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Abnormal Sterol Metabolism in Patients With Conradi-Hünermann-Happle Syndrome and Sporadic Lethal Chondrodysplasia Punctata

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The term, "chondrodysplasia punctata" (CDP) denotes a pattern of abnormal punctate calcification of dystrophic epiphyseal cartilage and certain other cartilaginous structures, such as the larynx. CDP occurs in a variety of genetic disorders associated with skeletal dwarfism and can also be caused by prenatal exposure to warfarin. Although the most studied clinical syndrome with CDP, rhizomelic chondrodysplasia punctata (RCDP), is known to be caused by several different abnormalities of plasmalogen biosynthesis, there are many other genetic disorders with CDP for which the biochemical cause is unknown. Because patients with Smith-Lemli-Opitz syndrome, a primary disorder of sterol biosynthesis, often have rhizomesomelic limb shortness and, less commonly, CDP, we assessed sterol levels and metabolism in patients with different clinical forms of CDP. By quantitative sterol analysis of a variety of tissues, we identified 5 patients with similar radiological findings and abnormally increased levels of 8-dehydrocholesterol and cholest-8(9)-en-3β-ol, suggesting a deficiency of 3β-hydroxysteroid-Δ^8,Δ^7-isomerase, a principal enzyme of cholesterol biosynthesis. Cultured cells available from one patient showed increased levels of the same two sterols, decreased synthesis of cholesterol, and a pattern of inhibition by triparanol and AY-9944 consistent with a deficiency of 3β-hydroxysteroid-Δ^8,Δ^7-isomerase. Clinical diagnoses among the 5 patients included X-linked dominant Conradi-Hünermann-Happle syndrome and nonspecific lethal CDP. We conclude that abnormal cholesterol biosynthesis is a characteristic of some clinical syndromes with rhizomesomelic dwarfing and CDP. Am. J. Med. Genet. 83: 213–219, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: chondrodysplasia punctata; cholesterol metabolism; rhizomelic dwarfism

INTRODUCTION

Chondrodysplasia punctata (CDP) is an uncommon but well-known skeletal abnormality characterized by irregular punctate calcification of dystrophic epiphyseal cartilage and certain other cartilaginous structures, such as the larynx. The most common forms of CDP include rhizomelic CDP ("RCDP") [Gilbert et al., 1976; Heymans et al., 1985]; X-linked dominant CDP ("CDPX2") [Happle, 1979]; X-linked recessive CDP (CDPX1) [Maroteaux, 1989]; and autosomal dominant Conradi-Hünermann syndrome [Silengo et al., 1980]. Common associated abnormalities include prenatal and postnatal growth retardation, rhizomelic or rhizomesomelic limb shortness, ichthyosis, cataracts, and mental retardation [Happle, 1981; Spranger et al., 1971]. Although classical RCDP has been known since 1985 to be caused by several different abnormalities of plasmalogen biosynthesis [Heymans et al., 1985], and X-linked recessive CDP was recently shown to be caused by a deficiency of arylsulfatase E [Daniele et al., 1998; Franco et al., 1995], the biochemical nature of other forms of CDP remains unknown.

An important metabolic disorder in which patients have rhizomesomelic limb shortness and, occasionally, calcific epiphyseal stippling [Gemme et al., 1978; Mei-

necke et al., 1987; Herman et al., 1993] is Smith-Lemli-Opitz syndrome (SLOS), or RSH syndrome, a defect of cholesterol biosynthesis caused by a genetic deficiency of 3β-hydroxysteroid-Δ^7-reductase [Fitzky et al., 1998; Tint et al., 1995]. An association between abnormal cholesterol biosynthesis and CDP was also suggested by Wanders and Romeijn [1998], who found that the levels of mevalonate kinase and phosphomevalonate kinase, two key peroxisomal enzymes of cholesterol biosynthesis, are markedly diminished in the liver of patients with classical RCDP. In addition, FitzPatrick et al. [1998] recently described a single premature infant with cleft palate, hypospadias, severe rhizomesomelic shortness of all 4 limbs, and markedly increased tissue levels of desmosterol (cholesta-8,24-dien-3β-ol), suggesting a defect of cholesterol biosynthesis at the level of 3β-hydroxysteroid-Δ^{24}-reductase. Because of these associations among CDP, rhizomesomelic shortness, and abnormal cholesterol biosynthesis, we screened for abnormalities of cholesterol metabolism among patients with biochemically undiagnosed forms of CDP. We report here on five patients with variably severe CDP, including two patients with a diagnosis of CDPX2, who had significantly increased plasma or tissue levels of 8-dehydrocholesterol ("8DHC") and cholest-8(9)-en-3β-ol ("8(9)-cholestenol"), suggesting an abnormality of cholesterol biosynthesis at the level of 3β-hydroxysteroid-Δ^8,Δ^7-isomerase.

MATERIALS AND METHODS

High purity derivatization solvents and reagents were purchased from Pierce (Rockford, IL) and other HPLC-grade organic solvents from VWR (San Francisco, CA). Silica gel GHL plates with 10% silver nitrate were supplied by Analtech. Sterol standards were purchased from Aldrich (Milwaukee, WI), except for authentic standards of the acetate esters of cholest-8(9)-en-3β-ol ("8(9)-cholestenol") and cholest-8(14)-en-3β-ol, which were generously provided by the late Dr. George Schroepfer, and authentic standards of cholesta-8(9),24-dien-3β-ol (zymosterol) and cholesta-8(14),24-dien-3β-ol, which were the gift of Dr. David Nes. Inhibitors of cholesterol biosynthesis, triparanol (MDL 5052) and AY-9944, were the gifts of Marion Merrell Dow Pharmaceuticals (Kansas City, MO) and Wyeth-Ayerst Laboratories (Wayne, PA), respectively. The purity of sterol standards was determined by gas chromatography combined with flame ionization quantification and mass spectrometry. All other reagents were of the highest purity and obtained from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI).

Plasma and EBV-transformed lymphoblasts were obtained from the diagnostic specimen archives of the Peroxisomal Diseases Laboratory of the Kennedy Krieger Institute. Additional tissue samples obtained from patients with biochemically diagnosed and undiagnosed forms of CDP were obtained from the International Skeletal Dysplasia Registry at Cedars-Sinai Medical Center. For abnormal controls, the same registry provided samples of periosseous soft tissue and cartilage from patients with type II osteogenesis imperfecta, lethal metatropic dysplasia, type II achondrogenesis, and Dyggve-Melchior-Clausen syndrome. All samples were obtained with informed consent.

Lymphoblasts were cultured in RPMI 1640 with 15% fetal calf serum for routine culture expansion. Prior to sterol analysis, lymphoblasts at saturation density were harvested by centrifugation and resuspended in twice the volume of fresh RPMI 1640 with 15% delipidated fetal calf serum, prepared as described [Kelley, 1995]. The cells were incubated at 37 °C in a 5% CO_2-humidified atmosphere for 72 hours, harvested, and washed twice in Dulbecco's phosphate buffered saline (PBS). After taking an aliquot for protein determination, the cells were analyzed for sterol content by gas chromatography with selected-ion mass spectrometry, as described [Kelley, 1995]. Sterols in tissues and dystrophic cartilage were measured in 5–10 mg samples processed in the same way as plasma and lymphoblasts, following homogenization of the tissue in 1–2 ml of sterol saponification solution in a ground glass homogenizer [Kelley, 1995]. Sterols were identified on the basis of the conformance of their mass spectra and their retention times on two different gas chromatographic liquid phases (methylsilicone and 5% phenyl-methylsilicone) with those of authentic standards. The diene sterol, 8DHC, (cholesta-5,8-dien-3β-ol), for which an authentic standard was not available, was identified based on its gas chromatographic, mass spectrometric, and silver-silica gel TLC equivalence with the compound identified as 8DHC in plasma of patients with SLOS [Axelson, 1991; Tint et al., 1995].

To evaluate the effect of AY-9944 and triparanol on cholesterol biosynthesis, 6 ml of normal and CDP lymphoblasts (patient 1) at saturation density in regular culture medium were harvested by centrifugation, resuspended in 12 ml cholesterol-free culture medium, and dispensed in 2 ml aliquots to 6-well (10 cm²) plates. After incubation for 24 h to induce cholesterol synthesis, 0.5 ml aliquots of 2.5 μmol/l AY-9944 or triparanol in cholesterol-free culture medium were added and the plates incubated for an additional 72 h. The cells were then harvested, washed twice with PBS, extracted for sterols, and derivatized and analyzed by gas chromatography/mass spectrometry, as described [Kelley, 1995].

RESULTS

Based on the hypothesis that CDP and rhizomesomelic dwarfism may be characteristics of as yet undescribed defects of cholesterol biosynthesis, we screened plasma and tissue samples from patients with biochemically undiagnosed CDP or other dysplasias with calcific stippling, including clinical diagnoses of CDPX2 (4), brachytelephalangic CDP (2), Greenberg dysplasia (2), Sheffield CDP (1) and unclassified severe CDP (11). The first plasma sample tested was from blood submitted for plasmalogen analysis from a patient suspected to have a congenitally lethal form of RCDP (Table I). Although the erythrocyte plasmalogen levels were normal, there were markedly increased plasma levels of 8DHC and 8(9)-cholestenol, an abnormal sterol profile not previously reported in any known genetic disorder or toxic condition. When cultured in

TABLE I. Clinical Findings*

Patient	Age	Clinical diagnosis	Cataracts	Skin lesions[a]	Skeletal asymmetry	Polyhy-dramnios	Family history[b]	Major areas of stippling
1	Term birth	Unclassified CDP	–	+	+	+	–	Spine, long bones, rib ends, ischium, ilium, pubis, carpals, tarsals, phalanges (larynx not visualized)
2	7 years	Conradi-Hünermann	+	+	+	–	+	None at 7 y, but laryngeal calcification, scoliosis, abnormal cervical vertebrae, and limb length discrepancy present
3	21 w fetus	Unclassified CDP	unk	unk	–	unk	–	Spine, long bones, ribs, scapula, carpals, tarsals (pelvis, larynx not on films)
4	30 w fetus	Unclassified CDP	+	–	+	+	+	Spine, long bones, rib ends, ilium, pubis, carpals, tarsals (larynx not on films)
5	25 w fetus	Conradi-Hünermann	+	+	+	–	+	Spine, long bones, rib ends, ilium, ischium, pubis, scapula, trachea, larynx

*CDP, chondrodysplasis punctata; unk, unknown.
[a]Ichthyosis, follicular atrophoderma, cicatricial alopecia.
[b]Sister, mother, or other female relative with skeletal and skin abnormalities characteristics of CDPX2.

cholesterol-depleted medium, lymphoblasts from the same patient developed a markedly increased level of 8(9)-cholestenol and an abnormally increased but, compared to the level in plasma, smaller amount of 8DHC, a sterol that is abundant in SLOS plasma but accumulates very slowly in cultured SLOS fibroblasts and lymphoblasts [Shefer et al., 1997; R. Kelley, unpublished observations]. The increased levels of these two sterols in plasma and the marked elevation of 8(9)-cholestenol in lymphoblasts suggested a deficiency of 3β-hydroxysteroid-Δ^8,Δ^7-isomerase ("sterol-Δ^8-isomerase"), which converts 8(9)-cholestenol to lathosterol (cholest-7-en-3β-ol) by isomerization of the 8–9 double

bond to a 7–8 double bond (Fig. 1). Samples of soft tissue or cartilage from patients with biochemically undiagnosed forms of CDP were then obtained and similarly analyzed for sterol content. Of these, samples from two patients with a diagnosis of CDPX2 and from two patients with unclassified severe CDP were also found to have abnormally increased levels of 8DHC and 8(9)-cholestenol (Tables I and II).

Although the 5 patients with increased levels of 8DHC and 8(9)-cholestenol had variably severe calcification and limb shortness, the sites of calcific stippling were similar and included the laryngeal cartilages and the epiphyseal cartilage of most long bones, carpals,

TABLE II. Sterol Levels in Plasma and Tissues of Patients With Chondrodysplasia Punctata*

Patient	Diagnostic sample	Cholesterol	8-Dehydro-cholesterol	Cholest-8(9)-en-3ß-ol	Desmosterol	7-Dehydro-cholesterol	Lathosterol
1	Plasma[a]	492	6.2	28.9	0.30	0.04	0.63
	Normal mean	758	<0.01	<0.01	0.66	0.04	0.59
	SD (N = 22)	200	<0.01	<0.01	0.23	0.04	0.27
	Lymphoblasts[b]	6.8	0.9	5.0	0.00	0.006	0.02
	Normal mean	12.2	<0.02	0.10	0.05	0.029	0.11
	SD (N = 21)	2.7	<0.02	0.10	0.04	0.016	0.07
2	Soft tissue[c]	99.2	0.19	0.09	0.03	0.001	0.17
3	Soft tissue[c]	94.4	2.07	2.30	0.07	0.070	0.62
4	Soft tissue[c]	97.7	1.23	0.70	0.18	0.002	0.43
	Control mean	99.6	0.02	<0.01	0.17	0.015	0.14
	SD (N = 7)	0.34	0.02	<0.01	0.10	0.006	0.05
5	Cartilage[c]	98.0	1.33	0.19	0.18	0.067	0.42
	RCDP mean	99.5	0.05	<0.01	0.15	0.011	0.16
	SD (N = 6)	0.37	0.06	<0.01	0.12	0.010	0.08
	DMCS mean	99.6	0.02	<0.01	0.07	0.013	0.17
	SD (N = 5)	0.25	0.04	<0.01	0.03	0.010	0.18

*SD, Standard deviation; RCDP, Rhyzomelic chondrodysplasia punctata; DMCS, Dyggve-Melchior-Clausen syndrome.
[a]Values: µg/ml; normal controls are neonates age 1–2 days.
[b]Values: µg/mg protein.
[c]Values: percent of neutral sterols.

tarsals, pelvis, and vertebral bodies (Table I). All patients were phenotypically female, and 4 of 5 had karyotyping and were found to be 46,XX. Patchy ichthyosis was reported in 3 patients, two of whom had been given a diagnosis of CDPX2 because of their asymmetric skeletal and dermal pathology and family histories consistent with X-linked dominant inheritance. The most severely affected patients, cases 3, 4, and 5, were fetuses from pregnancies first studied because of sonographic identification of polyhydramnios, retarded skeletal growth, or both. For case 3, the specific diagnosis of CDPX2 also was suggested by disproportionate short stature and asymmetric atrophic skin lesions in the mother, but skeletal films of her could not be obtained. Figure 4 shows AP and lateral views of patient 5, one of the more severely affected patients. A more comprehensive description of the clinical and radiological characteristics of these patients is in preparation.

Table II summarizes the sterol levels in specimens from the 5 patients and controls. All tissues tested had abnormal elevations of 8DHC and 8(9)-cholestenol compared to normal newborn plasma for case 1 and, for cases 2–5, disease-control tissues from patients with RCDP or other clinically or molecularly diagnosed skeletal dysplasias without CDP. The milder elevations of 8DHC and 8(9)-cholestenol in patient 2 compared to

the other 4 patients may be explained by her older age and her clinically less severe disease, which correlate with milder biochemical abnormalities in SLOS [Cunniff et al., 1997]. However, because that specimen was stored for almost 20 years longer than any other, decomposition of 8DHC and 8(9)-cholestenol is also possible. Despite its variation relative to cholesterol, the abnormally increased level of 8(9)-cholestenol was distinctive in all 5 patients.

As indicated in Figure 1, the biochemical abnormalities in these five patients suggested a block in cholesterol biosynthesis at the level of sterol-Δ^8-isomerase. The availability of cultured lymphoblasts for patient 1 permitted further testing of this hypothesis. As shown in Figure 2B, incubation of the lymphoblasts from patient 1 with AY-9944, a relatively specific inhibitor of 3β-hydroxysteroid-Δ^7-reductase (7DHC-reductase), caused no accumulation of 7DHC or other 7-ene sterols, a characteristic of normal lymphoblasts exposed to AY-9944 (Fig. 2B). Similarly (Fig. 3B), incubation of the same lymphoblasts with 0.5 μmol/l triparanol, an inhibitor of 3β-hydroxysteroid-Δ^{24}-reductase, led to the accumulation of zymosterol (cholesta-8(9),24-dien-3β-ol) but not desmosterol (cholesta-5,24-dien-3β-ol) or cholesta-7,24-dien-3β-ol, both of which require sterol-Δ^8-isomerase for synthesis (see Fig. 1). Because of the X-linked dominant nature of CDPX2, mildly increased
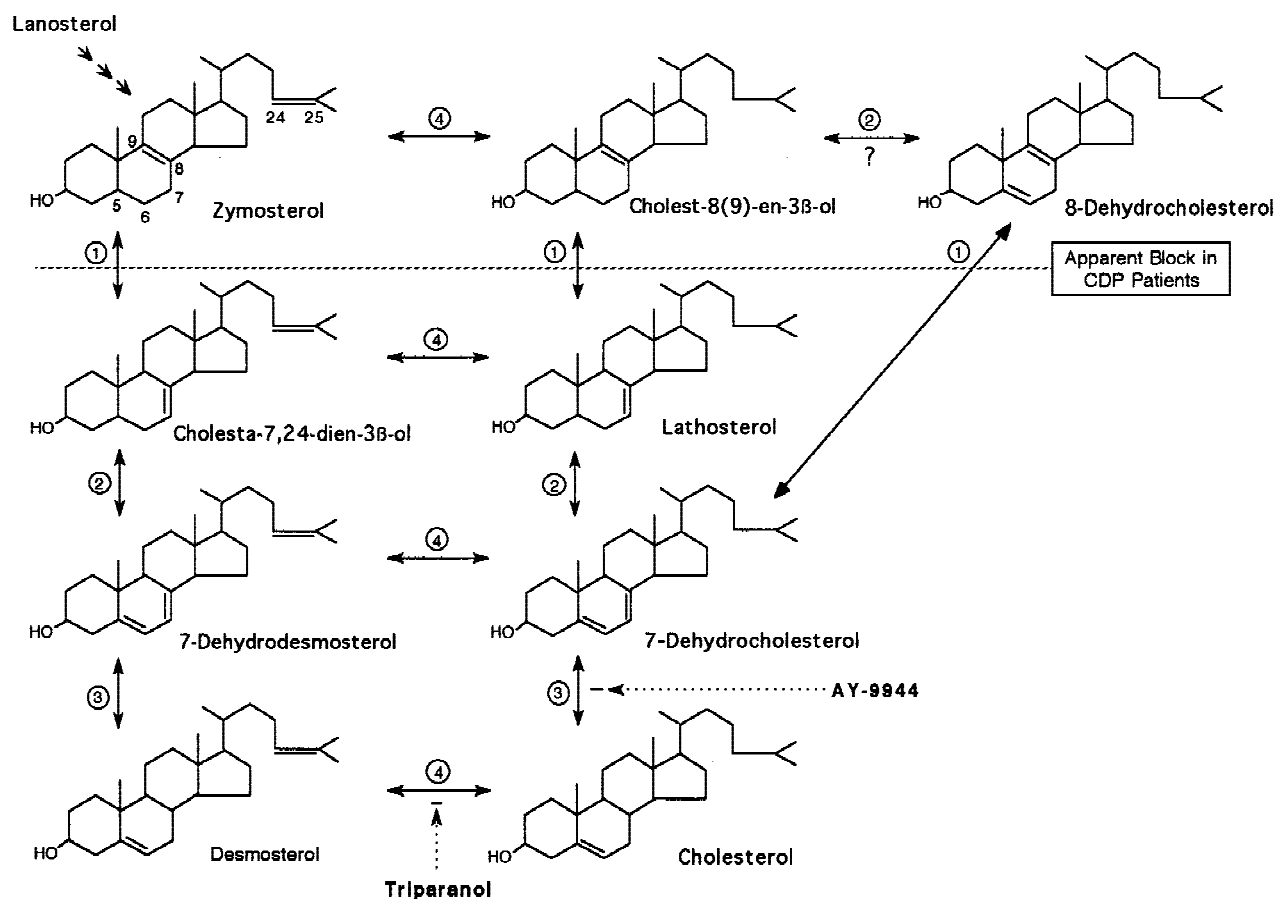

Fig. 1.   Enzymatic steps and 27-carbon sterol intermediates comprising the distal pathway for cholesterol biosynthesis. The denoted enzymatic steps are: 1, 3β-hydroxysteroid-Δ^8,Δ^7-isomerase, 2, 3β-hydroxysteroid-Δ^5-desaturase (lathosterol dehydrogenase); 3, 3β-hydroxysteroid-Δ^7-reductase (7DHC-reductase), inhibited by AY-9944; and 4, 3β-hydroxysteroid-Δ^{24}-reductase (desmosterol reductase), inhibited by triparanol.
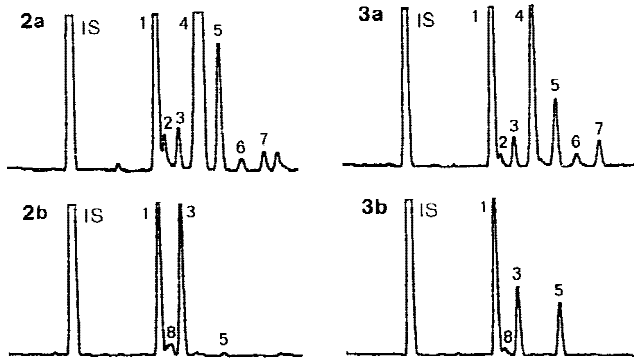
Figs. 2 and 3.   Gas chromatographic flame ionization profiles of sterol extracts of lymphoblasts of patient 1. The ordinate is the detector response in arbitrary units. Note that in untreated cells, cholesterol is the only sterol present above trace levels.

Figure 2:   normal (A) and patient 1 (B) lymphoblasts grown for three days in delipidated medium in the presence of 0.5 μmol/l AY-9944, an inhibitor of 3β-hydroxysteroid-Δ^7-reductase. The identified compounds are: IS, internal standard (epicoprostanol); 1, cholesterol; 2, cholesta-8,14-dien-3β-ol; 3, cholest-8(9)-en-3β-ol; 4, 7-dehydrocholesterol; 5, lathosterol; 6, cholesta-5,7,24-trien-3β-ol; 7, cholesta-7,24-dien-3β-ol; 8, 8-dehydrocholesterol. Note in (B) the marked deficiency of 7-ene sterols compared to (A).

Figure 3:   normal (B) and patient 1 (B) lymphoblasts grown for three days in delipidated medium in the presence of 0.5 μmol/l triparanol, inhibitor of 3β-hydroxysteroid-Δ^{24}-reductase. The identified compounds are: IS, internal standard (epicoprostanol); 1, cholesterol; 2, uncharacterized cholestadienol; 3, cholest-8(9)-en-3β-ol; 4, desmosterol; 5, zymosterol (cholesta-8(9),24-dien-3β-ol); 6, cholesta-5,7,24-trien-3β-ol; 7, cholesta-7,24-dien-3β-ol; 8, 8-dehydrocholesterol. Note in (B) the marked deficiency of 7-ene and 5-ene sterols compared to (A).

levels of desmosterol and 7-dehydrocholesterol might be expected in inhibitor-treated lymphoblast cultures presumed to contain a proportion of cells expressing the normal sterol-Δ^8-isomerase allele. However, patient 1 had unusually severe, congenitally lethal disease, and the derived lymphoblasts might have been especially adversely Lyonized as well. Indeed, the lymphoblasts of patient 1 grew more slowly and had lower cholesterol levels than lymphoblasts from the most severely affected patients with SLOS (R. Kelley, unpublished data).

DISCUSSION

We have described 5 patients with variably severe CDP and increased levels of 8DHC and 8(9)-cholestenol in plasma, lymphoblasts, soft tissue, or cartilage. Although the combined levels of 8DHC and 8(9)-cholestenol in the plasma and tissue samples amounted to only 1–5% of total sterols, and therefore were proportionately much less increased than the levels of 7-dehydrocholesterol ("7DHC") and 8DHC in most patients with SLOS [Cunniff et al., 1997], the levels were nonetheless substantially and significantly (p < 0.05) increased. Like 7DHC and lathosterol, 8(9)-cholestenol is a normal metabolite in the distal pathway for cholesterol biosynthesis from 3-hydroxy-3-methylglutarate. In normal plasma, 8(9)-cholestenol occurs only in trace amounts, and is present in slightly increased amounts (<0.5 μg/mg protein) in SLOS lymphoblasts cultured in cholesterol-free medium [R. Kelley, unpublished data]. The second sterol present at increased levels in our patients' tissues, 8DHC, is not

an intermediate of any known pathway for cholesterol biosynthesis. Rather, investigators have proposed that 8DHC, which is often increased 500-fold over normal in plasma of patients with SLOS, derives from the action of sterol-Δ^8-isomerase on 7DHC [Axelson, 1991; Tint et al., 1995; Wilton et al., 1969; Wolf et al., 1996]. Thus, analogous to the proposed synthesis of 8DHC from 7DHC, a possible alternate route for synthesis of 8DHC may be through the action of 3β-hydroxysteroid-Δ^5-desaturase on the unphysiologically high levels of 8(9)-cholestenol in our patients. If Δ^5-desaturation of 8(9)-cholestenol indeed occurs, then the combined increase of 8DHC and 8(9)-cholestenol could easily be explained by decreased activity of sterol-Δ^8-isomerase, as illustrated in Figure 1. However, we cannot exclude the possibility that accumulation of another undetected sterol or nonsterol compound inhibits cholesterol biosynthesis in a way that causes increased levels of these two sterols, either by inhibition of the isomerase or through other effects on sterol metabolism or transport.

The human sterol-Δ^8-isomerase is a monomeric, microsomal enzyme, the gene for which was recently localized to the human X-chromosome at p22.3 [Silve et al., 1996; Moebius et al., 1998]. Although the mildly increased levels of lathosterol in the tissues of cases 3 and 4 and of 7DHC in case 3 might seem inconsistent with a deficiency of sterol-Δ^8-isomerase, the level of lathosterol and, to a lesser degree, that of 7DHC, are increased in plasma whenever systemic synthesis of cholesterol is upregulated [Bjorkhem et al., 1987]. Thus, upregulation of cholesterol biosynthesis in tissues Lyonized in favor of the normal isomerase gene might lead to regionally increased levels of lathosterol if overall fetal cholesterol synthesis were substantially diminished. Other possible explanations for the mildly increased lathosterol levels include a primary synthetic defect at the level of 3β-hydroxysteroid-Δ^5-desaturase and inhibition of 3β-hydroxysteroid-Δ^5-desaturase by 8DHC or 8(9)-cholestenol taken up by cells expressing the normal isomerase allele.

In addition to the similar, if variable, biochemical abnormalities in these five patients, the associated skeletal disease and other clinical abnormalities are consistent with the variable expression of a single genetic disorder, specifically, CDPX2. All patients and affected relatives were females, two patients carried the diagnosis of CDPX2, and the other 3 had clinical or radiologic abnormalities consistent with severe forms of CDPX2. CDPX2 comprises rhizomesomelic dwarfism, CDP, cataracts, ichthyosiform erythroderma, follicular atrophoderma, and apparent early gestational lethality in 46,XY males. Because of the X-linked dominant inheritance of CDPX2 and the associated phenomenon of Lyonization in heterozygous females, the asymmetric distribution of pathology and high intrafamilial variability are important characteristics of CDPX2 and contrast the greater skeletal symmetry and relatively high phenotypic correlation of non–X-linked forms of CDP in sibs. CDPX2 also is clinically and biochemically distinguished from X-linked recessive ichthyosiform CDP (CDPX1), recently shown to be caused by a deficiency of warfarin-sensitive arylsulfa-
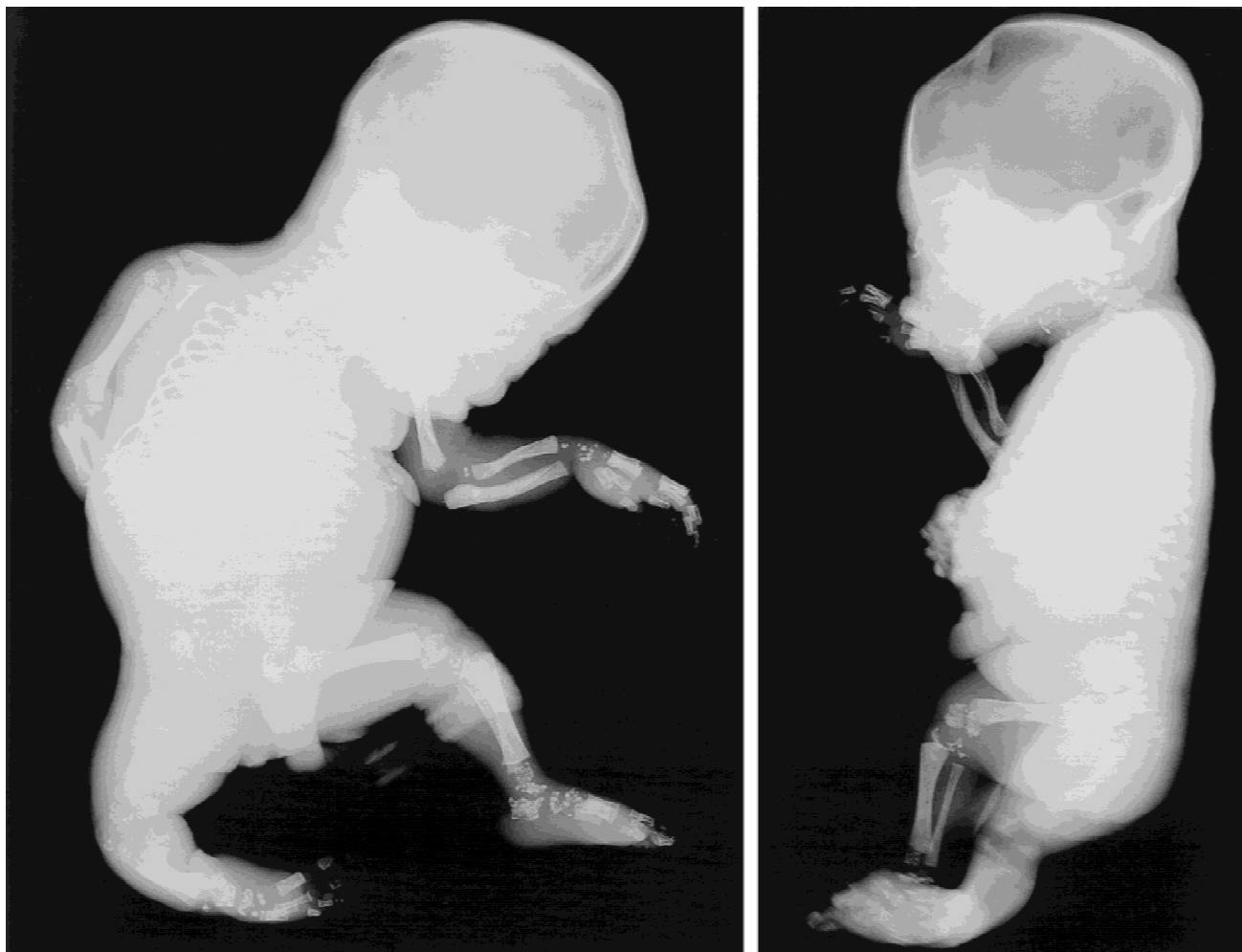
Fig. 4.   AP and lateral radiographs of patient 5. Note the limb length asymmetry and stippling of the spine, rib ends, pelvis, limbs, carpals, tarsals, and larynx.

tase E (ASE) localized to Xp22.1 [Daniele et al., 1998; Franco et al., 1995]. Although our patients were not tested for ASE deficiency, none had the distal phalangeal hypoplasia characteristic of CDPX1.

CDP has not previously been associated with a primary enzymatic defect of cholesterol biosynthesis. However, the asymmetric skeletal disease and dermal pathology of CDPX2 closely resemble those of the X-linked dominant "bare patches" (*Bpa*) mouse, which maps to a region of the mouse X chromosome syntenic with the q28 region of the human X chromosome [Herman and Walton, 1990]. *Bpa* has also recently been associated with apparently deleterious mutations of a novel gene homologous with eukaryotic 3β-hydroxysteroid dehydrogenase/Δ^5,Δ^4-isomerases [Herman et al., 1998]. Although the *Bpa* gene is expressed at high levels in gonadal and adrenal tissues and presumably has a role in murine steroid hormone biosynthesis [Herman et al., 1998], its expression at lower levels in all other adult tissues suggests a role of the enzyme in cholesterol biosynthesis, wherein homologous enzymes have a role in 4-demethylation of lanosterol [Billheimer et al., 1981; Billheimer et al., 1987]. Although a deficiency of a 3β-hydroxysteroid dehydrogenase/Δ^5,Δ^4-

isomerase, which isomerizes 3β-hydroxy-Δ^5-steroids with their homologous 3-keto-Δ^4-steroids, might impair sterol 4-demethylation and lead to increased levels of 28 to 30-carbon cholesterol precursors, such a deficiency should not cause increased levels of 27-carbon 8DHC and 8(9)-cholestenol. However, because the p22.3 region of the X-chromosome, which contains the gene for sterol-Δ^8-isomerase, also is subject to Lyonization, sterol-Δ^8-isomerase should be considered a strong candidate for the deficient enzyme causing the biochemical and asymmetric physical abnormalities in our patients.

In summary, as part of a search for defects of cholesterol biosynthesis among patients with biochemically undiagnosed forms of CDP, we identified five patients with abnormal plasma or tissue sterol profiles characterized by increased levels of 8DHC and 8(9)-cholestenol. The patients had physical and skeletal abnormalities and, in some cases, genetic histories consistent with the diagnosis of X-linked dominant CDPX2, or Conradi-Hünermann-Happle syndrome. Although the sterol abnormalities in our patients and the results of inhibitor studies in cultured lymphoblasts suggest a deficiency of sterol-Δ^8-isomerase as the cause

of these abnormalities, more detailed biochemical and mutational studies will be necessary to establish the genetic basis of this apparently new biochemical disorder.

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