Review

Clinical importance of the cytochromes P450

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The human cytochrome P450 (CYP) superfamily comprises 57 genes. These genes code for enzymes that can have a role in: metabolism of drugs, foreign chemicals, arachidonic acid and eicosanoids; cholesterol metabolism and bile-acid biosynthesis; steroid synthesis and metabolism; vitamin D_3 synthesis and metabolism; retinoic acid hydroxylation; and those of still unknown function. Cytochrome P450 was once believed to be mainly a hepatic drug detoxication system, but is now understood to include a myriad of enzymic reactions implicated in important life processes. Mutations in many *CYP* genes cause inborn errors of metabolism and contribute to many clinically relevant diseases.

Cytochrome P450, a cellular chromophore, was first named in 1961, because the pigment (P) has a 450-nm spectral peak when reduced and bound to carbon monoxide. P450 was thought to be one enzyme in the early 1960s, and by the mid 1960s it was associated with drug and steroid metabolism. By the late 1970s, as many as six P450 enzymes were speculated to exist; however, the membrane-associated and hydrophobic nature of the enzyme system impeded purification, and the number of proteins involved could not be accurately counted. Advances in mRNA purification in the early 1980s allowed Gonzalez and colleagues[1] to isolate the first cDNA encoding a complete cytochrome P450 (CYP) protein, and thereafter, results of many cloning studies have revealed dozens of different enzymes. Sequence comparisons indicated extensive similarity between cytochromes P450 identified in man and bacteria, and suggested that the superfamily originated from a common ancestral gene some three billion years ago.[2] A systematic nomenclature scheme for the *CYP* gene superfamily, based on divergent evolution, has been in place for 15 years, and continues to be developed on the internet.[3,4]

Cytochrome P450 proteins are conveniently arranged into families and subfamilies on the basis of percentage aminoacid sequence identity.[2-4] Enzymes that share ≥40% identity are assigned to a particular family designated by an Arabic numeral, whereas those sharing ≥55% identity make up a particular subfamily designated by a letter. For example, the sterol 27-hydroxylase enzyme and the vitamin D_3 24-hydroxylase enzyme are both assigned to the CYP27 family because they share ≥40% sequence identity. Sterol 27-hydroxylase is further assigned to the CYP27A subfamily, and vitamin D_3 24-hydroxylase assigned to CYP27B, because their protein sequences are <55% identical. If an additional enzyme were to be discovered that shared ≥55% identity with the sterol 27-hydroxylase, then it would be named CYP27A2, etc. Development and application of this beautifully logical system of nomenclature has eliminated the confusion that frequently plagues naming of other gene families and superfamilies.

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Presently, there are more than 270 different *CYP* gene families, with 18 recorded in mammals.[4] Because diversity of small molecules in plants is enormous, these organisms were predicted to contain many cytochrome P450 enzymes.[5,6] This expectation was met in the genome of the tiny mustard plant *Arabidopsis thaliana*, which contains 249 active *CYP* genes and 24 non-functional pseudogenes, a remarkable 1% of its total gene number. The genome of the rice plant is similar, with at least 324 functional genes reported up to now.[4] By contrast, human beings have 57 *CYP* genes and 33 pseudogenes arranged into 18 families and 42 subfamilies (panel 1), and this number is not likely to change—unless active members of the human *CYP2G* and *CYP2T* subfamilies are found.[7]

Advances in molecular biology and genomics facilitated the biochemical characterisation of individual P450 enzymes, which in turn revealed many surprises about an enzyme system once believed to metabolise drugs mainly in the liver. First, the cytochromes P450 act on many endogenous substrates, introducing oxidative, peroxidative, and reductive changes into small molecules of widely different chemical structures. Substrates identified to date include saturated and unsaturated fatty acids, eicosanoids, sterols and steroids, bile acids, vitamin D_3 derivatives, retinoids, and uroporphyrinogens. Second, many cytochrome P450 enzymes can metabolise various exogenous compounds including drugs, environmental chemicals and pollutants, and natural plant products.[5,6] Third, metabolism of foreign chemicals frequently results in successful detoxication of the irritant; however, the actions of P450 enzymes can also generate toxic metabolites that contribute to increased risks of cancer, birth defects, and other toxic effects. Fourth, expression of many P450 enzymes is often induced by accumulation of a substrate.[8,9] For example, hepatic concentrations

Search strategy and selection criteria

Medline and PubMed databases from 1966 until April, 2002, were searched for P450 reviews and primary articles related to the *CYP* genes, CYP enzyme metabolism of both endogenous and exogenous substrates, and relevance to clinical disease. Specific keywords we used included cytochrome P450 metabolism and: drugs, xenobiotics, environmental chemicals, toxicity, cancer, endogenous substrates, evolution, arachidonic acid, eicosanoids, cholesterol, bile acids, steroids, sterols, vitamin D_3, cholecalciferol, retinoic acid, gene polymorphisms. Citations were chosen on the basis of relevance to the topics covered, without any bias toward author or journal.

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of the human CYP3A enzymes are increased by consumption of drugs such as rifampicins, which are prescribed for bacterial infection. This induction not only increases rifampicin metabolism but also leads to enhanced clearance of other drugs that are CYP3A substrates. Because rifampicin also induces several CYP2C enzymes, this process would lead to more rapid clearance of CYP2C substrates. The ability of one P450 substrate to affect the concentrations of another in this manner is the basis for so-called drug-drug interactions, which complicate treatment.[10]

Availability of cloned genes, and the biochemical and immunochemical probes derived from these cDNAs, has given us new insight into the diverse biological and clinical roles of individual cytochromes P450. Their biological functions include metabolism of endogenous substrates and synthesis of endogenous hydrophobic lipids such as cholesterol, bile acids, steroid hormones, and fatty acids. Below, we summarise the physiological roles of the various human cytochromes P450 and how these enzymes affect clinical outcomes.

Metabolism of foreign chemicals, arachidonic acid, and eicosanoids

Foreign chemicals

Foreign chemicals (sometimes called xenobiotics) include drugs, plant-derived or fungal-derived secondary metabolites consumed with food, and thousands of environmental pollutants—eg, halogenated hydrocarbons, polycyclic aromatic hydrocarbons, arylamines, ingredients of combustion, industrial complex mixtures, herbicides, pesticides, etc. Human cytochromes P450 that metabolise these foreign chemicals are almost exclusively in the *CYP1*, *CYP2*, *CYP3*, and to a lesser degree, *CYP4* families. Many allelic variants exist within each of these gene families, resulting in pharmacogenetic heterogeneity between individuals.

Panel 1: **Substrates and functions of human *CYP* gene families**

Family	Number of subfamilies	Number of genes	Substrates and functions
CYP1	2	3	Foreign chemicals, arachidonic acid, eicosanoids
CYP2	13	16	Foreign chemicals, arachidonic acid, eicosanoids
CYP3	1	4	Foreign chemicals, arachidonic acid, eicosanoids
CYP4	5	12	Fatty acids, arachidonic acid, eicosanoids
CYP5	1	1	Thromboxane A_2 synthase
CYP7	2	2	Cholesterol, bile acid synthesis
CYP8	2	2	Prostacyclin synthase, bile-acid synthesis
CYP11	2	3	Steroidogenesis
CYP17	1	1	Steroid 17α-hydroxylase, 17/20-lyase
CYP19	1	1	Aromatase to form oestrogen
CYP20	1	1	Unknown
CYP21	1	1	Steroid 21-hydroxylase
CYP24	1	1	Vitamin D_3 24-hydroxylase
CYP26	3	3	Retinoic acid hydroxylation
CYP27	3	3	Bile-acid biosynthesis, vitamin D_3 hydroxylations
CYP39	1	1	24-hydroxycholesterol 7α-hydroxylase
CYP46	1	1	Cholesterol 24-hydroxylase
CYP51	1	1	Lanosterol 14α-desmethylase

A database of human allelic variants of *CYP* genes is maintained on the internet,[11] which uses a consistent classification system. In brief, the consensus, or reference, sequence (**1* allele) generally encodes an efficient-metabolism phenotype, whereas variant alleles encode a poor-metabolism phenotype, with low or no enzyme activity towards a particular drug. On occasion, because of one or more gene duplications, a variant genotype might indicate a very high ultra-metabolism phenotype. Different alleles of the *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *CYP2A6*, and *CYP2B6* drug-metabolising genes can thus result in treatment failure, toxic effects, and even death in rare cases.[12–15] Similarly, rates of detoxication, and sometimes of metabolic activation, of environmental chemicals can be strikingly different between individuals with different *CYP* haplotypes. Examples of metabolic activation can be seen in the CYP1A1, CYP1A2, CYP1B1, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 enzymes: results of studies have shown that, for these enzymes, the efficient-metabolism or high-activity phenotype is associated with an increased risk of certain types of cancer or toxic effects. These drawbacks can be further exacerbated by co-inheritance of other polymorphic enzymes in the same metabolic pathway of a particular drug or chemical.[16–18] Although data are very convincing in animals, confirmation of such associations in clinical populations remains difficult.

CYP1 gene family—Expression of the *CYP1* gene family is induced by the aryl hydrocarbon receptor, a transcription factor that is activated by binding of polycyclic aromatic hydrocarbons, such as those found in industrial incineration products, cigarette smoke, and charcoal-grilled food. CYP1A1 and CYP1B1 are expressed in varying amounts in different tissues, and are most efficient metabolising polycyclic aromatic hydrocarbons, whereas CYP1A2 preferentially metabolises arylamines and N-heterocyclics. CYP1A1, and possibly CYP1A2 and CYP1B1, metabolise an as-yet-to-be-identified endogenous ligand for the aryl hydrocarbon receptor,[19] and CYP1A1 also inactivates prostaglandin G_2.[20] CYP1A2 and CYP1B1 hydroxylate oestrogen at the carbon-2 and carbon-4 positions, respectively, and CYP1A2 oxidises uroporphyrinogen[21] and melatonin.[22] CYP1A2 metabolises about 10–20 different drugs, whereas CYP1A1 and CYP1B1 do not seem to act mainly on drugs. Reasons for the unusually high expression of CYP1B1 in some types of solid tumours are not known,[23] but this occurrence might be useful in designing drugs for chemotherapeutic intervention. All three CYP1 enzymes detoxify or activate many environmental carcinogens.

Although many alterations in carcinogen and drug metabolism are recorded in mice without the *Cyp1a1*, *Cyp1a2*, and *Cyp1b1* genes, such animals are viable, suggesting that the encoded P450 enzymes are either redundant or do not have an essential role in metabolism of endogenous compounds.[24–26] On the other hand, mutations in the human *CYP1B1* gene cause primary congenital glaucoma (buphthalmos)[27] (panel 2); this clinical observation suggests that development of the anterior chamber of the eye during embryogenesis requires metabolism of an important endogenous substrate by CYP1B1. Since CYP1B1 seems to have a role in retinoic acid biosynthesis and degradation,[28] this fact might help to explain the cause of primary congenital glaucoma. However, in view of their overlapping substrate specificities, if CYP1B1 has a role in the retinoic acid

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pathway, what about CYP1A1 and CYP1A2? Possibly related to this discussion, mice with a disruption in the *Ahr* gene accumulate liver retinoids, and metabolism of retinoic acid is reduced.[29]

CYP2 gene family—*CYP2* is the largest P450 family in mammals (panel 1). Human CYP2C8, CYP2C9, CYP2C18, and CYP2C19 together metabolise—to varying amounts—greater than half of all frequently prescribed drugs, and arachidonic acid and some steroids. Results of in-vitro biochemical assays show that CYP2D6 metabolises more than 75 drugs.[15] CYP2A6, CYP2A13, CYP2B6, CYP2E1, CYP2F1, and CYP2J2 also help to metabolise some drugs.[30,31] Functions of other members of this family—including CYP2A7, CYP2R1, CYP2S1, CYP2U1, and CYP2W1—are presently unknown. Although enzymes of the *CYP2C* subfamily are generally thought to have a role in drug metabolism, a β-naphthoflavone-inducible CYP2C enzyme is implicated in synthesis of the vasodilator 11,12-epoxyeicosatrienoic acid by the vascular endothelium.[32] This association represents a recurrent theme in P450 published work,[8]—ie, most *CYP* gene products in vertebrates probably first evolved for important life functions, before then developing plant-metabolite-degradation and drug-metabolism abilities. Although the *CYP2G* and *CYP2T* subfamilies encode functional genes in rodents, they only seem to encode pseudogenes in man, suggesting that whatever functions these genes had about 80 million years ago (at the time of the mammalian radiation), they are no longer needed in man. Mice deficient in the *Cyp2e1* gene seem to be outwardly normal, but are very resistant to benzene toxic effects,[26] indicating a role in xenobiotic metabolism for this subfamily.

CYP3 gene family—The *CYP3* family has four members (panel 1). CYP3A4 and CYP3A5 are the most abundantly expressed P450 enzymes in the human liver and gastrointestinal tract, and are known to metabolise more than 120 frequently prescribed drugs,[12–17] and endogenous substrates such as steroids and bile acids.[33,34] Of particular clinical importance, metabolism of certain antifungal and immunosuppressive drugs by CYP3A4 and CYP3A5 could lead to insufficient amounts of these drugs in extensive-metaboliser patients, and excessive concentrations in those with the poor-metabolism phenotype, when either type of patient is given the recommended prescribed dose. The function of hepatic CYP3A43 is not yet known. CYP3A7 is expressed in fetal liver and the uterine endometrium, but its role in these tissues is not known.

In the *CYP3A* subfamily, an important regulatory pathway controlling expression of these enzymes in the liver and gut has been reported.[35] Evidence suggests that drugs of diverse structure can induce members of this family, and that the capacity of a particular compound to induce CYP3A enzymes varies between species.[36,37] The molecular basis of induction was traced to a ligand-activated transcription factor, which is known as the pregnane X receptor (PXR), pregnane-activated receptor, or steroid and xenobiotic receptor. This protein is a member of the nuclear hormone receptor superfamily, which binds small molecules and activates transcription of *CYP3A* genes containing a particular DNA motif or response element sequence in their regulatory regions. Interspecies differences in induction of CYP3A enzymes by a particular drug show ability of the compound to interact with the ligand-binding domain of the PXR receptor.[38,39]

Existence of this regulatory system and its pharmacological properties explains the long mysterious ability of certain drugs to protect an organism from the toxic effects of other compounds.[40] For example, derivatives of the steroid pregnenolone can attenuate the hepatotoxicity associated with ingestion of indometacin and digitoxin. Pregnenolone-related compounds are ligands for the PXR and induce synthesis of *CYP3A* subfamily members, which in turn inactivate the offending substance. A similar situation exists with the compound hypericum, the active agent in St John's wort, a herbal remedy widely used for treatment of depression. Hypericum activates the PXR and *CYP3A* genes, leading to enhanced metabolism of many compounds—including prescription drugs, and their attendant adverse clinical events.[41] Our future ability to predict drug-drug interactions and to design new treatments should be greatly aided by screens using the PXR and its *CYP3A* target genes.

Some *CYP2B* and *CYP3A* genes are induced by phenobarbital and other drugs by another member of the nuclear hormone receptor superfamily—the constitutive androstane receptor (CAR)—and by PXR. Although CAR and PXR response elements or DNA motifs in the regulatory regions of these genes are distinct—it has now been established that CAR can activate *CYP3A* genes via PXR response elements and that PXR can regulate *CYP2B* genes via the CAR or phenobarbital response element.[42,43] This cross-talk between receptor transcription factors suggests an extra layer of protection against the harmful effects of toxic compounds such as plant

Panel 2: Diseases associated with mutations in a *CYP* gene

Gene	Disorder
CYP1B1	Primary congenital glaucoma (buphthalmos)
CYP4A, 4B	(?)Defects in salt metabolism, water balance leading to arterial hypertension
CYP5A1, 8A1	Defects leading to clotting and inflammatory disorders, coronary artery disease, and pulmonary hypertension
CYP7A1	Hypercholesterolaemia, resistance to statin drugs
CYP7B1	Severe hyperoxysterolaemia and neonatal liver disease
CYP11A1	Lipoid adrenal hyperplasia; occasional congenital adrenal hyperplasia (CAH)
CYP11B1	Occasional CAH
CYP11B2	Corticosterone methyloxidase deficiency type I, or type II; occasional CAH
CYP11B1, 11B2	Chimeric enzymes causing glucocorticoid-remediable aldosteronism; occasional CAH
CYP17A1	Mineralocorticoid excess syndromes, glucocorticoid and sex hormone deficiencies; association with increased risk of prostate cancer and benign prostatic hypertrophy; occasional CAH
CYP19A1	Loss-of-function: virilisation of females, hypervirilisation of males, occasional CAH Gain-of-function: gynaecomastia in young males
CYP21A2	More than 90% of all CAH
CYP24A1	(?)Hypervitaminosis D
CYP27A1	Cerebrotendinous xanthomatosis
CYP27B1	Vitamin D-dependent rickets type I

?=strong evidence of disease in animals, but not yet convincing in clinical studies.

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metabolites or drugs but, at the same time, increases the propensity for drug-drug interactions.

CYP4 gene family—The *CYP4* family has 12 members (panel 1). CYP4A11, CYP4B1, CYP4F2, and CYP4F3 metabolise some drugs but mainly have a role in metabolism of fatty acids, arachidonic acid, leukotrienes, prostaglandins, epoxyeicosatrienoic acids, (EETs) hydroxyeicosatetraenoic acids (HETEs), and hydroperoxyeicosatetraenoic acids (HPETEs). CYP4F8, CYP4F11, CYP4F12, and CYP4F22 seem to be implicated in arachidonic acid and fatty-acid metabolism.[7] Functions of CYP4A20, CYP4A22, CYP4V2, and CYP4X1 are unknown. Peroxisome proliferator-activated receptors (PPARα, β, γ, and δ classes), which are also members of the nuclear receptor superfamily, play a part in regulation of *CYP4A* and *CYP4B* genes.[43] Several CYP4A and CYP4B enzymes are expressed in the distal convoluted tubules of the kidney, and defects in some *CYP4* genes cause alterations in salt metabolism, water balance, and arterial blood pressure[44] (panel 2). Most studies of blood pressure have been done in rats, although human kidney CYP4A11 and CYP4F2 have also been shown to convert arachidonic acid to 20-HETE.[45]

Arachidonic acid and eicosanoids

All cytochrome P450 enzymes probably have one or more endogenous functions, in addition to metabolism of foreign chemicals and plant compounds from which almost all drugs are derived.[8] Examples of this dual function are seen in the cytochrome P450 enzymes that metabolise arachidonic acid, which is converted into more than 102 eicosanoid metabolites (figure 1). Up to now, at least 14 cytochrome P450 enzymes in the *CYP1*, *CYP2*, *CYP3*, and *CYP4* families have been shown to participate in epoxidation, reduction, and oxidation of these second-messenger molecules. Prostaglandins D_2, $F_{2\alpha}$, E_2 and EETs, HETEs, and HPETEs have a role in many life processes, including: renal vasoconstriction; bronchodilation; bronchoconstriction; oedema; intestinal vasodilation; smooth-muscle contraction; allergic response; mitogenesis; chemotaxis; inhibition of platelet aggregation and declumping; bone resorption; fever generation; modulation of ion transport; enhanced peptide secretion; mobilisation of intracellular calcium; modulation of the sodium and potassium ATPase; egg formation; and pain response.[46,47] Diseases caused by mutations in P450 enzymes that participate in metabolism of arachidonic acid have yet to be described, but almost certainly exist.

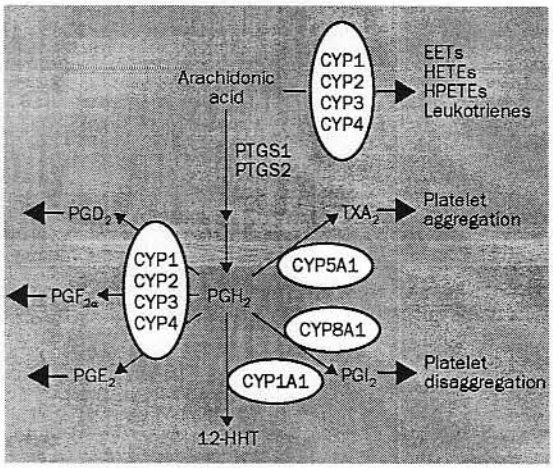

Figure 1: **Cytochrome P450 enzymes implicated in arachidonic acid cascade**

PTGS1=prostaglandin G/H synthase-1, PTGS2=prostaglandin G/H synthase-2; PGH_2=prostaglandin H_2; PGD_2=prostaglandin D_2; $PGF_{2\alpha}$=prostaglandin $F_{2\alpha}$; PGE_2=prostaglandin E_2; 12-HHT=12(S)-12-hydroxy-5,8,10-heptadecatrienoic acid; TXA_2=thromboxane A_2; PGI_2=prostacyclin. The more than 102 eicosanoids in this cascade include about 40 EETs, HETEs, and HPETEs, at least 28 leukotriene derivatives, and more than 30 prostaglandins (denoted by thick arrows). TXA_2 is formed by CYP5A1 and further metabolised to at least five more distal compounds. PGI_2 is formed by CYP8A1 and further metabolised to at least five additional prostanoids. PGD_2, $PGF_{2\alpha}$, and PGE_2 are converted into at least 18 additional EETs, HETEs, and HPETEs. Formation of the leukotrienes from arachidonic acid begins with arachidonate lipoxygenase-5.

Cholesterol metabolism and bile-acid biosynthesis

At least seven, possibly nine, cytochrome P450 enzymes have a role in conversion of acetate into sterols and bile acids (figure 2). Lanosterol 14α-desmethylase, which is encoded by the *CYP51A1* gene, is pivotal in the synthesis of cholesterol, removing two methyl groups via oxidative reactions from the intermediate lanosterol. The CYP51A1 enzyme is the target of antifungal drugs such as ketoconazole, and is one of the most evolutionarily conserved of all cytochromes P450. Genes encoding this enzyme are found in plants, fungi, animals, and even in the primitive prokaryote *Mycobacterium tuberculosis*. The *CYP51* gene seems to have been lost in other phylogenetic branches, such as the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the sea squirt *Ciona savignyi*.[4] The widespread distribution of this enzyme across phyla and kingdoms has led to speculation that it might represent an ancestor to all eukaryotic cytochromes.[4,50,51]

Synthesis of bile acids from cholesterol represents the major catabolic route for disposal of cholesterol in mammals. Hydroxylation of the ring structures in cholesterol, plus oxidation and shortening of the eight-carbon side-chain, produces water-soluble bile acids with powerful detergent properties. These metabolic transformations are catalysed in part by at least seven different P450 enzymes, including members of the *CYP3*, *CYP7*, *CYP8*, *CYP27*, *CYP39*, and *CYP46* families (figure 2). CYP7A1, CYP7B1, and CYP39A1 initiate the synthesis of bile acids from cholesterol and oxysterol substrates by introduction of a hydroxyl group in the α-configuration at carbon-7 of the B-ring. The farnesoid X receptor (also called the bile acid receptor), which is a member of the nuclear hormone receptor superfamily, is implicated in regulation of the *CYP7A1* gene; mice without the *Fxr* gene have increased concentrations of bile acids, cholesterol and triglycerides, and proatherogenic serum lipoprotein.[52] CYP8B1 is a sterol 12α-hydroxylase that is essential for synthesis of the primary bile acid, cholate.[53] CYP27A1 is a sterol 27-/26-hydroxylase with a role in synthesis of oxysterols and oxidation of the sterol side-chain.[54] CYP46A1 also catalyses formation of oxysterols.[55]

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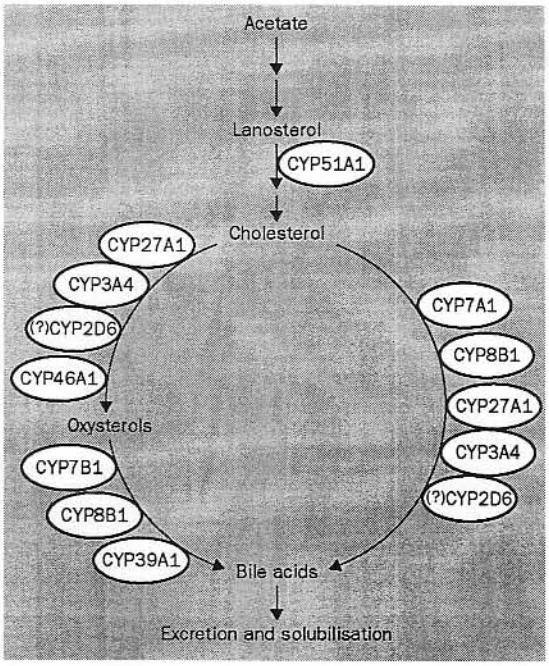

Figure 2: **Cytochrome P450 enzymes with a role in cholesterol and bile-acid synthesis**

Numbers and letters in ovals denote the nine cytochrome enzymes possibly implicated in these pathways. Six steps take place between acetate and lanosterol, and another six (starting with CYP51A1) between lanosterol and cholesterol. Bile acids include cholic acid, chenodeoxycholic acid, and at least ten other derivatives.

CYP46A1 is an unusual member of the P450 superfamily because it is expressed almost exclusively in neurons of the central nervous system.[55] In these cells, the enzyme converts cholesterol into the oxysterol, 24*S*-hydroxycholesterol, which—unlike cholesterol—is freely permeable to the blood-brain barrier.[56] The synthesis of this oxysterol and its excretion into the circulation represents a major pathway of cholesterol turnover in the brain and a form of reverse cholesterol transport, because the secreted 24*S*-hydroxycholesterol is converted into bile acids by the liver.

Mutations in three of five P450 genes involved in bile-acid synthesis have been characterised. Loss of *CYP7A1* function causes hypercholesterolaemia and is associated with resistance to cholesterol-lowering statins.[57] A mutation in the *CYP7B1* gene caused severe hyperoxysterolaemia in a male infant.[58] More than 50 different mutations are known in the *CYP27A1* gene in people with the genetic disease cerebrotendinous xanthomatosis, the clinical features of which include accelerated atherosclerosis and severe neurological impairment.[56] CYP27A1 deficiency is treatable with cholic acid, which restores the bile-acid pool and prevents synthesis of toxic sterol intermediates in the bile-acid pathway.

Steroid synthesis and metabolism

Six cytochrome P450 enzymes participate in steroidogenesis (figure 3). During sexual differentiation of the genital ridge in early embryogenesis, the transcription factor steroid-factor-1, a member of the nuclear hormone receptor gene family, is pivotal in upregulation of P450 genes implicated in steroid hormone synthesis—including members of the *CYP11*, *CYP17*, *CYP19*, and *CYP21* families.[59] CYP11A1, CYP11B1, and CYP11B2 are mitochondrial enzymes. CYP17A1 is needed for synthesis of cortisol and testosterone and oestrogen, whereas CYP19A1 converts androgenic precursors into oestrogens. Both CYP17A1 and CYP19A1 are located within the endoplasmic reticulum.

CYP17A1 is a dual-function enzyme, catalysing 17α-hydroxylation of steroid substrates and cleavage and oxidation of their side-chains. Mutations in *CYP17A1* that impair both these enzymic activities lead to deficiencies in production of glucocorticoids and sex steroids, whereas those that prevent oxidation and shortening of the side-chain lead to deficiencies in sex steroids only[60] (panel 2). Mutations in *CYP11A1* are the cause of lipoid adrenal hyperplasia, whereas defects in *CYP11B1* produce 11β-hydroxylase deficiency.[61] Allele-specific mutations in *CYP11B2* cause either corticosterone methyloxidase type I deficiency or corticosterone methyloxidase type II deficiency, and recombination events between the two closely-linked *CYP11B1* and *CYP11B2* genes on chromosome 8 that encode functional chimeric enzymes cause glucocorticoid-remediable aldosteronism[61] (panel 2).

CYP19A1 synthesises oestrogen by aromatisation of the A ring of the androgenic steroid substrates. Loss-of-function mutations in *CYP19A1* cause androgen excess, which leads to improper virilisation in females and hypervirilisation in males; affected males and females also have skeletal abnormalities, indicating the essential role of oestrogens in bone formation. Rare gain-of-function mutations in *CYP19A1* produce gynaecomastia in males.[62]

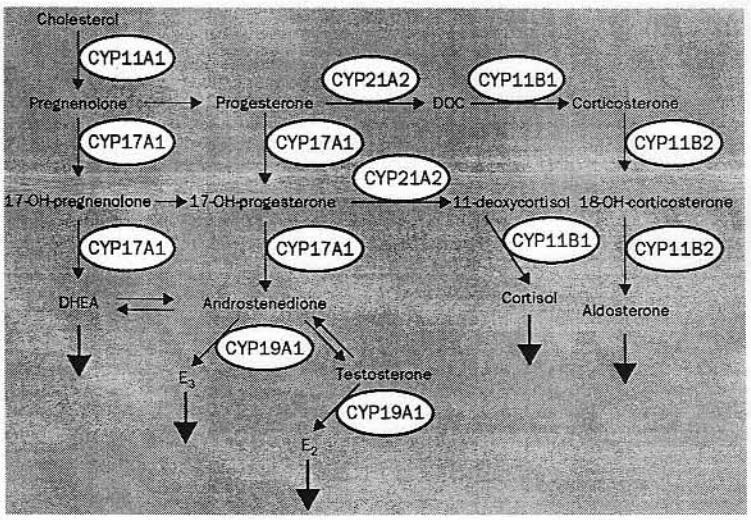

Figure 3: **Metabolic pathway of steroidogenesis**

CYP11A1=cholesterol side-chain cleavage; CYP11B1=11β-hydroxylase; CYP11B2=aldosterone synthase; CYP17A1=17α-hydroxylase; CYP21A2=21-hydroxylase; CYP19A1=aromatase; DOC=deoxycorticosterone; DHEA=dehydroepiandrosterone; E_3=oestriol; E_2=oestradiol. Numbers and letters in ovals denote six cytochrome P450 enzymes. Steps to form progesterone and androstenedione are catalysed by 3β-hydroxysteroid dehydrogenase, and testosterone is formed by 17-ketosteroid reductase. DHEA, E_3, E_2, testosterone, cortisol, and aldosterone are further degraded (hydroxylated) by members of the CYP1, CYP2, and CYP3 families (thick arrows).

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Hydroxylation of steroid precursors at carbon-21 is an essential step in biosynthesis of glucocorticoids and mineralocorticoids, and is catalysed by CYP21A2 (figure 3). Mutations that disrupt 21-hydroxylation underlie more than 90% of cases of congenital adrenal hyperplasia, an exceptionally prevalent genetic disease. Three categories of this disorder are known, including salt-wasting with masculinisation of females and life-threatening low sodium, high potassium, and hypovolaemia (classic); simple virilising congenital adrenal hyperplasia; and minor impairment of CYP21 activity (non-classic). Congenital adrenal hyperplasia can also be caused by mutations in the *CYP11A1, CYP11B1, CYP11B2, CYP17A1*, or *CYP19A1* genes[63] (panel 2).

Vitamin D_3 synthesis and metabolism

Four P450 enzymes, including three located in mitochondria and one in the endoplasmic reticulum, participate in synthesis and breakdown of 1α,25-dihydroxyvitamin D_3, the ligand of the vitamin D_3 receptor, which is a member of the nuclear hormone receptor superfamily. This receptor-ligand system is responsible for promotion of the export of calcium from bone and absorption of calcium from the gastrointestinal tract (figure 4). CYP27A1 and porcine CYP2D25 are mitochondrial and microsomal enzymes, respectively, that form 25-hydroxyvitamin D_3 from the vitamin D_3 precursor, colecalciferol.[64] Both enzymes are abundantly expressed in the liver, in which CYP27A1 also has a major role in synthesis of bile acids (see above). In the pig, CYP2D25 seems to be the more important of the two 25-hydroxylating enzymes. Mutations in human beings that eliminate CYP27A1 activity cause a bile-acid-deficiency phenotype (cerebrotendinous xanthomatosis) but have little or no effect on vitamin D_3 metabolism.[56]

Five *CYP2D* genes have been reported in the mouse and in the rat, an unknown number in the pig, but only one (*CYP2D6*) in man.[4,50] Because the *CYP2D* subfamily has existed for more than 450 million years,[50] it seems highly probable that human CYP2D6 has retained the steroid 25-hydroxylation function similar to that of the pig CYP2D25 enzyme. A nuclear receptor transcription factor, hepatic nuclear factor-4α (HNF4α), controls expression of the human *CYP2D6* gene; curiously, an 18-fold rise in serum bile acids was reported in mice without the *Hnf4a* gene, which is associated with loss of CYP2D enzyme activity.[65] Perhaps this effect might be explainable by a yin-yang relation between the *CYP27A1* and *CYP2D6* genes: loss of *CYP2D6* expression might upset the balance and lead to CYP27A1 overexpression with resultant increases in bile acid production (figure 2), whereas loss of CYP27A1 activity causes bile acid deficiency,[56] as mentioned above. A study of bile-acid metabolites and 25-hydroxyvitamin D_3 concentrations would be informative, comparing patients with the CYP2D6 efficient-metabolism, poor-metabolism, and ultra-metabolism phenotypes, to establish if alterations in the CYP2D6 enzyme that affect drug metabolism might also extend to changes in formation of bile acids, 25-hydroxyvitamin D_3, or both.

Alternatively, a member of the CYP3A instead of the CYP2D subfamily might have taken on the part played by porcine CYP2D25. CYP3A4 has been shown to be a 25-hydroxylase and 26-hydroxylase of 5β-cholestane-3α,7α,12α-triol and a 23*R*-hydroxylase, 24*S*-hydroxylase, and 27-hydroxylase of 5β-cholestane-3α,7α,12α,25-tetrol; in mice the equivalent CYP3A activity and mRNA are increased in *Cyp27a1(-/-)* knockout animals.[66] No increase in liver microsomal CYP3A4 activity was seen in a patient with cerebrotendinous xanthomatosis, suggesting that this species difference explains why the *Cyp27a1(-/-)* knockout mouse does not show signs and symptoms of cerebrotendinous xanthomatosis. Therefore, in figure 2 and figure 4 we have included both CYP3A4 and (?)CYP2D6 as possible participants, along with CYP27A1, in the hydroxylations of 25, 26, 23*R*, 24*S*, and 26 in the bile-acids pathway, and in the 25-hydroxylation of colecalciferol.

The CYP27B1 enzyme catalyses 1α-hydroxylation of 25-hydroxy-vitamin D_3 to form the active ligand of the vitamin D_3 receptor (figure 4). Mutations in *CYP27B1* encoding this mitochondrial P450 underlie vitamin D-dependent rickets type I.[67] The 24-hydroxylation of vitamin D_3 and its intermediates, which is catalysed by the mitochondrial CYP24A1 enzyme, prevents the ligand's subsequent binding to the receptor, and represents the major catabolic pathway of vitamin D_3. Transcription from the human *CYP24A1* gene is increased by calcium ions and by excess amounts of 1α,25-dihydroxyvitamin D_3.[61,68] Defects in the mouse *Cyp24a1* gene lead to a build-up of vitamin D_3 and an associated hypervitaminosis D phenotype[69] (panel 2).

Retinoic acid hydroxylation

The *CYP26* gene family has three genes, one in each of three subfamilies, suggesting that these genes arose from a common ancestor at least 150–200 million years ago. All three catalyse hydroxylation of retinoic acid (vitamin A). CYP26A1 is an all-*trans*-retinoic acid hydroxylase that does not act on 9-*cis* or 13-*cis* retinoic acid. Retinoic acid is an important morphogen during vertebrate development, operating via several retinoic acid receptors and retinoid X receptors.[70] As is true of many cytochromes P450 and other drug-metabolising enzymes,[8] CYP26A1 might catabolise vitamin A, degrading the ligand for these retinoic acid receptors, and thus turn off the powerful developmental signals sent by retinoids. CYP26B1 and CYP26C1 also seem to have a role in metabolism of retinoic acid or its derivatives,[7] but the biological parts played by these enzymes have not yet been elucidated.

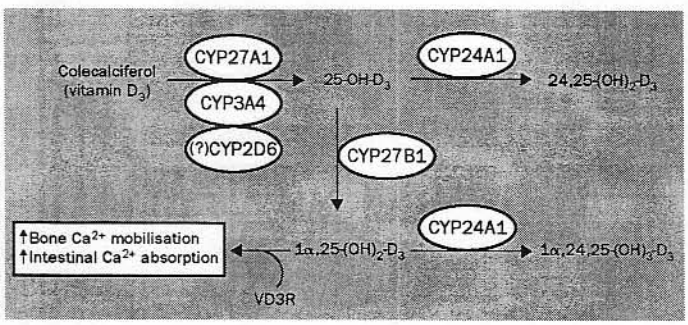

Figure 4: **Cytochrome P450 enzymes implicated in vitamin D_3 action**
Numbers and letters in ovals denote the five cytochrome enzymes possibly with a role in this pathway. Colecalciferol is derived from cholesterol. Order of hydroxylation is not absolutely crucial—ie, 1α-hydroxy-D_3 can undergo 25-hydroxylation.

Cytochrome P450 enzymes of unknown function

Functions of several cytochromes P450—including CYP20A1, CYP27C1, CYP4A20, CYP4F11, CYP4F12, CYP4F22, CYP4V2, CYP4X1, CYP26B1, and CYP26C1 enzymes—are unknown or, at best, sketchy.

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The dearth of information associated with these proteins is mainly attributable to their method of identification, which largely concerned database searches of the human genome.[4,7] Some of these genes might have only very limited tissue-specific or cell type-specific distributions, be transiently expressed, or both during embryogenesis or fetogenesis.

Looking to the future

As more and more *CYP* gene products are analysed, it seems highly likely that their roles in diverse biological systems will expand. Genes in the *CYP* superfamily are highly polymorphic,[71] as is true of most (if not all) other genes in the human genome,[71,72] and with P450 genetic differences will come interindividual variation in phenotype, with the attendant results for medicine and treatment. In the near future, many more studies are anticipated that will show associations between *CYP* variant alleles and myriad genetic diseases, environmental toxic effects and cancer, and other complex diseases.

Conflict of interest statement

None declared.

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