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Mutations in the 3β-Hydroxysterol Δ^{24}-Reductase Gene Cause Desmosterolosis, an Autosomal Recessive Disorder of Cholesterol Biosynthesis

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Desmosterolosis is a rare autosomal recessive disorder characterized by multiple congenital anomalies. Patients with desmosterolosis have elevated levels of the cholesterol precursor desmosterol, in plasma, tissue, and cultured cells; this abnormality suggests a deficiency of the enzyme 3β-hydroxysterol Δ^{24}-reductase (DHCR24), which, in cholesterol biosynthesis, catalyzes the reduction of the Δ^{24} double bond of sterol intermediates. We identified the human *DHCR24* cDNA, by the similarity between the encoded protein and a recently characterized plant enzyme—DWF1/DIM, from *Arabidopsis thaliana*—catalyzing a different but partially similar reaction in steroid/sterol biosynthesis in plants. Heterologous expression, in the yeast *Saccharomyces cerevisiae,* of the *DHCR24* cDNA, followed by enzyme-activity measurements, confirmed that it encodes DHCR24. The encoded DHCR24 protein has a calculated molecular weight of 60.1 kD, contains a potential N-terminal secretory-signal sequence as well as at least one putative transmembrane helix, and is a member of a recently defined family of flavin adenine dinucleotide (FAD)–dependent oxidoreductases. Conversion of desmosterol to cholesterol by DHCR24 in vitro is strictly dependent on reduced nicotinamide adenine dinucleotide phosphate and is increased twofold by the addition of FAD to the assay. The corresponding gene, *DHCR24,* was identified by database searching, spans ~46.4 kb, is localized to chromosome 1p31.1-p33, and comprises nine exons and eight introns. Sequence analysis of *DHCR24* in two patients with desmosterolosis revealed four different missense mutations, which were shown, by functional expression, in yeast, of the patient alleles, to be disease causing. Our data demonstrate that desmosterolosis is a cholesterol-biosynthesis disorder caused by mutations in *DHCR24.*

Introduction

In recent years, several inherited disorders have been linked to enzyme defects in the isoprenoid/cholesterol biosynthetic pathway, by the discovery of abnormally increased levels of intermediate metabolites in patients, followed by the demonstration that there are disease-causing mutations in genes encoding the implicated enzymes (Schafer et al. 1992; Fitzky et al. 1998; Wassif et al. 1998; Waterham et al. 1998; Braverman et al. 1999; Derry et al. 1999; Drenth et al. 1999; Houten et al. 1999; Konig et al. 2000). The isoprenoid/cholesterol biosynthetic pathway produces numerous molecules that are involved in a variety of cell processes, including cell growth and differentiation, glycosylation, signal transduction, and electron transport (Goldstein and Brown 1990). Most currently identified enzyme defects specifically affect the biosynthesis of cholesterol and lead to multiple congenital, skeletal, and/or skin abnormalities (Herman 2000; Kelley 2000; Kelley and Hennekam 2000; Waterham and Wanders 2000).

Isoprenoid/cholesterol biosynthesis starts with the C_2 compound acetyl-CoA, which, in a series of six different enzyme reactions, is converted to isopentenyl-PP, the basic C_5 isoprene unit used for the synthesis of all subsequent isoprenoids (Goldstein and Brown 1990; Waterham and Wanders 2000). The first intermediate committed to the production of sterols is C_{30} squalene (composed of six isoprene units), which, after cyclization, is converted to lanosterol (4,4,14α-trimethylcholesta-8(9),24-dien-3β-ol). To eventually produce cholesterol from lanosterol, a series of enzyme reactions is required, including one demethylation at C-14, two demethylations at C-4, one isomerization of $\Delta^{8(9)}$ to Δ^{7},

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three reductions of the Δ^{24}, Δ^{14}, and Δ^{7} double bonds, and one desaturation between C-5 and C-6 (fig. 1*A*). Although the various enzyme reactions required for the conversion of lanosterol to cholesterol have been established, their preferred sequence may vary. Consequently, two major routes involving the same enzymes have been proposed that postulate, as the ultimate precursor of cholesterol, either 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol) or desmosterol (cholesta-5,24-dien-3β-ol), depending mainly on the timing of the reduction of the Δ^{24} double bond (Frantz and Schroepfer 1967; Bae and Paik 1997; Waterham and Wanders 2000). It is assumed that the various enzymes involved in the conversion of lanosterol to cholesterol, are rather nonspecific and can handle different intermediates.

The majority of the human genes encoding the various enzymes involved in cholesterol biosynthesis have been identified only very recently, as a corollary of the identification and elucidation of the biochemical and molecular bases of inherited defects of cholesterol biosynthesis. In most cases, the gene identification was aided by the similarity, in sequence and/or function, between the human proteins and their orthologs in other organisms, including yeasts and plants.

We now report the molecular basis of autosomal recessive desmosterolosis (MIM 602398), a severe multiple-congenital-anomaly syndrome caused by a defect in cholesterol biosynthesis, as indicated, in patients, by elevated plasma and tissue levels of the cholesterol precursor desmosterol (FitzPatrick et al. 1998). Since this

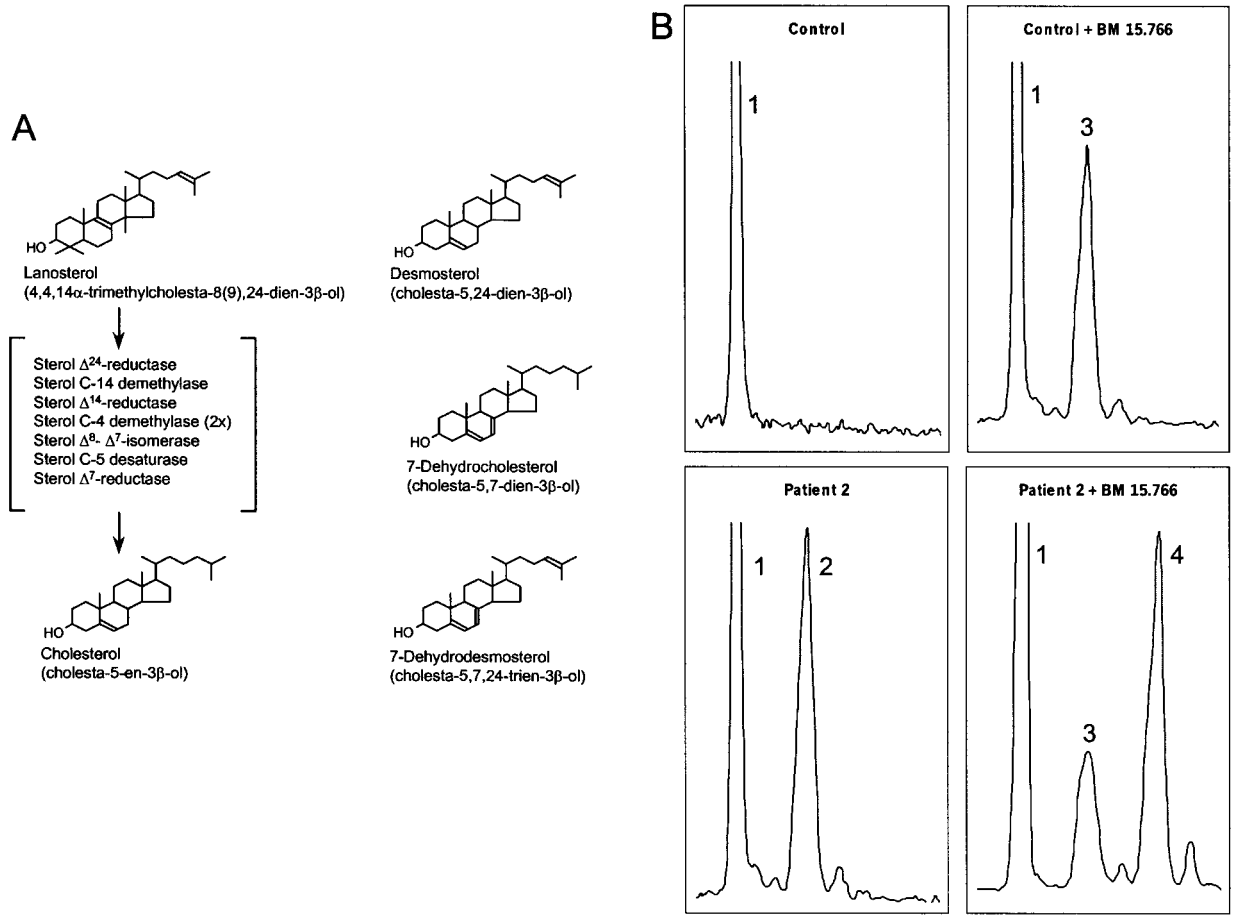

Figure 1 Sterol synthesis in lymphoblasts from the control and from patient 2. *A,* Synthesis of cholesterol from lanosterol, the first sterol intermediate produced in the isoprenoid-biosynthetic pathway, in a series of enzymatic steps catalyzed by two demethylases, three reductases, one isomerase, and one desaturase. Deficiency of either DHCR24 or DHCR7 (as in SLOS or as induced by BM 15.766) results in accumulation of the sterol intermediates desmosterol or 7-dehydrocholesterol, respectively. A combined deficiency of both sterol reductases results in accumulation of 7-dehydrodesmosterol. *B,* GC/MS sterol analysis of cultured lymphoblasts. Sterol analysis of lymphoblasts from a control and from patient 2, cultured in lipoprotein-deficient medium, revealed, in the absence of BM 15.766, accumulation of desmosterol (2) in cells from the patient, whereas only accumulation of cholesterol (1) was observed in cells from the control. When cultured in the presence of the DHCR7 inhibitor BM 15.766, accumulation of both 7-dehydrocholesterol (3) and 7-dehydrodesmosterol (4) was observed in cells from the patient, indicating a partial deficiency of DHCR24.

biochemical abnormality suggested a deficiency of the cholesterol-biosynthetic enzyme 3β-hydroxysterol Δ^{24}-reductase (DHCR24), we undertook to identify the human gene encoding this enzyme. Although no sterol Δ^{24}-reductase gene had been reported for any organism, we were able to identify the human gene by the sequence similarity between its encoded protein and a recently characterized plant enzyme catalyzing a different but partially similar reaction in steroid/sterol biosynthesis in plants. Mutation analysis of the *DHCR24* gene in two patients with desmosterolosis revealed different disease-causing mutations, as demonstrated by expression in *Saccharomyces cerevisiae*.

Subjects and Methods

Patients

Two patients previously diagnosed with desmosterolosis were included in this study: Patient 1 was a female infant of European descent, born to unrelated parents, at 34 wk gestation, who died 1 h after birth; she exhibited multiple congenital anomalies, including macrocephaly, frontal bossing, hypoplastic nose, posteriorly rotated low-set ears, cleft palate, micrognathia, ambiguity of external genitalia, rhizomelic shortening of the limbs, malformation of several internal organs, and generalized osteosclerosis (FitzPatrick et al. 1998). Patient 2 is a 4-year-old male child of European descent, born to parents not known to be related, who exhibits a phenotype less severe than that exhibited by patient 1; his clinical presentation includes dysmorphic facial features, microcephaly, limb anomalies, and profound developmental delay (Andersson et al. 2000). Informed consent to the study of both patients and of parental material was obtained.

Sterol Analysis of Cultured Cells

Lymphoblasts from a control and from patient 2 were cultured, for 5 d, in HAM-F10 medium (Gibco) containing 10% delipidated fetal-calf serum (Roche Biochemicals), in the absence or presence of 20 μM BM 15.766 (3β-hydroxysterol Δ^7-reductase [DHCR7] inhibitor). For sterol analysis, the lymphoblasts were harvested by centrifugation and were saponified, for 2 h at 70°C, in alkaline ethanol, after which the sterols were extracted with hexane, converted to trimethylsilyl derivatives by bis(trimethylsilyl)trifluoroacetamide-trimethylchlorosilane, and analyzed by gas chromatography/mass spectrometry (GC/MS).

Expression, in S. cerevisiae*, of human* DHCR24 *cDNA*

Construction of expression plasmids.—For functional expression studies of DHCR24 proteins, the coding regions of control and mutant *DHCR24* cDNA were PCR amplified from first-strand cDNA synthesized from RNA isolated either from cultured primary skin fibroblasts from the control or from cultured lymphoblasts from patient 2, as described elsewhere (IJlst et al. 1994). Control *DHCR24* was amplified from fibroblast cDNA by primers $DHCR24_{-4-19}$ (5′-ATTAAGCTTCCACCATGGAGCCCGCCGTGTCGC-3′; introducing a *Hin*dIII site) and $DHCR24_{1555-1577}$ (5′-ATTGAATTCGTCTGTCTCTCCAGGCGGGCTCC-3′; introducing an *Eco*RI site). The same primer set was used to amplify the E191K allele from cDNA prepared from lymphoblasts from patient 2. Since, for patient 1, no cDNA was available, the Y471S and N294T+K306N (combined and separate) mutations were introduced into the *DHCR24* coding region by means of the megaprimer procedure (Barik 1995). In the first round of PCR amplification, control *DHCR24* cDNA was used as template, primer $DHCR24_{-4-19}$ was used in conjunction with mutation primer $DHCR24_{1412A\rightarrow C}$ (5′-GTAGCAGTCGGCAGACAGCATCTGG-3′; introducing Y471S), and primer $DHCR24_{1555-1577}$ was used in conjunction with mutation primers $DHCR24_{881A\rightarrow C}$ (5′-CAGCAAGCTGACTAGCATTGGC-3′; introducing N294T) and $DHCR24_{918G\rightarrow C}$ (5′-CCGTGGTTCTTTAACCATGTGGAGAAC-3′; introducing K306N). In the second round, to generate, by PCR amplification, the full-length coding regions, control *DHCR24* cDNA was used as template, and the amplicons from the first round were used in conjunction with either primer $DHCR24_{1555-1577}$ (for Y471S) or primer $DHCR24_{-4-19}$ (for N294T+K306N). To obtain the combined N294T+K306N allele, the constructed N294T allele was used, in the second round of amplification, as a template, with the K306N amplicon and with primer $DHCR24_{-4-19}$. All PCR products used for expression studies were entirely sequenced—for verification and to exclude PCR-introduced errors.

Expression in S. cerevisiae.—For expression in *S. cerevisiae*, control and mutant *DHCR24* coding sequences were cloned as *Hin*dIII-*Eco*RI fragments, under transcriptional control of the *GAL1* promoter, in the yeast-expression vector pYES2 (Invitrogen). The resulting expression plasmids were transformed into *S. cerevisiae* strain INVSC2 (Invitrogen), and the expression strains were cultured at 30°C, in yeast-nitrogen base medium (Difco) supplemented with 20 μg histidine/ml, with 2% galactose (for induction of the *GAL1* promoter) as carbon source. After the yeast cultures reached, at 600 nm, an optical density of ~1 (i.e., after 15–20 h of growth), the cells were harvested by centrifugation, washed with 5 mM Tris/HCl (pH 7.5) and 50 mM NaCl buffer, and resuspended in 500 μl NaCl buffer. Cells were disrupted by vigorous vortexing (5 × 60 s, at 4°C), after addition of 250 μl glass beads.

DHCR24 enzyme-activity measurements.—To assay for DHCR24 activity, the production of cholesterol from

desmosterol was measured by incubation of 25 μl of the resulting homogenates in 225 μl assay mix, for 4 h at 37°C. Final concentrations in the enzyme assay were 100 mM Tris/HCl (pH 7.23), 0.1 mM EDTA, 1 mM dithiothreitol, 30 mM nicotinamide, 3.5 mM nicotinamide adenine dinucleotide phosphate (NADP), 30 mM glucose-6-phosphate, 2 U glucose-6-phosphate dehydrogenase/ml (for reduced-nicotinamide-adenine-dinucleotide-phosphate [NADPH] [re]generation), 0.5 mg bovine serum albumin/ml, and 168 μM desmosterol (Sigma; prepared, as 420 μM stock in 1.25% methyl-β-cyclodextrin [Fluka], in Tris/HCl [pH 7.23]). After incubation, cholesterol and desmosterol levels in the assay were analyzed by GC/MS, by the procedure described above. To correct for interassay differences between homogenate concentrations, the production of cholesterol from desmosterol was normalized for the cytosolic phosphoglucose isomerase (PGI) activity, measured in the same type of yeast homogenates as that which has been described by Bergmeyer et al. (1983). For study of the effect that NADPH and flavin adenine dinucleotide (FAD) have on DHCR24 activity, yeast homogenates were incubated in the assay mix described above—with NADP, without NADP, without NADP but with the addition of 20 μM FAD, and with NADP with the addition of 20 μM FAD.

Mutation Analysis

Genomic DNA from the patients, from their parents, and from control subjects was isolated by the Wizard genomic DNA purification kit (Promega). The protein-encoding portions of exons 1 and 9 and the entirety of exons 2–8 plus flanking intron sequences from the *DHCR24* gene were PCR amplified by primer pairs tagged with either −21M13 (5′-TGTAAAACGACGGCCAGT-3′) or M13rev (5′-CAGGAAACAGCTATGACC-3′). Primer sequences are given in table 1. PCR fragments were sequenced, in two directions, by "−21M13" and "M13rev" fluorescent primers, on an Applied Biosystems 377A automated DNA sequencer, according to the manufacturer's protocol (Perkin Elmer).

Results

Identification of Human DHCR24 *cDNA*

Previous analysis of postmortem tissues from patient 1 (FitzPatrick et al. 1998) and of plasma from patient 2 (Andersson et al. 2000) revealed a relative deficiency of cholesterol and markedly elevated levels of its biosynthetic precursor, desmosterol, indicating a specific impediment to the conversion of desmosterol to cholesterol. Sterol analysis of lymphoblasts from patient 2, cultured in delipidated medium in the presence or absence of the DHCR7 inhibitor BM 15.766, revealed de novo synthesis of desmosterol and of cholesterol, indicating that, although significant, the impediment in this patient is partial (fig. 1). The abnormal sterol patterns observed in both patients suggested a deficiency of DHCR24, the enzyme catalyzing the reduction of the Δ^{24} bond of desmosterol (as well as other Δ^{24}-bond–containing sterol intermediates), producing cholesterol (fig. 2*B*). To demonstrate this at the molecular level, we undertook to identify the gene encoding human DHCR24.

In our search for candidate genes, we noticed two recent reports describing the biochemical characterization of DWARF1/DIMINUTO mutants of *Arabidopsis thaliana*—that is, of plants displaying dwarfism and reduced fertility (Klahre et al. 1998; Choe et al. 1999). The mutated *DWF1* (or *DIM*) gene encodes an integral endoplasmic-reticulum–membrane protein—DWF1—involved in the biosynthesis of plant sterols and of brassinosteroids; DWF1 catalyzes both the isomerization of the $\Delta^{24(28)}$ bond to a $\Delta^{24(25)}$ bond and the subsequent reduction of the $\Delta^{24(25)}$ bond in various sterol/brassinosteroid precursors (fig. 2*A*). Both the striking resemblance of this latter reduction to that of the Δ^{24} bond of desmosterol (and to that of other Δ^{24}-bond–containing sterol intermediates) required for cholesterol production (fig. 2*B*) and the existence of a putative human ortholog of DWF1 (Nomura et al. 1994; Takahashi et al. 1995) prompted us to study whether this ortholog is human DHCR24. Comparison of the predicted amino acid se-

Table 1

Primers Used for Mutation Analysis of *DHCR24*

	PRIMER (5′→3′)	
EXON(S)	Forward	Reverse
1	[−21M13]GAACCTCGGCGACCCGAG	[M13rev]CTCCCGTCGCCACCTCGC
2	[−21M13]TGCTGGCCAGGGAGGTGC	[M13rev]CTGTCCACTCTGCAATGCCC
3 and 4	[−21M13]AGCTCCCCTTGGGCCTGG	[M13rev]ACCTCCCTGACCTCAGGATC
5	[−21M13]TTGCTCCCCCACTGACTGC	[M13rev]TCAGGATAGGGGAACCGGC
6	[−21M13]TGAAAAGGCCAGGAGTGCTG	[M13rev]CTAAGAGCCTGCTGCTTGAAC
7 and 8	[−21M13]CCCTTGCACTTGGAGACATTC	[M13rev]GCTGGCCTCATTCCCCTGG
9	[−21M13]GCTGCAGAATGAGCAGTTGG	[M13rev]TGCCCCCTGGAAGCCAGG

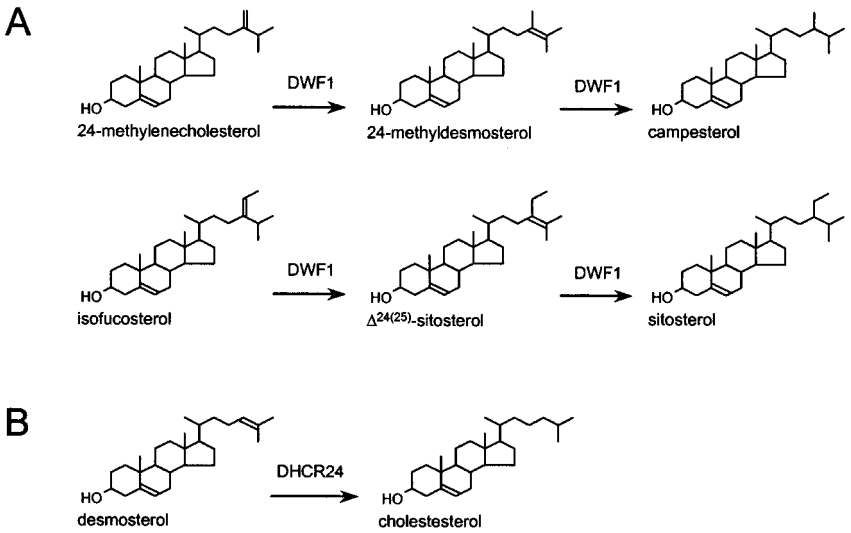

Figure 2 Sterol/steroid biosynthesis in plant and in human. *A*, DWF1 function in the biosynthetic pathway for plant sterols and steroids (brassinolide). The enzyme catalyzes both the isomerization of the $\Delta^{24(28)}$ bond into a $\Delta^{24(25)}$ bond and the subsequent reduction of the $\Delta^{24(25)}$ bond from plant-sterol intermediates 24-methylenecholesterol and isofucosterol, to produce campesterol (plant-steroid precursor) and sitosterol (major plant sterol), respectively. *B*, Reduction, by DWF1, of the $\Delta^{24(25)}$ bond from plant-sterol intermediates, which resembles the reduction, catalyzed by DHCR24 and required for the synthesis of cholesterol, of the Δ^{24} bond from desmosterol.

quence of the original human-KIAA0018-cDNA sequence (GenBank), with an open reading frame (ORF) of 1,172 bp, to that of DWF1 suggested a sequence error (i.e., a cytidine omission at position 1129) leading to the prediction of a truncated protein species of 391 amino acids. Indeed, sequencing of part of the 4,149 bp of cDNA PCR amplified from reverse-transcribed skin-fibroblast mRNA established an ORF of 1,548 bp predicted to encode a polypeptide of 516 amino acids.

To determine whether the DWF1 ortholog is DHCR24, we expressed the coding sequence in *S. cerevisiae*, a eukaryotic organism devoid of endogenous sterol Δ^{24}-reductase activity and incapable of cholesterol synthesis. When we incubated homogenates of the expressing strain with desmosterol, we observed an efficient conversion of desmosterol to cholesterol (fig. 3). This conversion was strictly dependent on NADPH, a result that is in accordance with earlier enzymological reports (Steinberg and Avigan 1969; Bae and Paik 1997) and that indicated that we had identified human DHCR24.

Characterization of the Human DHCR24 *Gene and of the Encoded Protein*

Using the *DHCR24* cDNA sequence as query, we searched the GenBank database (National Center for Biotechnology Information) by the BLAST algorithm (Altschul et al. 1990) and discovered that the complete *DHCR24* gene was part of a 169,072-bp, human genomic DNA clone composed of chromosome 1 sequence. The *DHCR24* gene spans 46,415 bp and comprises nine exons and eight introns (table 2). Although it had been predicted that the *DHCR24* gene would be located on chromosome 20 (Croce et al. 1974), it actually appeared to be located on chromosome 1p31.1-p33.

Analysis of normalized poly(A^+) RNA isolated from 68 different human tissues, on a commercially available multiple-tissue–expression array from Clontech Laboratories, revealed, in adult and fetal tissues, an almost-ubiquitous expression of *DHCR24* mRNA, similar to the expression of *DHCR7* mRNA, which encodes DHCR7 (Moebius et al. 1998; H. R. Waterham, unpublished data). Highest expression was observed in adult adrenal gland and, to a lesser extent, in adult spinal cord, liver, medulla oblongata, and pons as well as in fetal liver (H. R. Waterham, unpublished data).

DHCR24 mRNA comprises an ORF of 1,548 bp predicted to encode a polypeptide of 516 amino acids with a calculated molecular weight of 60.1 kD, with a potential N-terminal secretory signal sequence, and with at least one putative transmembrane helix (fig. 4). DHCR24 shows 97%, 49%, and 4% amino acid identity with its orthologs in mouse, *Caenorhabditis elegans*, and *A. thaliana*, respectively. No sequence similarity to other previously identified human or yeast sterol reductases was observed, nor did we find a consensus sequence for NADPH binding. As is the case with plant DWF1, DHCR24 contains a conserved domain (at residues 96–233) that is postulated to be involved in noncovalent FAD binding, which is characteristic of a recently defined family of FAD-dependent oxidoreductases (Mushegian

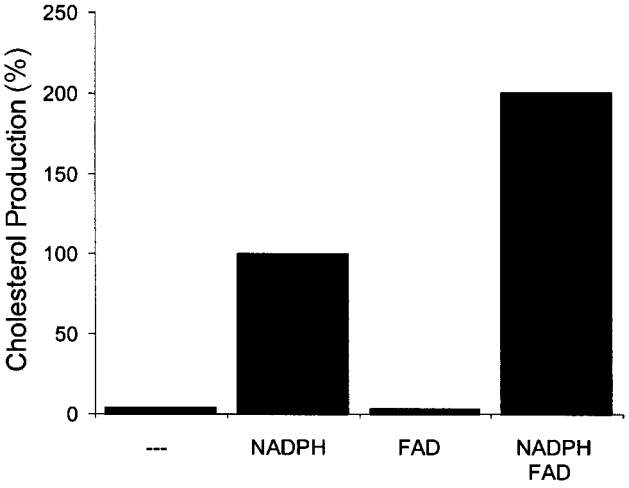

Figure 3 Dependence of DHCR24 activity on NADPH and on FAD. Yeast homogenates were incubated with the enzyme-assay mix with the addition of desmosterol (see "Subjects and Methods"), in the absence or presence of NADPH and/or FAD, as indicated. After 4 h incubation at 37°C, the production, in the assay, of cholesterol from desmosterol was determined by GC/MS. The production of cholesterol from desmosterol measured in the presence of NADPH was set as 100%.

and Koonin 1995; Fraaije et al. 1998). When we repeated the desmosterol-to-cholesterol–conversion assay, in the presence of 20 μM FAD, in homogenates of the DHCR24-expressing yeast strain, we did observe a twofold increase in activity, indicating that FAD has a functional role in DHCR24 enzyme activity (fig. 3).

Sequence Analysis of DHCR24 *in Patients with Desmosterolosis*

To determine whether desmosterolosis is due to mutations in *DHCR24,* we PCR amplified and sequenced the coding parts of the nine exons, as well as their intron junctions, from genomic DNA from the two patients. In patient 1, three different missense mutations were found: one (Y471S) was inherited from the mother, and the other two (N294T and K306N) were inherited from the father (table 3). Both Y471 and N294 are located in the carboxy-terminal half of DHCR24 and are invariantly conserved among the orthologs in mouse, *C. elegans,* and *A. thaliana* (fig. 4). K306 also is located in the carboxy-terminal half but is conserved only in the ortholog of mouse, whereas a T and a Q are found at this position in the orthologs in *C. elegans* and *A. thaliana,* respectively, leaving the possibility that K306N is a polymorphic variant (fig. 4). Patient 2 was a homozygote for an E191K mutation, which was confirmed by the discovery that both parents were heterozygotes for this mutation (table 3). E191 is located in the FAD-binding domain and is conserved among most members of the oxidoreductase family, including the orthologs in mouse, *C. elegans,* and *A. thaliana* (fig. 4).

Heterologous Expression, in S. cerevisiae, *of* DHCR24 *cDNA from Patients*

To study the effects that the mutations have on DHCR24 activity, we expressed, in *S. cerevisiae,* the various alleles in the patients and measured the ability of the mutant proteins to convert desmosterol to cholesterol. As in the severe clinical phenotype, neither of the mutant alleles in patient 1 produced active DHCR24 (table 3). To determine whether one of the two paternal mutations in patient 1 is a common polymorphic variant, we analyzed 50 alleles of controls of European descent, but we did not detect either of the two mutations. Therefore, the effect that either of the individual mutations has on DHCR24 activity was studied by separately expressing them in *S. cerevisiae.* This revealed that both mutations affect DHCR24 activity, although the residual activity was three to four times higher in the K306N allele than in the N294T allele. The combination of both mutations is required for complete deficiency (table 3). Expression, in *S. cerevisiae,* of the E191K mutant allele in patient 2 revealed ~20% residual activity (table 2), which is in good accordance with the relatively mild clinical and biochemical phenotype of this patient.

Discussion

In this study, we have reported the human *DHCR24* gene, identified by the similarity between the DHCR24 protein and the recently characterized DWARF1/DIMINUTO gene product of *A. thaliana,* which is involved in plant steroid/sterol biosynthesis. We have confirmed that *DHCR24* encodes DHCR24, by functional expression, in *S. cerevisiae,* of the *DHCR24* cDNA. In addition, we have demonstrated—by expression, in *S. cerevisiae,* of alleles in patients—that enzyme-inactivating mutations in *DHCR24* are the cause of the cholesterol-biosynthesis disorder desmosterolosis.

No sequence similarity between DHCR24 and other, previously identified human, plant, or yeast sterol reductases exists, which, in retrospect, explains the difficulty of identifying both the protein and its encoding gene. Except for DHCR24, all sterol reductases—including sterol Δ^{7}-, sterol Δ^{14}-, and sterol $\Delta^{24(28)}$-reductases—belong to one family of proteins sharing a high degree of sequence homology. These sterol reductases all have been localized to the membrane of the endoplasmic reticulum, and all require NADPH for their activities; both of these conditions are also true of DHCR24. As suggested by the presence of a putative

Table 2

Genomic Structure of *DHCR24* at 1p31.1-p33

Exon	cDNA Sequence (size [bp])	Splice Site		Intron (size[bp])
		Acceptor	Donor	
1	−87–231 (269)	...	AAGCAGgtgagcggcg	IVS1 (3,115)
2	232–387 (156)	gtggttgcagGTGCGG	AAACAGgtgagagtag	IVS2 (7,559)
3	388–493 (106)	gtgtcaacagATTGTC	CAGTGGgtgaggaccc	IVS3 (730)
4	494–612 (119)	gtctctgcagGGGGTC	ACTCCGgtgagttcag	IVS4 (3,479)
5	613–876 (264)	tgtctcacagTCCGAA	AGCAAGgtaagccagg	IVS5 (14,759)
6	877–1,020 (144)	gtcatttcagCTGAAT	CTCCAGgtgaggcttg	IVS6 (11,065)
7	1,021–1,218 (198)	tgttttccagGACATT	ATCCACgtgagtgggg	IVS7 (424)
8	1,219–1,397 (179)	gtctacccagGTCTAC	GCATGGgtgagtggcc	IVS8 (1,048)
9	1,398–4,149 (2,752)	gcctctgcagCTTCCA	...	...

Note.—Data are for part of human clone RPII-12C17, sequenced by Genome Sequencing Center, Washington University School of Medicine, Seattle.

secretory-signal sequence, subcellular localization and topology studies of *A. thaliana* DWF1 revealed a localization to the endoplasmic-reticulum membrane, with the carboxy terminus oriented toward the cytoplasm (Klahre et al. 1998). In addition, Greeve et al. (2000) recently reported a localization, to the endoplasmic-reticulum membrane, of the human seladin-1 protein, which appears identical to DHCR24 (also see below).

As is the case for the activities of the other sterol reductases, the conversion of desmosterol to cholesterol by DHCR24 appears strictly NADPH dependent, although no common consensus sequence for an NADPH-binding site can be found in the DHCR24 amino acid sequence. In contrast, DHCR24 appears to be a member of a recently defined family of FAD-dependent oxidoreductases that all share a conserved domain postulated to be involved in either covalent or noncovalent binding of FAD. Although FAD could not substitute for NADPH in our enzyme-activity assay, we noted a twofold increase in activity after the addition of FAD, strongly suggesting that, in the case of DHCR24, FAD is noncovalently linked.

Desmosterolosis is only the fourth inherited disorder of postsqualene cholesterol biosynthesis for which the molecular basis has been solved; the first three include autosomal recessive Smith-Lemli-Opitz syndrome (SLOS [MIM 270400]), which is caused by deficiency of DHCR7 (encoded by the *DHCR7* gene, at 11q13 [Fitzky et al. 1998; Wassif et al. 1998; Waterham et al. 1998; Witsch-Baumgartner et al. 2000]), and the X-linked dominant inherited disorders Conradi-Hünermann-Happle syndrome (CDPX2 [MIM 302960]) and congenital hemidysplasia with ichthyosis and limb defects (CHILD) syndrome (MIM 308050), which are caused by deficiencies of sterol Δ^8-Δ^7 isomerase (encoded by the *EBP* gene, at Xp11.22-23 [Braverman et al. 1999; Derry et al. 1999]) and of sterol C-4 demethylase (encoded by the *NSDHL* gene, at Xq28 [Konig et al. 2000]), respectively. SLOS is clinically characterized by multiple morphogenic and congenital abnormalities, including craniofacial, organ, limb/skeletal, and urogenital anomalies, as well as by growth retardation and mental retardation (Kelley 2000; Kelley and Hennekam 2000; Waterham and Wanders 2000). Both CDPX2 and CHILD syndrome clinically present with skeletal and skin abnormalities, including stippling of epiphyses, rhizomelia (shortening of long bones), ichthyosis, and hyperkeratosis (Herman 2000; Kelley 2000). The two patients with desmosterolosis exhibit clinical features of all three aforementioned syndromes. Since the clinical presentation of both patients was, however, rather different, the identification of additional patients will be required for further delineation of the clinical phenotype of desmosterolosis.

The overlap, in clinical phenotype, between the different cholesterol-biosynthesis disorders suggests a common mechanism underlying their pathogenesis. Indeed, the involvement of cholesterol in embryonic development and morphogenesis, recently discovered by its role in the hedgehog-protein signal–transduction pathways, provides a potential key to the pathogenesis of these disorders (Porter et al. 1996; Hammerschmidt et al. 1997; Mann and Beachy 2000; McMahon 2000; Villavicencio et al. 2000). The hedgehog proteins comprise a family of secreted embryonic-signaling molecules involved, in both vertebrates and invertebrates, in embryonic patterning. In higher vertebrates, including humans, three different hedgehog proteins (Sonic, Desert, and Indian) have been identified and have been implicated in an increasing number of different developmental processes (Hammerschmidt et al. 1997; McMahon 2000; Villavicencio et al. 2000), many of which are defective in patients with one of the cholesterol-biosynthesis disorders. Although the exact role of cholesterol in the pathways is still elusive and may occur at multiple

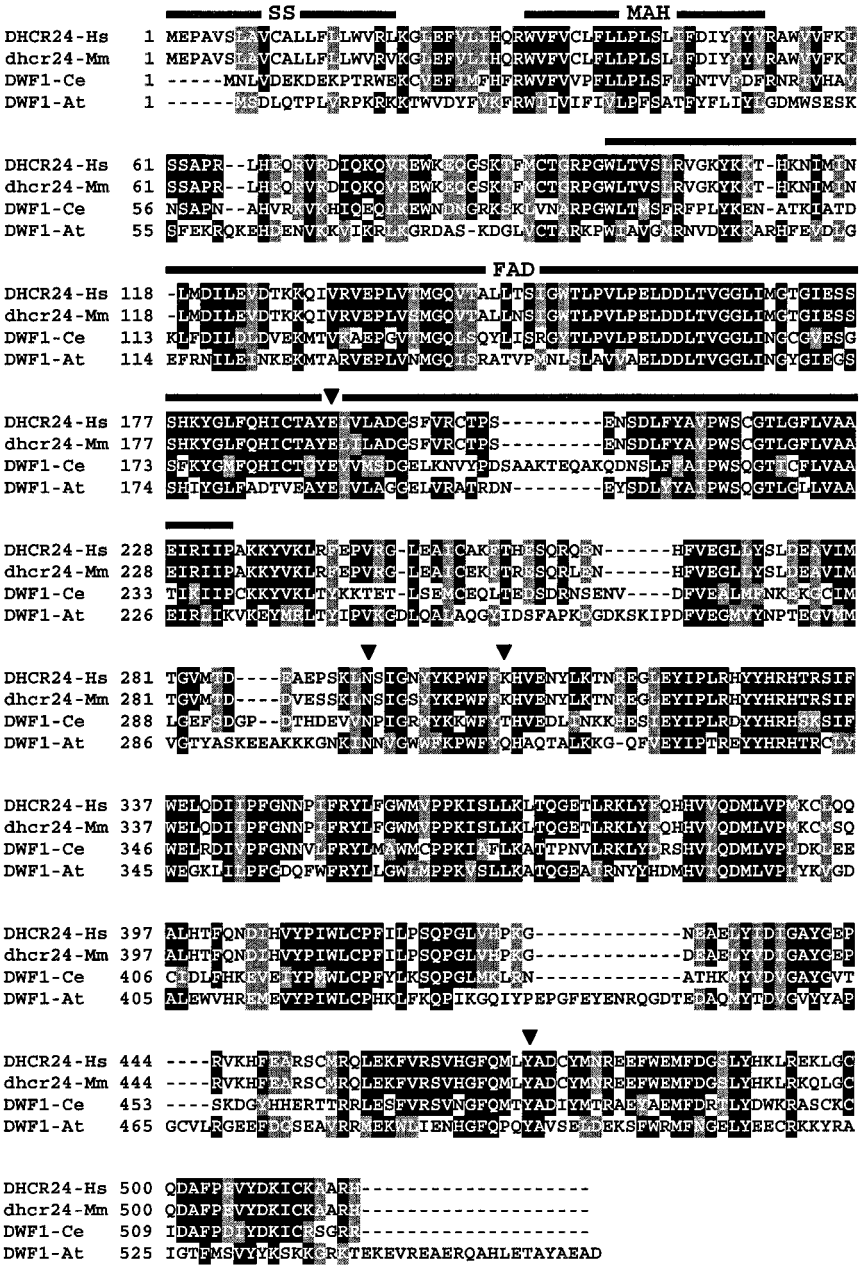

Figure 4 Sequence alignment of human (Hs) DHCR24 and its orthologs in mouse (Mm), *C. elegans* (Ce), and *A. thaliana* (At), by the CLUSTAL W program (Thompson et al. 1994). Only amino acids that are identical and conserved in at least three sequences are indicated, by black boxes and gray boxes, respectively. Indicated above the DHCR24 sequence are conserved domains predicted to encode a secretory-signal sequence (SS), an FAD-binding domain characteristic of the family of FAD-dependent oxidoreductases (FAD), and a membrane-associated helix (MAH); arrowheads indicate mutations identified in patients with desmosterolosis.

levels (i.e., hedgehog-protein–signal generation, reception, transduction, and/or transport), it has been assumed that perturbations of these pathways provide an explanation of most of the congenital abnormalities associated with these disorders.

Recently, a human DWF1 homolog was reported to exhibit a relatively low expression in affected neurons of patients with Alzheimer disease (AD) (Greeve et al. 2000). This homolog, which, because of its lack of physiological function, was named "seladin-1" (for selective Alzheimer disease indicator 1), appears identical to DHCR24. Overexpression of seladin-1 in PC12 cells and in human neuroglioma H4 cells resulted in resistance against AD-associated amyloid-β peptide–induced

Table 3

Mutations in *DHCR24* in Patients with Desmosterolosis—and the Effects of Those Mutations on DHCR24 Activity

Subject and Nucleotide[a] (Amino Acid) Change	DHCR24 Activity[b] (%)
Patient 1:	
1412A→C^{m} (Y471S)	ND
881A→C + 918G→C^{p} (N294T + K306N)	.8
881A→C^{p} (N294T)	14.4
918G→C^{p} (K306N)	49.8
Patient 2:	
571G→A^{m+p} (E191K)	19.9

[a] Mutations inherited from the father are denoted by a superscript "p," and mutations inherited from the mother are denoted by a superscript "m."

[b] Determined by expression in *S. cerevisiae,* normalized to PGI activity, and expressed as percentage of activity of wild-type DHCR24 protein; values are the means of two experiments; ND = not detectable.

toxicity and in protection from apoptotic cell death, respectively. On the basis of these in vitro observations, it was suggested that high seladin-1 levels might delay and/or prevent neurodegeneration in AD (Greeve et al. 2000); however, given our current demonstration of seladin-1/DHCR24's function in cholesterol biosynthesis, it is unclear how to interpret these results, in light of recent data indicating that, in fact, a reduction of cholesterol biosynthesis leads to a decrease in the production and accumulation of amyloid-β peptide and, as a consequence, to the development of AD (Wolozin et al. 2000; Fassbender et al. 2001; Kojro et al. 2001; Wolozin 2001).

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for genomic clone RPII-12C17 containing *DHCR24* [accession number AC009946] and for human *DHCR24* KIAA0018 cDNA [accession number D13643], human *DHCR24* (seladin-1) cDNA [accession number AF261758], human *DHCR24* gene [accession numbers AF398336–AF398342], mouse *Dhcr24* cDNA [accession number AY039762], *C. elegans DWF1* ortholog [accession number AF026214], and *A. thaliana DWF1* [accession number U12400])

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/BLAST/ (for BLAST algorithm)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for desmosterolosis [MIM 602398], SLOS [MIM 270400], CDPX2 [MIM 302960], and CHILD syndrome [MIM 308050])

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