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Abnormal Sterol Metabolism in a Patient With Antley-Bixler Syndrome and Ambiguous Genitalia

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Antley-Bixler syndrome (ABS) is a rare multiple anomaly syndrome comprising radiohumeral synostosis, bowed femora, fractures of the long bones, premature fusion of the calvarial sutures, severe midface hypoplasia, proptosis, choanal atresia, and, in some, ambiguous genitalia. Of fewer than 40 patients described to date, most have been sporadic, although reports of parental consanguinity and affected sibs of both sexes suggests autosomal recessive inheritance in some families. Known genetic causes among sporadic cases of ABS or ABS-like syndromes are missense mutations in the IgII and IgIII regions of *FGFR2*, although the assignment of the diagnosis of ABS to such children has been disputed. A third cause of an ABS-like phenotype is early in utero exposure to fluconazole, an inhibitor of lanosterol 14-alpha-demethylase. The fourth proposed cause of ABS is digenic inheritance combining heterozygosity or homozygosity for steroid 21-hydroxylase deficiency with effects from a second gene at an unknown locus. Because fluconazole is a strong inhibitor of lanosterol 14-alpha-demethylase (CYP51), we evaluated sterol metabolism in lymphoblast cell lines from an ABS patient without a known *FGFR2* mutation and from a patient with an *FGFR2* mutation and ABS-like manifestations. When grown in the absence of cholesterol to stimulate cholesterol biosynthesis, the cells from the ABS patient with ambiguous genitalia but without an *FGFR2* mutation accumulated markedly increased levels of lanosterol and dihydrolanosterol. Although the abnormal sterol profile suggested a deficiency of lanosterol 14-alpha-demethylase, mutational analysis of its gene, *CYP51*, disclosed no obvious pathogenic mutation in any of its 10 exons or exon-intron boundaries. Sterol metabolism in lymphoblasts from the phenotypically unaffected mother was normal. Our results suggest that ABS can occur in a patient with an intrinsic defect of cholesterol biosynthesis at the level of lanosterol 14-alpha-demethylase, although the genetic nature of the deficiency remains to be determined.

KEY WORDS: Antley-Bixler syndrome; lanosterol; cholesterol; CYP51; 14-alpha-demethylase; radiohumeral synostosis; FGFR2

INTRODUCTION

In 1975, Antley and Bixler described a new multiple anomaly syndrome in a child with "trapezoidocephaly" and multiple other anomalies including, radiohumeral synostosis, bowed femora, and fractures of the long bones [Antley and Bixler, 1975]. Since then, the list of anomalies noted in Antley-Bixler syndrome (ABS) phenotype has expanded to include craniosynostosis, severe midface hypoplasia, proptosis, choanal atresia, arachnodactyly, multiple joint contractures, rib anomalies, renal agenesis, cardiac malformations, and

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ambiguous genitalia [Hassell and Butler, 1994; Crisponi et al., 1997]. Among the fewer than 40 patients described to date, most have been sporadic cases, although reports of affected sibs of both sexes and parental consanguinity in a few case studies suggests that ABS can be inherited as an autosomal recessive trait [Yasui et al., 1983; Suzuki et al., 1987; LeHeup et al., 1995]. However, in 1998, Chun et al. [1998] reported a de novo Ser351Cys missense mutation in the FGFR2 gene in a child with some anomalies characteristic of ABS, but without the femoral bowing, fractures, and autosomal recessive inheritance characteristic of ABS. Although the diagnosis of ABS in that child and several subsequent patients with *FGFR2* mutations [Schaefer et al., 1998; Reardon et al., 2000] has been questioned [Gorlin, 1999; Gripp et al., 1999], an association of ABS-like craniosynostosis and elbow ankylosis with de novo mutations in *FGFR2* is clear [Tsai et al., 2001].

Among the more unusual patients with characteristics of ABS have been 4 infants born following early gestational exposure to fluconazole, a commonly used antifungal medication that inhibits the p450 cytochrome-bearing enzyme, lanosterol 14-alpha-demethylase (P45014DM; CYP51) [Pursley et al., 1996; Aleck and Bartley, 1997]. Although Aleck and Bartley [1997] delineated several minor phenotypic differences between "classic" ABS patients and the fluconazole-exposed infants, the similarities between the two groups of patients are nonetheless striking and suggest an embryologically and possibly biochemically related pathogenesis. Because CYP51 catalyzes 14-alpha demethylation of lanosterol in the normal biosynthesis of cholesterol [Rozman et al., 1996; Stromstedt et al., 1996], Aleck and Bartley [1997] speculated that some cases of ABS may be caused by defects of CYP51 or a related step of cholesterol biosynthesis, although plasma sterol analysis of their patients and of a second ABS child showed no abnormalities (R. Kelley, unpublished data). The same theme linking ABS with abnormal steroid or sterol metabolism was next taken up by Reardon et al. [2000], who studied 16 patients with ABS syndrome and found a variety of mild to moderate steroid abnormalities in 7 ABS patients, including one with a Thr290Cys mutation in *FGFR2*. Because minor steroid abnormalities were also found in several fathers of the ABS patients, Reardon and colleagues speculated that some cases of ABS, especially those associated with genital abnormalities, are caused by digenic inheritance. In their digenic model, an abnormal FGFR2 or an abnormal FGFR2-associated protein combines with a deficiency in an unknown enzyme of steroid metabolism to cause the ABS phenotype.

From our experience with Conradi-Hünermann syndrome (CDPX2) caused by sterol Δ^8-isomerase deficiency and of CHILD syndrome caused by NSDHL (4-methylsterol demethylase) deficiency, we have learned that affected patients may have minimal or no sterol abnormalities in plasma, but diagnostic abnormalities in cultured lymphoblasts [Kelley et al., 1999; Kelley, 2000]. We therefore undertook an analysis of sterol metabolism in lymphoblastoid cell lines from a patient with apparently autosomal recessive ABS and from a second patient with a S351C *FGFR2* mutation previously reported with an ABS phenotype [Okajima et al., 1999]. We report here lymphoblast sterol abnormalities consistent with a functional deficiency of CYP51 in a patient with ABS and genital abnormalities but lacking a known mutation in *FGFR2*.

CLINICAL REPORTS

Patient 1

The patient was born to a 22-year-old primagravid mother and 24-year-old father by planned Caesarean section at 38 weeks gestation following a pregnancy complicated by oligohydramnios and breech presentation. Birth weight was 2,595 g, length 52 cm, and head circumference (OFC) 33 cm. At birth, a number of craniofacial and skeletal anomalies were found. The elbow joints were immobile secondary to radiohumeral synostosis, but there were no other joint contractures or limitations. Arachnodactyly of the fingers and rocker bottom feet also were noted, and radiographic studies disclosed bilateral femoral fractures and a unilateral tibial fracture. Craniofacial anomalies included a bulbous nose and unilateral choanal atresia, but the eyes were not prominent. There was micrognathia with no cleft of the palate. An echocardiogram disclosed an ASD versus a stretched patent foramen ovale. Renal ultrasound findings were normal. Anus was perforate. The genitalia were ambiguous, and initial sex assignment was female. However, when the peripheral karyotype was found to be 46,XY, the parents chose to raise the child as a boy.

Neonatally, the patient had significant respiratory complications including apnea. Seizures were suspected, and the EEG showed right temporal spike wave discharges. At age 12 months, length was at the 25th centile, weight at the 5th, and OFC at the 25th. Head shape was unusual with palpable fusion of the lambdoid sutures and a large, anteriorly placed anterior fontanel. There was marked frontal and parietal bossing and midface hypoplasia, but the eyes were not prominent. The ears were overfolded superiorly, cup-shaped, and low set and had a simple architecture with no tags and with ear canals that were small but patent. The philtrum was normal in length and the palate highly arched but intact. The hands measured at the 50th centile and middle fingers at the 25th centile in length. The fingers were tapered and had hypoplastic nails, but they no longer had the appearance of arachnodactyly. The palmar creases were very unusual, having many fine axial lines and no transverse creases. The genitalia were ambiguous with fusion of the labioscrotal folds, chordee, micropenis, third degree hypospadias, and no palpable corporal tissue. There was a strawberry hemangioma at the frontal hairline and a nuchal nevus flammeus. At 23 months, the length was at the 90–95th centile, weight at the 75th, and OFC between the 10th and 25th. Ocular proptosis had developed and the head shape was still abnormal. Psychomotor development was age-appropriate apart

from the motor limitations imposed by the elbow ankylosis. There is no known consanguinity between the parents, whose families are of mixed European ancestry. The parents have had together only the two pregnancies described here. A maternal half brother was stillborn with no known cause or described anomalies, and a maternal cousin with anencephaly and absent kidneys was stillborn. The family history is otherwise non-contributory.

Patient 2

One year after the birth of the propositus (patient 1), the parents requested prenatal diagnosis by sonography for a subsequent pregnancy. Sonography performed at 18 weeks gestation disclosed a fetus with dolichocephaly with frontal bossing, radiohumeral synostosis, and femoral bowing. Sonographic dating was about two weeks behind LMP dating. The amniocyte karyotype was 46,XX. The parents elected to terminate the pregnancy, and an autopsy was performed. The fetus had a weight of 140 g and a length and head circumference consistent with 18–20 weeks of gestation. There was dolichocephaly and a large anterior fontanel. The right ear was abnormally modeled. The nasal bridge was depressed, both choanae were probepatent, and the palate was intact. In addition to apparent rhizomelic shortness of the arms, skeletal abnormalities included micrognathia, bilateral radiohumeral synostosis, femoral bowing, and arachnodactyly of the hands and, possibly, the feet. The genitalia were ambiguous with a prominent clitoris. In addition, there was a sacral meningomyelocele and a ventral wall defect causing protrusion of the bowel near the umbilicus. The similar phenotype of the fetus and her sib, patient 1, supports autosomal recessive inheritance of their disorder. (No photographs of either sib are available.)

Patient 3

This infant was reported previously as case 1 of Okajima et al. [1999] with a de novo *FGFR2* S351C mutation. The term infant had a number of clinical abnormalities suggesting ABS syndrome, including brachycephaly with apparent sagittal craniosynostosis, hydrocephalus, large anterior fontanel, frontal bossing, and choanal stenosis. Except for a "narrow" penis, the genitalia were normal. Although the infant had only elbow ankylosis without synostosis, another patient with the same *FGFR2* mutation had radiohumeral synostosis typical of classical ABS syndrome [Chun et al., 1998].

MATERIALS AND METHODS

Patient Material

EBV-lymphoblasts obtained under IRB-approved protocols of Johns Hopkins University were available on two patients with ABS or an ABS-like phenotype, patient 1 and her mother and a second, patient 3, who had a de novo S351C mutation in *FGFR2* (case 1 of Okajima et al. [1999]). Control lymphoblasts, also collected under IRB-approved protocols, were from parents and sibs of patients with defects of sarcomeric proteins, individuals who would be expected to have normal cholesterol metabolism.

Sterol Analysis

High purity derivatization solvents and reagents were purchased from Pierce, Rockford, IL, and other HPLC-grade organic solvents from VWR, San Francisco, CA. Sterol standards were purchased from Aldrich, Milwaukee, WI. Triparanol (MDL 5052), an inhibitor of sterol 24-reductase, was the gift of Marion Merrell Dow Pharmaceuticals. The purity of the sterol standards was determined by flame ionization quantification gas chromatography as well as gas chromatography-mass spectrometry. All other reagents were of the highest purity available from Sigma, St. Louis, MO, or Aldrich, Milwaukee, WI. Tissue culture media and other tissue culture reagents were obtained from Sigma, St. Louis, MO, and tissue culture plasticware from Corning, Corning, NY. Fetal bovine serum was purchased from GIBCO BRL, and Cab-O-Sil for serum delipidation was obtained from Eastman Chemicals, Rochester, NY. For routine culture expansion, EBV-transformed lymphocytes were grown in bicarbonate buffered RPMI 1640 with 10% fetal calf serum in a humidified 5% carbon dioxide atmosphere.

Sterols were quantified by flame ionization gas chromatography for cholesterol and by selected ion monitoring, ion-ratio gas chromatography/mass spectrometry for lanosterol, dihydrolanosterol, and other cholesterol precursor sterols, as described [Kelley, 1995]. For analysis of cellular sterol biosynthesis, 3 ml of lymphoblasts at saturation density were collected by centrifugation and resuspended in 6 ml of the same culture medium except with 10% delipidated fetal calf serum having a concentration of cholesterol less than 1 μg/ml, prepared as described [Gibson et al., 1990]. After 72 h further incubation, the cells were harvested by centrifugation, washed with buffered saline, resuspended in 600 μl saline, and aliquots taken for sterol analysis and for protein determination by a Lowry method. After digestion of the cell material in ethanolic KOH, the sterols were extracted with hexane, derivatized with BSTFA/1% TMCS, and then analyzed by gas chromatography/mass spectrometry, as described [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% phenylmethylsilicone) with those of authentic standards.

To evaluate the effect of Triparanol on cholesterol biosynthesis, 6 ml of normal and ABS 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 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].

Mutation Screening

Genomic DNA from the patient was isolated from whole blood. Human genomic DNA from Roche Molecular Biochemicals was used as a control. Each of the 10 exons and the intron/exon boundaries of the CYP51 gene were amplified from the patient using the polymerase chain reaction (Table I) with 100 ng of genomic DNA in each reaction. PCR reactions were run in a total volume of 40 μl containing 0.2 mM dNTPs, 0.5 μM each primer, 1.5 mM $MgSO_4$, 4 μl of 10X High Fidelity PCR Buffer (Gibco BRL), 1.25 units Platinum Taq DNA polymerase High Fidelity (Gibco BRL), and 10% DMSO, when needed. Amplification conditions were: initial denaturation at 94°C for 6 minutes, 94°C for 1 minute, specific annealing temperature (Table I) for 1 minute, and extension at 68°C for either 45 or 70 seconds. After amplification, the reaction products were purified using the GFX PCR DNA and Gel Band Purification kit (Amersham Pharmacia Biotech Inc.). PCR products from the patient were sequenced in the forward and reverse directions by the Johns Hopkins Genetic Research Core Facility using a 3700 automated DNA sequencer (Applied Biosystems). For any discrepancies between the patient's sequence and the published sequence, control DNA was amplified for that exon and sequence as a comparison (Genbank entry AC000120, [Rozman et al., 1996]). For Southern analysis,10 μg of genomic DNA were digested with Eco RI and Pvu II, electrophoresed, and blotted per the manufacturers' recommendations. The membrane was probed with ^{32}P-labeled PCR products generated for exons 2 and 9, hybridized and washed under stringent conditions, and autoradiographed. Screening of the *FGFR2* gene for mutations in the IgII and IgIII region was performed as described [Meyers et al., 1996].

RESULTS

Sterol Analysis

We anticipated that growth of ABS lymphoblasts under conditions that stimulate cholesterol biosynthesis might elicit an underlying sterol abnormality not evident in plasma. Indeed, whereas lymphoblasts from patient 3 showed precursor sterol levels comparable to those of control lymphoblasts, the lymphoblasts of patient 1 showed (Fig. 1) markedly increased levels of lanosterol (4,4′,14-trimethylcholesta-8(9),24-dien-3β-ol) and dihydrolanosterol (4,4′,14-trimethylcholest-8(9)-en-3β-ol), the C30 sterols that lead the sterol pathway for synthesis of cholesterol (Fig. 2). Similarly increased levels of lanosterol and dihydrolanosterol were not found in the same cells grown in the presence of 10% untreated fetal calf serum, which contains sufficient cholesterol to supply at least two doublings of lymphoblasts under the chosen culture conditions (data not shown). When expressed as a percentage of total cholesterol, the level of lanosterol + dihydrolanosterol in the cells of patient 1 was approximately 10-fold higher than that of the control cells or patient 3 (Fig. 3). The likely partial nature of the apparent block at the level of lanosterol-14-alpha-demethylase was illustrated by the growth of the cells in the presence of the sterol 24-reductase inhibitor, Triparanol, which caused an increase in the level of the 24-unsaturated C27 sterol, desmosterol (cholesta-5,24-dien-3β-ol) as a percent of cholesterol from 0.20 to 27.8% (N = 2), similar to the increase from 0.81 to 30.3% observed in control lymphoblasts (N = 4). Desmosterol is a C27 sterol formed by an alternate route of cholesterol biosynthesis, but still several steps distal to the removal by lanosterol 14-alpha-demethylase of the C14 carbon of lanosterol (Fig. 2). A similar result occurs in other human sterol disorders in which the defect appears to be partial and often substantial residual cholesterol synthetic capacity remains [Kelley, 2000].

Molecular Analysis

DNA from patient 1 was screened as described previously for mutations in *FGFR1* exon IIIa [Meyers et al., 1996], *FGFR2* exons IIIa and IIIc [Meyers et al., 1996], and *FGFR3* exon IIIa [Paznekas et al., 1998], but no mutations were found. These are the *FGFR* regions in which mutations have been found in various craniosynostosis syndromes, including ABS.

CYP51 is a member of the cytochrome P450 superfamily of genes. Rozman et al. [1996] determined that the gene maps to 7q21.2-21.3, spans 22 kb of genomic

TABLE I. Primers Used for the Amplification of Exons From CYP51 in Patient and Control

Exon number	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Product size (bp)	Annealing temperature (°C)	Extension time (s)
1[a]	CAGCTTCTCTCGTTCCGTC	CGTCTAGGATCAGATACACAGC	540	55	45
2	TTTGCAATTTGGCTGTTCTC	CCTCAATGGTGTCTCAAAGACG	493	60	45
3	AAAGTTACTGTCGTAGTGTTGC	CCTGGGTTCAAGCGATTC	546	59	45
4	TCTTGTTTCTAGTGATGGG	AATCATTACATAACCCTCCC	550	51	45
5	TTGGCTGTTTAGAGGGAC	TGTTAAGGCAAAGCATACC	454	55	45
6 & 7	GATGCTTTATGCTCGGTCC	ATCTCTAAGCCATCAACCC	1047	56	70
8	GGACAGTGGTAGAGCATG	TTGTAGAGATGAGGTCAGTC	441	51	45
9	TGTGTACTTCCAACCCAACG	TTGACTAACGATCGAGAAGAG	881	56	45
10	CCCAAACTAGATTACCATC	GTACACTTCATTCTCTTCG	551	49	45

Primer sequence based on Genbank entry AC000120.
[a]DMSO added to reaction.

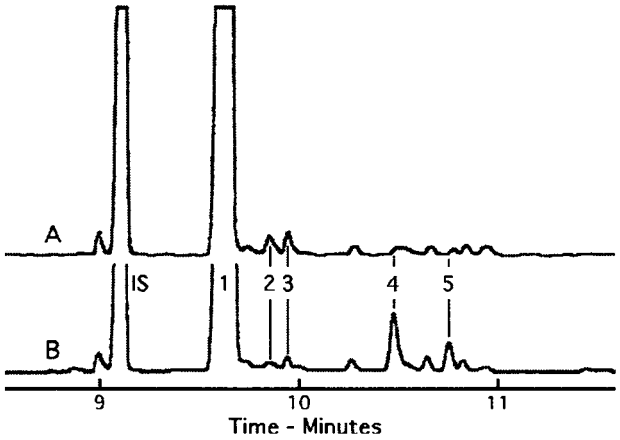

Fig. 1. Gas chromatographic flame ionization profiles of sterol extracts of lymphoblasts of control (**A**) and patient 1 (**B**). The ordinate is the detector response in arbitrary units. The denoted compounds are: IS, internal standard (epicoprostanol); 1, cholesterol (cholest-5-en-3β-ol); 2, desmosterol (cholesta-5,24-dien-3β-ol); 3, lathosterol (cholest-7-en-3β-ol); 4, dihydrolanosterol (4,4′,14-trimethylcholest-8(9)-en-3β-ol), 5, lanosterol (4,4′,14-trimethylcholesta-8(9),24-dien-3β-ol). Note relatively diminished levels of the distal pathway C27 sterols, desmosterol and lathosterol. The C30 sterols, dihydrolanosterol and lanosterol, are present in control plasma (A) only as shoulders of small peaks of C28 and C29 sterols.

DNA, and contains 10 exons. No difference was seen in the hybridization of the CYP51 gene products to the DNA of patient 1 as compared to her mother and control DNA, suggesting that the patient is neither rearranged nor deleted for the CYP51 gene. Moreover, no mutations were found in the patient's DNA after sequencing all 10 exons and the intron/exon boundaries. No variants were found in any splice site consensus sequence or branchpoint consensus sequence. A one base pair deletion (delT) 16 base pairs downstream of exon 2 was found in the patient, the patient's unaffected mother, and one control and is therefore an unlikely cause of the disease. A one base pair insertion (insA) 130 base pairs upstream of exon 2 was found only in the patient. However, because branchpoint consensus sites are usually located approximately 30 bp upstream of any exon, it is also unlikely that the single base pair insertion was the cause of the disease.

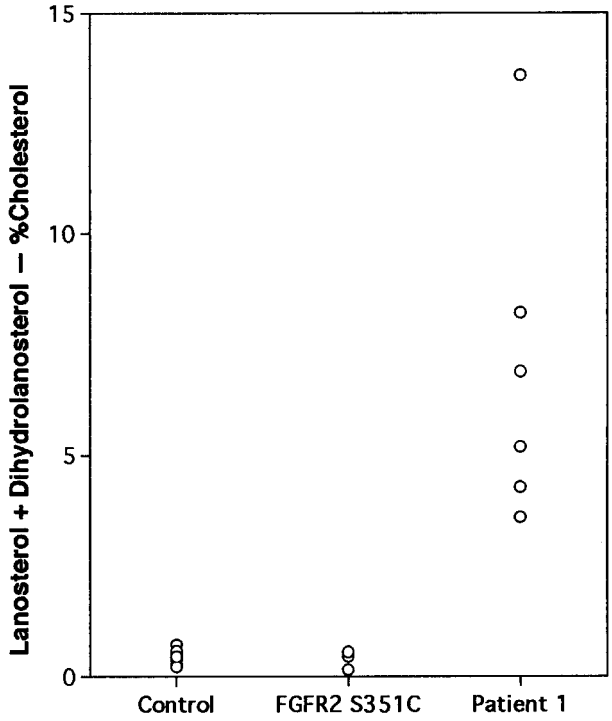

Fig. 3. Sum of lanosterol and dihydrolanosterol expressed as a percentage of cholesterol for lymphoblasts from controls, Antley-Bixler syndrome phenotype associated with *FGFR2* mutation, and Antley-Bixler syndrome patient 1.

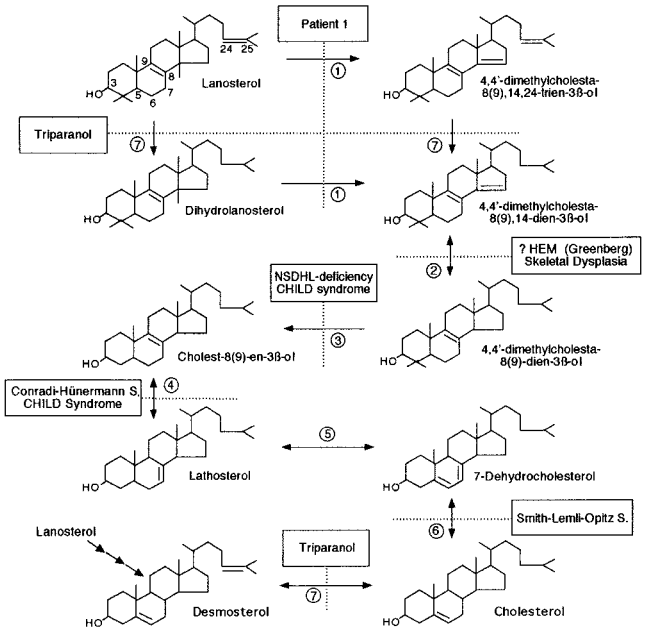

Fig. 2. Apparent biochemical block in patient 1, the enzymatic steps and principal intermediates comprising the distal pathway for cholesterol biosynthesis, and other human disorders or chemical agents associated with inhibition at each step. The denoted enzymatic steps are: 1, lanosterol-14α-demethylase; 2, 3β-hydroxysteroid-Δ^{14}-reductase; 3, 4α-methylsterol-4-demethylase complex; 4, 3β-hydroxysteroid-Δ^{8}, Δ^{7}-isomerase; 5, 3β-hydroxysteroid-Δ^{5}-desaturase (lathosterol dehydrogenase); 6, 3β-hydroxysteroid-Δ^{7}-reductase (7-dehydrocholesterol reductase); and 7, 3β-hydroxysteroid-Δ^{24}-reductase (desmosterol reductase), inhibited by Triparanol.

DISCUSSION

ABS is one of the rarest skeletal dysplasias. Yet, even among the very few cases reported with the distinctive phenotype of elbow ankylosis, long-bone bowing and fragility, and craniosynostosis, the cause of ABS appears to be very heterogeneous. Indeed, there is evidence for at least 4 different causes of the ABS phenotype: dominant mutations in *FGFR2* [Chun et al., 1998], as yet unidentified autosomal recessive mutations [Schinzel et al., 1983], in utero exposure to fluconazole [Pursley et al., 1996; Aleck and Bartley, 1997], and a digenic model of inheritance involving, in part, biochemical lesions suggesting steroid 21-hydroxylase deficiency [Reardon et al., 2000]. Because of the known potent inhibitory effect of fluconazole on CYP51,

it seemed reasonable to speculate that the apparently recessively inherited cases of ABS are caused by mutations in *CYP51* or a gene encoding a related protein involved in sterol biosynthesis. Thus, it was not surprising that our patient, having an apparently autosomal recessive form of ABS and lacking an identifiable mutation in *FGFR2,* had sterol abnormalities supporting a block in cholesterol synthesis at the level of CYP51. Although the oxidative decarboxylation of the C4 methyl groups of lanosterol is invested in a four-protein complex [Gachotte et al., 2001], all of the chemical steps involved in oxidative removal of the C14 methyl group of lanosterol and dihydrolanosterol are believed to be catalyzed entirely by CYP51. We were therefore surprised not to find an obviously disabling mutation of *CYP51* by our mutational studies, although we cannot exclude functional impairment of CYP51 as the cause of the sterol abnormalities in our patient. Interestingly, Aoyama and Yoshida [1986] have shown evidence that the immediate product of 14-alpha-demethylation is the 14-unsaturated sterol, 4,4′-dimethylcholesta-8(9),14,24-trien-3β-ol, and that AY-9944, an inhibitor of sterol Δ^{14}-reductase activity in yeast, inhibits the action of CYP51. Thus, although we did not find an abnormal accumulation of a 14-unsaturated, C29-trienol sterol in the lymphoblasts of our patient, which would point to a more distal block in the pathway as the primary metabolic lesion, it is possible either that such a compound is unstable; that there is substantial product inhibition of CYP51 at relatively low levels of the expected 8,14,24-dimethyltrienol sterol; or that there is substrate channeling between CYP51 and a sterol Δ^{14}-reductase. Although the molecular identity of the human sterol Δ^{14}-reductase participating in cholesterol biosynthesis is unknown, a genetic deficiency of sterol Δ^{14}-reductase may be the cause of the hydrops-ectopic calcification-moth-eaten skeletal dysplasia (Greenberg dysplasia) [Kelley et al., 2000]. If so, then the impaired function of CYP51 in our patient may lie in a deficiency of an unknown regulatory protein or structural protein, similar to the ERG28 "scaffolding" protein associated with the sterol 4-demethylase complex [Gachotte et al., 2001].

Patient 1 appears to have a definite abnormality of sterol metabolism that may indirectly affect steroid metabolism. However, it is difficult to see a direct relationship between the apparent deficiency of CYP51 in our patient and the steroid abnormalities reported by Reardon et al. [2000] in 7 patients with an ABS phenotype, in whom mutations in *FGFR2* were found in one and excluded in 3 others. Four of the patients without *FGFR2* mutations had increased levels of 17-hydroxyprogesterone—slight in two and greater than 3 times upper limit of normal in the other two—which the authors speculated may be caused by heterozygosity for steroid 21-hydroxylase (CYP21B) deficiency or other defect of steroidogenesis. Roth et al. [2000] recently reported a severely virilized female infant with signs of ABS born to a mother with a pregnancy luteoma. During pregnancy, there was virilization of mother and fetus. After birth, the infant was found to have moderately increased levels of progesterone and 17-hydroxyprogesterone suggesting mild 21-hydroxylase deficiency. However, the infant had no identifiable mutation in the gene encoding CYP21B, and her virilization was much in excess of that predicted for the relatively mild nature of her steroid abnormality.

To explain the findings in their patients, Reardon et al. [2000] proposed a digenic genetic model. In their model, some patients with ABS are heterozygotes for a disorder of steroidogenesis, such as 21-hydroxylase deficiency, which in some unspecified manner raises an otherwise non-deleterious *FGFR2* mutation or other genetic trait to a pathogenic role in causing the ABS phenotype. Validated digenic models are rare, but could explain the widely divergent phenotypes for certain *FGFR2* mutations, such as the finding of a single *FGFR2* mutation, Cys342Ser, in patients with conditions as divergent as ABS, Crouzon syndrome, and Pfeiffer syndrome. Also notable is that in each of the three ABS cases of Reardon et al. [2000] and in the case of Roth et al. [2000], the parent with similar steroid abnormalities was the father. Moreover, as shown by Lindenthal et al. [2001], 17-hydroxyprogesterone at biological levels is a significant inhibitor of sterol 4-demethylation. Unfortunately, steroid metabolites were not measured in our patient 1 with the ABS phenotype.

Although fibroblast growth factors and their receptors are not known to interact directly with pathways of cholesterol biosynthesis, it is interesting to note that FGF8 in some species has signaling functions complementary to the highly sterol-sensitive Sonic Hedgehog (SHH) signaling cascade at the site of laterality determination at Henson's node [Meyers and Martin, 1999]. Moreover, FGF8 has an important role in the development of the midface [White et al., 1995], which is severely hypoplastic in typical ABS, and that the interaction of certain isoforms of FGF8 and FGFR2 may influence limb skeletal development [MacArthur et al., 1995]. FGF8 as well as SHH may be involved in the abnormal lateralization of defects in CHILD syndrome caused by two different primary X-linked dominant defects of cholesterol biosynthesis, CDPX2 deficiency and NSDHL deficiency [Grange et al., 2000]. In the absence of a demonstrated mutation in *CYP51* in our patient, we must consider the possibility that the increased levels of lanosterol and dihydrolanosterol represent a secondary effect on CYP51. However, because the abnormal biochemistry of our patient 1 was measured in cultured lymphoblasts, which do not synthesize adrenocorticosteroids, a digenic model involving a mutation in *CYP21* does not seem very likely.

Skeletal dysplasia is a common theme among all known disorders of cholesterol biosynthesis, from mild rhizomelia without significant dwarfism in mevalonic aciduria and SLOS, to osteosclerosis in desmosterolosis, to severe chondrodystrophy in CDPX2, CHILD syndrome, and Greenberg dysplasia [Kelley, 2000]. To this list may now be added the several novel skeletal defects of Antley-Bixler syndrome: elbow ankylosis, craniosynostosis, and long-bone diaphyseal bowing and fragility. However, the abnormal skeletal development among inborn errors of cholesterol biogenesis appears

to be a congress of pathogenetically diverse defects, since CDPX2 is essentially a chondrodystrophy with evidence of apoptosis of prechondrocytes (W. Wilcox, personal communication), whereas desmosterolosis and ABS are characterized more by defects of diaphyseal osteogenesis. With the assignment of at least a functional block in ABS to the level of CYP51 in our patient, there are now syndromes described with proven or apparent blocks at each major step in post-squalene cholesterol biosynthesis other than lathosterol dehydrogenase. Several of these reported sterol defects, including that manifested by our ABS patient, remain to be demonstrated on a molecular level, as do defects of three of the four proteins known to constitute the sterol-4-demethylase complex, the enzymatic step deficient in one form of X-linked dominant CHILD syndrome (NSDHL deficiency) [Konig et al., 2000]. In considering other possible molecular defects causing ABS in our patient or in Greenberg dysplasia with increased 14-unsaturated sterols [Kelley, 2000], it is important to note that some SLOS patients with two *DHCR7* null mutations and absent 7-dehydrocholesterol reductase activity synthesize substantial amounts of cholesterol (R. Kelley, unpublished observations). Thus, the inability to find a CYP51 mutation in patient 1 or a mutation in the sterol Δ^{14}-reductase gene in Greenberg dysplasia [Kelley et al., 2000] could be explained by the existence of an alternate pathway, possibly peroxisomal [Appelkvist et al., 1990] or nuclear [Silve et al., 1998], for cholesterol biosynthesis.

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