Cytochromes P450: Roles in Diseases

The cytochrome P450 superfamily consists of a large number of heme-containing monooxygenases. Many human P450s metabolize drugs used to treat human diseases. Others are necessary for synthesis of endogenous compounds essential for human physiology. In some instances, alterations in specific P450s affect the biological processes that they mediate and lead to a disease. In this minireview, we describe medically significant human P450s (from families 2, 4, 7, 11, 17, 19, 21, 24, 27, 46, and 51) and the diseases associated with these P450s.

Cytochrome P450 enzymes are found in all biological kingdoms and a majority of phyla so far examined. More than 17,000 genes are currently known, and this number increases each year as new genome sequences are reported. In humans, 57 P450s have been identified (1), and we still continue to learn about the properties of these enzymes and their roles in different diseases. In general, P450s of medical relevance are the enzymes acting on important endogenous substrates and in many cases controlling their levels. Hence, alteration in the activity of these P450s often leads to a serious human disease. In this minireview, we summarize the underlying reasons for human P450 deficiencies and focus mainly on established monogenic diseases associated with four critical types of endogenous compounds: steroid hormones, cholesterol, vitamin D3, and eicosanoids. We do not discuss the clinical relevance of xenobiotic-metabolizing P450s and refer readers to excellent prior reviews (2, 3).

P450-dependent Diseases of Steroid Biosynthesis and Metabolism

CYP11A1 (cholesterol side chain cleavage enzyme, Mendelian Inheritance in Man (MIM) database number 18485) is a mitochondrial monoxygenase converting cholesterol to pregnenolone, the precursor of all steroid hormones (Fig. 1A). It is one of the more essential P450s because placental progesterone produced during steroidogenesis is required to maintain human pregnancy, as it suppresses uterine contractility and produced during steroidogenesis is required to maintain human pregnancy, as it suppresses uterine contractility and prevents spontaneous abortion (4). In very rare cases, however, fetuses with a complete or partial lack of CYP11A1 activity reach late gestation (5–11), and the babies born have either minimal or no serum concentrations of all steroids. All CYP11A1-deficient individuals develop congenital partial or complete adrenal insufficiency with 46,XY sex reversal (MIM 613743), a severe endocrine disorder lethal without hormone replacement therapy. Ten different variations in CYP11A1 were found in individuals with reduced or no cholesterol side chain cleavage activity. These are three homozygous or heterozygous nucleotide insertions or deletions in exons or introns and seven missense mutations, either single homozygous (A359V and L222P) or compound heterozygous (A189V/R353W, L141W/V4125E, and 835delA/A269V) (5–11). The A189V mutation does not affect CYP11A1 activity but creates a novel splice site, resulting in truncated inactive protein (6). The L141W mutation likely deteriorates cholesterol binding because, in the crystal structure of CYP11A1 (12, 13), Leu-141 points inside the active site. The other five affected amino acid residues are located outside the regions involved in the P450 function and are probably important for protein folding or stability.

CYP21A2 (steroid 21-hydroxylase; MIM 613815) is a microsomal P450 expressed predominantly in the adrenal cortex, where it is required for the biosynthesis of aldosterone from progesterone and cortisol from 17-hydroxyprogesterone. Cortisol is the major human glucocorticoid needed for glycemic control and response to stress, whereas aldosterone is the major human mineralocorticoid controlling salt balance and body fluid volume. Mutations in CYP21A2 lead to congenital adrenal hyperplasia (CAH2; MIM 201910), a family of autosomal recessive diseases arising from defects in the enzymes downstream of CYP11A1 in the pathway of steroidogenesis. The symptoms of CAH vary depending upon which steroidogenic P450 has reduced activity. CYP21A2 is located in the major histocompatibility locus of human chromosome 6 (14). In the same location is CYP21A1, a pseudogene. A significant number of CAH cases arise from crossover between these two genes (15). Additional causes include point mutations leading to single amino acid changes in CYP21A2 (15). Being the most common P450-related disease, CYP21A2 deficiency accounts for 90–95% of cases of CAH predicted to occur in 1:15,000 births (15) and ranging from complete to mild loss of monoxygenase activity. The most severe form of CAH arising from CYP21A2 mutations is known as salt-wasting, involving complete or nearly complete loss of enzymatic activity, significant reduction in glucocorticoid and mineralocorticoid biosynthesis, and elevated androgen levels. A somewhat less profound loss of CYP21A2 activity leads to the simple virilizing form of CAH. In this case, an increase in androgen production leads to impaired fertility. Females with either salt-wasting or simple virilizing forms have ambiguous genitalia, whereas in males, elevated levels of androgens enhance genitalia. The most common form of
CAH (1:1000 births) is the nonclassical form, which sometimes results in hirsutism or impaired fertility. Although a large number of mutations have been identified and classified (at least 115), we believe that many more of mutations have not yet been discovered. The 3.0 Å structure of bovine CYP21A2 was recently determined and represents an excellent model of human CYP21A2 because 85% of the reported amino acids mutated in the human enzyme are identical in the bovine ortholog (16). Using this structure and identified mutations, amino acids specific for substrate access (G65E), substrate binding (G291C), proton transfer (T294N), protein stability (G227X), heme binding (R425H), the EXXR structural motif (R354C), overall structural stability (Q481P), and reductase binding (K75X) have been predicted (16). Presumably different mutations at the same or nearly same site can result in the different forms of CAH. Also, it is conceivable that mutations in certain locations (such as heme binding) will very likely lead to only severe forms of CAH.

CYP17A1 (17α-hydroxylase/17,20-lyase cytochrome P450; MIM 609300) is a microsomal P450 found in several tissues, including the adrenal cortex and gonads. It is a particularly interesting P450, with two distinct enzymatic properties in different tissues: 17α-hydroxylase in the adrenal cortex for glucocorticoid biosynthesis and both 17α-hydroxylase and 17,20-lyase activities in gonads for androgen biosynthesis. The substrate for glucocorticoid biosynthesis is progesterone, which is converted to 17α-hydroxyprogesterone by the 17α-hydroxylase activity. In gonads, pregnenolone is converted to 17α-hydroxypregnenolone and progesterone to 17α-hydroxyprogesterone by the 17α-hydroxylase activity and then to the androgens dehydroepiandrosterone and androstenedione, respectively, by the lyase activity. Thus, the lyase activity converts 21-carbon steroids to 19-carbon androgens. However, the following questions remain unanswered. 1) What is the basis of one activity in one cell type and both in other cell types? 2) Is the product of the first reaction released in one cell type and not in others? 3) What is the structure-function relationship of the two distinct activities? More than 50 point mutations were found in the human enzyme, leading to different forms of CAH (17). Of these mutations, the most interesting are R347H and R358Q, which inhibit the 17,20-lyase activity but not the 17α-hydroxylase activity (18). Because protein-protein interaction between CYP17A1 and cytochrome b5 enhances the lyase activity (19, 20), both mutations probably inhibit cytochrome b5 binding to CYP17A1. CYP17A1 is a target in anti-prostate cancer therapy. Inhibition of the lyase activ-
ity that leads to reduced androgen synthesis inhibits testosterone production and therefore the development of prostate cancer. Drugs that are effective in inhibition of CYP17A1 have been developed (21, 22). Now that the enzyme structure co-crystallized with such inhibitors is available (17), even better drugs that would be able to inhibit the lyase activity without an effect on hydroxylation activity could probably be designed. CYP11B1 (steroid 11β-hydroxylase; MIM 610613) and CYP11B2 (aldosterone synthase; MIM 124080) are two human mitochondrial P450s, each with 11β-hydroxylase activity, and are required for cortisol and aldosterone biosynthesis, respectively (23, 24). Both are expressed in the adrenal cortex, with 11-deoxycortisol being the substrate for CYP11B1 and 11-deoxy cortisol for CYP11B2. In addition to the 11β-hydroxylase activity, CYP11B2 also catalyzes 18-hydroxylase and 18-oxidase activities, leading to aldosterone production. Genes encoding these two proteins are near one another in chromosome 8, and defects in both activities result from their unequal crossover (25), leading to glucocorticoid-remediable aldosteronism (MIM 103900) manifested in hypertension and other symptoms. Also, for each P450, amino acid mutations that cause CAH are found when occurring in CYP11B1 and corticosterone methyl oxidase deficiencies of types I and II (MIM 203400 and 610600, respectively) when occurring in CYP11B2. Examples of mutations in CYP11B1 are W116G (affects substrate recognition), A165D (alters protein surface polarity), and R366C and R453Q (reduce the binding of the electron donor adrenodoxin). These effects are predicted based on a CYP11B1 structure model (26). Likewise, those in CYP11B2 include S315R and R374W (alter P450 stability), S308P (affects active site), and L415H (affects heme binding) as suggested by a CYP11B2 structure model (27). CAH arising from CYP11B1 deficiency is characterized by androgen excess and hypertension, whereas CYP11B2 deficiency is presented with life-threatening salt loss, leading to failure to thrive.

CYP19A1 (CYP19 aromatase; MIM 107910) is a microsomal P450 essential for the synthesis of estrogen. It is expressed in several different tissues, including the ovary, breast, vasculature, and brain, and is responsible for the local production of estrogen. A particularly interesting feature of CYP19A1 is five different first exons functional in different tissues (28, 29). For example, alterations in exon 1.3 in the ovary inhibit aromatase expression in that tissue. In rare cases, nucleotide mutations in these first exons can lead to increased transcription of CYP19A1 in men and women, resulting in a rare aromatase excess syndrome (MIM 139300) characterized by heterosexual precocity in males and homosexual precocity in females. Other nucleotide mutations in the noncoding exons lead to aromatase deficiency (MIM 613546) manifested in pseudohermaphroditism in females and eunuchoid body proportions with excess adiposity in males. In addition to the essential role in reproduction and other biological processes, estrogen has a well-known enhancing effect on breast tumor growth. This is due to exon 1.4 in adipose tissue, which can enhance aromatase activity in the breast. Hence, aromatase is an important drug target, and its inhibitors anastrozole and letrozole are used in treatment of CYP19A1-dependent forms of breast cancer, as they bind tightly to the enzyme active site (30). Because the x-ray structure of human aromatase has recently been determined (31), new drugs for treatment of breast cancer will likely be developed in the future. It has also been discovered that aromatase deficiency can lead to metabolic syndrome and insulin resistance (28).

P450-dependent Diseases of Cholesterol Biosynthesis and Metabolism

CYP51 (sterol 14α-demethylase; MIM 601637) is the only P450 involved in cholesterol biosynthesis, a physiological process requiring >30 different enzymatic reactions. CYP51, a microsomal enzyme, catalyzes one of these reactions, 14α-demethylation of the cholesterol precursors lanosterol and 24,25-dihydrolanosterol, an essential step in the formation of cholesterol. Mutations in CYP51 completely abolishing the enzymatic activity have not been reported, probably because they are embryonically lethal, as indicated by the prenatal lethality in Cyp51 knockout mice (32). However, the CYP51 variant (National Center for Biotechnology Information Single Nucleotide Polymorphism database ID rs2229188; http://www.ncbi.nlm.nih.gov/projects/SNP/) leading to the V13E amino acid substitution and a yet unknown effect on enzymatic activity was recently identified. This SNP was found among the SNPs in 39 other genes suggested to likely determine the levels of serum high density lipoprotein cholesterol (33), a protective factor against cardiovascular disease. We predict that the V13E amino mutation should not significantly disrupt the CYP51 activity but may affect membrane binding because the N-terminal part of the CYP51 molecule encompassing this mutation is involved in the interaction with the lipid bilayer, as in any microsomal P450.

CYP7A1 (cholesterol 7α-hydroxylase; MIM 118455) is the rate-limiting enzyme in the major (also called classical) pathway of cholesterol elimination from the human body. This pathway takes place in the liver and involves, besides microsomal CYP7A1, 16 other enzymes that collectively convert cholesterol to bile acids, the end products of cholesterol degradation. CYP7A1 catalyzes the first step in hepatic bile acid biosynthesis and plays a critical role in the maintenance of the whole body cholesterol homeostasis as indicated by the phenotype of individuals with a complete lack of cholesterol 7α-hydroxylase activity (34). Only three such individuals, all from the same family and all having a homozygous 2-bp deletion mutation in CYP7A1, have been described so far. The identified frameshift mutation produces the truncated enzyme lacking part of the active site and hence is inactive. Because cholesterol cannot be degraded to bile acids via the pathway, which accounts for the majority of cholesterol elimination, plasma total and low density lipoprotein cholesterol, both risk factors for cardiovascular diseases, are significantly elevated, and cholesterol content in the liver is doubled. The homozygous mutation carriers also have a markedly reduced rate of bile acid production and excretion and a premature gallstone disease, which is likely a result of unstable bile supersaturated with cholesterol (34). Genetic variations are also found in the promoter region of CYP7A1, with the rs3808607 polymorphism being the most frequent and found in ~45% of the population. This polymorphism was studied for the effect on plasma lipids, response to...
cholesterol-lowering drugs and plant sterols, and a number of diseases such as atherosclerosis, gallstone disease, and colorectal cancer (reviewed in Refs. 35 and 36). More investigation is required to draw conclusions about the effect of this SNP on human health.

CYP27A1 (sterol 27-hydroxylase; MIM 606530) is a ubiquitously expressed multifunctional mitochondrial monooxygenase (37). Enzymatic activities of importance to cholesterol metabolism include C27 hydroxylation of bile acid intermediates in the liver and cholesterol in many extrahepatic tissues. The former activity is essential for the degradation of the steroid side chain to bile acids, whereas the latter initiates cholesterol removal from extrahepatic organs or the so-called alternate pathway of bile acid biosynthesis. Complete inactivation of CYP27A1 due to genetic mutations leads to a reduced production of bile acids (predominantly chenodeoxycholic acid), increased formation of cholestane (reduced form of cholesterol), and increased formation of bile acid alcohols (38). Consequently, affected individuals develop the multisymptom disease cerebrotendinous xanthomatosis (CTX; MIM 213700) (39). The initial manifestations of CTX frequently include diarrhea (due to bile acid deficiency) and juvenile bilateral cataracts, followed by the appearance later in life of tendon xanthomas, multiple progressive neurological dysfunctions, and premature atherosclerosis due to cholestane and cholesterol deposition in the lens, tendons, brain, and blood vessels. Fewer than 400 CTX cases have been reported so far (40), yet CTX is believed to be underdiagnosed, and it has been suggested not to be as rare as previously thought, with the estimated prevalence of 1/1,800,000 to 3–5/50,000 (41, 42) depending on the geographic region. More than 60 different types of mutations have been identified in CYP27A1, with studies (40, 42, 43) collectively describing 58 of them. The majority of CYP27A1 mutations (62%) are missense and nonsense amino acid substitutions. The splice site and deletion mutations account for ~17% each, and insertion mutations are rare (~2%). This spectrum of mutations is distributed throughout all nine CYP27A1 exons and a number of introns affecting regions critical for the enzymatic activity: heme binding (R441W/Q/C/G and R446C/G), the putative active site (R104W, P368R, N370K, R372W/Q, T306M), interaction with the redox partner adrenodoxin (P351L), membrane binding (K226R), and protein folding (G112R, A183P, R362C/H, P408S, and G439A). These predictions are based on our analysis of a CYP27A1 model (data not shown) generated based on the crystal structure of CYP11A1 (12), which, like CYP27A1, is a mitochondrial P450. Most CYP27A1 mutations likely completely inactivate the P450. Early diagnosis of CTX is essential because the treatment (chenodeoxycholic acid alone or in combination with the cholesterol-lowering drug statin) may limit or prevent the onset of neurological and cardiovascular complications.

CYP46A1 (cholesterol 24-hydroxylase; MIM 604087) is a microsomal monooxygenase expressed mainly in the brain, where it converts cholesterol to (24S)-hydroxycholesterol (44, 45). (24S)-Hydroxycholesterol then crosses the blood-brain barrier and is delivered to the liver for further degradation to bile acids. CYP46A1-mediated cholesterol 24-hydroxylation initiates the major pathway of cholesterol removal from the brain and determines the rate at which cholesterol is turned over in this organ (44–46), which was unexpectedly found to play a role in memory and cognition (47). (24S)-Hydroxycholesterol is also suggested to affect the processing of the amyloid precursor protein (48, 49). Accordingly, frequent polymorphisms in CYP46A1 are investigated for the association with Alzheimer disease and effect on cognition. These are rs754203, rs3742376, rs7157609, and rs4900442, which are found in either the introns or CYP46A1 promoter region and affect 29–44% of the population. The results are still conflicting (reviewed in Ref. 50) and preclude definite conclusions about the medical significance of CYP46A1. Possible involvement of the rs754203 polymorphism in developing the blinding eye disease glaucoma is also not confirmed (51, 52).

CYP7B1 (oxysterol 7α-hydroxylase; MIM 603711) is expressed in the endoplasmic reticulum in many organs, including the liver, brain, kidney, and reproductive tract, where it acts on different cholesterol metabolites (53–56). The function of CYP7B1 in the liver is 7α-hydroxylation of 25- and 27-hydroxycholesterols (57–59), which cannot be converted to bile acids without this obligatory modification of the steroid nucleus. The role in the brain is still under investigation but likely pertains to the metabolism of steroid hormones and their precursors such as pregnenolone and dehydroepiandrosterone (54, 60). An important substrate in the reproductive tract is the estrogen receptor ligand 5α-androstane-3β,17β-diol, which is inactivated by CYP7B1 via 6α-hydroxylation (61, 62). Complete lack of CYP7B1 activities due to the same homozygous mutation R388X was found to underlie two different diseases: congenital bile acid synthesis defect 3 (MIM 613812) (59) and autosomal recessive spastic paraplegia 5A (SPG5A; MIM 270800) (63). The former is characterized by neonatal onset of cholestasis, cirrhosis, and liver failure and is consistent with a role of CYP7B1 in hepatic bile acid biosynthesis (59). The liver problems are believed to be due to the accumulation of the oxysterols and unusual unsaturated monohydroxy bile acids and a lack of the normal primary bile acids (59). In contrast, SPG5A is a neurodegenerative disease with a variable age of onset and the main clinical feature being progressive weakness and contraction (spasticity) in the lower limbs. The mechanism whereby loss of CYP7B1 function affects motor neurons in the corticospinal tract is currently unknown. Also unclear is why the same null mutation leads to different clinical manifestations. In addition to the R388X variant, 16 other mutations have been identified in CYP7B1: one (R112X) in an infant with congenital bile acid synthesis defect 3 (64) and 15 in patients with SPG5A (63, 65–67). These variants are summarized in Ref. 68. Of them, three are nucleotide deletion or insertions in the CYP7B1 coding regions, four are nonsense mutations, and 10 are missense mutations. The effect of the missense mutations on enzyme function remains to be established.

**P450-dependent Diseases of Activation and Inactivation of Vitamin D₃**

CYP2R1 (MIM 608713) is found in the endoplasmic reticulum in many tissues, including the liver and pancreas, with the highest expression in the testes (69, 70). The role in the liver is 25-hydroxylation of the biologically inert vitamins D₂ and D₃,
the first and obligatory step in the production of the active form of these vitamins (69). Although a number of other P450 enzymes (CYP27A1, CYP2C11, and CYP2J3) can 25-hydroxylate vitamin D$_3$, available evidence points to CYP2R1 as the major vitamin D$_3$ 25-hydroxylase (71–73). Mutations in CYP2R1 lead to low serum levels of 25-hydroxyvitamin D$_3$ and are associated with rickets (softening and weakening of bones in children, leading to bone deformities and fractures). CYP2R1 deficiency is designated as vitamin D-dependent rickets type 1B (MIM 600081) (71, 74–76). The identified gene defects are very rare and include one missense mutation (L99P), one intronic single nucleotide change, and one single nucleotide insertion in exon 3. The former likely affects heme binding and protein folding, whereas the latter two are predicted to lead to truncated inactive protein (76, 77).

CYP27B1 (25-hydroxyvitamin D$_3$, 1α-hydroxylase; MIM 609506) is expressed in mitochondria of a variety of human tissues but at low levels (reviewed in Ref. 78). In the kidney, CYP27B1 1α-hydroxylates 25-hydroxyvitamin D$_3$ to form bioactive 1α,25-dihydroxyvitamin D$_3$ (79). Inactivating mutations in CYP27B1 cause vitamin D-dependent rickets type 1A (MIM 264700), a rare autosomal recessive disorder characterized by the early onset and severe syndrome of rickets (80, 81). To date, 47 mutations in CYP27B1 have been identified (summarized in Ref. 82). Of them, 28 are missense, three are nonsense, eight are deletion, two are insertion, two are deletion/insertion, and four are splice site mutations. Missense mutations are distributed throughout the primary sequence and either significantly reduce CYP27B1 activity (by >78%; G57V, G73W, E189G, T321R, L333F, L343F, R432C, R459C, and R492W) or completely abolish it (R107H, G125E, R335P, P382S, and R389H/G) or indicated by *in vitro* enzyme assays of mutant recombinant proteins (81–85).

CYP24A1 (1α,25-hydroxyvitamin D$_3$, 24-hydroxylase; MIM 126065) is a mitochondrial enzyme detected in many tissues at low levels but undergoing rapid induction in response to active forms of vitamins D$_3$ and D$_2$, which are inactivated by the enzyme through multiple oxidations of the sterol side chain (reviewed in Ref. 1). Loss of CYP24A1 function usually leads to idiopathic infantile hypercalcaemia (MIM 143880), also characterized by failure to thrive, vomiting, dehydration, and nephrocalcinosis. The disease is rare, and its transmission (autosomal recessive or autosomal dominant with partial penetrance of the trait) is not yet clear (86–89). The identified underlying mutations (missense as well as insertion or deletion variants) either completely (A475fsX490, E143del, R159Q, E322K, and R396W) or significantly (L409S) ablate CYP24A1 activity (86). In the crystal structure of rat CYP24A1 (90), the affected amino acid residues are located near the heme (Arg-159 and Arg-396), inside the active site (Glu-322), or on the protein surface outside the functionally important regions (Glu-143 and Leu-409) and are probably involved in heme and substrate binding or protein folding and stability.

**P450-dependent Diseases of Eicosanoid Synthesis**

CYP4A11 (fatty acid ω-hydroxylase; MIM 601310) oxidizes arachidonic acid to 20-hydroxyicosatetraenoic acid (20-HETE) in the endoplasmic reticulum of kidney tubules (Fig. 1B). 20-HETE regulates salt and water balance in this organ; therefore, a mutation in CYP4A11 (F434S) profoundly reducing 20-HETE biosynthesis was found in three different cohorts of individuals with elevated blood pressure (essential hypertension; MIM 145500) (91–94). Elevated blood pressure was more significant in males than in females in an African-American cohort, suggesting androgen regulation of CYP4A11 transcription (94). This effect of androgens has been previously reported to exist in rodents (93). 20-HETE is also synthesized from arachidonic acid in the kidney by another human P450 (CYP4A22) found to have 13 sequence variations in the coding sequence in a Japanese cohort (95). However, it remains to be determined whether any mutations in CYP4A22 influence blood pressure. In contrast, in the case of CYP4A11, efforts to establish clinical management of CYP4A11-dependent hypertension are under way (93).

**Concluding Remarks**

Herein, we have described 15 human P450s and 14 monogenic diseases associated with these P450s, which by no means represent all medically relevant P450s and P450-dependent diseases. The development of new DNA-sequencing platforms and genome-wide association studies have revealed previously unanticipated associations and P450 contributions to a number of polygenic diseases. There is no doubt that, within the next decade, our knowledge about the roles of P450s in different diseases will be significantly expanded.

**REFERENCES**


MINIREVIEW: Medical Significance of P450s


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