

# CYP7B1: One Cytochrome P450, Two Human Genetic Diseases, and Multiple Physiological Functions\*

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The CYP7B1 cytochrome P450 enzyme hydroxylates carbons 6 and 7 of the B ring of oxysterols and steroids. Hydroxylation reduces the biological activity of these substrates and facilitates their conversion to end products that are readily excreted from the body. CYP7B1 is expressed in the liver, reproductive tract, and brain and performs different physiological functions in each tissue. Hepatic CYP7B1 activity is crucial for the inactivation of oxysterols and their subsequent conversion into bile salts. Loss of CYP7B1 activity is associated with liver failure in children. In the reproductive tract, the enzyme metabolizes androgens that antagonize estrogen action; mice without CYP7B1 have abnormal prostates and ovaries. The role of CYP7B1 in brain is under investigation; recent studies show that spastic paraplegia type 5, a progressive neuropathy, is caused by loss-of-function mutations in the human gene.

There are two general types of cytochrome P450 enzymes, those that act on endogenous substrates like lipids and those whose primary function is to act on exogenous substrates like drugs and environmental chemicals. Deciding whether a new P450 acts on endogenous *versus* exogenous substrates is difficult in the absence of experimental analysis. This was the case for the P450 now known as CYP7B1, whose cDNA was initially isolated from a rat hippocampal library and described in the *Journal of Biological Chemistry* as a new P450 with 39% sequence identity to cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) (1). The latter enzyme acts on an endogenous substrate (cholesterol) and plays an important role in bile salt synthesis (2), but what role did CYP7B1 play? Subsequent biochemical experiments showed that CYP7B1 also acted on endogenous substrates, hydroxylating dehydroepiandrosterone, pregnenolone, and other steroids (3) as well as two oxysterols, 25-hydroxycholesterol (4) and 27-hydroxycholesterol (5).

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Here, we review the biochemistry, physiology, and genetics of CYP7B1, which reveal the enzyme to be a multifunctional P450.

## CYP7B1 Gene, mRNA, and Enzyme

The CYP7B1 gene encodes an evolutionarily conserved P450 and is found in the genomes of organisms ranging from humans to the Japanese fire-bellied newt to the fungus *Aspergillus niger*. The human gene spans ~220 kb on chromosome 8q21.3 and encompasses six exons separated by five introns (Fig. 1A). Transcription is initiated ~200 bp 5' of the initiation codon in exon 1 and produces an mRNA of ~9 kb that has a long (~7 kb) 3'-untranslated sequence (6). The tissue-specific expression pattern of the CYP7B1 gene differs between species. The human mRNA is present in many organs, with the highest levels detected in kidney and brain (6). The mRNA is also widely distributed among different rat tissues (7). Expression is restricted to the liver, lung, kidney, brain, and reproductive tract in the mouse, with the amount of mRNA being highest in liver and lung.<sup>4</sup> As with several other P450 enzymes, the CYP7B1 mRNA, protein, and enzyme activity in liver and kidney are higher in male mice than in female mice, and this sexually dimorphic expression requires androgen receptor signaling (8). The biological significance of this male-female difference and whether a similar pattern of expression exists in other species are not known.

The human mRNA encodes a 506-amino acid enzyme (EC 1.14.13.100) that catalyzes the 6 $\alpha$ - or 7 $\alpha$ -hydroxylation of several steroids and oxysterols (Fig. 1B). NADPH and cytochrome P450 oxidoreductase serve as cofactors in these reactions, and based on the latter requirement, CYP7B1 is presumed to be located in the endoplasmic reticulum. An anti-peptide antibody raised in a rabbit against amino acids 266–281 of mouse CYP7B1 recognizes a protein with an  $M_r$  of ~58,000 on immunoblots (4), which is in good agreement with that ( $M_r = 58,359$ ) predicted from conceptual translation of the mouse cDNA. The enzyme has not been purified and kinetically characterized; thus, whether steroids are better substrates than oxysterols or whether preferences exist within these two classes remains to be determined. The properties of CYP7B1 isolated from different species may differ, as nafimdone (1-(2-naphthoylethyl)imidazole) selectively inhibits the mouse but not the human enzyme (4). The three-dimensional structure of CYP7B1 has yet to be solved, but a structure at 2.15 Å is available for cholesterol 7 $\alpha$ -hydroxylase (CYP7A1, Protein Data Bank code 3DAX), which, as noted above, shares sequence identity and sterol substrate partiality with the enzyme.

## Physiological Roles of CYP7B1

**Bile Salt Synthesis**—A major metabolic fate of cholesterol is conversion into conjugated bile salts in liver (2). Once synthesized, bile salts act in the intestine to facilitate solubilization of hydrophobic nutrients from the diet, including fat-soluble vitamins and cholesterol. In liver, bile salts stimulate bile flow and the excretion of metabolites such as porphyrins

<sup>4</sup> J. Repa, personal communication.

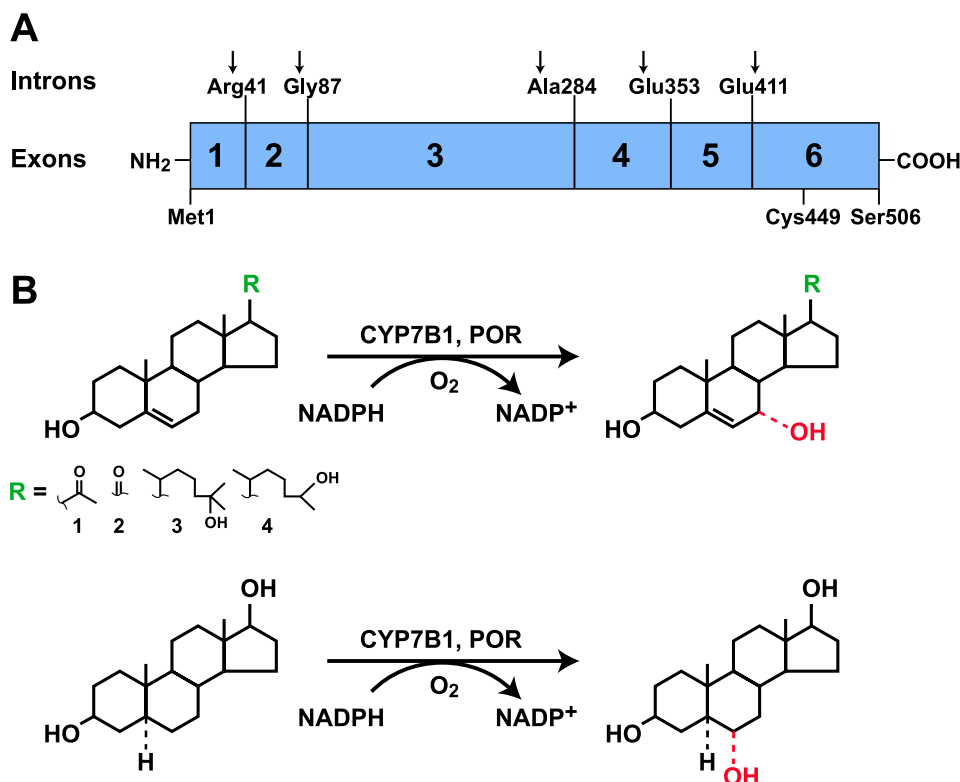


FIGURE 1. **CYP7B1 and reactions catalyzed.** A, schematic of the 506-amino acid enzyme showing regions encoded by the six exons of the gene and the positions marked by *arrows* at which five introns interrupt individual codons. The heme cofactor is covalently bound to Cys<sup>449</sup>. B, 7 $\alpha$ -hydroxylation of substrates (1, pregnenolone; 2, dehydroepiandrosterone; 3, 25-hydroxycholesterol; 4, 27-hydroxycholesterol) and 6 $\alpha$ -hydroxylation of 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol. POR, cytochrome P450 oxidoreductase.

**TABLE 1**  
Physiological roles of CYP7B1

Role/tissue	Substrates	Refs.
<b>Bile salt synthesis</b> Liver	25-Hydroxycholesterol, 27-hydroxycholesterol	8, 14, 29
<b>Steroid hormone metabolism</b> Brain	Pregnenolone, dehydroepiandrosterone	3, 7
<b>Metabolism of estrogen receptor ligands</b>		
Prostate	5 $\alpha$ -Androstane-3 $\beta$ ,17 $\beta$ -diol	19, 20
Prostate	Dehydroepiandrosterone?	23, 24
Vasculature	27-Hydroxycholesterol	21, 22
<b>Immunoglobulin production</b>		
Immune cells	25-Hydroxycholesterol	25, 26

that arise from the breakdown of heme. The identification and cDNA cloning of the enzymes that participate in bile salt synthesis began in the late 1980s (9), and *inter alia*, these studies showed that both cholesterol and oxysterols are substrates for bile salt synthesis. The first and rate-limiting step in the cholesterol pathway involved 7 $\alpha$ -hydroxylation, a reaction catalyzed by cholesterol 7 $\alpha$ -hydroxylase (10). Björkhem and co-workers (11) later showed that a different enzyme was involved in 7 $\alpha$ -hydroxylating oxysterols, and the finding that CYP7B1 shared sequence identity with cholesterol 7 $\alpha$ -hydroxylase suggested that CYP7B1 might be an oxysterol 7 $\alpha$ -hydroxylase. Genetic, pharmacological, and

biochemical experiments subsequently confirmed this hypothesis (Table 1) (4, 12).

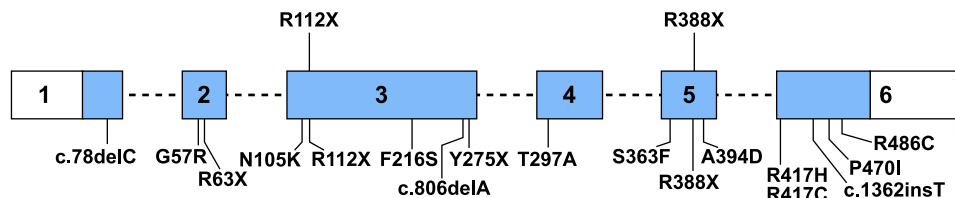
A majority of mice lacking cholesterol 7 $\alpha$ -hydroxylase die from vitamin and caloric deficiencies in the first 18–21 days of life (13); thereafter, the expression of CYP7B1 is induced in liver, resulting in the synthesis of bile salts and the prevention of further premature death (12). Adult cholesterol 7 $\alpha$ -hydroxylase-deficient mice contain ~30% of the normal amount of bile salts, suggesting that oxysterols are precursors for about one-third of the bile salt pool in this species (14). Cholesterol balance studies support this estimate and indicate that cholesterol 7 $\alpha$ -hydroxylase expression is ~30% higher in CYP7B1 knock-out mice<sup>5</sup>; this increase in expression produces a normal bile salt pool (8). Both 25- and 27-hydroxycholesterol accumulate in the sera and tissues of CYP7B1 mutant mice (8), a finding that supports their *in vivo* roles as enzyme substrates. Unexpectedly, the related side chain oxysterol, 24-hydroxycholesterol, does not

accumulate; this sterol is metabolized to a bile salt precursor by another oxysterol 7 $\alpha$ -hydroxylase, CYP39A1 (15).

**Steroid Hormone Metabolism**—CYP7B1 was initially identified as a steroid 7 $\alpha$ -hydroxylase with activity toward pregnenolone, a 21-carbon steroid, and dehydroepiandrosterone, a 19-carbon steroid (3). This result, the isolation of the cDNA from a hippocampal library (1), and histochemical data (7) suggested that CYP7B1 had a function in brain (Table 1). Pregnenolone and dehydroepiandrosterone are so-called neurosteroids, an enigmatic class of compounds that are ascribed broad regulatory, functional, and metabolic roles (16); thus, one hypothesis currently under investigation is that CYP7B1 inactivates neurosteroids in brain. There is as yet no evidence that pregnenolone or dehydroepiandrosterone accumulates in CYP7B1 knock-out mice; consequently, the importance of CYP7B1 in clearing these steroids from the brain and body *versus* other enzymes that utilize these substrates such as 3 $\beta$ -hydroxysteroid dehydrogenases is unknown. A case for CYP7B1 action in the central nervous system can be made given the clinical presentation of subjects with spastic paraplegia type 5 (see below), but whether this disease is caused by abnormali-

<sup>5</sup> Two lines of CYP7B1 knock-out mice are described in the literature (7, 8). The phenotypes of these two lines are not always similar. Whether differences arise from genetic background, diet, infection, investigator error, or a combination of these variables has not been determined. References indicate the line of mice in which a particular observation was made.

## Liver failure in newborns



## Spastic paraplegia type 5

FIGURE 2. Mutations in human CYP7B1 gene. Exons are indicated by shaded boxes drawn to scale. Introns are indicated by dashed lines and are not drawn to scale. Transcript coding regions are shaded blue; 5'- and 3'-untranslated regions are white. Mutations in subjects with liver failure are shown above the gene; those found in spastic paraplegia type 5 are shown below the gene.

ties in steroid *versus* oxysterol metabolism remains to be determined.

**Metabolism of Estrogen Receptor Ligands**—A third steroid substrate of CYP7B1 is  $5\alpha$ -androstane- $3\beta,17\beta$ -diol (7), a 19-carbon steroid that is an agonist for the estrogen receptor (17). Unlike with other substrates, CYP7B1 prefers to  $6\alpha$ -hydroxylate  $5\alpha$ -androstane- $3\beta,17\beta$ -diol (Fig. 1B) (18), but the net effect of this hydroxylation is the same as that for  $7\alpha$ -hydroxylation, namely inactivation of the steroid. Loss of CYP7B1 in males derived from one line of knock-out mice (7) leads to smaller prostates (19) and to early ovarian failure in female mice (20). In both sexes, these reproductive tract abnormalities are ascribed to excess  $5\alpha$ -androstane- $3\beta,17\beta$ -diol, which is postulated to accumulate in the absence of CYP7B1 and to drive pathologic activation of the estrogen receptor (19, 20).

The oxysterol 27-hydroxycholesterol is a selective estrogen receptor modulator, antagonizing estrogen-mediated activation of the receptor in the vascular wall (21) and activating the receptor in the absence of estrogen in breast cancer and other cell lines (22). In agreement with this selective estrogen receptor modulator activity, CYP7B1 knock-out mice (8), which accumulate 27-hydroxycholesterol in serum and tissues, show abnormalities in estrogen-mediated gene expression in the vasculature and defects in re-endothelialization (21). It is not yet known whether these phenotypes arise from a failure of CYP7B1 to catabolize 27-hydroxycholesterol locally (*i.e.* in the vascular wall) or systemically (*i.e.* in liver).

Conflicting evidence exists regarding the ability of  $7\alpha$ -hydroxydehydroepiandrosterone, a product of CYP7B1, to selectively activate the estrogen receptor  $\beta$  subtype. One report suggested that this steroid was made in human prostatic cells and therein activated the receptor (23), whereas a second report failed to replicate the receptor activation in human embryonic kidney 293 cells (24). Both studies relied on *in vitro* transfection approaches, and to date, there is no *in vivo* evidence to suggest a physiological role for  $7\alpha$ -hydroxylated dehydroepiandrosterone in estrogen receptor function.

**Immunoglobulin Production**—Activation of macrophages via Toll-like receptors induces cholesterol 25-hydroxylase and the subsequent synthesis of 25-hydroxycholesterol (25, 26). The oxysterol is secreted from the macrophage and suppresses the production of IgA by B cells via two mechanisms: suppression of cytokine-mediated B cell proliferation and repression of

a gene (activation-induced cytidine deaminase) that is required for IgA synthesis. This immunoregulatory role is supported by the findings that cholesterol 25-hydroxylase knock-out mice, which cannot synthesize 25-hydroxycholesterol, have higher than normal levels of IgA in their sera and mucosa, whereas CYP7B1 knock-out mice, which accumulate the oxysterol (8), have low levels of this immunoglobulin. CYP7B1 expression is induced in mouse lung and human joints by proinflammatory stimuli that are

released upon activation of macrophages and other cells of the innate immune system (27, 28). This induction may reflect the need to inactivate 25-hydroxycholesterol produced by macrophages.

## Genetics of CYP7B1

**Liver Failure in Children**—As noted above, a role for CYP7B1 in hepatic bile salt synthesis was suggested by studies in the mouse. This function was confirmed with the description of an infant who presented with liver failure arising from an inherited mutation in the CYP7B1 gene (29). Chemical analyses revealed that serum oxysterols and other bile salt intermediates were markedly elevated in this individual, whereas mature bile salts were lacking. DNA sequencing revealed a homozygous nonsense mutation at codon 388 in exon 5 of the gene (Fig. 2), which produced a truncated protein that lacked enzyme activity. A second subject with a similar clinical presentation but resulting from a homozygous nonsense mutation at codon 112 in exon 3 of the CYP7B1 gene was reported (30). The accumulation of oxysterols and other bile salt intermediates in these subjects was thought to have caused irreparable damage to the liver, ultimately necessitating transplantation. Together, these results underscored the importance of CYP7B1 in bile salt synthesis and the role of the enzyme in the inactivation of otherwise hepatotoxic oxysterols.

**Neuropathy in Adults**—Unexpected insight into CYP7B1 came in 2008 from a team of neurologists who reported that the autosomal recessive disorder spastic paraplegia type 5 was also caused by mutations in the CYP7B1 gene (31). These results were confirmed (32–35), and to date, 17 different mutations in >20 unrelated families have been identified in the gene (Fig. 2). The spastic paraplegias are a clinically heterogeneous group of disorders characterized by lower limb spasticity and weakness associated with degeneration of motor neuron axons in the spinal cord (36). Over 41 spastic paraplegia loci have been mapped in the human genome, and mutations in 17 different genes have now been identified in individuals affected with the various forms of the disease. Spastic paraplegia type 5 has a variable age of onset, appearing in children as young as 1 and adults as old as 58 years (33). Most type 5 cases are said to be “pure” in that progressive lower limb spasticity is the only clinical symptom observed; however, in two families, the disease was manifest in

“complex” form and presented in association with ataxia, mental retardation, and other neurological symptoms (34).

### One Gene, Two Diseases

How can mutations in the same gene give rise to two diseases with symptoms as diverse as liver failure in newborns and progressive neuropathy in children and adults? These syndromes are not caused by different mutations in the gene, as the same lesions are found in subjects with liver failure and spastic paraplegia type 5 (Fig. 2), nor are they caused by varying amounts of residual enzyme activity because complete loss of function mutations (e.g. homozygous nonsense mutations) are found associated with both presentations. A testable hypothesis is that the two diseases result from the accumulation of different CYP7B1 substrates, e.g. oxysterols in liver failure and steroids or another lipid in spastic paraplegia type 5. An infection in a newborn infant might lead to increased synthesis of the oxysterol 25-hydroxycholesterol (25, 26), which, in the absence of catabolism by CYP7B1, could lead to liver damage. Similarly, a different subclinical episode causing increased steroid synthesis might initiate spinal cord damage by an as yet unknown mechanism, which over time would progress to spastic paraplegia type 5.

Differential manifestations of cytochrome P450 deficiency are not unique to CYP7B1. Loss of CYP27A1, a sterol 27-hydroxylase that participates in oxysterol and bile salt synthesis (2), causes liver disease in some infants (37, 38) but a progressive central nervous system neuropathy and other symptoms (cerebrotendinous xanthomatosis) in a majority of affected adults (39). Different substrates build up in these presentations; bile alcohols accumulate in children with liver disease, whereas the hydrophobic sterol cholestanol accumulates in the myelin of adults.

### Perspectives

There are straightforward questions to be answered by future CYP7B1 research. First, which substrates of the enzyme amass in spastic paraplegia type 5? Second, is infection and a concomitant elevation of 25-hydroxycholesterol a precipitating condition of liver failure in infants with CYP7B1 deficiency? Third, is childhood liver disease a common but unreported symptom in older subjects with spastic paraplegia type 5? Fourth, do CYP7B1 knock-out mice display symptoms of neurodegeneration and the accumulation of previously defined or new enzyme substrates in the central nervous system? Fifth, what are the biochemical properties of purified CYP7B1? Sixth, how does the three-dimensional structure of CYP7B1 compare with cholesterol 7 $\alpha$ -hydroxylase (CYP7A1), and what features of the enzymes determine their substrate specificities? The 14 years since the identification of CYP7B1 have provided a wealth of information concerning this P450, and there is more still to learn.

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