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DHCR7: A VITAL ENZYME SWITCH BETWEEN CHOLESTEROL AND VITAMIN D PRODUCTION

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TABLE OF CONTENTS

ABSTRACT

The conversion of 7-dehydrocholesterol to cholesterol, the final step of cholesterol synthesis in the Kandutsch-Russell pathway, is catalyzed by the enzyme 7-dehydrocholesterol reductase (DHCR7). Homozygous or compound heterozygous mutations in *DHCR7* lead to the developmental disease Smith-Lemli-Opitz syndrome, which can also result in fetal mortality, highlighting the importance of this enzyme in human development and survival. Besides serving as a substrate for DHCR7, 7-dehydrocholesterol is also a precursor of vitamin D via the action of ultraviolet light on the skin. Thus, DHCR7 exerts complex biological effects, involved in both cholesterol and vitamin D production. Indeed, we argue that DHCR7 can act as a switch between cholesterol and vitamin D synthesis. This review summarizes current knowledge about the critical enzyme DHCR7, highlighting recent findings regarding its structure, transcriptional and post-transcriptional regulation, and its links to vitamin D synthesis. Greater understanding about DHCR7 function, regulation and its place within cellular metabolism will provide important insights into its biological roles.

Abbreviations: 7DHC, 7-dehydrocholesterol; AEBS, antiestrogen binding site; DHCR7, 7-dehydrocholesterol reductase; DHCR14, Δ14-sterol reductase; DHCR24, 24-dehydrocholesterol reductase; EBP, emopamil binding protein; Hh, Hedgehog; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; LBR, lamin B receptor; MaSR1, *Methylomicrobium alcaliphilum* 20Z sterol reductase; NADPH, nicotinamide adenine dinucleotide phosphate; NADSYN1, nicotinamide adenine dinucleotide synthetase 1; SNP, single nucleotide polymorphism; SLOS, Smith-Lemli-Opitz syndrome; SRE, sterol-regulatory element; SREBP-2, SRE binding protein-2; SSD, sterol-sensing domain; TM, transmembrane domain; UVB, ultraviolet B.

Keywords: 7-dehydrocholesterol, 7-dehydrocholesterol reductase, cholesterol, Smith-Lemli-Opitz syndrome, vitamin D_3

1. INTRODUCTION

Cholesterol synthesis is a complex, multi-step pathway that has many layers of regulation to ensure homeostasis. As an important rate-limiting enzyme, the regulation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is the best understood [1,2]. HMGCR is the well-known target of the statin class of drugs, which are prescribed to lower cholesterol levels, and are one of the most successful pharmaceuticals in history [3]. However, with over 20 enzymes involved in the endogenous production of cholesterol, many more regulatory processes exist post-HMGCR than previously appreciated [4], making the pathway more complicated and intriguing.

Cholesterol is the precursor for steroid hormones and bile acids, but not vitamin D (specifically vitamin D_3, which is also known as cholecalciferol), as is often incorrectly asserted. That role belongs to 7-dehydrocholesterol (7DHC), which is also a substrate of the enzyme 7-dehydrocholesterol reductase (DHCR7, E.C. 1.3.1.21) to form cholesterol. However, 7DHC inhabits a special place in the pathway, connecting cholesterol to the vitamin D_3 synthetic pathway (Figure 1). Conversion of 7DHC to cholesterol can occur ubiquitously in the body, but in the skin, exposure to ultraviolet B (UVB) light from the sun causes the cleavage of the C(9-10) bond in 7DHC to form vitamin D_3 [5] (Figure 1).

Here, we focus on the important role of DHCR7 in health and disease, particularly in fetal development and the regulation of vitamin D_3 synthesis. In addition, we review recent work on the characterization of this enzyme, in terms of its structure, function and regulation, and how this may affect two biologically essential molecules – cholesterol and vitamin D_3.

1.1 The History of DHCR7

Cholesterol synthesis utilizes six isoprene units from acetyl-CoA to form the isoprenoid hydrocarbon squalene, which is cyclized to form the sterol backbone, first found in lanosterol. A complex series of oxidative and reductive steps follow, which include the loss of three methyl groups to finally form the 27-carbon cholesterol [6]. Integral to elucidating this process was Andrew Kandutsch, who reported the enzymatic reduction of 7DHC at the C(7-8) double bond to form cholesterol [7]. Together with Alice Russell, he worked on tumors from the preputial gland – an accessory sex gland found in mice [8,9]. Notably, they observed that the preputial gland produced large quantities of lanosterol that could be radiolabeled *in vitro* and the enzymatic conversion to cholesterol monitored. Through this method, a novel sequence of sterols was observed which formed what is now known as the Kandutsch-Russell pathway [9] (Figure 2). This was an alternative to the previously established Bloch pathway of cholesterol synthesis – named after Kandutsch's mentor, the Nobel prize-winning Konrad Bloch [10,11].

Over time, the pathways were studied further but the physiological need for two alternative pathways remains unclear. Each enzyme of the Kandutsch–Russell pathway is utilized in the Bloch

pathway, albeit in a different order, excluding a compensatory role (Figure 2). However, each pathway produces distinct sterol intermediates which can have potent effects on cholesterol homeostasis and other cellular processes, independently of cholesterol. For example, 7DHC of the Kandutsch-Russell pathway is the precursor to vitamin D_3, and desmosterol of the Bloch pathway is an activator of the liver X receptor [12].

Remarkably, it was not until 2015 when modern flux-tracing methods largely validated the 1960 findings of Kandutsch and Russell [9]. The study performed by Mitsche *et al*. [13] identified that the major divergent point of the pathways occur at zymosterol rather than lanosterol (Figure 2). They also confirmed that the Kandutsch-Russell pathway was highly active in the preputial glands of the mouse, as well as in the skin. Together with the prevalence of 7DHC in the skin [13], this suggests that the main role of the Kandutsch-Russell pathway in the skin may be to provide 7DHC for vitamin D_3 synthesis [13]. Tissues where the Kandutsch-Russell or Bloch pathway is preferentially active are indicated in Figure 3. However, preference for a pathway may also be age-dependent, with another study identifying that the Bloch pathway was more active in the brain of young mice, compared to the Kandutsch-Russell pathway, which was more critical in the adult brain [14].

Knowledge of the DHCR7 enzyme advanced largely in the past 50 years due to its links to the developmental disorder Smith-Lemli-Opitz syndrome (SLOS, OMIM #270400) (Section 2.1), which is caused by mutations in the *DHCR7* gene. In 1964, physicians David Smith, Luc Lemli and John Opitz first reported a disorder characterized by several congenital abnormalities, including underdeveloped external genitals and facial features [15]. Initially, they designated the disease RSH syndrome, derived from the surnames of the first three patients [15]. At the time, it was not known that DHCR7 played a role, but occurrence of the disease in siblings suggested it was an inheritable disease.

Many cases emerged over the next few decades, highlighting the relative prevalence of the disease. However, it was not until 1994 that Stephen Tint and colleagues [16] measured low cholesterol and high 7DHC levels in SLOS patients, and determined it was the result of a defect in the enzymatic reduction of 7DHC [7]. Further measurements of the abnormal sterol profile in SLOS models [17,18], including undetectable levels of urinary bile acids due to low cholesterol [19], helped to cement the importance of DHCR7 in SLOS. At the turn of the millennium, multiple groups successfully cloned human and rodent DHCR7 [20–24], with the chromosomal location of *DHCR7* identified as 11q13.4 [20–22]. Together with the identification of specific DHCR7 mutations in SLOS patients [20,22,25], a deficiency of DHCR7 enzymatic activity was confirmed to be responsible for the disease. This work finally offered answers to previously inexplicable symptoms in SLOS patients, such as pseudohermaphroditism which is now known to be due to the lack of cholesterol-derived steroid hormones [26]. Today, ongoing research into finding treatments and a cure for SLOS, as well as increasing interest in the link between DHCR7 and vitamin D_3 (discussed in Section 2.4), highlight the importance of DHCR7 in human health and disease.

2. IMPLICATIONS IN HUMAN HEALTH AND DISEASE

2.1. Smith-Lemli-Opitz syndrome

SLOS is a developmental disorder where patients exhibit morphogenic and congenital abnormalities, mental retardation, and behavioral problems. SLOS has been extensively studied and reviewed [27–29], including in a special 50th anniversary article in 2015 [30]. SLOS results from homozygous or compound heterozygous mutations in the gene encoding DHCR7, causing insufficient functional enzyme, with a subsequent lack of cholesterol and accumulation of 7DHC.

Proposed as the third most common autosomal recessive disorder in Caucasians [31], SLOS has an incidence of 1 in ~40,000 and a carrier frequency of ~1% [32]. The combined carrier rate of the two most frequent mutations (a null mutation caused by the splice site IVS8-1G>C, and the nonsense mutation W151X) ranges from ~1 to 2.3% [33]. The majority of other mutants are missense mutations, of which at least 110 exist (Figure 4A). The prevalence of specific mutations in certain European populations can be explained by genetic drift over hundreds of generations [33], with the splice site IVS8-1G>C carrier rate approximately 1% for North American Caucasians, but may be as high as 3.3% in Central European populations [34]. However, the observed incidence of SLOS is much lower than expected from the carrier rate, suggesting high fetal losses may be involved, as well as an under-diagnosis of milder cases.

Currently, the link between genotype and phenotype is poor [27,35], making it difficult to predict the severity of the disease in affected individuals. These correlations are further confounded by the maternal genotype, where, for example, variations in apolipoprotein E [36] and ATP-binding cassette transporter A1 [37] can also affect the severity of SLOS in the offspring. By contrast, two individuals recently identified as homozygous carriers of the common splice mutation IVS8-1G>C have been found to be “resilient” to SLOS [38], with the study suggesting that some unknown genetic variation protects the individuals from acquiring the disease.

The lack of correlation between genotype and phenotype certainly indicates that other factors influence the severity of the disease. One likely factor is the amount of cholesterol available to the fetus, as it is critical for embryonic development. The major effects of SLOS occur during gestation and are currently irreversible. It has been noted that the SLOS phenotype is often not as severe as other inborn errors of cholesterol synthesis [39], such as desmosterolosis (OMIM #602398), caused by mutations in the gene *24-dehydrocholesterol reductase* (*DHCR24*). This may be due to greater maternal transfer of cholesterol *in utero* [40], but why this would occur in SLOS and not alternative disorders is unknown. Fetal intravenous transfusions of cholesterol have been explored, albeit not recently [41], likely due to technical and ethical considerations. Alternative therapies *in utero* are being explored [42], but cholesterol supplementation via the diet remains the standard treatment option for SLOS patients [43,44]. Some of the potential biochemical treatments that are in use or being explored for SLOS are outlined in Table 1. Non-biochemical processes such as surgery and/or

behavioral therapy have also proved important for the effective management and treatment of the disease [45], but are not included here.

2.2. Health Effects of 7-dehydrocholesterol and its Derivatives

In addition to low cholesterol levels, it has been suggested that the levels of circulating 7DHC also influence the severity of the SLOS phenotype [46,47]. However, these findings are not always consistent [48]. 7DHC itself is known to decrease activity of the major rate-limiting enzyme of cholesterol synthesis, HMGCR [49], which could exacerbate the negative consequences of low cholesterol in a SLOS setting. Furthermore, accumulated 7DHC can be converted to other metabolites by enzymatic and non-enzymatic reactions, with certain metabolites proposed to contribute to SLOS pathogenesis [35,46,50]. Figure 5 depicts the direct products of 7DHC that are produced enzymatically and found to be elevated in SLOS patients (e.g. [35,50–55]), some of which may have biological activity. For example, 7-ketocholesterol may be involved in immune functions [56], 25-hydroxy-7DHC can activate the liver X receptor and the vitamin D receptor [51], and 27-hydroxy-7DHC has been reported to inhibit sterol synthesis and activate the liver X receptor [51,53]. Thus, these metabolites may contribute to SLOS pathology, and further work is warranted to elucidate their (patho)physiological effects.

7DHC is 200 times more reactive towards free radical chain oxidation than cholesterol [57]. The oxidation products of 7DHC can be harmful to health, causing severe cytotoxic effects in cell culture [46], as well as retinal degeneration [58] and enhanced photosensitivity in SLOS patients [59]. Treatment with antioxidants is currently being explored (Table 1) [60], with preliminary results showing a significant decrease in the levels of harmful 7DHC-derived oxysterols in a SLOS mouse model [61].

Similar to mutations in *DHCR7*, the inhibition of DHCR7 also causes accumulation of 7DHC. Recently, Herron *et al.* [62] identified that benzalkonium chloride, a common antimicrobial agent found in many consumer products, can potently inhibit DHCR7 activity. Considering the potential generation of harmful 7DHC-derived oxysterols, the study proposes that environmental exposure to these compounds could also contribute to various problems in embryogenesis. Moreover, screening of DHCR7 inhibition itself has been proposed as a method to detect off-target, teratogenic effects in drug development [63].

TABLE 1. Summary of potential biochemical treatments for Smith-Lemli-Opitz syndrome (SLOS)*

Treatment	Description	Clinical Stage	Results/Comments
Cholesterol supplementation	Additional cholesterol provided through: 1. Cholesterol-rich diet 2. Pharmaceutical grade solutions 3. Fresh frozen plasma [64]	In use in patients. Clinical trials completed (IDs: NCT00114634, NCT00272844) Further clinical trials underway (e.g. ID: NCT01773278)	▪ Increased plasma cholesterol levels, and often decreased 7DHC leading to an overall improved sterol profile [65] ▪ Some studies show little improvement in behavior or development [44,66,67] ▪ Prenatal cholesterol supplementation may aid healthy embryogenesis and prevent the SLOS phenotype entirely [41]
Statin therapy	Use of statin drugs, which inhibit HMGCR and thus cholesterol synthesis, to reduce the accumulation of 7DHC and its toxic by-products	Tested in mild cases of SLOS. Clinical trials completed (ID: NCT00064792).	▪ Simvastatin treatment of SLOS fibroblasts with residual DHCR7 activity, increased DHCR7 expression and increased cholesterol synthesis [68] ▪ Treatment in SLOS patients, in conjunction with cholesterol-supplementation, also reduced 7DHC and improved behavior [69,70] ▪ However, one study has found increased aggression with statin therapy and little improvement in behavior or development [67]
Antioxidant therapy	Antioxidant mixture, with vitamin E proposed as the active component, to reduce toxic 7DHC-derived oxysterols	Clinical trial currently recruiting patients (ID: NCT01773278)	▪ Antioxidant mixture decreased formation of 7DHC-derived oxysterols in human SLOS fibroblasts [60] ▪ Antioxidant-enriched diet reduced toxic oxysterol levels in brain and liver tissues of newborn DHCR7-knockout mice [61]
Genetic transfer of *DHCR7*	Use of adeno-associated virus vector and intrathecal injection to deliver *DHCR7* gene to the liver and CNS to produce functional DHCR7 enzyme	*In vivo* studies in two mouse models with partial deletions of *DHCR7* [42,71,72]	▪ Delivery of functional *DHCR7* gene through the blood-brain barrier and into the CNS could help restore cholesterol homeostasis ▪ Whether a healthy sterol profile can be maintained in the long-term, and if it can be utilized in all cases of SLOS remains to be seen
Activation of Wnt signaling	Stabilization of β-catenin, and other methods to promote the Wnt signaling pathway for healthy development	*In vitro* study in induced pluripotent stem cells taken from patients with mild and severe cases of SLOS	▪ Preliminary findings suggest that the loss of cholesterol binding to the Wnt receptor complex destabilizes β-catenin and prevents the transcription of important developmental genes [47]. Therefore, targeting the Wnt/β-catenin signaling pathway could help avoid severe SLOS phenotypes

* Please note that many of these treatments remain at the pre-clinical or clinical stage, and the information presented should not be used to adjust any current treatment plans for SLOS patients

2.3. Role of Sterols in Embryogenesis

The accumulation of 7DHC, and corresponding lack of cholesterol, can have serious consequences for embryogenesis. For example, cholesterol is required for the activation of the canonical Wnt signaling pathway [73], a highly conserved pathway that regulates many aspects of cell fate determination and organogenesis. The enrichment of cholesterol in the membrane recruits the scaffold protein Dishevelled, and subsequently enables formation of the Wnt signaling complex. Thus, a lack of cholesterol, which has approximately 20 times more affinity for Dishevelled than 7DHC [47], has severe effects on Wnt signaling in SLOS patients. This was recently demonstrated by Francis *et al*. [47], who utilized an induced pluripotent stem cell model of SLOS to show that accumulated 7DHC is detrimental to Wnt signaling and contributes to the neuronal defects observed in SLOS patients. Further research into targeting the Wnt signaling pathway could provide promising therapies for SLOS (Table 1).

Similarly, the Ret signaling pathway utilizes cholesterol-rich lipid rafts in the development and maintenance of the genitourinary and nervous systems [74]. Thus, defective Ret signaling was also proposed to be responsible for the congenital abnormalities seen in SLOS patients. However, this was found not to be the case, with the finding that 7DHC effectively supports the Ret signaling pathway in the absence of cholesterol [75].

Cholesterol is also well-known to play a vital role in normal Hedgehog (Hh) signaling, which is important for vertebrate development. Several groups have implicated DHCR7 as both a positive and negative regulator of Hh signaling. The inhibition of DHCR7 activity impaired Hh signaling under various conditions [31,76,77], which was attributed to decreased Smoothened activity caused by a deficit in cholesterol rather than the accumulation of 7DHC or oxysterols [31]. On the other hand, studies in *Xenopus laevis* found that DHCR7 negatively regulates Hh signaling at, or downstream of, Smoothened, and that this was not contingent on DHCR7's enzymatic activity [78]. Supporting this finding in a mammalian system, overexpression of wild-type or mutant DHCR7 in NIH3T3 cells decreased Hh signaling, likely downstream of Smoothened [79]. Thus, DHCR7 has been proposed to play a dual, yet opposing role in Hh signaling [80,81], and further work is clearly needed to elucidate the details.

2.4. Vitamin D_3 Status

Cholesterol plays a crucial role in the skin, contributing to its waterproof properties and helping to make it impermeable [82]. As mentioned previously, the Kandutsch-Russell pathway was recently identified as the major active pathway of cholesterol synthesis in the skin [13], perhaps to generate 7DHC for vitamin D_3 synthesis (Figure 2). Vitamin D_3 is best known for maintaining calcium homeostasis and bone health, but its deficiency is increasingly associated with a number of different diseases [83].

SLOS patients may be expected to have higher than normal levels of vitamin D_3 due to their accumulation of 7DHC. The mutations could offer a heterozygous advantage to carriers, helping to prevent low vitamin D_3 [39], which could explain the prevalence of certain SLOS mutations in populations located in areas of low sunlight. To the best of our knowledge, only one study has examined vitamin D_3 status in SLOS patients, finding no difference in samples from fifteen patients [84]. However, this could be due to abnormal vitamin D_3 metabolism and enhanced photosensitivity in SLOS patients [59,84], which minimizes their exposure to sunlight and thus, is likely to prevent overproduction of vitamin D_3.

Certainly, genetic factors are known to contribute to the variability in vitamin D_3 status [85]. This includes SNPs associated with genes in the vitamin D_3 metabolic pathway, such as the *vitamin D binding protein*, *vitamin D receptor*, and *vitamin D 25-hydroxylase* [85,86]. In addition, in 2010, two independent groups performed large-scale genome-wide association studies (GWAS) to determine genetic factors contributing to vitamin D_3 status, and identified *DHCR7/NADSYN1* as a novel locus [87,88]. Specifically, the minor alleles of the nine SNPs they identified (three by [87], and six by [88], specified in Table 2) are associated with decreased vitamin D_3 levels compared to the major allele. The chromosomal locations of the SNPs in Table 2 are indicated in Figure 6. *DHCR7* has biological relevance to vitamin D_3 levels, with its activity directly decreasing the amount of available 7DHC for vitamin D_3 synthesis. Its neighboring gene, *NADSYN1* (NAD synthetase 1), catalyzes the formation of NAD, which is an important cofactor in many redox reactions but has no direct relatinoship to DHCR7 or vitamin D_3.

TABLE 2. *DHCR7/NADSYN1*-associated SNPs and effects on vitamin D_3 status

DHCR7/NADSYN1 SNP variant	*DHCR7/NADSYN1* nucleotide[a]		Location[a]	Effect on vitamin D_3[b] (No. of studies [Refs])		
	Major	Minor (MAF)		Decrease	No effect	Increase
rs12785878[c]	G	T (0.35)	Intron	4 [88–91]	10 [92–101]	2 [102,103]
rs3829251	G	A (0.27)	Intron	5 [87,89,104–106]	4 [93–95,101]	
rs1790349[d]	T	C (0.25)	Intron	2 [87,106]	3 [96,97,107]	
rs4944957	A	G (0.43)	Intron	1 [88]	3 [94,95,100]	1 [102]
rs12800438	G	A (0.40)	Non-coding variant	1 [88]	3 [94,95,100]	1 [102]
rs3794060	C	T (0.35)	3’ UTR variant	1 [88]	3 [94,95,100]	1 [102]
rs7944926	A	G (0.35)	Intron	1 [88]	2 [94,95]	1 [102]
rs4945008	A	G (0.35)	Intron	1 [88]	1 [95]	1 [102]
rs11234027	G	A (0.29)	Intron	1 [87]	1 [95]	
rs11233570	C	G (0.04)	Intron		1 [107]	
rs1540130	C	G (0.28)	Intron		1 [107]	
rs1540129	G	C (0.50)	Intron		1 [107]	
rs12419279	T	A (0.39)	Upstream gene variant		1 [107]	
rs1792272	T	C (0.26)	Intron		1 [107]	
rs7122671	G	A (0.11)	Intron		1 [107]	
rs1790334	G	A (0.19)	Synonymous (T69)		1 [107]	
rs1790373	G	A (0.20)	Non-coding variant		1 [107]	

[a] From Ensembl, note that minor allele frequencies (MAFs) are different in some studies due to population differences

[b] Decrease = minor allele is associated with lower vitamin D_3 levels, Increase = minor allele is associated with higher vitamin D_3 levels

[c] One study found that the minor allele was nominally associated with reduced risk of vitamin D_3 insufficiency [92]

[d] This SNP is listed in the Ensembl database as a T/C SNP, but it is most likely an A/G SNP, based on publications [96,97,106,107]

TABLE 3. Summary of studies involving DHCR7 SNPs and vitamin D_3 status

Sample size	Study Participant Information	Ref
33,996	Europeans from Europe, Canada and USA	[88]
9,528	Norwegians	[104]
4,501	Europeans from Finland and USA	[87]
3,210	Han Chinese from Beijing and Shanghai (aged 50-70)	[106]
2,857	Dutch (aged ≥ 65)	[90]
2,100	Germans (aged 35-65)	[103]
1,959	1,605 Hispanic and 354 non-Hispanic white women	[96]
1,787	Healthy, non-Hispanic whites (aged 45-75)	[93]
1,549	Arabs, South Asians, and Southeast Asians from Kuwait	[94]
1,322	Finnish	[91]
1,204	European post-menopausal women	[107]
1,057	652 African American and 405 European American men	[95]
993	Africans in Southwest USA; Utah residents with ancestry from Northern and Western Europe; Chinese in Denver, Colorado; Gujarati in Houston, Texas; Japanese and Han Chinese from Tokyo or Beijing; Luhya in Kenya; Mexicans in Los Angeles; Maasai in Kenya; Tuscans in Italy; Yoruba in Nigeria	[102]
758	Danish (from 201 families)	[97]
743	Yup'ik Alaskans	[100]
712	Southern Chinese women from Hong Kong	[92]
600	300 Uyghur + 300 Kazak ethnicity from Xinjiang, China	[98]
506	Han Chinese children from Northeastern China	[89]
484	Pre-diabetic adults from Norway	[105]
222	Londoners (aged 48-94)	[101]
180	Adults from Virginia, USA	[99]

Subsequent studies have included SNPs at the *DHCR7/NADSYN1* locus as part of their investigations to determine its genetic effects on vitamin D_3 status. We reviewed 21 studies, each with at least 150 subjects (Tables 2 and 3) that measured circulating levels of 25-hydroxyvitamin D, which is the sum of 25-hydroxyvitamin D_3 (derived from sunlight, see Figure 1), and 25-hydroxyvitamin D_2 (derived from dietary plants). These studies reported inconsistent effects of *DHCR7/NADSYN1* SNPs on circulating 25-hydroxyvitamin D levels (Table 2). For example, for rs3829251, five studies reported that the minor allele is associated with decreased 25-hydroxyvitamin D, while another four studies reported no effect (Table 2). Of note, studies with larger sample sizes (and hence, with greater statistical power) were more likely to find an association (e.g. [87,88,104,106], Table 2), whereas many of the smaller studies (n < 2000) reported no effect (e.g. [97,99–101], Table 2). Perhaps due to this, another study with a smaller sample size in the pilot cohort (n = 229) [108], which was not

included in our assessment as the specific SNP was not stipulated, found no association between *DHCR7/NADSYN1* SNPs and 25-hydroxyvitamin D levels.

In an interesting finding, the rs3829251 SNP has been identified as important for calcium metabolism [104], where homozygotes of the major allele were ~2 cm taller compared to the homozygotes of the minor allele. It is tempting to speculate that this effect is occurring via increased vitamin D_3 levels offering greater calcium absorption and skeletal health, but the mechanism behind this process remains to be elucidated.

Also noteworthy is that different SNPs may have different effects on 25-hydroxyvitamin D levels in certain sub-populations. For instance, the minor allele of rs12800438 was associated with vitamin D_3 deficiency in African Americans but not in European Americans [95]. Another example is rs12785878, where the minor allele is associated with vitamin D_3 deficiency in the Kazak ethnic population, but not in Uyghurs [98].

The two original genome-wide association studies reported *DHCR7/NADSYN1* variants in subjects of European descent. Kuan *et al*. [102] conducted a follow up study on *DHCR7/NADSYN1* SNPs likely to undergo natural selection in ten populations. While the minor alleles of these six SNPs were originally found to be associated with decreased 25-hydroxyvitamin D levels [88] (Table 2), Kuan and colleagues [102] reported that they were associated with increased 25-hydroxyvitamin D levels. Moreover, they found that the frequency of these minor alleles was higher in Europeans compared to the other populations, and determined that these particular variants were positively selected for in populations living in northern latitudes – suggested to have evolved to help early humans inhabit areas of low sunlight.

While each study generally accounted for factors that may affect vitamin D_3 status other than genetic variance (e.g. subject age, sex, season, sunlight exposure, and vitamin D_3 supplementation), findings from studies where subjects consist of a defined sub-population may still be limiting. For example, studies that consist of only older adults (e.g. [90,101,107]) may be confounded since vitamin D_3 levels tend to decrease with age [109]. There is also continued dispute over the reliability of measuring circulating 25-hydroxyvitamin D as a biomarker of vitamin D_3 status [110,111]. The inaccuracies typically arise from the level of 25-hydroxyvitamin D that is bound to the vitamin D receptor protein [112], which can vary greatly between ethnicities and populations [113]. Thus, careful consideration of the 25-hydroxyvitamin D assays used and the development of more robust analytical methods are required to accurately assess vitamin D_3 levels.

In addition, many studies focus on *DHCR7/NADSYN1* SNPs implicated in vitamin D_3 status and risk of disease, including overall mortality [114]. For example, genetically low vitamin D_3 levels (including SNPs in *DHCR7/NADSYN1*) were associated with type I diabetes [115], whereas another study found no correlation [116], indicating that further work is required. While the link between *DHCR7/NADSYN1* SNPs and vitamin D_3 levels is widely accepted (e.g. [86,114]), we found that this may be equivocal and whether these SNPs have a functional effect on DHCR7 remains to be

determined. In support of such a connection, we have recently found that DHCR7 activity levels can influence production of vitamin D_3 in cell studies ([117], Section 4.2).

3. CHARACTERIZATION OF THE DHCR7 PROTEIN

3.1. DHCR7 Topology and Structure

DHCR7 is a 55 kDa protein containing 475 amino acids, and was predicted to contain nine transmembrane domains (TMs) [24]. Recently, the crystal structure of a DHCR7 homolog, the sterol reductase from the haloalkaliphilic bacterium *Methylomicrobium alcaliphilum* 20Z (MaSR1) was solved to 2.7 Å [118]. This bacterial protein catalyzes the reduction of the double bond between C(14-15) in the sterol D-ring, whereas DHCR7 acts on C(7-8) in the B-ring. Despite these functional differences, MaSR1 shares 37% identity and 51% similarity with human DHCR7 using EMBOSS Needle [119]. Interestingly, the 3-dimensional structure (RCSB Protein Data Bank entry: 4QUV) shows that MaSR1 has a 10-TM topology [118]. Given the high similarity between the two proteins, it is likely that DHCR7 also crosses the membrane ten times, which differs from the predicted 9-TM model [24]. In light of this, we further analyzed the topology of DHCR7 *in silico*.

Using a panel of 12 currently available online servers, we found that DHCR7 was predicted to have 6-10 TMs (Table 4). All predicted TMs share at least 46% similarity with that of MaSR1 (Table 5). In particular, the C-terminus of MaSR1 covering TMs 5-10 (residues 196-475) is highly similar to DHCR7 (residues 235-475), sharing 49% identity and 63% similarity with very few gaps in the loop region when aligned [119]. Therefore, it is very likely that this region of DHCR7 is 3-dimensionally like MaSR1. It is interesting to note the unusual arrangement of TM helices 9 and 10 in the context of TM predictions. The relatively short TM helix 9 (15 residues) barely emerges from the membrane before looping back to the equally short TM helix 10 via a minimal linker (AAFGSP) in MaSR1 [118]. It is therefore not surprising that 8 out of 12 programs failed to identify this region as two TM helices because the length likely falls below the threshold for a TM by these algorithms.

TABLE 4. Predicted transmembrane domains

Method	No. of TMs Predicted
MaSR1, PPM*	10
TOPCONS	10
PolyPhobius	10
PHDhtm	9
Philius	9
TMPred	9
CCTOP	8
SACS MEMSAT	8
SCAMPI	8
OCTOPUS	7
Sosui	7
SPOCTOPUS	6
TMHMM	6

*The membrane boundary of MaSR1 was determined by the server PPM [120] using the PDB entry 4QUV.

TABLE 5. Comparison of DHCR7 putative transmembrane domains with MaSR1

TM	Residues based on MaSR1 Alignment	Identity with MaSR1 (%)	Similarity with MaSR1 (%)	No. of Programs that predicted the TM (out of 12)
1	41-60	30	65	12
2	94-115	36	46	10
3	145-164	25	60	12
4	176-197	27	48	12
5	235-256	55	82	8
6	268-288	43	71	10
7	302-326	72	76	6
8	332-353	36	50	12
9	408-424	47	65	8
10	426-442	53	82	7

Taken together, DHCR7 should be considered a 10-TM protein. Furthermore, the number of positively charged arginine and lysine residues is 30 on one side of the membrane, and four on the other side. According to the 'positive inside' rule [121], the side with more positively charged amino acid residues is assigned to the cytosol. This orientation is also supported by our own experimental results where the location of the C-terminus was confirmed by tryptic digestion of an epitope tag (AJ Brown *et al.* unpublished data). Accordingly, we present a predicted topology map and 3-dimensional structure in Figure 4A and 4B.

Highlighted in Figure 4A are residues known to be mutated in at least one SLOS patient, based on publications [28,122–126] and a comprehensive database of SLOS-causing mutations in DHCR7, including those that are unpublished [127]. We predicted that SLOS missense mutations are enriched

in the membrane regions of DHCR7, since mutations in the membrane-associated regions of DHCR7 may prevent correct protein folding and result in its degradation. Indeed, we [117] and others [20,128] have found that a number of common SLOS missense mutations destabilize DHCR7 protein. However, when we analyzed if SLOS mutations are overrepresented in TMs (based on similarity to MaSR1), this did not reach statistical significance (61/110 total, $p = 0.1$ Fischer's exact test). This possibility will become clearer as the structure and TMs become more certain.

3.2. Important Domains of DHCR7

It is well-established that the conversion of 7DHC to cholesterol requires the reduced pyridine nucleotide, NADPH [24,129]. Dempsey *et al*. [129] first included NADPH *in vitro* and identified it was needed in this reductive process. Although a classic Rossmann-fold sequence domain for NADP-binding [130] does not exist in DHCR7, the role of NADPH has been experimentally confirmed with human and rat DHCR7 [23,24]. Enzymatic assays using microsomal preparations with active DHCR7 determined that NADPH (and not NADH or FAD) was necessary for its activity [24]. Further studies with rat DHCR7 suggested cytochrome P450-reductase as the redox partner for this NADPH-dependent reaction [131]. However, more recent evidence from hepatic cytochrome P450-reductase-null mice ruled this out, with high DHCR7 activity still observed in its absence [132].

The MaSR1 structure supports a dependence on NADPH. While the role of TMs 1-4 serves as a scaffold, TMs 5-10 comprise the catalytic region containing the NAPDH binding site [118]. The residues identified for NADPH binding are completely conserved between MaSR1 and DHCR7, and located in cytosolic loop 4 and the C-terminus (Figure 4A). This structural motif contains arginine and lysine residues which steers negatively charged NADPH to the active site by electroattraction, and an aspartic acid residue which is commonly found forming hydrogen bonds with the hydroxyl group of the ribose moiety in ATP binding cassettes [133]. However, it remains to be confirmed that NADPH binds DHCR7 in the same way.

DHCR7 is predicted to contain a sterol-sensing domain (SSD), a conserved motif involved in sterol-regulation that is found in several membrane proteins related to cholesterol metabolism [134]. This domain is predicted to be localized to TMs 4-8 of DHCR7 [24], which is reasonable considering that the corresponding region in MaSR1 is the predicted binding pocket for the hydrophobic sterol substrate [118]. However, this has not been verified experimentally. The SSD plays an important role in HMGCR, mediating its sterol-induced degradation [135], and thus, the feedback regulation of sterol synthesis. The putative cholesterol binding sites predicted from MaSR1 could be involved in the interactions between cholesterol and DHCR7, but we have found that they are not implicated in the cholesterol-mediated degradation of DHCR7 (discussed further in Section 4.2) [117]. Furthermore, we have shown that the predicted SSD in DHCR7 does not need Insig to mediate sterol-regulated degradation [117], as it is for HMGCR [135]. Although the putative SSD is quoted as having significant similarity to SSDs from other proteins [24,134], our comparison of SSD similarity has

found that this is not the case (Figure 4C). All the SSDs had at least 32% similarity with at least one other SSD, whereas the putative DHCR7 SSD had a maximum of 11% similarity with the Dispatched SSD. Therefore, we recommend caution regarding the designation of this region as an SSD, pending its experimental verification.

3.3. Evolution of DHCR7

The reductive steps in the cholesterol synthesis pathway are catalyzed by four sterol reductases. In addition to DHCR7 and DHCR24, Δ14-sterol reductase (DHCR14) and the lamin B receptor (LBR) both reduce the C(14-15) double bond in the process of making cholesterol. LBR helps maintain the structural integrity of the inner nuclear envelope, a specialized extension of the endoplasmic reticulum [136]. It has also been ascribed a key role in cholesterol synthesis, perhaps even more important than DHCR14 [137]. DHCR7 shares high sequence identity with LBR and DHCR14, at 24% and 35% respectively using EMBOSS Needle [119], suggesting they are homologs and likely to be similarly regulated. In contrast, DHCR24 shares only 9% sequence identity with DHCR7 [119].

Human LBR and DHCR14 complement ERG24, a C14 sterol reductase in *Saccharomyces cerevisiae* [138], however DHCR7 is absent in this species [23]. Nonetheless, phylogenetic analysis reveals that DHCR7 is present in all three major eukaryotic groups (animals, fungi and plants) [136]. Analysis of DHCR7 protein sequences present in SWISS-PROT [139], showed that there is a high level of conservation of the DHCR7 protein, with greater than 70% identity between human DHCR7 and each species listed, except for *Acanthamoeba polyphaga mimivirus* (Figure 7). It is surprising that an intra-amoebic virus would carry the *DHCR7* gene, but it is proposed to have been acquired from a eukaryotic group of green plants (viridiplantae) via horizontal gene transfer [140]. Among viruses, this mimivirus possesses the largest viral genome to date, possibly due to its increased ability to transfer and acquire genetic material [141]. Moliner *et al*. [140] suggest that DHCR7 may even play a role in these parasites, with cholesterol needed for efficient replication and survival within its host.

In all species indicated in Figure 7, 75% of residues with high mutation frequencies in SLOS were conserved (cf. Figure 4A). The medium and low frequency mutations had 65% or 53% conservation, respectively, suggesting that mutation of highly conserved residues are more likely to cause SLOS due to their importance in DHCR7 function. Similarly, 100% of the NADPH binding sites were conserved. Other regions of high conservation could indicate important regulatory domains within DHCR7.

4. REGULATION OF DHCR7

4.1. Transcriptional Regulation of DHCR7

Like many cholesterol synthetic genes, *DHCR7* is under the control of the transcription factor, sterol-regulatory element binding protein 2 (SREBP-2), assisted by nuclear factor-Y [142,143]. In

conditions of low cholesterol, SREBP-2 cleavage enables its active N-terminus to migrate into the nucleus where it binds to sterol regulatory elements (SREs) in the proximal promoters of its target genes. This process increases *DHCR7* transcription and overall cholesterol synthesis.

The human *DHCR7* proximal promoter possesses two SRE sites – one which is highly conserved, whereas the other, located 100 base pairs downstream, is only present in higher organisms [143]. In the rat *DHCR7* promoter, one SRE site was identified and found to be sterol responsive [144], but the corresponding site in humans was not [143], suggesting that the location of this SRE appears to have drifted over evolutionary time.

In addition, the two SREs in the human *DHCR7* promoter work cooperatively to activate gene expression only when sufficient SREBP-2 is present [143]. Since cholesterol is an energetically expensive molecule to synthesize, the cooperative behavior of the dual SREs requires strong activation of target genes before commitment to cholesterol synthesis. Four out of five enzymes known to possess dual SREs are cholesterol synthetic genes (*DHCR7*, *DHCR24*, *HMG CoA synthase* and *squalene synthase*) [143,145–147], and likely to undergo this economical mode of regulation. Our own survey of the literature indicates that several enzymes of the cholesterol synthesis pathway are yet to have their SREs reliably mapped in their human promoters [4], and it will be interesting to determine whether any others exhibit cooperative behavior.

Cholesterol homeostasis is also under the control of several epigenetic mechanisms [148], which adds another layer of regulation. For instance, male mice fed a low-protein diet produced offspring with several upregulated cholesterol synthetic genes, including *DHCR7*, which increased 4-fold [149]. This presents a way in which environmental information can be transferred via the epigenome. Schulz *et al*. [150] identified that mouse *DHCR7* is an imprinted gene, preferentially expressed from the maternal allele in the placenta, but biallelically expressed in the embryo. Paternal expression of *DHCR7* is thought to be repressed in the placenta [151]. Although imprinting analysis on human placenta found that maternal *DHCR7* was not solely expressed, *DHCR7* transcription from the paternal allele was at a lowered level [150]. This could have implications on cholesterol synthesis *in utero*, which is an important process in fetal development and in the potential occurrence of SLOS. As mentioned in Section 2.1, maternal features such as variations in apolipoprotein E and ATP-binding cassette transporter A1 [36,37], or the level of maternal to fetal cholesterol transfer [152] may influence severity of SLOS. Taken together, this may suggest that maternally inherited mutations have a stronger effect on the SLOS clinical phenotype. Further work is needed to ascertain the effect of *DHCR7* epigenetic regulation on SLOS, and whole-body cholesterol metabolism.

In addition, the methylation of CpG islands in the promoter suppresses transcription of rat *DHCR7* [153]. Whether this methylation pattern exists in the human *DHCR7* promoter remains to be determined. Intriguingly, methylation levels of the *DHCR7* promoter were reduced in a male, African-American population with severe vitamin D_3 deficiency [154], suggesting that the methylation-mediated inhibition of *DHCR7* transcription is necessary for adequate vitamin D_3 synthesis. Can this

methylation be consistently observed in populations with high vitamin D_3 status? Further insights into the transcriptional regulation of human *DHCR7* could help elucidate its role in these important biological processes.

4.2. Post-transcriptional Regulation of DHCR7

Although regulation at the transcriptional level plays a crucial role in modulating expression, it acts relatively slowly to influence DHCR7 levels. In contrast, post-transcriptional regulatory mechanisms can provide more acute control of DHCR7. At the level of the mRNA transcript, Sen *et al*. [155] found that DHCR7 and Scap, a master regulator of cholesterol homeostasis, are direct targets of the serine/arginine-rich splicing factor 3 family of RNA binding proteins. Atypical splicing events can affect enzyme levels, with aberrant splicing and the subsequent misfolded protein inducing the immediate degradation of the DHCR7 transcript. A previous study found that five distinct isoforms of the mouse DHCR7 transcript were produced and differentially expressed in a tissue- and age-dependent manner [156], which may help achieve specific sterol synthesis rates for specific purposes. However, the same study found only one DHCR7 isoform in a human liver cell line, suggesting that isoforms are likely tissue- and species-dependent.

Excessive levels of DHCR7 are also known to physically damage the cell structure by causing a significant expansion of the endoplasmic reticulum and perinuclear space [157], although this is unlikely to occur physiologically. We recently identified that DHCR7 protein levels are controlled by its products, cholesterol and desmosterol, in an example of negative feedback regulation [117]. Cholesterol induces the proteasomal degradation of DHCR7, and this rapid turnover could serve to direct accumulated 7DHC to alternative products. Considering the potential relationship between DHCR7 and vitamin D_3 (Section 2.4), it is possible that decreased DHCR7 levels allow greater flux into the vitamin D_3 pathway. Indeed, this was the case in cultured human skin cells, where cholesterol induced the loss of DHCR7 function and increased vitamin D_3 production [117]. Therefore, we argue that DHCR7 can be a switch between cholesterol and vitamin D_3 synthesis. Vitamin D_3 itself has also been identified to decrease DHCR7 activity in a potential vitamin D_3 positive feedback loop [158]. The extent to which the resulting switch from cholesterol to vitamin D_3 synthesis occurs in an *in vivo* system remains to be determined.

The instability of DHCR7 protein is in stark contrast to DHCR24 [117,159]. Interestingly, we identified that DHCR24 physically interacts with DHCR7, with DHCR24 knockdown and overexpression decreasing and augmenting DHCR7 activity, respectively [160]. This may suggest the existence of a cholesterol metabolon, where sequential cholesterol enzymes, particularly in the later stages of the synthetic pathway, interact with each other to facilitate substrate channeling [160].

DHCR7 also physically interacts with another cholesterol synthetic enzyme – the emopamil binding protein (EBP, also known as 3β-hydroxysteroid-Δ^8-Δ^7-isomerase), which forms a hetero-oligomeric complex with DHCR7 [161]. DHCR7 and EBP act as the regulatory and catalytic subunits

of the antiestrogen binding site (AEBS), respectively [161]. Located in the endoplasmic reticulum, AEBS is a target of tamoxifen [162], the breast cancer therapy that can also competitively inhibit the estrogen receptor. AEBS was found to promote the activity of cholesterol epoxide hydrolase, which catalyzes the hydration of cholesterol-5,6-epoxides into cholestane-3β,5α,6β-triol [161]. Cholesterol-5,6-epoxides can be conjugated with histamine to produce dendrogenin A, which induces cancer cell death and may be a natural tumor suppressor metabolite [163]. Together, these findings suggest that DHCR7 and EBP play additional roles in promoting cell growth and differentiation, which may be important in a cancer setting.

The activity of DHCR7 and other cholesterol synthetic enzymes can also be acutely affected by post-translational modifications such as phosphorylation [164]. While phosphorylation inactivates HMGCR [165], inhibition of phosphorylation decreases DHCR24 [159] and rat DHCR7 activity [166]. Providing yet another layer of regulation, we found that the phosphorylated S14 residue of DHCR7, and several kinase inhibitors, can regulate the activity of DHCR7 [167]. Beyond these findings, it is worthwhile noting that there are six published phosphorylation sites and six published ubiquitination sites in DHCR7 [168] (Figure 4A). These were identified in large-scale proteomics studies and require dedicated investigation to determine their role(s) in DHCR7. Considering the high sequence similarity between DHCR7, LBR and DHCR14, we are currently investigating if LBR and DHCR14 are rapidly turned over like DHCR7, and if so, what triggers this degradation. With this information, further insights can be gained into the regulation of DHCR7, and that of the entire cholesterol synthesis pathway.

5. CONCLUDING REMARKS

The vast array of DHCR7 mutations that lead to SLOS confounds attempts to delineate a clear genotype/phenotype relationship. Current treatments can, at best, manage some symptoms of the disease, but a cure is unlikely until *in utero* therapeutics or gene modification is feasible and ethical. The lability of 7DHC is likely a significant problem in SLOS patients, as it can readily accumulate and be converted to toxic products.

However, this quality is also what enables 7DHC to be effectively converted to vitamin D_3, another important product for human health. A relationship between DHCR7 and vitamin D_3 exists, with DHCR7 acting as the switch between cholesterol and vitamin D_3. It is logical that *DHCR7/NADSYN1*-associated SNPs may influence vitamin D_3 levels, but our survey of the literature indicates that the jury is still out.

Recent advances in the determination of the structure of DHCR7 will undoubtedly assist in further characterizing the regions of the enzyme that are critical for its function. It may also help to test how the enzyme interacts with cholesterol and other sterols. The mechanisms that regulate DHCR7 activity and function at the transcriptional and post-translational levels are continuing to be

uncovered. DHCR7 is an intriguing enzyme, and further study will likely provide insights into its role in both cholesterol and vitamin D_3 homeostasis.

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FIGURE LEGENDS

FIGURE 1. 7-dehydrocholesterol (7DHC) is the immediate precursor of cholesterol and vitamin D_3. The C(7-8) double bond of 7DHC can be reduced by DHCR7 in an NADPH-dependent reaction to form cholesterol. Alternatively, exposure of the skin to ultraviolet B (UVB) light can open the B-ring of 7DHC, which then undergoes isomerization to form vitamin D_3 (also known as cholecalciferol). This is then transported to the liver where cytochrome P450 (CYP) 2R1 or CYP27A1 act to convert it to 25-hydroxyvitamin D_3 (also known as calcidiol). Finally, this is transported to the kidneys where it is hydroxylated by CYP27B1 to 1,25-dihydroxyvitamin D_3 (also known as calcitriol) – the active form of vitamin D_3.

FIGURE 2. Simple schematic of cholesterol and vitamin D_3 synthesis. The cholesterol synthesis pathway leads to zymosterol, which can be diverted into either the Bloch or Kandutsch-Russell pathway. 24-dehydrocholesterol reductase (DHCR24) or 7-dehydrocholesterol reductase (DHCR7) catalyze the terminal steps of each pathway, respectively. Modified from [117].

FIGURE 3. Utilization of the Bloch and Kandutsch-Russell pathways. Selected tissues where the Bloch or Kandutsch-Russell pathways are preferentially used. Information is based on data from mice presented by Mitsche *et al*. [13]. *BAT/WAT, brown and white adipose tissue.*

FIGURE 4. DHCR7 topology and 3-dimensional structure based on MaSR1, and homology with known sterol-sensing domains (SSDs). A) A 10-TM model of DHCR7 based on a sequence alignment with MaSR1 [118]. Residues forming the α-helix of TMs are stacked. SLOS mutations based on frequency (low to high as indicated), and phosphorylation, ubiquitination and NADPH binding sites are indicated. The loops in the lumen (LL) and cytosol (CL) are numbered sequentially from the N- to C-terminus. B) A predicted 3-dimensional structure of DHCR7 (residues 42-475) using Modeller [169]. The α-carbons of amino acid residues for SLOS mutations, phosphorylation and ubiquitination sites are shown with the same color coding as in (A). C) The whole protein and respective SSDs of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), Patched, DHCR7, Dispatched, SREBP cleavage-activating protein (SCAP), and Niemann-Pick C1 (NPC1) were aligned pairwise with every other protein or SSD and plotted with the % similarity.

FIGURE 5. Metabolites enzymatically derived from 7DHC elevated in SLOS patients. 7-dehydrocholesterol (7DHC) can be interconverted to 8-dehydrocholesterol (8DHC) by emopamil binding protein (EBP) [170]. 7DHC can also be converted to 24-hydroxy-7DHC (24OH-7DHC) by CYP46A1 [171]; to 25-hydroxy-7DHC (25OH-7DHC) by CYP46A1 [171] or CYP27A1 [51]; to 4α- and 4β-hydroxy-7DHC (4OH-7DHC), likely by CYP3A4 [50]; to steroid hormones by CYP11A1 [172]; to 7-ketocholesterol (7KC) by CYP7A1, with 7α,8α-epoxycholesterol (7,8EC) produced as a minor product [173]; and to 27-hydroxy-7DHC (27OH-7DHC; also known as 26-hydroxy-7DHC) by CYP27A1 [51].

FIGURE 6. Chromosomal location of *DHCR7* and *NADSYN1*-associated SNPs. The *DHCR7* gene is located at chromosome location 11q13.4 and is transcribed in the reverse direction, from telomere to centromere. The neighboring gene *NADSYN1* is transcribed in the forward direction. The location of *DHCR7/NADSYN1* single nucleotide polymorphisms (SNPs) putatively associated with changing vitamin D_3 levels (in Table 2) are indicated by a black vertical line, and their accession numbers provided.

FIGURE 7. Evolutionary conservation of the DHCR7 protein. DHCR7 protein sequences, taken from SWISS-PROT [139], were aligned for the indicated species. 100% conservation is shown above the alignment in green. Please refer to Figure 4A for SLOS mutations and other important residues.

FIGURE 1

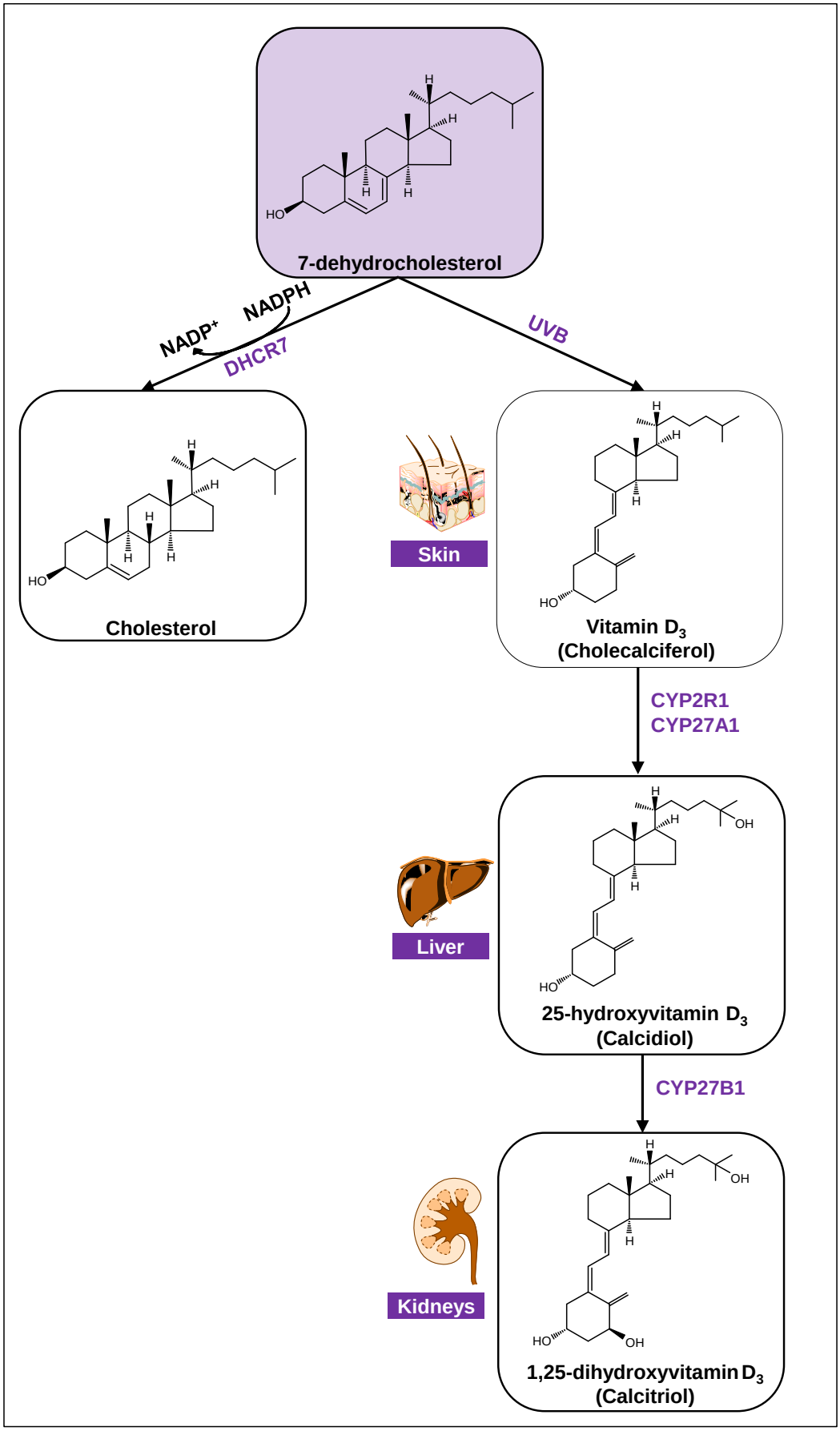

FIGURE 2

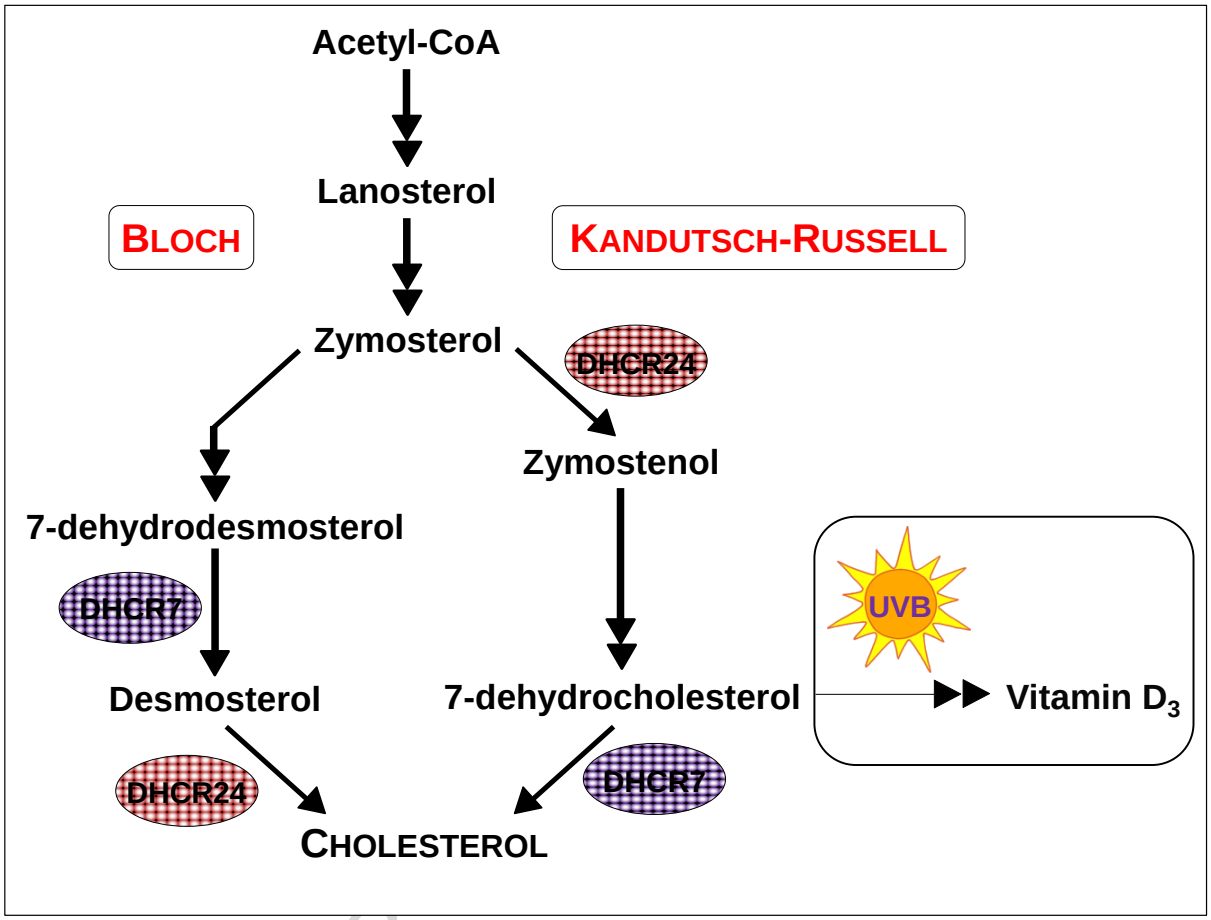

FIGURE 3

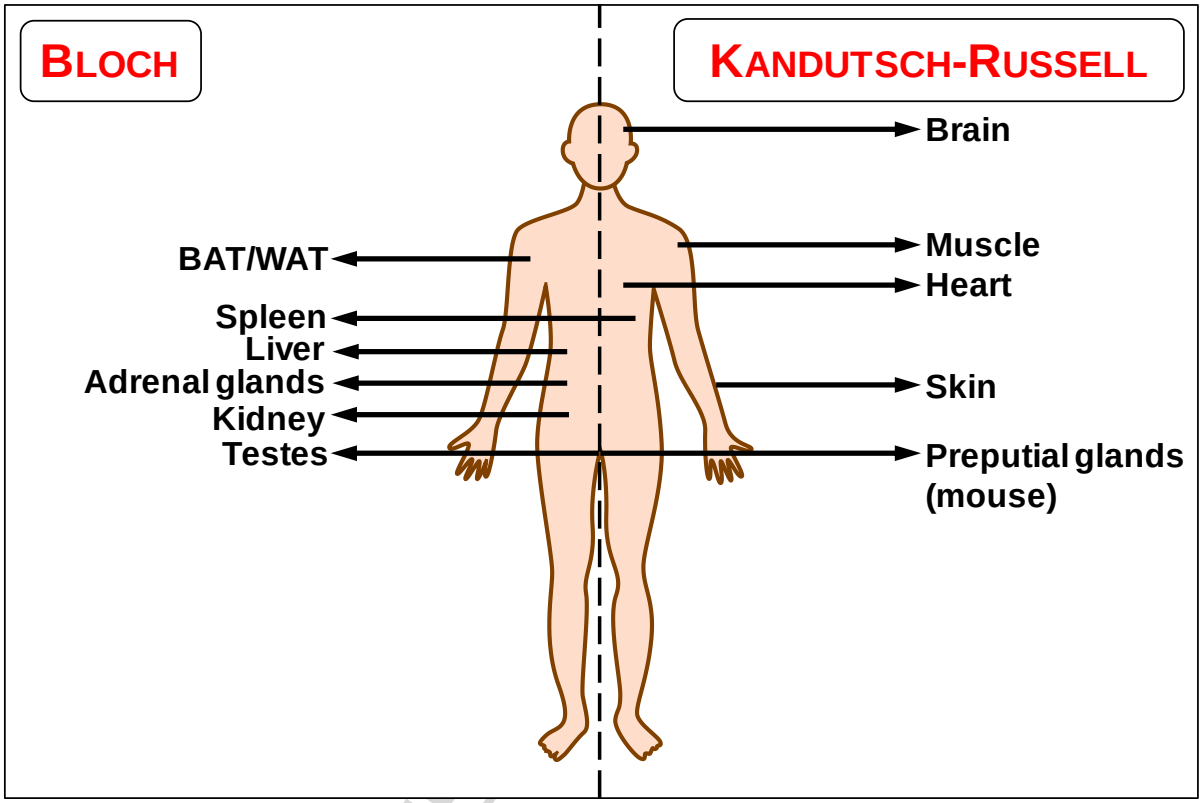

FIGURE 4

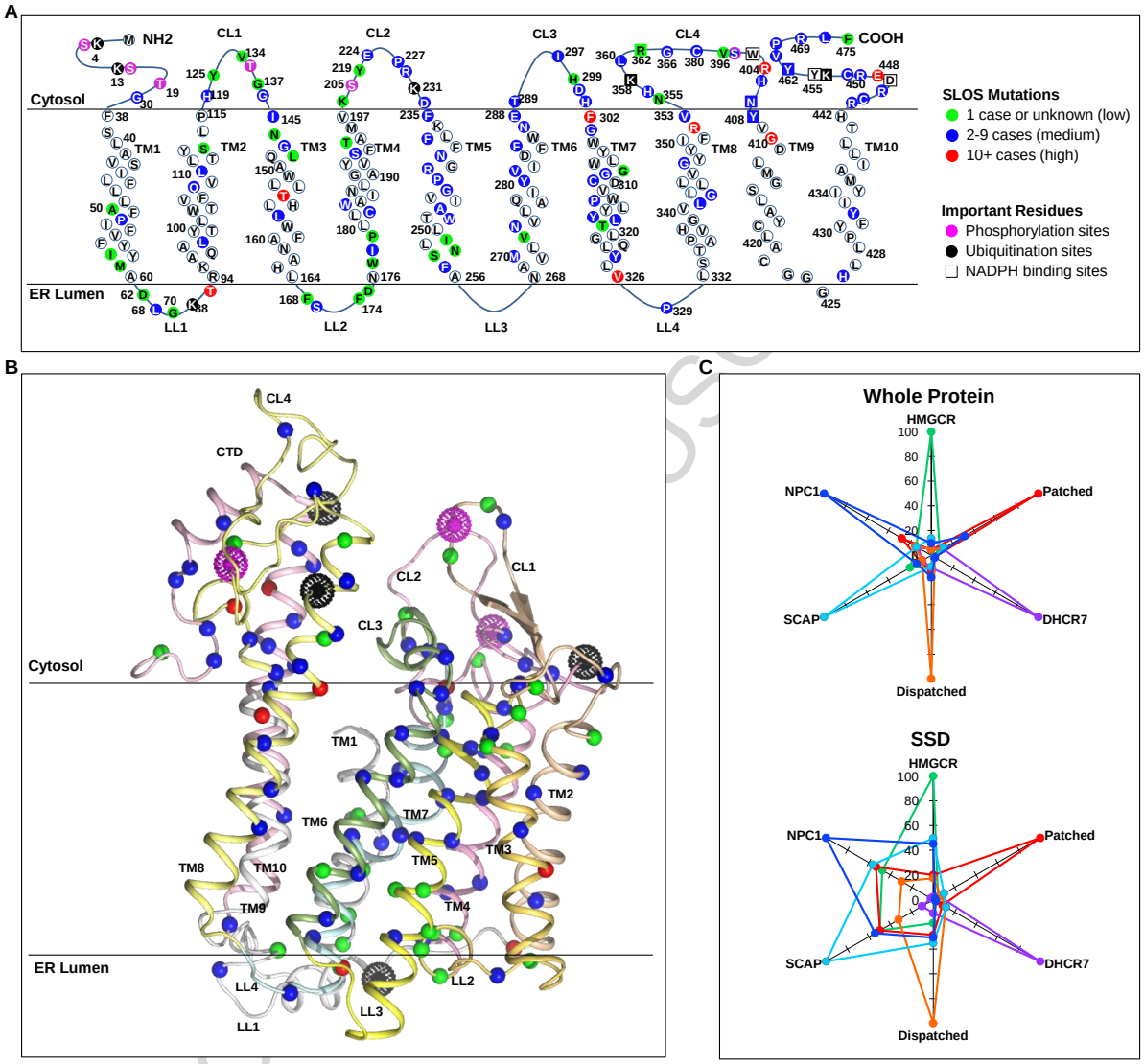

FIGURE 5

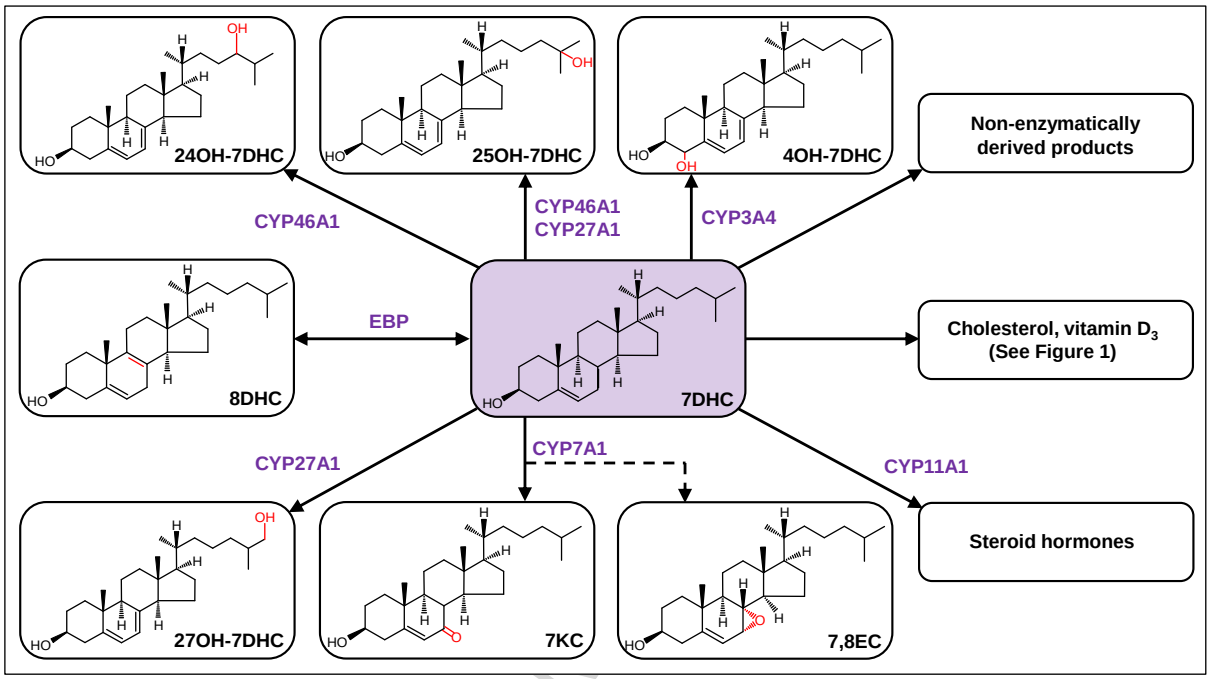

FIGURE 6

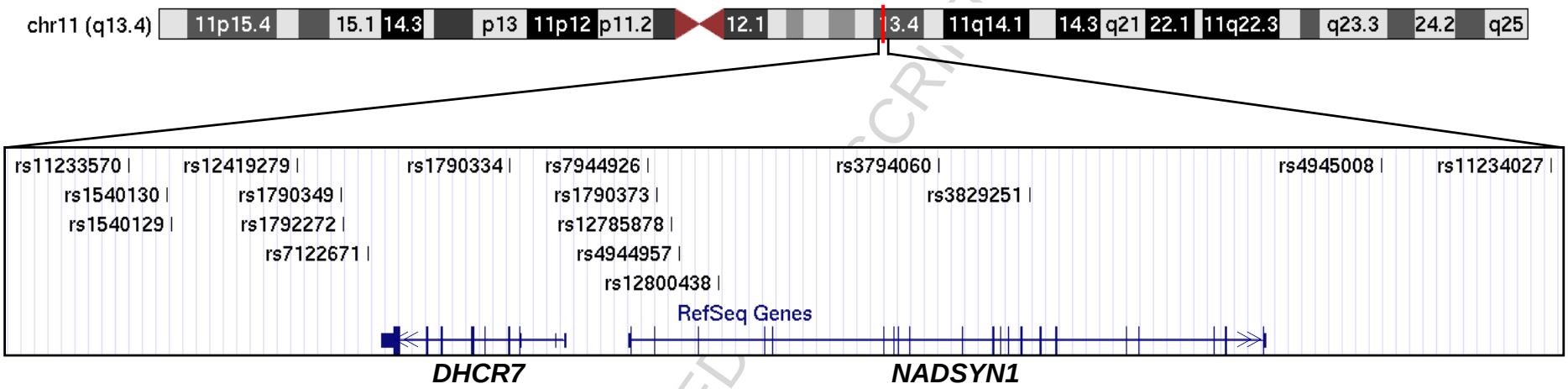

FIGURE 7