerning the diagnosis and specific treatment of endocrine abnormalities in ABS/POR deficiency, the reader is referred to several excellent recent reviews (313, 320, 324).

Abnormalities of sterol metabolism

A phenotype similar to ABS with DSD was reported in 1997 in an infant with in utero exposure to the antifungal agent fluconazole and compared with three previously published cases (325). Fluconazole is a potent inhibitor of lanosterol-14α-demethylase encoded by the *CYP51* gene (Fig. 2). Based on these findings, Kelley et al. (326) examined possible defects in cholesterol synthesis in cultured lymphoblasts from an infant with ABS and DSD and an infant with an ABS phenotype, heterozygous *FGFR2* mutation, and normal genitalia. Only cells from the first infant

accumulated lanosterol and dihydrolanosterol compared with control cells, consistent with a partial block in cholesterol synthesis at the level of lanosterol-14α-demethylase (Figs. 2 and 8). However, no mutations were identified upon sequencing the 10 exons and flanking introns of the lanosterol-14α-demethylase *CYP51* gene, nor were genomic rearrangements detected by Southern blotting. Similar accumulations of sterol intermediates have now been documented in additional cases of ABS caused by POR deficiency (321) and are explained by the fact that POR serves as an electron donor for microsomal CYP51, as it does for CYP21A2 and CYP17A1 (Fig. 8). However, patient serum cholesterol levels are usually normal, and detection of an abnormal sterol profile often requires culturing mutant cells in lipid-depleted media.

The P450 oxidoreductase gene and protein

The human *POR* gene, encoding a P450 oxidoreductase, is located at 7q11.2. It spans ~72 kb, contains 15 coding exons, and encodes a protein of 681 amino acids. With the recognition that the steroid and sterol abnormalities in ABS and related syndromes result from altered function of multiple cytochrome P450 enzymes, in 2004, Fluck et al. (311) demonstrated mutations in the *POR* gene, the electron donor for each affected P450 enzyme, in four unrelated individuals with phenotypes ranging from classic ABS with DSD to an adult female with amenorrhea. Subsequently, others have demonstrated additional mutations in approximately 50 unrelated patients overall (312, 317, 327, 328). Two recurrent missense mutations are very common: A287P and R457H account for ~40% and 50% of mutant alleles among those of European and Japanese ancestry, respectively, although R457H is also present among other ethnic groups. Although the majority of mutant alleles are missense mutations, compound heterozygotes with one missense and one frameshift or splice site mutation have been described. To date, no individuals have been described with two null alleles, and it has been suggested that this combination would be lethal, similar to the *Por* knockout mouse (see below) (312, 319).

The POR protein (P450 oxidoreductase) serves an electron donor to 50 cytoplasmic (Type II) P450 enzymes found in the ER. Domains of the POR protein include an NADPH binding site and sites for FAD and FMN. The FAD moiety accepts electrons directly from NADPH and can transfer them through FMN to an acceptor cytochrome P450. This is in contrast to the seven mitochondrial P450s where transfer of electrons occurs from NADPH via the separate proteins ferredoxin and ferredoxin reductase to acceptor P450 cytochromes [(329) and reviewed in (313)]. For several missense mutations, expression studies in yeast or bacteria have demonstrated reduced POR enzymatic function (308, 311, 328).

Some genotype/phenotype correlations have been made, particularly for the more common missense mutations. In particular, Fukami et al. (317) compared the phenotype of 35 Japanese *POR* deficient patients that were homozygous for R457H/R457H (Group A), heterozygous for p.R457H and a null allele (Group B), or had other mutations (Group C). In general, the p.R457H homozygotes had milder disease with no instances of craniosynostosis, elbow synostosis, choanal stenosis, or adrenal crisis. Only 50% had any reported skeletal abnormalities, and none had hypospadias or cryptorchidism, although one male did have micropenis. None of the males in Group A had pubertal delay or failure, although females were likely to have some abnormality of the genitalia at birth, and all had some pubertal delays. All of the patients with a null allele or other combinations of mutations had some skeletal manifestations and 95% had overt craniosynostosis. Pubertal delays and DSD were found in many individuals within groups B and C.

Pathogenesis and Por mouse models

It is clear that DSD and endocrine abnormalities in human *POR* deficient individuals result from partial deficiency of P450 enzymes of steroidogenesis. The etiology of the skeletal and other anomalies in POR deficiency has not been proven; however, it is likely that partial deficiency of lanosterol-14α-demethylase with concomitant accumulation of sterol metabolites, found in the most severe cases, is at least partially responsible. The evidence supporting sterol involvement is multifold and includes: prominent skeletal and other anomalies found in other disorders of cholesterol biosynthesis, the very similar bony phenotype found with exposure to fluconazole in utero, and the phenotypes of mice with targeted mutations in the murine *Por* locus.

Two targeted null mutations in *Por* have been developed. Homozygous null mutants die at E9.5 (330) or E11.5-E13.5 (331) with anomalies of the heart, neural tube, eye, and limb, and defective vasculogenesis and hematopoeisis. The early prenatal lethality and patterning defects noted in homozygous null embryos has been attributed to altered retinoic acid metabolism (330, 332). Retinoic acid is an important morphogen involved in axis formation, patterning, and organogenesis of the early mammalian embryo [reviewed in (333)]. The cytochrome P450 enzyme sterol 26-hydroxylase is involved in the degradation of retinoic acid in the developing embryo, and $Por^{-/-}$ embryos have elevated levels and ectopic expression of retinoic acid. Mice with a homozygous hypomorphic mutation producing 75–95% reduction in enzyme activity have slightly reduced viability (80% of expected), with reduced body weight and fertility as adults, similar to human patients with mild POR deficiency (334). Finally, Schmidt et al. (335) generated a conditional targeted *Por* allele using Cre-lox technology (336, 337) and subsequently inactivated the locus in the developing limb bud using *Prx1-cre* mice (338). Although viable, the mice were smaller than wild-type littermates, with shorter forelimbs. On skeletal preparations, all four limbs were malformed, and joint fusions were noted. Transcriptional studies of E12.5 mutant and wild-type limbs using microarrays demonstrated altered expression of genes involved in both retinoic acid and cholesterol metabolic pathways. The presence of excess retinoic acid and cholesterol deficiency was confirmed by biochemical analysis of limbs from affected

embryos. The authors concluded that impaired cholesterol synthesis with cholesterol deficiency was likely responsible for the majority of skeletal defects in the mice.

Summary

Subsequent to identifying a defect of cholesterol synthesis as the cause of SLOS, a series of other human malformation syndromes, as discussed in this review, have been shown to be due to defects in cholesterol synthesis. The inborn errors of cholesterol synthesis are unique in that they are truly the first congenital malformation syndromes identified to be due to a metabolic cause. The pathophysiological processes underlying the developmental, behavioral, and cognitive problems found in these disorders are complex in that both the potential effects of cholesterol deficiency and specific toxic effects of biologically active sterol precursors need to be considered. In addition the inborn errors of cholesterol synthesis are both developmental disorders and inborn errors of metabolism. Cognitive and behavioral problems due to the biochemical disturbance may be amendable to therapeutic intervention. Although these are rare genetic disorders, further study of these single gene disorders is warranted. Further study may give insight into biological mechanisms that function in fundamental developmental processes and contribute to our understanding of more common multifactorial human diseases such as Autism Spectrum Disorder.

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