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# Human cytochromes P450 in health and disease

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There are 18 mammalian cytochrome P450 (*CYP*) families, which encode 57 genes in the human genome. *CYP2*, *CYP3* and *CYP4* families contain far more genes than the other 15 families; these three families are also the ones that are dramatically larger in rodent genomes. Most (if not all) genes in the *CYP1*, *CYP2*, *CYP3* and *CYP4* families encode enzymes involved in eicosanoid metabolism and are inducible by various environmental stimuli (i.e. diet, chemical inducers, drugs, pheromones, etc.), whereas the other 14 gene families often have only a single member, and are rarely if ever inducible or redundant. Although the *CYP2* and *CYP3* families can be regarded as largely redundant and promiscuous, mutations or other defects in one or more genes of the remaining 16 gene families are primarily the ones responsible for P450-specific diseases—confirming these genes are not superfluous or promiscuous but rather are more directly involved in critical life functions. P450-mediated diseases comprise those caused by: aberrant steroidogenesis; defects in fatty acid, cholesterol and bile acid pathways; vitamin D dysregulation and retinoid (as well as putative eicosanoid) dysregulation during fertilization, implantation, embryogenesis, foetogenesis and neonatal development.

## 1. Introduction

As the field of 'cytochrome P450 research' began in the late 1950s and 1960s, originally 'P-450' was thought to be a single cytochrome (detectable by spectrophotometry) which was believed to exist almost exclusively in liver [1–3], and the reason for its existence was to metabolize drugs and other foreign chemicals [4,5]. That these enzyme activities were inducible suggested they would be of clinical importance in pharmacology and therapeutics [4–7]. A few laboratories also recognized that P450 enzymes participated in steroid and fatty acid hydroxylation in both adrenal cortex and liver [8,9]. Because the correct definition of 'cytochrome' is 'an iron-containing protein that participates in cell respiration as a catalyst of oxidation-reduction', in the 1970s some emphasized that a better name for 'cytochromes P450' would be 'haem-thiolate monooxygenases' [10–13].

### (a) Cytochrome P450 nomenclature based on evolution

With the explosion of molecular biology in the 1980s, it became clear that P450 genes existed in virtually all species—from prokaryotes to rodents and humans [14], and alignment of deduced amino acid sequences led to the first proposal of a gene superfamily nomenclature system based on evolutionary divergence [15]. This evolutionary concept also implied that all *CYP* genes today arose from a single ancestor, which originated probably more than 3 billion years ago. The 30 genes originally reported in 1987 were derived from six vertebrates (rat, mouse, human, rabbit, cow and chicken), yeast and *Pseudomonas putida* [15]. Twenty-five years later, the superfamily has now expanded to a nomenclature system online which, as of 22 August 2012, totals 18 687 named protein-coding P450 genes having putative functions (http://drnelson.uthsc.edu/cytochromeP450.

**Table 1.** Historical format for the assignment of *CYP* gene families (http://drnelson.uthsc.edu/cytochromeP450.html).

| | |
|---|---|
| *CYP1–49* | animals |
| *CYP51–69* | yeast, fungi |
| *CYP71–99* | plants |
| *CYP101–299* | bacteria |
| *CYP301–499* | animals |
| *CYP501–699* | lower eukaryotes |
| *CYP701–999* | plants |
| *CYP3001–4999* | animals |
| *CYP5001–6999* | lower eukaryotes |

html): 5442 in animals; greater than 6800 in plants; greater than 4800 in fungi; 247 in Protozoa; greater than 1200 in Eubacteria; 48 in Archaebacteria and two in viruses. Table 1 describes the historical numbering system for the *CYP* gene families, established around 1990.

CYPs are conveniently arranged into families and subfamilies, based on percent amino acid sequence identity [14–16]. Enzymes sharing about greater than or equal to 40 per cent identity are assigned to a particular family designated by an Arabic numeral, whereas those sharing about greater than or equal to 55 per cent identity make up a particular subfamily designated by a letter. For example, both sterol 27-hydroxylase and 25-hydroxy-D 1α-hydroxylase are assigned to the CYP27 family because they share greater than 40 per cent sequence identity. However, sterol 27-hydroxylase is assigned to the 'A' subfamily and 25-hydroxy-$D_3$ 1α-hydroxylase to the CYP27 'B' subfamily of CYP27 because their protein sequences are less than 55 per cent identical. If an additional enzyme is discovered that shares greater than 55 per cent identity with the sterol 27-hydroxylase, then it would be named CYP27A2 (as in *Takifugu rubripes*, the puffer fish), etc.

Members of the mammalian *CYP2*, *CYP3* and *CYP4* families are named chronologically, regardless of species, according to their time of discovery; this explains why, for example, the four human *CYP3A* genes are *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43*. Development and application of this beautifully logical system of nomenclature has eliminated much of the confusion that often plagues the naming of genes, gene families and superfamilies. In fact, similar rules have now been followed for nomenclature of hundreds of other gene superfamilies in dozens of vertebrate and invertebrate databases (http://www.genenames.org/).

The human genome thus contains 18 *CYP* families, divided into 41 protein-coding subfamilies encoding 57 genes (table 2). As previously noted [18], the *CYP2*, *CYP3* and *CYP4* families contain far more genes than the other 15 families. Intriguingly, these three families are also the ones that are much larger in the mouse (which totals 103 genes) and other mammalian genomes; this expansion is also associated with the apparent 'increased responsiveness' of these three families to the 'environment' (diet, chemical inducers, drugs, pheromones, etc.). In fact, many of the genes in these three families, plus the *CYP1* family, are inducible by many various stimuli, whereas genes in the remaining 14 families often have only a single member, are rarely if ever 'inducible', are often not redundant and appear to be involved more directly in critical life functions [14,18,19]. It therefore follows that these remaining 14 families are also more likely to be associated with serious human diseases—if the important gene is mutated or missing.

## (b) Appreciation that CYP enzymes participate in endogenous functions

Throughout the 1970s and 1980s—with an increase in the number of reports of cytochromes P450 associated with seemingly unrelated various life processes in many organisms (including plants)—it was proposed that CYP enzymes are important upstream in the synthesis and degradation of virtually all non-protein ligands that bind to receptors or activate second-messenger pathways regulating growth, differentiation, apoptosis, homeostasis and neuroendocrine functions [19]. This article is intended to update the clinical importance of P450 functions (table 2) in critical life processes associated with normal human health.

## (c) Role of CYP enzymes in human disease

As with many other genes and enzymes associated with a critical life function, certain mutations or other variability in that gene and, hence, the gene product will result in pathology. Moreover, if the gene product is not redundant, the risk of serious disease is much higher. Another purpose of this article is to update the importance of P450 functions (table 2) as they relate to clinical disease.

## (d) Roles of electron-donating redox partners in CYP activities

Cytochromes P450 found in bacteria and eukaryotic mitochondria are termed 'type I', whereas those found in eukaryotic endoplasmic reticulum (ER; microsomes) are termed 'type II' (figure 1). Products of the *CYP11A1*, *CYP11B1*, *CYP11B2*, *CYP24A1*, *CYP27A1*, *CYP27B1* and *CYP27C1* genes are the seven P450 enzymes exclusively located in mitochondria and are therefore type I. In the mitochondrion, NADH or NADPH can donate electrons to the membrane-bound flavoprotein ferredoxin reductase (FDXR), which then passes them to a soluble iron–sulphur protein ferredoxin (FDX), which donates the electrons to type I P450s.

In the ER, NADPH donates electrons to the di-flavin (FAD–FMN) protein P450 oxidoreductase (POR), which then passes them on to the type II P450 (figure 1). The FAD group of POR accepts electrons from NADPH, eliciting a conformational change in POR so that the FAD becomes aligned with, and donates the electrons to, the FMN moiety—inducing the POR to return to its original conformation, thereby permitting the FMN domain of POR to interact with the type II P450 [20,21].

Haem iron in both types of P450 ultimately receives the electrons and donates them to molecular oxygen as the terminal electron acceptor, mediating catalysis. In some cases, cytochrome $b_5$ (CYB5) acts as an allosteric factor to facilitate interaction of POR with type II P450, and may rarely act as an alternative donor for the second, but not the first, electron in the P450 cycle; thus, the action of POR is essential for all type II P450s. Human POR deficiency causes dysregulated steroidogenesis and the Antley–Bixler skeletal malformation syndrome, whereas clinically apparent disorders of drug metabolism are rare [22–24]. CYB5 deficiency causes disordered sex steroid

**Table 2.** Functions and/or diseases associated with mutations in a *CYP* gene. 'Metabolism of eicosanoids' includes arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid (no P450 enzyme has been examined for all possible eicosanoid substrates). As discussed in the text, variants in genes of the *CYP2* and *CYP3* families (sometimes *CYP1* and *CYP4* families) can profoundly affect drug or metabolite levels in blood or urine; this can lead to therapeutic failure, toxicity or even death—when the patient receives the commonly recommended dosage of a drug. Variants in the *CYP1*, *CYP2A6*, *CYP2B6*, *CYP2C*, *CYP2D6*, *CYP2E1* and *CYP3A* genes have also been reported to be associated with increased risk of cancer, birth defects or adverse drug reactions; however, whereas laboratory animal data are convincing, most clinical studies showing associations with *CYP* variant alleles are statistically too underpowered to arrive at an unequivocal conclusion [17].

| gene | function(s) | disease(s) |
|---|---|---|
| *CYP1A1* | metabolism of eicosanoids; foreign chemicals | |
| *CYP1A2* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP1B1* | metabolism of eicosanoids; foreign chemicals | primary congenital glaucoma (buphthalmos) |
| *CYP2A6, 2A13* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP2A7* | nitrosamine metabolism | |
| *CYP2B6* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP2C8, 2C9, 2C18, 2C19* | metabolism of eicosanoids; drugs and foreign chemicals; *2C9* contributes to warfarin metabolism | |
| *CYP2D6* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP2E1* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP2F1* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP2J2* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP2R1* | vitamin D 25-hydroxylase | vitamin D 25-hydroxylase deficiency |
| *CYP2S1* | metabolism of eicosanoids; drugs and foreign chemicals; retinoids | |
| *CYP2U1* | metabolism of eicosanoids, long-chain fatty acids; drugs and foreign chemicals | |
| *CYP2W1* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP3A4* | metabolism of eicosanoids, sex steroids; greater than or equal to 60% of all clinically used drugs; foreign chemicals | |
| *CYP3A5, 3A7, 3A43* | metabolism of eicosanoids, sex steroids; drugs and foreign chemicals | |
| *CYP4A11* | metabolism of eicosanoids, medium- and long-chain fatty acids; drugs and foreign chemicals | association with hypertension, coronary artery disease |
| *CYP4A22* | function(s) unknown | |
| *CYP4B1* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP4F2* | metabolism of eicosanoids; drugs and foreign chemicals; contributes to warfarin metabolism | |
| *CYP4F3, 4F8* | metabolism of eicosanoids; drugs and foreign chemicals | |
| *CYP4F11* | function(s) unknown | |
| *CYP4F12* | metabolism of eicosanoids, long chain-fatty acids; drugs and foreign chemicals | |
| *CYP4F22* | function(s) unknown | lamellar ichthyosis type-3 |
| *CYP4V2* | function(s) unknown | Bietti crystalline corneoretinal dystrophy |
| *CYP4X1* | function(s) unknown; anandamide metabolism in brain | |
| *CYP4Z1* | metabolism of eicosanoids, fatty acids; drugs and foreign chemicals | |
| *CYP5A1* | eicosanoid metabolism (thromboxane $A_2$ synthase), participates in platelet aggregation (official gene name: *TBXAS1*) | Ghosal haemato-diaphyseal syndrome |
| *CYP7A1* | associated with elevated LDL-cholesterol levels; cholesterol 7$\alpha$-hydroxylase, biosynthesis of bile acids, metabolism of oxysterols | |
| *CYP7B1* | oxysterol 7$\alpha$-hydroxylase, neurosteroid 7$\alpha$-hydroxylase | neonatal cholestasis; hereditary spastic paraplegia 5A |

(*Continued*.)

**Table 2.** (*Continued.*)

| gene | function(s) | disease(s) |
|---|---|---|
| *CYP8A1* | eicosanoid metabolism (prostacyclin ($PGI_2$) synthase), participates in platelet disaggregation (official gene name: *PTGIS*) | one cause of essential hypertension |
| *CYP8B1* | sterol 12α-hydoxylase | |
| *CYP11A1* | cholesterol side-chain cleavage | congenital adrenal insufficiency with 46,XY sex reversal; postnatally lethal in mouse |
| *CYP11B1* | steroid 11β-hydroxylase | occasional congenital adrenal hyperplasia (CAH) |
| *CYP11B2* | steroid 11β- and 18-hydroxylase and 18-oxidase | corticosterone methyloxidase deficiency type I, type II; increased aldosterone-to-renin ratio |
| *CYP11B1-11B2* | chimeric enzyme (due to unequal crossovers) | causes glucocorticoid-remediable hyperaldosteronism |
| *CYP17A1* | steroid 17α-hydroxylase, 17,20-lyase | steroid 17α-hydroxylase, 17,20-lyase deficiency |
| *CYP19A1* | androgen aromatase, oestrogen synthetase | aromatase deficiency; aromatase excess syndrome |
| *CYP20A1* | function(s) unknown | |
| *CYP21A2* | steroid 21-hydroxylase | causes greater than 95% of all CAH; (adrenal insufficiency with hyperandrogenism), classic and nonclassic types |
| *CYP24A1* | vitamin D 24-hydroxylase | idiopathic infantile hypercalcaemia |
| *CYP26A1* | retinoid acid (RA) inactivation (hydroxylase) | embryolethal in mouse |
| *CYP26B1* | RA inactivation (hydroxylase) | Antley–Bixler-like syndrome; embryolethal in mouse |
| *CYP26C1* | RA inactivation (hydroxylase) | embryolethal in mouse |
| *CYP27A1* | bile acid biosynthesis, sterol 27-hydroxylase, vitamin D 25-hydroxylase | cerebrotendinous xanthomatosis |
| *CYP27B1* | 25-hydroxy-vitamin D 1α-hydroxylase | vitamin D-dependent rickets type 1A; infantile hypocalcaemia |
| *CYP27C1* | function(s) unknown | association with avascular necrosis of femoral head |
| *CYP39A1* | oxysterol 7α-hydroxylase | |
| *CYP46A1* | cholesterol 24-hydroxylase (brain) | |
| *CYP51A1* | lanosterol 14α-demethylase | embryolethal GD15 in mouse |

biosynthesis and mild methaemoglobinemia [25]. Deficiency states for FDXR or FDX have not been reported.

Some (or perhaps all) of the 'traditionally microsomal' P450 proteins in the *CYP1*, *CYP2*, *CYP3* and *CYP4* families can be targeted to mitochondria as well, and this phenomenon appears to be age- and organ-specific. Trafficking of these P450 proteins requires either proteolysis by a cytosolic peptidase [26] or phosphorylation of CYP2 and CYP3 proteins [27] as major mechanisms involved in mitochondrial import. The significance of these enzymes in mitochondria currently is not yet understood.

## 2. Drug, foreign chemical, fatty acid, arachidonic acid and eicosanoid metabolism: CYP1, 2, 3, 4, 5 and 8 families

Historically, P450-associated enzyme activities were studied predominantly in liver; this was because liver is the largest, most easily accessible organ in laboratory animals. Inducing chemicals or drugs were usually administered intraperitoneally; this was also carried out mostly as a method of convenience: chemicals in large doses could be injected into the peritoneal cavity with ease. Hence, intraperitoneal administration of 3-methylcholanthrene [28], phenobarbital [29], pregnenolone 16α-carbonitrile [30], steroid hormones [31], ethanol [32], rifampicin [33] and clofibrate [34] were among the seven earliest 'foreign signals' or 'stimuli' reported to enhance mammalian P450-mediated enzyme activities. In retrospect, these 'inducers' cause increases in P450 enzyme activities derived almost exclusively from members of the *CYP1*, *CYP2*, *CYP3* and *CYP4* gene families.

### (a) CYP1, CYP2, CYP3 and CYP4 enzymes in health and disease

Throughout the 1980s, it became appreciated that drugs are often derived from plants, and plant metabolites are substrates

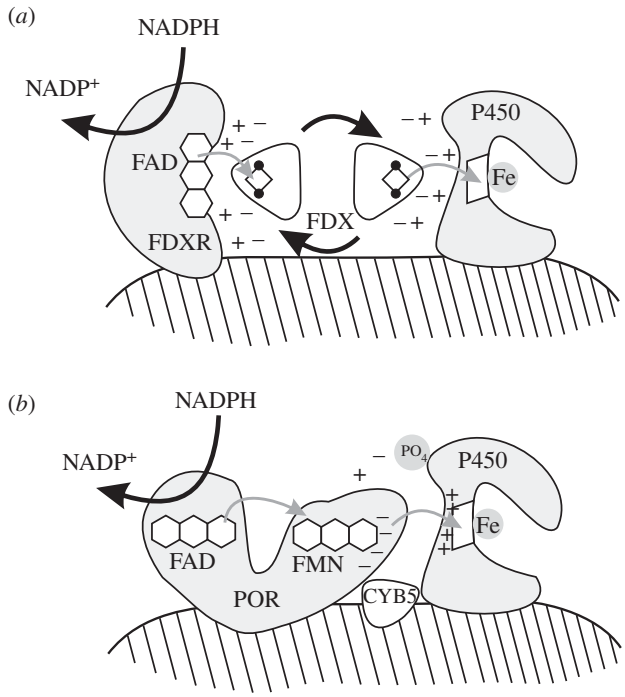


**Figure 1.** Schematic of (*a*) type I P450 and (*b*) type II P450. FAD, flavin adenine dinucleotide; FDX, ferrodoxin; FDXR, ferrodoxin reductase; FMN, flavin mononucleotide; POR, P450 oxidoreductase; CYB5, cytochrome $b_5$. Scheme copyrighted by WL Miller.

for mammalian P450 enzymes [19]—setting up a case for 'animal–plant warfare' being waged, and with P450 genes playing a central role [35]. A form of cytochrome P450 was proposed to be responsible for alveolar hypoxic pulmonary vasoconstriction in dogs [36]. P450-mediated peroxidation of acetaminophen and prostaglandin endoperoxide synthetase was reported in rabbit kidney [37]. Thus began the realization that arachidonic acid [38], and many of the metabolites downstream of arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid (all of them now collectively termed 'eicosanoids'), can be substrates for cytochromes P450 [39].

It is now understood that perhaps every member of the *CYP1*, *CYP2*, *CYP3* and *CYP4* families might participate in one or more specific steps during eicosanoid synthesis and degradation (figure 2); although not yet known, participation of CYP enzymes in the eicosanoid pathway is likely to be redundant with lipoxygenases and other peroxidases [40]. There are now more than 150 identified eicosanoids [40], which participate in virtually every imaginable critical life process (table 3). In all likelihood due to redundancy, allelic variants or other mutations leading to absence of activity in any specific *CYP2* or *CYP3* gene do not appear to be specifically associated with serious human disease. (Concerning the *CYP1* family, primary congenital primary glaucoma is an autosomal recessive disorder caused by *CYP1B1* mutations, as discussed later.)

There are many published genome-wide association (GWA) studies to date in which one or several genes in the *CYP1*, *CYP2*, *CYP3* or *CYP4* families are shown to be significantly associated with increased risk of a particular human complex disease. Unless it can be demonstrated otherwise, the 'connection' in many, if not all, of these GWA studies most probably reflect P450 involvement in the eicosanoid cascade. In mouse studies, for example, increased thirst, renal dysfunction and injury and inflammation associated with angiotensin II-induced hypertension all depend on CYP1B1 activity [41]. These findings most probably reflect CYP1B1-mediated specific eicosanoid synthesis or degradation in the kidney.

Further evidence for redundancy comes from mouse *Cyp* knockout lines. Ablation of all three *Cyp1* genes results in a phenotype: incomplete penetrance for greater risk of embryolethality before gestational day(GD)-11, hydrocephalus, hermaphroditism and cystic ovaries; intriguingly, however, about one of every 40 $F_1$ pups survive and eventually breed, leading to loss of the above-mentioned traits [42]. Because this improvement in phenotype happens in only one or two generations, it is likely that epigenetics is involved in such selective breeding [42,43]. Nevertheless, because defects in implantation, growth *in utero*, hydrocephalus, hermaphroditism and cystic ovaries all are very likely to reflect disturbances in eicosanoid actions (table 3), data from the *Cyp1a1/1a2/1b1*(−/−) triple-knockout mouse line reinforce the tenet that removal of the entire *Cyp1* gene family is able to perturb eicosanoid-mediated functions in dramatic fashion.

Entire subfamilies of the mouse *Cyp3a* [44,45], *Cyp2d* [46] and *Cyp2c* [47] gene clusters have now been ablated, and these lines are reported as viable and fertile. However, alterations in drug metabolism profiles and hepatic gene expression patterns have been noted. Whereas ablation of the *Cyp1* family with its three members indeed results in a profound phenotype, it appears that removal of (at least) the *Cyp2* and *Cyp3* subfamily clusters do not. Hence, we conclude that, whereas genes in mammalian *CYP2* and *CYP3* subfamilies are substantially redundant, genes in the *CYP1* family are significantly less so.

The *CYP4* family appears to be more like the *CYP1* family, i.e. individual mutated genes can cause autosomal recessive disorders. For example, in 23 of 25 unrelated patients with Bietti crystalline corneoretinal dystrophy, an autosomal recessive disorder leading to progressive night blindness and constriction of the visual field, a total of 13 missense mutations was found in the *CYP4V2* gene [48]. By linkage analysis of 12 consanguineous families with lamellar ichthyosis type-3, seven distinct mutations in the *CYP4F22* gene were reported [49]. Mutations in other *CYP4* genes have been reported to alter the metabolism of various eicosanoids. *Cyp4a10*(−/−) knockout mice were found to be normotensive on a low-salt diet, but become hypertensive when fed normal or high-salt diets; these mice also show altered eicosanoid metabolite profiles [50].

## (b) CYP1, CYP2, CYP3 and CYP4 enzymes in metabolism of drugs and other foreign chemicals

There are many single-nucleotide polymorphisms (SNPs) in the *CYP1A2* gene and in the *CYP2*, *CYP3* and *CYP4* gene families shown to be correlated with important differences in pharmacokinetic (PK) parameters of drug substrates relatively (but not completely) specific for a particular P450 enzyme (http://www.cypalleles.ki.se/). PK parameters (absorption, distribution, metabolism, excretion) include variations in drug or metabolite levels in blood or urine, as well as therapeutic failure. Thus, interindividual differences in the 'recommended effective drug dosage' can be associated with some of these SNPs.

CYP2D6 metabolizes more than 20 per cent of all prescribed drugs, and the *CYP2D6* gene is exceedingly polymorphic; indeed, the 'ultra-metabolizer' (UM) phenotype has

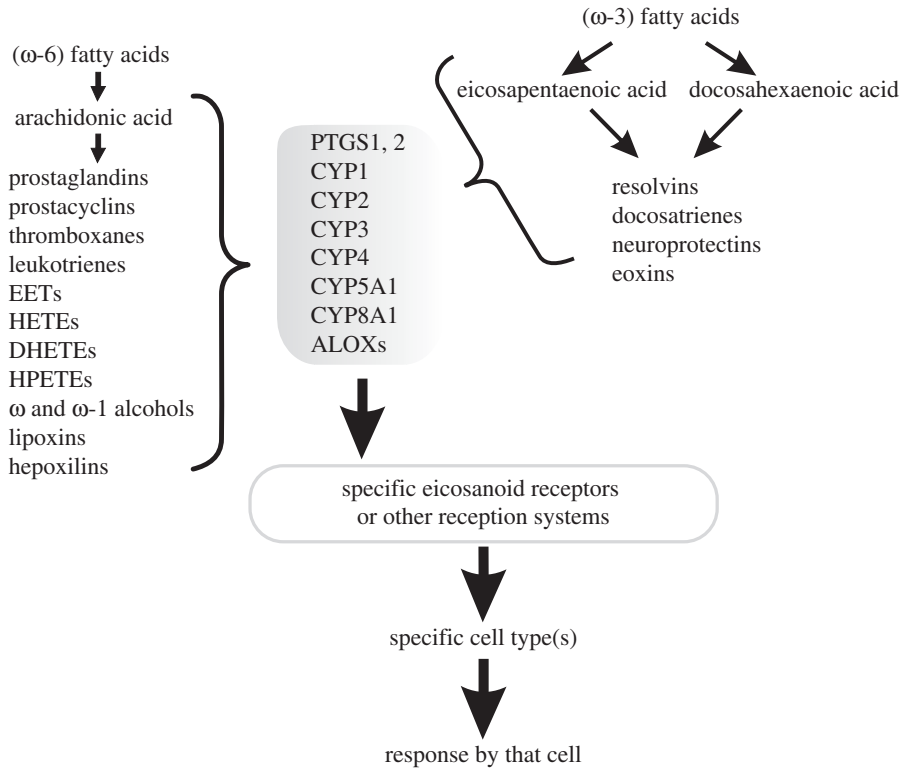


**Figure 2.** Scheme to show the relationship of eicosanoid synthesis and degradation, catalysed by cyclooxygenases-1 and -2 (PTGS1, PTGS2), possibly every member of the P450 families CYP1, CYP2, CYP2 and CYP4, plus the six arachidonate lipoxygenases (human ALOX5, ALOX12, ALOX12B, ALOX15, ALOX15B and ALOXE3). All of this metabolism occurs upstream of eicosanoid binding to specific receptors, activity in specific cell types, resulting in innumerable critical life processes. Whereas most eicosanoids are derived via arachidonic acid from ω-6 fatty acids, an important group of resolvins are derived via eicosapentaenoic acid and docosahexaenoic acid from ω-3 fatty acids. EETs, epoxyeicosatrienoic acids; HETEs, hydroxyeicosatetraenoic acids; DHETEs, dihydroxyeicosatrienoic acids; HPETEs, hydroperoxyeicosatetraenoic acids.

been shown to be caused by multiple copies—from two to 13 copies—of the *CYP2D6* gene [51]. The UM phenotype can cause serious side effects. The 'commonly recommended prescribed dose' of codeine, when given to a child or pregnant mother having the UM phenotype, can result in narcosis, apnoea, brain damage and even death due to excessive amounts of codeine being transformed quickly to morphine [52,53].

For the most part, however, therapeutic efficacy and adverse drug reactions represent *multifactorial traits* (traits which reflect the contribution of many genes plus environmental effects plus transgenerational and other epigenetic effects) wherein any one gene almost always contributes far less than 1 per cent to the phenotype. Thus, therapeutic efficacy and adverse drug reactions are really no different from complex traits such as obesity, schizophrenia, dementia, cancer, heart disease, stroke or height. In GWA studies, it is therefore difficult—if not impossible—to demonstrate a truly statistically significant association between any multifactorial trait and one or several SNPs in any gene of the *CYP1*, *CYP2*, *CYP3* or *CYP4* families [54]. The same conclusion was made when analysing all previous SNP studies that had purported to show an unequivocal association between cancer and any gene of the *CYP1*, *CYP2*, *CYP3* or *CYP4* families [17].

## (c) CYP5A1 and CYP8A1 enzymes in health

Along with most (if not all) members of the CYP1, CYP2, CYP3 and CYP4 families, CYP5A1 and CYP8A1 participate in eicosanoid metabolism. More than three decades ago, prostacyclin synthetase was shown to have P450 spectral properties [55]. In the evolutionary scheme of *CYP* gene nomenclature, thromboxane $A_2$ synthase [56,57], involved in platelet aggregation, and prostacyclin ($PGI_2$) synthase [55,57], which participates in platelet disaggregation, are encoded by the *CYP5A1* and *CYP8A1* genes, respectively. Hence, CYP5A1 and CYP8A1 provide a yin-yang system during the normal process of blood coagulation. Officially, these two genes are named *TBXAS1* and *PTGIS*, respectively (http://www.genenames.org/), because these genes and functions of their gene products were studied for many years prior to formal acceptance of the *CYP* gene superfamily nomenclature system. The same scenario can be stated for cyclooxygenase-1 and -2 (COX1, COX2), encoded by genes whose official names are *PTGS1* and *PTGS2*, respectively.

Beyond the scope of this paper, each of the seven 'classes' of P450 inducers mentioned earlier for genes in the *CYP1*, *CYP2*, *CYP3* and *CYP4* families has a mechanism-of-action involving one or more receptors: e.g. AHR, CAR, PXR and other members of the nuclear hormone receptor superfamily. Likewise, the *TBXA1R* and *TBXA2R* genes code for thromboxane synthase receptors-1 and -2, respectively, which regulate the *TBXA1* gene in platelets; and the *PTGIR* gene encodes the prostacyclin receptor that governs the *PTGIS* gene.

## (d) CYP5A1 and CYP8A1 enzymes in disease

Four published missense mutations in the *TBXAS1* gene have been shown to be associated with Ghosal haemato-diaphyseal syndrome. Originally discovered as a rare autosomal recessive trait [58], patients from two inbred families exhibit increased

**Table 3.** Eicosanoids and their effects on critical life processes.[a]

| class of eicosanoid | life processes |
|---|---|
| prostaglandins | maintenance of blood pressure and renal function ($PGI_2$, $PGE_2$, $PGD_2$, $PGF_{2\alpha}$ and $TXA_2$) |
| | regulation of airway smooth muscle |
| | regulation of cardiovascular smooth muscle |
| | organic anion transport |
| | inflammation and resolution of inflammation |
| | anti- and pro-inflammatory effects in eosinophils ($PGD_2 \longrightarrow$ 15-deoxy-$\Delta$-12,14-$PGJ_2$) |
| | thermoregulation ($PGE_2$) |
| | gastroduodenal defence |
| | neurological defence ($PGE_2$, $PGD_2$, $PGF_{2\alpha}$, $PGI_2$) |
| | ocular pressure, glaucoma |
| | blastocyst implantation, decidualization |
| | proton transport |
| | bone formation ($PGE_2$), destruction, joint formation and disease |
| prostacyclins | vascular relaxation |
| | inhibition of platelet aggregation which can promote pre-atherosclerotic vascular disease |
| thromboxanes | regulation of vascular tone and blood pressure |
| | tumour progression and cancer metastasis |
| | platelet activation (disaggregation) and promotes thrombus formation |
| leucotrienes | pro-inflammatory processes |
| | increase in production of pro-inflammatory cytokines |
| | increase in microvascular permeability |
| | potent chemotactic agent (of eosinophils, neutrophils, monocytes in inflamed tissue) |
| | enhancer of superoxide generation |
| EETs (epoxyeicosatrienoic acids) | activation of smooth-muscle large-conductance $Ca^{2+}$-activated $K^+$ channels, producing hyperpolarization and vasorelaxation |
| | cation (Na, K, Ca) transport |
| | modulation of ion channel activity |
| | anion transport |
| | smooth-muscle contraction of intra-lobar pulmonary arterial wall |
| | renal vasoconstriction |
| | renal vasodilation (anti-hypertensive) |
| | intestinal vasodilation, vasoconstriction |
| | decrease in ischaemia-reperfusion injury |
| | anti-inflammatory effects within the vasculature |
| | anti-atherogenesis |
| | stimulation of lipid metabolism |
| | regulation of insulin sensitivity |
| | activation of PPAR$\alpha$ and PPAR$\gamma$ |
| | vasodilation in coronary circulation |
| | enhanced local blood flow in CNS |
| | anti-proliferative and pro-apoptotic effects on PDGF-stimulated 3T6 fibroblasts |
| | stimulation of c-*Fos*, c-*Jun* |
| | mitogenesis |
| | stimulation of tyrosine phosphorylation |
| | cell proliferation |

(*Continued*.)

**Table 3.** (*Continued.*)

| class of eicosanoid | life processes |
|---|---|
| | cell migration |
| | angiogenesis |
| | inhibition of platelet aggregation (anti-thrombotic) |
| | fibrinolysis |
| | neurohormone secretion (or release) |
| | G-protein signalling |
| | modulation of NF$\kappa$B |
| | decreases in non-neuropathic pain |
| HETEs (hydroxyeicosatetraenoic acids) | inhibition of $Na^+$-$K^+$ ATPase activity |
| | inhibition of $Na^+$-$K^+$/$2Cl^-$ cotransporter |
| | vascular $O_2$ sensor |
| | mediator of mitogenesis |
| | mediator of angiogenesis |
| | vasoconstriction in retina |
| | renal vasoconstriction |
| | chemotaxis |
| | pro-inflammatory effects during re-perfusion of the heart |
| | vasoconstriction of coronary circulation |
| | regulation of vascular tone in brain, kidney, heart and splanchnic beds |
| | action on vascular smooth-muscle cells to cause vasoconstriction |
| | depolarization of vascular smooth-muscle cells by blocking $Ca^{2+}$-activated $K^+$ channels |
| DHETEs (dihydroxyeicosatrienoic acids) | stimulation of lipid metabolism |
| | regulation of insulin sensitivity |
| | inhibition of platelet aggregation (anti-thrombotic) |
| | fibrinolysis |
| HPETEs (hydroperoxyeicosatetraenoic acids) | induction of apoptosis in tumour cells |
| | inhibition of growth of solid tumours in nude mice |
| | inhibition of lung fibrosis and inflammation |
| | mediator of insulin levels |
| | anti-inflammatory |
| $\omega$-alcohols, $\omega$-1 alcohols | stimulation of $Na^+$-$K^+$ ATPase activity |
| | vasoconstriction |
| | bronchoconstriction |
| | activation of PPAR$\alpha$ |
| lipoxins | inflammation resolution: inhibition of granulocyte chemotaxis, adherence, diapedesis and activation; upregulation of monocyte/macrophage chemotaxis and non-phlogistic uptake of apoptotic granulocytes |
| | anti-inflammatory: inhibition of chemokine production; suppression of Th1 and Th2 immune responses; upregulation of haem oxygenase-1; inhibition of ERK and JNK activation (T cells, astrocytes) |
| | inhibition of signalling through toll-like receptors and TNF receptor family members in dendritic cells via SOCS2-mediated degradation of TRAF6 and TRAF2 |
| | inhibition of fibroblast proliferation and angiogenesis |
| | inhibition of mesangial cell proliferation |
| | protection in experimental models of: asthma, cystic fibrosis, pleuritis, peritonitis, ischaemia-reperfusion injury, periodontitis, colitis, corneal injury, glomerulonephritis, graft-versus-host disease |

(*Continued.*)

**Table 3.** (*Continued.*)

| class of eicosanoid | life processes |
|---|---|
| resolvins: E-series and D-series | resolution of inflammation: inhibition of granulocyte trans-endothelial and trans-epithelial migration; upregulation of non-phlogistic phagocytosis of apoptotic granulocytes by monocyte/macrophages |
| | anti-inflammatory: inhibition of pro-inflammatory cytokine production by dendritic cells, microglia, T cells |
| | inhibition of apoptosis of retinal pigment epithelial cells |
| | protection in experimental models of: asthma, ischaemia-reperfusion injury, stroke, retinopathy, peritonitis, colitis |
| eoxins | pro-inflammatory action by modulating and enhancing vascular permeability |
| hepoxilins (fatty acid epoxy-alcohols) | involvement in skin barrier function in the outer epidermis |
| | diacylglycerol release in neutrophils, insulin secretion in pancreatic beta-cells and synaptic actions in brain |
| | key regulator of mucosal inflammation; release $Ca^{2+}$ from intracellular stores |

[a]EETs can be converted quickly to DHETEs and to ω- and ω-1 alcohols. HPETEs can be reduced to HETEs and converted to hepoxilins. Thus, a critical life function attributed to EETs or to HPETEs might actually reflect the activity of one or more of their downstream products; the same can be said for many of the other categories of eicosanoids (reviewed, with detailed references, in [40]).

bone density, progressive sclerosing dysplasia in the midsection of long bones, refractory anaemia, and a specific deficit in arachidonic acid-produced platelet aggregation.

When 200 'essential hypertension' patients were screened for mutations in the *PTGIS* gene, one patient was found to have a splice-site mutation in intron 9, which caused a truncated protein having a deletion in the P450 haem-binding region; two of three other siblings of the proband also carried the same splice-site mutation and were similarly hypertensive [59]. Another manifestation of this splicing defect was a substantial decrease in urinary prostacyclin metabolites.

## 3. Biogenic and neurogenic amines

Human recombinant CYP2D6 is capable of converting tyramine to dopamine [60]. Tyramine is an endogenous compound that exists in the brain as a trace amine, but is also found in foods, such as cheese and wine. CYP2D6 is expressed in liver but also in other tissues. In human brain, membrane-bound CYP2D6 is localized in pyramidal cells of the cortex and hippocampus, as well as Purkinje cells of the cerebellum. CYP2D-mediated synthesis of dopamine from tyramine has recently been demonstrated to function in brain of the intact rat [61].

Data from experiments using recombinant human CYP2D6 enzyme and humanized CYP2D6-transgenic mice have provided evidence that 5-methoxytryptamine, a metabolite and precursor of melatonin, is a specific endogenous substrate for CYP2D6 [62]. This potent serotonergic neuromodulator is *O*-demethylated to serotonin by CYP2D6. CYP2D6 was demonstrated to be a specific 5-methoxyindolethylamine *O*-demethylase [63]. These findings remain somewhat controversial, because the affinities of CYP2D6 for these putative endogenous substrates are not as high as affinities for endogenous substrates generally are.

CYP2D6 action on substrates with activity in the brain has important clinical implications. Polymorphic *CYP2D6* gene expression is absent in liver and brain in up to 10 per cent of human populations. For this reason, there may be possible differences in the handling of neurotransmitters between individuals carrying an active *CYP2D6* gene and those deficient in CYP2D6 activity due to mutant *CYP2D6* alleles [63,64]. The *CYP2D6* polymorphism may contribute to personality differences and also to disease risk; in fact, analysis of 121 clinically depressed patients (113 CYP2D6 efficient metabolizers and eight poor metabolizers) suggested a significant relationship between behaviour and CYP2D6 activity levels [65].

## 4. Cholesterol and bile acids: biosynthesis and metabolism

Cholesterol is present in cellular membranes and serves as the principal precursor for synthesis of biologically active compounds, such as bile acids, oxysterols and steroid hormones. Maintenance of adequate cholesterol levels in tissues and cells requires the complex interaction of a number of physiological factors. Disturbances of cholesterol homeostasis are associated with diseases, such as atherosclerosis and neurological disorders.

Biosynthesis of cholesterol from acetate occurs in most cell types; a large part of endogenously formed cholesterol is synthesized in the liver. Cholesterol is eliminated from the body by conversion to bile acids, the end products of cholesterol catabolism (figure 3). Bile acids are powerful detergents that promote intestinal absorption of dietary lipids, including fat-soluble vitamins. At the cellular level, bile acids are important signalling molecules that coordinately control a network of metabolic pathways [66]. Bile acids regulate important genes in lipid homeostasis and drug metabolism by way of activating nuclear receptors (e.g. farnesoid X receptor (FXR), pregnane X receptor (PXR), vitamin D receptor (VDR)) and cell-signalling pathways.

Oxysterols are monooxygenated cholesterol metabolites. They are intermediates in bile acid biosynthesis, participate in

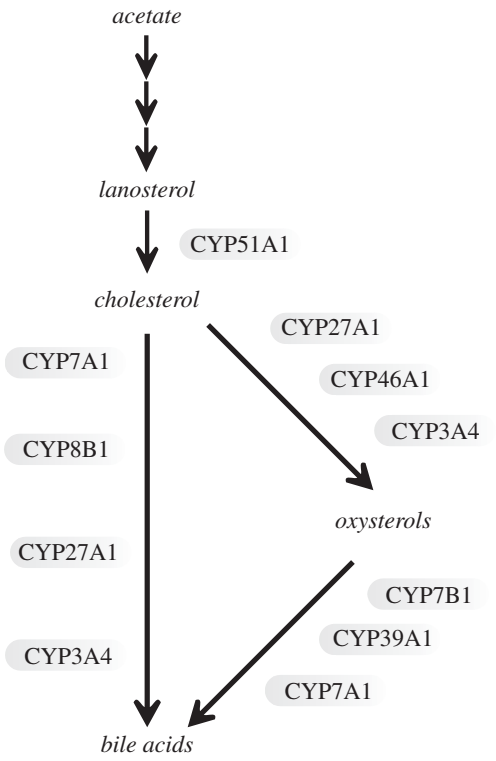


**Figure 3.** Summary of P450 enzymes in cholesterol and bile acid biosynthesis. Biosynthesis of cholesterol from acetyl-CoA involves approximately 30 enzymatic steps. There is a single P450 (CYP51A1) in cholesterol biosynthesis. In the classic (neutral) pathway of bile acid biosynthesis, cholesterol is converted to two primary bile acids in human liver—cholic acid and chenodeoxycholic acid. In the alternative (acidic) pathway and other pathways involving oxysterols, mainly chenodeoxycholic acid is formed. The key regulated enzymes in the bile acid biosynthetic pathways are CYP7A1, CYP8B1, CYP27A1 and CYP7B1.

cholesterol transport, and are potent regulators of genes in cholesterol homeostasis via activation of the liver X receptor (LXR). Excess levels of some oxysterols can cause cytotoxicity and have been implicated in disease. High concentrations of some oxysterols are found in atherosclerotic plaques.

Biosynthesis and metabolism of cholesterol, bile acids and oxysterols involve several CYP enzymes—including CYP51A1, CYP7A1, CYP7B1, CYP8B1, CYP27A1, CYP46A1, CYP39A1 and CYP3A4.

## (a) CYP51A1 in health and disease

CYP51A1 is the only P450 enzyme involved in cholesterol biosynthesis; this enzyme catalyses the oxidative 14α-demethylation of lanosterol. Demethylation is carried out by three consecutive monooxygenation reactions. Distribution of CYP51A1 is very widespread among living organisms; genes encoding CYP51 are found in yeast, plants, fungi, animals and even prokaryotes, suggesting this is among the oldest of the P450 genes [67]. CYP51 is a common target of antifungal drugs (e.g. miconazole and ketoconazole), which inhibit CYP51 activity and formation of ergosterol.

The *Cyp51a1*(−/−) knockout mouse is embryolethal at GD15 [68] and exhibits several prenatal signs resembling the Antley–Bixler syndrome (ABS). ABS represents a group of heterogeneous disorders characterized by skeletal, cardiac and urogenital abnormalities, as well as disorders in steroidogenesis.

## (b) CYP7A1 in health and disease

The *CYP7A1* gene encodes cholesterol 7α-hydroxylase, catalysing the first and major rate-limiting step in the classical, neutral pathway for bile acid biosynthesis. CYP7A1 is considered to be liver-specific [69,70]. CYP7A1 also participates in 7α-hydroxylation of 27-hydroxycholesterol and other oxysterols [71]. The *CYP7A1* gene is regulated by bile acids and other factors [66]. Studies using *Cyp7a1*(−/−) knockout mice have demonstrated a crucial role for this enzyme in bile acid biosynthesis [72,73]; however, mice and humans with cholesterol 7α-hydroxylase deficiency exhibit different phenotypes [70].

A metabolic disorder presenting with elevated plasma cholesterol levels, caused by a homozygous frameshift mutation in the *CYP7A1* gene, resulting in no enzyme activity, has been described [74]. High levels of LDL-cholesterol in these CYP7A1-deficient subjects were found to be resistant to treatment with hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. Heterozygotes for this mutation were also found to be hypercholesterolemic [74]. Associations between plasma LDL-cholesterol levels and mutations in the *CYP7A1* 5′-flanking region have also been described [75,76]. One *CYP7A1* promoter polymorphism has also been reported to be a risk factor for gallbladder cancer [77] and decreased risk of proximal colon adenomas [78]; however, these studies are statistically very underpowered and in need of replication in much larger cohorts before unequivocal conclusions can be made.

## (c) CYP7B1 in health and disease

The *CYP7B1* gene codes for a 7α-hydroxylase involved in metabolism of oxysterols, sex hormones and neurosteroids. The enzyme is associated with several physiological processes including brain function, immune system, cholesterol homeostasis and cellular viability and growth. Human CYP7B1 shares 40 per cent amino acid sequence identity with human CYP7A1. CYP7B1 is expressed in many human tissues, such as brain, kidney, liver and prostate [79].

The enzyme was originally found to catalyse 27-oxygenated sterols in the alternative acidic pathway for bile acid biosynthesis [80]. Moreover, several of the products formed by CYP7B1 are reported to have physiological effects; metabolic actions of CYP7B1 affect endocrine signalling and govern cellular steroid levels in various cell types such as those in the central nervous system (CNS) [81]. CYP7B1 also participates in 7α-hydroxylation of 27-hydroxycholesterol and other oxysterols [82].

A homozygous mutation in the *CYP7B1* gene of a newborn resulted in severe neonatal cholestasis (congenital bile acid defect, type 3); the condition was postnatally lethal [83]. Pathological findings were consistent with accumulation of hepatotoxic unsaturated monohydroxy bile acids. The patient had dramatically elevated 27-hydroxycholesterol levels, and liver samples showed no 27-hydroxycholesterol 7α-hydroxylase activity. An Asian child with neonatal cholestatic liver disease, having a *CYP7B1* mutation, showed similar symptoms [84].

Mutations in the *CYP7B1* coding region have also been found in patients with a form of hereditary spastic paraplegia (HSP) type 5A. HSP is an upper-motor-neuron degenerative disease that affects lower limb movement, resulting in weakness and spasticity, and characterized by axonal

degeneration of spinal cord neurons. *CYP7B1* mutations have been correlated with a pure form of autosomal recessive HSP in several families [85]. The pathogenic basis for this disease may be related to effects on either cholesterol homeostasis in the brain (i.e. on CYP7B1-mediated control of 27-hydroxycholesterol levels) or metabolism of dehydroepiandrosterone and other neurosteroids.

### (d) CYP8B1 in health

*CYP8B1* encodes a sterol 12α-hydroxylase catalysing a reaction unique for cholic acid biosynthesis and expressed exclusively in liver. The human and mouse *CYP8B1* genes are intronless, a feature unique among mammalian P450 genes [86]. CYP8B1 is active towards certain intermediates in the neutral pathway of bile acid synthesis. Because CYP8B1 is required for cholic acid formation, CYP8B1 activity determines the primary bile acid composition, which may affect intestinal cholesterol absorption. To date, no *CYP8B1* mutations have been associated with disease.

### (e) CYP27A1 in health and disease

CYP27A1 is a sterol 27-hydroxylase and vitamin D 25-hydroxylase, which is localized in the inner mitochondrial membrane. The purified enzyme [87] and cDNA [88] encoding for CYP27A1 were first isolated from rabbit liver. Human CYP27A1 is expressed in numerous tissues, including liver, kidney, lung, macrophages, brain, vascular endothelium, skin and prostate. This enzyme catalyses important reactions in both cholesterol homeostasis and vitamin D metabolism.

The anti-atherogenic CYP27A1 enzyme participates in several different metabolic reactions in cholesterol homeostasis. CYP27A1 catalyses the 27-hydroxylation of cholesterol and several 27-carbon sterol intermediates in bile acid biosynthesis. The 27-hydroxylated sterol is further oxidized by CYP27A1 to the corresponding 27-carbon acid by three consecutive monooxygenations at carbon-27. CYP27A1 initiates degradation of the 27-carbon sterol side chain in both the classical neutral pathway and the alternative acidic pathway for bile acid biosynthesis. In addition, CYP27A1 participates in other processes in cholesterol homeostasis, such as oxysterol formation and cholesterol transport [70]. The enzyme is known to produce 27-hydroxycholesterol in many different cells, and small amounts of 24- and 25-hydroxycholesterol also may be formed.

Sterol 27-hydroxylase deficiency leads to cerebrotendinous xanthomatosis (CTX), a rare autosomal recessive sterol storage disorder with neurological dysfunction—characterized by the accumulation of cholesterol and cholestanol in most tissues, particularly in tendon and brain xanthomas. Serious neurological symptoms, dementia, cerebellar ataxia and spinal cord paresis, are thought to be caused by the brain xanthomas.

CTX patients have a decreased capacity to synthesize bile acids. Specific missense mutations in the *CYP27A1* gene were found to cause this disorder [89]; approximately 50 *CYP27A1* mutations leading to CTX have now been reported. In addition to neurological impairment, other manifestations of sterol 27-hydroxylase deficiency include accelerated atherosclerosis and osteoporosis. Although the disorder is rare, the incidence is substantially greater than previously recognized—due to a phenotypic gradient in severity. CTX can be successfully treated by oral bile acid therapy, which prevents synthesis of toxic sterol intermediates in the bile acid pathway.

### (f) CYP46A1, CYP39A1 and CYP3A4 in health and disease

CYP46A1 (cholesterol 24-hydroxylase) converts cholesterol to 24*S*-hydroxycholesterol; the *CYP46A1* gene is primarily expressed in brain. The retina expresses CYP46A1 and is enriched in 24*S*-hydroxycholesterol. The 24-hydroxylation is important for cholesterol elimination from the brain, because cholesterol transport in and out of neurons is prevented by the blood-brain barrier; 24*S*-cholesterol is ultimately converted to bile acids in the liver [90]. There is a possible association of *CYP46A1* polymorphisms as a risk factor for age-related macular degeneration [91].

The *CYP39A1* gene encodes a specific 24-hydroxycholesterol 7α-hydroxylase, expressed mainly in the liver ER membrane. Hepatic CYP39A1 mRNA levels are not altered in response to dietary cholesterol, bile acids or a bile acid-binding resin [92]; to date, CYP39A1 has not been associated with any human disorder.

CYP3A4 catalyses 6α-hydroxylation of the hepatotoxic lithocholic acid and 25-hydroxylation of the cholic acid precursor 5β-cholestane-3α,7α,12α-triol. These CYP3A4 substrates are ligands for PXR, a regulator of the *CYP3A4* gene [93–96]. Oxysterol 4β-hydroxycholesterol is formed by CYP3A4, and this oxysterol has been suggested to be used as a marker for CYP3A4/5 enzymatic activity [97]. As described earlier, no human disease is correlated with *CYP3A4* mutations.

## 5. Steroid synthesis and degradation

Studies of steroidogenesis have played a central role in elucidating P450 biochemistry, genetics and physiology: the first activity specifically ascribed to a P450 enzyme was steroid 21-hydroxylase [8], a key enzyme in the production of both mineralocorticoids and glucocorticoids; missense mutations in the gene occur in about 1 in 15 000 persons. Six P450 enzymes—three type I enzymes in the *CYP11* family and three type II enzymes in the *CYP17*, *CYP19* and *CYP21* families—play indispensable roles in the production of steroid hormones that are essential to life and reproduction (figure 4). The biochemistry, genetics and physiology of all steroidogenic enzymes have recently been reviewed in detail [98].

### (a) CYP11A1: conversion of cholesterol to pregnenolone by P450scc

The mitochondrial cholesterol side-chain cleavage enzyme P450scc, encoded by *CYP11A1*, is the first and rate-limiting step in production of all steroid hormones (figure 4); designation of a cell as being 'steroidogenic' requires that *CYP11A1* is expressed. The location of P450scc on the inner mitochondrial membrane, and paucity of cholesterol there, necessitates a complex mitochondrial cholesterol import mechanism based on the steroidogenic acute regulatory protein (StAR), which facilitates cholesterol influx into mitochondria [99]. StAR deficiency causes congenital lipoid adrenal hyperplasia—which is fairly common in Japan and Korea [100].

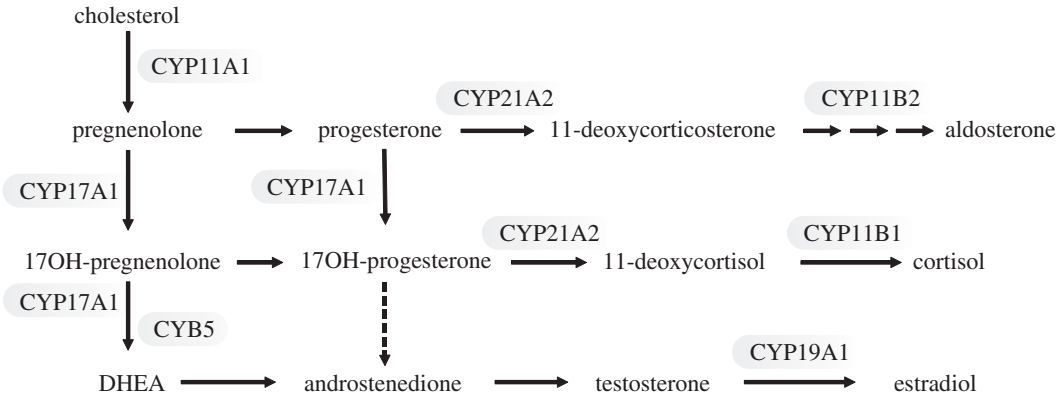


**Figure 4.** Simplified scheme illustrating the P450 enzymes involved in steroidogenesis. CYB5, cytochrome $b_5$; DHEA, dehydroepiandrosterone.

By contrast, P450scc deficiency is rare, probably because P450scc is needed for placental biosynthesis of progesterone, which prevents spontaneous abortion. *Cyp11a1(−/−)* knockout mice, which die early in postnatal life [101], do not model this biology, because the rodent ovary produces the needed progesterone throughout pregnancy, unlike humans. Nevertheless, more than a dozen patients with *CYP11A1* mutations have been described [102–106], including late-onset 'non-classical' forms secondary to mutations that retain partial activity [107–109]. These patients are clinically and hormonally indistinguishable from those with StAR mutations. The physiologic basis of the survival of P450scc-deficient foetuses is unknown—but many have been born prematurely following unsuppressible labour, suggesting that maternal ovarian steroidogenesis may simply persist longer than usual in these pregnancies. Like lipoid CAH, P450scc deficiency is characterized by impaired synthesis of mineralocorticoids, glucocorticoids and sex steroids, causing partial or complete 46,XY sex reversal.

## (b) CYP17A1: 17α-hydroxylation and 17,20-lyase activities of P450c17

P450c17, encoded by *CYP17A1*, principally catalyses steroid 17α-hydroxylation and subsequent 17,20-lyase activity in which 21-carbon 17-hydroxysteroids are cleaved to 19-carbon precursors of sex steroids, i.e. oestrogens and androgens. Thus, P450c17 is essential for reproduction in all mammals. There are substantial species differences in substrate preference for 17,20-lyase activity: human P450c17 principally catalyses this reaction with 17α-hydroxypregnenolone, whereas rodents use 17α-hydroxyprogesterone, and ungulates use both [110]. Human 17,20-lyase activity is stimulated 10-fold by CYB5 (figure 4), acting as an allosteric factor rather than as an electron donor [110]. Human P450c17 is unusual in that it may be serine/threonine-phosphorylated, which substantially augments its 17,20-lyase activity [111,112].

Severe defects in *CYP17A1* eliminate the synthesis of cortisol and sex steroids, while glucocorticoid deficiency is rare because of compensatory production of corticosterone. The typical phenotype is a sexually infantile female with either 46,XX or 46,XY karyotype who has hypertension due to overproduction of deoxycorticosterone; mild *CYP17A1* mutations are associated with genital ambiguity [113]. Many *CYP17A1* mutations have been identified, and Arg362Cys and Try406Arg founder effects make this disorder common in Brazil [114]. Rare mutations can cause isolated 17,20-lyase deficiency, sparing the 17α-hydroxylase activity; most are found in the domain of P450c17 that interacts with POR—especially Arg347His and Arg358Gln [115,116]—although one active-site mutation, Glu305Gly, causes an equivalent phenotype [117].

## (c) CYP21A2: 21-hydroxylation and the most common form of CAH

Microsomal P450c21, encoded by *CYP21A2*, catalyses the 21-hydroxylation of progesterone and 17α-hydroxyprogesterone in biosynthesis of mineralocorticoids and glucocorticoids, respectively. The nature of this reaction has been of great medical interest, because 21-hydroxylase deficiency has an incidence of 1 in 15 000 births, resulting in greater than 90 per cent of all cases of CAH [118]. Decreased cortisol and aldosterone synthesis may result in potentially lethal sodium loss, potassium retention and hypotension. Decreased cortisol synthesis *in utero* leads to overproduction of ACTH and consequent overstimulation of adrenal steroid synthesis, resulting in overproduction of androgens that virilize female foetuses [119]. Although CYP2C9, CYP3A4, CYP2C19 and possibly other enzymes catalyse low levels of progesterone 21-hydroxylation, predominantly in liver [120], there is only one adrenal P450c21.

The *CYP21* gene locus is complex: the functional *CYP21A2* gene and the *CYP21A1P* pseudogene lie on chromosome 6 within the *HLA* locus. The gene and pseudogene are duplicated (*CYP21A* plus *CYP21B* loci), in tandem, along with the *C4A* and *C4B* genes encoding the fourth component of serum complement. Although the *CYP21A* locus is transcribed, the resultant mRNAs do not encode protein; only the *CYP21B* locus codes for functional P450c21. *CYP21A2* and *CYP21A1P* each span approximately 3.4 kb and differ by only 87 or 88 bp; this high degree of sequence similarity indicates that, following duplication, these two genes are evolving in tandem by means of intergenic exchange of DNA. The *CYP21* genes of cattle and mice are also duplicated and linked to leucocyte antigen loci. However, while only the *CYP21B* locus functions in the human, only the *Cyp21a* locus functions in the mouse and both loci function in cattle.

Because the *HLA* locus is highly recombinogenic, exchange between *CYP21A1P* and *CYP21A2* is common. Thus, approximately 75–80 per cent of cases of 21-hydroxylase deficiency are caused by gene conversion events, in which some, or all, of the *CYP21A1P* pseudogene replaces the corresponding area of the *CYP21A2* gene, thus decreasing

expression of the encoded P450c21 protein and/or impairing its activity [98].

Further complexity of the *CYP21* locus includes a pair of genes, *TNXA* and *TNXB*, located on the DNA strand opposite the *C4-CYP21* locus and overlapping the 3′ ends of the *CYP21* genes [121]. The *TNXB* gene encodes tenascin-X, a large extracellular matrix protein that stabilizes collagen fibrils; TNXB deficiency causes a severe form of Ehlers–Danlos syndrome [122].

CAH is among the most common inborn errors of metabolism. Complete absence of P450c21 activity prevents synthesis of aldosterone and cortisol (figure 4). In CAH, low foetal cortisol upregulates ACTH secretion, which stimulates adrenal hyperplasia and transcription of genes for all the steroidogenic enzymes; subsequently, there is accumulation of steroids that are converted to testosterone, resulting in virilization of affected female foetuses. The degree of virilization generally correlates with the severity of the *CYP21A2* mutation [123]. Because normal male foetuses produce abundant testosterone in the testes, the additional testosterone produced in the adrenals with 21-hydroxylase deficiency does not produce a detectable phenotype. There is a broad spectrum of clinical manifestations of 21-hydroxylase deficiency, reflecting a continuous spectrum of enzymatic impairments [119].

Patients with 21-hydroxylase deficiency can have gene deletions, gene conversions and apparent point-mutations. Many mutant *CYP21A2* genes have been sequenced, showing that a relatively small number of mutations normally found in the *CYP21A1P* pseudogene cause most cases of CAH; hence, most apparent point-mutations are actually microconversions, meaning that gene conversions account for approximately 85 per cent of the lesions in 21-hydroxylase deficiency. Most patients with 21-hydroxylase deficiency are compound heterozygotes, i.e. carrying different lesions on their two alleles. Gene deletions and large conversions eliminate all *CYP21A2* transcription; therefore, homozygotes will have salt–losing CAH. Microconversions that create frameshifts or premature translational termination also cause salt–losing CAH. Simple virilizing and non-classical CAH are associated with amino acid replacements in the P450c21 protein caused by microconversion events. Affected patients are usually compound heterozygotes bearing a severely defective allele, plus a mildly impaired allele, so that the clinical manifestations are primarily dependent on the nature of the more active allele.

Two unusual and related features of the 21-hydroxylase locus complicate its analysis. First, the gene deletions in this locus are quite unusual in that they extend approximately 30 kb from one of several points in the middle of *CYP21A1P* to precisely the homologous point in *CYP21A2*. Second, gene conversions are extremely common in this locus. Two types of gene conversions commonly cause 21–hydroxylase deficiency: large gene conversions that can be mistaken for gene deletions, and small microconversions that resemble point mutations. A study of 3200 French patients [124] found an intron-2 mutation in 30 per cent, deletions and large gene conversions in 25 per cent, Ile172Asn in 17 per cent and Gln318Xaa in 7 per cent of alleles causing classic CAH; in non-classic CAH, Val281Leu was found in 55 per cent, intron-2 mutations in 9 per cent, large rearrangements in 8 per cent, Ile172Asn in 4 per cent, and Gln318Xaa in 3 per cent. However, there is an ascertainment bias in favour of the more severely affected patients, and most studies address populations primarily of European origin.

## (d) CYP11B1 and CYP11B2: final steps in glucocorticoid and mineralocorticoid biosynthesis

The final steps in synthesis of glucocorticoids and mineralocorticoids are catalysed by two closely related (93% amino acid sequence identity) mitochondrial enzymes, P450c11β and P450c11AS, encoded by the tandemly duplicated *CYP11B1* and *CYP11B2* genes [125]. There are substantial differences in this enzyme system among various mammals: cattle have a single gene and enzyme that produces both cortisol and aldosterone, whereas rats (but not mice) have three functional *CYP11B* genes [126].

P450c11β is the classical 11β–hydroxylase that converts 11–deoxycortisol to cortisol and is expressed predominantly in the adrenal zona fasciculata; P450c11AS is much less abundant and is confined to the adrenal zona glomerulosa, where it catalyses the three separate reactions needed to convert deoxycorticosterone to aldosterone (figure 4). Mutations in *CYP11B1* cause 11β-hydroxylase deficiency, in which cortisol synthesis is disrupted but aldosterone is still produced; as with 21-hydroxylase deficiency, affected females overproduce androgens during foetal life, causing virilization. Defects in *CYP11B2* cause rare forms of aldosterone deficiency, while retaining the ability to produce cortisol.

Although the genetic anatomy of the two *CYP11B* genes is similar to that of *CYP21A1P* and *CYP21A2*, gene conversions are rare because chromosome 8q24 appears not to experience the unusually high rate of genetic recombination that characterizes 6p21. An unusual gene duplication, caused by unequal crossing over between the *CYP11B1* and *CYP11B2* genes, results in one allele having a third, hybrid *CYP11B* gene in which the ACTH-regulated promoter and the first few exons of *CYP11B1* are fused in frame to most of the exons of *CYP11B2*. This results in abundant expression of a chimeric protein with aldosterone synthase activity, causing a glucocorticoid-suppressible form of hyperaldosteronism [127,128], which may account for as much as 2 per cent of patients having hypertension.

The amino acid sequences of P450c11β and P450c11AS differ by only 37 residues, of which 24 are conservative replacements [129]. Multiple studies have located residues that distinguish the activities of these two enzymes. Residues 288, 296, 301, 302, 320 and 325 are critical [130,131]; these residues lie in or near the I-helix, and are expected to alter active-site geometry. Studies *in vitro* suggest that changing Ser288 and Val320 would be sufficient to distinguish between the two enzyme activities [130].

## (e) CYP19A1: aromatization of androgens to oestrogens

Oestrogens are produced via aromatization of androgens (figure 4) by a complex series of reactions catalysed by the microsomal aromatase, P450aro [132], encoded by *CYP19A1*. This gene spans greater than 75 kb and uses several different promoter sequences, transcriptional start-sites and alternative first-exons to transcribe aromatase mRNA in varying tissues under different hormonal regulation. CYP19A1 is expressed in steroidogenic tissues (ovarian granulosa cells, placenta), in brain and in non-steroidogenic tissues—especially fat and bone. The oxidative demethylation of androgens consumes three equivalents of molecular $O_2$ and

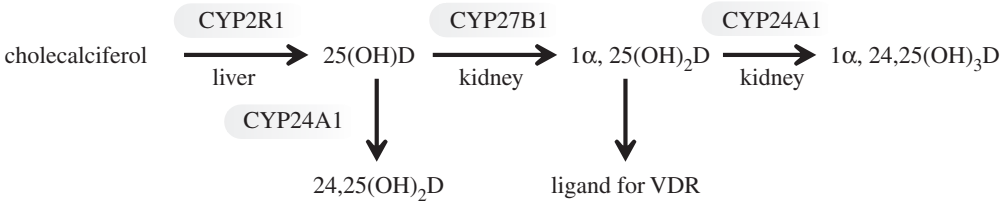


**Figure 5.** Diagram showing the fundamental steps and P450 enzymes involved in vitamin D biosynthesis and degradation pathway. Cholecalciferol ($D_3$) is converted via two steps to make the potent active ligand for vitamin D receptor (VDR). CYP24A1 participates in degradation (at two possible steps), the *CYP24A1* gene being activated when $Ca^{2+}$ levels are sufficient and thus less VDR action is needed.

NADPH, sequentially donated by three molecules of POR. Studies of patients with aromatase deficiency have shown that biologically significant oestrogen synthesis is derived entirely from P450aro and that foeto-placental oestrogen is not needed for normal foetal development [133].

Extra-glandular P450aro expression, especially in fat, also converts androgens to oestrogens. Aromatase in the epiphyses of growing bone converts testosterone to oestradiol, which fuses the epiphyses and terminates growth; tall stature, delayed epiphyseal maturation and osteopenia in males with aromatase deficiency, and their rapid reversal with oestrogen replacement therapy, demonstrate that oestrogen, not androgen, is responsible for epiphyseal closure in males. Patients with P450aro deficiency grow normally during childhood, but continue to grow after puberty; when treated with oestrogens, however, aromatase-deficient subjects fuse their epiphyses and cease linear growth.

A great deal of attention has focused recently on the class of environmental contaminants termed 'endocrine-disrupting chemicals' (EDCs). The most common mechanism for EDC disorders is that various chemicals bind directly to one or both oestrogen receptors. However, perturbation of P450aro expression and function by EDCs also can modify the rate of oestrogen production—disturbing local and systemic levels of oestrogens; these effects can cause alterations in sexual differentiation, behaviour and regulation of the reproductive cycle not only in the human but also other mammals, reptiles, amphibians and fish [134]. Genes encoding StAR and CYP17A1 are also known to be targets of EDCs [135].

## 6. Vitamin D synthesis and metabolism

Vitamin D is essential for mammalian calcium metabolism. In cholesterol biosynthesis, the final step is conversion of 7-dehydrocholesterol to cholesterol by 7-hydroxycholesterol reductase; alternatively, 7-dehydrocholesterol may be converted to cholecalciferol (vitamin $D_3$) in human skin by the action of ultraviolet radiation at 270–290 nm—which directly cleaves the 9–10 carbon–carbon bond of the B ring [136]. Plants produce a closely related, biologically nearly equivalent sterol, ergocalciferol (vitamin $D_2$). Both forms of vitamin D are pro-hormones which are then activated by 25- and 1α-hydroxylations and subsequently inactivated by 24-hydroxylation by a series of P450 enzymes. The active ligand for the VDR is the 1α,25-dihydroxy metabolite (figure 5). Nutritional vitamin D deficiency (rickets) is common, whereas genetic disorders in vitamin D biosynthesis are rare.

### (a) Vitamin D 25-hydroxylases

Vitamin D is rapidly 25-hydroxylated in liver by one or more enzymes that are not physiologically regulated; thus, circulating concentrations of 25(OH)D are primarily determined by dietary intake of vitamin D and exposure to sunlight. The principal 25-hydroxylase appears to be CYP2R1, which has a high affinity for vitamin D [137]. A homozygous *CYP2R1* mutation was identified in one family as an autosomal recessive trait responsible for vitamin D deficiency, low levels of circulating 25(OH)D and negligible 25-hydroxylase activity [138].

Vitamin D 25-hydroxylation can also be catalysed by CYP27A1 (figure 5); however, patients with *CYP27A1* mutations suffer from cerebrotendinous xanthomatosis without a disorder in calcium metabolism [89]. No other genetically proved cases of 25-hydroxylase deficiency have been reported, suggesting that both CYP2R1 and another enzyme (possibly CYP27A1), are the most effective 25-hydroxylases *in vivo*, and symptomatic disease is only seen when there appears to be a *CYP2R1* mutation in the presence of another stressor.

### (b) Vitamin D 1α-hydroxylase

The active hormonal form of vitamin D is 1α,25-dihydroxy vitamin D (1,25(OH)$_2$D), which is produced via 1α-hydroxylation of 25(OH)D by kidney mitochondrial CYP27B1; this reaction is rate-limiting and tightly regulated. The exquisitely low abundance of this highly active enzyme frustrated efforts to clone the gene until 1997 [139–143]. Vitamin D 1α-hydroxylase deficiency is a genetic form of rickets characterized by infantile hypocalcaemia. Patients display a variety of *CYP27B1* mutations, with very low serum concentrations of 1,25(OH)$_2$D despite normal concentrations of 25(OH)D, and patients respond to physiologic replacement doses of 1,25(OH)$_2$D [144]. More than 100 genetically confirmed cases and at least 38 different *CYP27B1* mutations have been reported, with a broad range of severity in enzymatic activity [144–147]. Intriguingly, a recent report shows a strong association between heterozygosity for *CYP27B1* mutations and increased risk of multiple sclerosis [148].

### (c) Vitamin D 24-hydroxylase

Calcitriol can be inactivated by CYP3A4, but the most important mechanism of inactivation is via mitochondrial P450c24, encoded by *CYP24A1*; evolutionarily not too distant from *CYP27A1*. CYP24A1 deficiency is a recently described cause of severe infantile neonatal hypercalcaemia [149,150].

## 7. Metabolism of retinoic acid and other putative morphogens

Retinoic acid (RA) is a vitamin A-derived morphogen important for axial patterning and organ formation in developing vertebrates, as well as invertebrate chordates.

Retinoids are regulators of cell differentiation, cell proliferation and morphogenesis.

### (a) *CYP26* gene family

All three members of the *CYP26* gene family—*CYP26A1*, *CYP26B1* and *CYP26C1*—are involved in RA synthesis and degradation. Normal or deficient expression of one or another of these genes would probably be associated with alterations in spatial distribution and abnormal RA concentrations at critical times during embryonic and foetal development [151]. CYP2S1 is another enzyme that has been recently shown to be involved in retinoid metabolism [152].

RA is required for induction of several *Hox* genes involved in hindbrain and spinal cord patterning, as neuroectoderm emerges from the primitive streak. RA is also required to ensure bilaterally symmetrical generation of left versus right somites, as the pre-somitic mesoderm emerges from the primitive streak [153]. Studies have identified functional RA response elements within flanking sequences of some of the most 3′-ward *Hox* homeobox genes [154], suggesting a direct interaction between *Hox* genes and RA.

*Cyp26a1(−/−)* knockout mice exhibit anomalies that include caudal agenesis, similar to those induced by administering excess RA; in the tail bud there is downregulation of the *T* (*brachyury*) and *Wnt3a* genes—which may be the cause of the caudal truncation [155]. *Tbx1(−/−)* mice, having the T-box 1 gene ablated, result in downregulation of the *Cyp26a1*, *Cyp26b1* and *Cyp26c1* genes; *Tbx1(−/−)* knockout mice show a loss of the caudal pulmonary artery and pharyngeal arch arteries, small otic vesicles, loss of head mesenchyme and, at later stages, DiGeorge syndrome-like heart defects, including common arterial trunk and perimembranous ventricular septal defects [156]. In fact, *Por(−/−)* knockout mice having the P450 oxidoreductase gene ablated are embryolethal with elevated RA levels and show defects in vasculogenesis, brain and limb patterning linked to RA homeostasis [157]; *Por(−/−)* mice bear a striking resemblance to *Cyp26a1(−/−)* [155] and *Cyp26b1(−/−)* [158] knockout mice.

Although knockout of the mouse *Cyp26b1* gene causes embryonic lethality, hypomorphic missense mutations of human *CYP26B1* cause a severe skeletal dysplasia that resembles ABS. In one family, CYP26B1 Arg363Leu homozygosity was embryonic lethal and was devoid of virtually all activity *in vitro*; in a second family, CYP26B1 Ser146Pro homozygosity caused an Antley–Bixler skeletal phenotype. Experiments in *Cyp26b1* hypomorphic mice and zebrafish showed CYP26B1 expression in regions of joint formation, and that CYP26B1 deficiency or retinoic acid excess causes increased endochondral bone formation at these sites. These data suggest the hypofunction of this POR-dependent retinoic acid-degrading enzyme is responsible for the skeletal disorder in severe POR deficiency [159].

During embryogenesis, the abundance of RA is determined by the balance between RA synthesis by retinaldehyde dehydrogenases (ALDH1A1, 1A2 and 1A3) and RA degradation by the CYP26 enzymes [160]; pregastrulation mouse embryos express the CYP26 but not the ALDH1 enzymes. Mice lacking all three *Cyp26* genes show a duplication of the body axis [161] as the result of elevated RA levels.

### (b) *CYP1B* gene subfamily

Mutations in the *CYP1B1* gene represent a major cause of primary congenital glaucoma [162]; this report was the first example in which defects in P450 metabolism are realized to be attributable to a primary developmental disorder. Since that first discovery, close to 20 *CYP1B1* mutations have clinically been associated with glaucoma. *Cyp1b1/Tyr(−/−)* double-knockout mice exhibit defects in the ocular drainage structure and trabecular meshwork, which are similar to those reported in human primary glaucoma patients [163]. These data are consistent with the CYP1B1 enzyme performing a critical function in the anterior chamber of the eye during embryogenesis; absence of CYP1B1 perturbs normal differentiation. Interestingly, apparently there is no redundant enzyme that can replace CYP1B1 in that embryonic space during that developmental window. Because eicosanoids are in all likelihood to be involved in this process (cf. table 3), we suggest the unknown substrate of CYP1B1 is probably one of greater than 150 eicosanoids being synthesized or degraded.

## 8. Cytochromes P450 still having unknown functions

There are still about eight so-called ‘orphan’ cytochromes P450 that currently have not been unequivocally assigned a biological function: e.g. CYP2A7, CYP4A22, CYP4F11, CYP4F22, CYP4V2, CYP4X1, CYP20A1 and CYP27C1 (table 2). CYP4X1, however, has been suggested to be involved in anandamide signalling in brain [164].

*CYP20A1* is fascinating because this gene is highly conserved from genomes of the sea anemone and sponge to the human; therefore, CYP20 is one of the original 11 P450 clans (cf. Nelson *et al*. [165]). CYP20A1 mRNA expression is present in human liver, as well as the nasopharynx [166] and CNS regions such as substantia nigra, hippocampus and amygdala [167]. CYP20A1 mRNA expression has also been found in rat CNS, heart and liver. The endogenous metabolic function of P450 20A1 remains unknown.

Although CYP27A1 and CYP27B1 have well known distinct functions, the third member of this family, CYP27C1, remains a mystery. Using array comparative-genomic hybridization and qRT-PCR methods in blood samples from 18 patients having avascular necrosis of the femoral head compared with controls, *CYP27C1* was among the top three most statistically significant, most highly expressed genes [168]. The physiological as well as metabolic functions of the CYP27C1 enzyme, however, remain unknown.

## 9. Conclusions

P450 genes are pivotal in many critical life processes and, as a consequence, defects in these genes can lead to various severe diseases. There is a gradient of phenotypic responses that occur when both copies of a *CYP* gene carry missense mutations or are completely missing: (a) embryolethality (e.g. *CYP51A1*, *CYP26A1*); (b) serious disease in the neonatal period (e.g. *CYP7B1*, *CYP21A2*); (c) congenital defect (e.g. *CYP1B1*, *CYP4V2*); (d) classical inborn errors of metabolism (e.g. *CYP2R1*, *CYP4F22*, *CYP5A1*, *CYP27A1*, *CYP27B1*); (e) suggestive associations with particular complex diseases such as altered behaviour (e.g. *CYP2D6*), hypertension (e.g. *CYP4A11*, *CYP8A1*), age-related macular degeneration (e.g. *CYP46A1*); and (f) no overt phenotype presumably due to redundancy (e.g. *CYP1A2*, *CYP2A6*, *CYP2B6*, *CYP2E1*,

*CYP3A4*, *CYP4F11*). This last category involves most of the P450 genes that metabolize endogenous eicosanoids as well as drugs and other environmental chemicals. The first five of the six above-mentioned categories are probably commonly observed for many other genes among various gene families.

## Note added in proof

It should be noted that there exist other opinions as to 'how many of the remaining P450 enzymes still have no established substrate/function'. Guengerich and Cheng have recently suggested that—in addition to the eight we have listed herein—five others should be considered as orphan P450s: CYP2S1, CYP2U1, CYP2W1, CYP3A43 and CYP4Z1 [169]. We would propose, however, that all five of these most likely metabolize one or another of the more than 150 eicosanoids.

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## Glossary

| | |
|---|---|
| $1,25(OH)_2D$ | 1$\alpha$,25-dihydroxy vitamin D |
| 25(OH)D | 25-hydroxy vitamin D |
| ABS | Antley–Bixler syndrome |
| ALDH1A1, 1A2 and 1A3 | retinaldehyde dehydrogenases |
| ALOXs | arachidonate lipoxygenases |
| CAH | congenital adrenal hyperplasia |
| CNS | central nervous system |
| CTX | cerebrotendinous xanthomatosis |
| CYB5 | cytochrome $b_5$ |
| CYP | cytochrome P450 (*CYP*, human genes; *Cyp*, mouse genes) |
| DHETEs | dihydroxyeicosatrienoic acids |
| EDCs | endocrine-disrupting chemicals |
| EETs | epoxyeicosatrienoic acids |
| ER | endoplasmic reticulum |
| FAD | flavin adenine dinucleotide |
| FDX | ferrodoxin |
| FDXR | ferrodoxin reductase |
| FMN | flavin mononucleotide |
| FXR | farnesoid X receptor |
| GD | gestational day |
| HETEs | hydroxyeicosatetraenoic acids |
| HMG-CoA | hydroxymethylglutaryl coenzyme A |
| HPETEs | hydroperoxyeicosatetraenoic acids |

| | |
|---|---|
| HSP | hereditary spastic periplegia |
| LXR | liver X receptor |
| PDGF | platelet–derived growth factor |
| PG | prostaglandin ($I_2$, $E_2$, $D_2$, $F_{2\alpha}$) |
| $PGI_2$ | prostacyclin |
| PK | pharmacokinetic |
| POR | P450 oxidoreductase (*Por*, mouse gene encoding POR) |
| *PTGIS* gene (human) | encodes prostacyclin synthase (also called CYP8A1) |
| PTGS1 and 2 | cyclooxygenase-1 and -2 (COX1, COX2) |
| PXR | prostane X receptor |
| RA | retinoic acid |
| SNPs | single-nucleotide polymorphisms |
| StAR | steroidogenic acute regulatory protein |
| *T* gene (mouse) | encodes brachyury transcription factor |
| *Tbx1* gene (mouse) | encodes T-box 1 transcription factor |
| $TBXA_2$ | thromboxane $A_2$ |
| *TBXAS1* gene (human) | encodes thromboxane synthase (also called CYP5A1) |
| *Tyr* gene (mouse) | encodes tyrosinase |
| UM | ultra-metabolizer phenotype |
| VDR | vitamin D receptor |
| *Wnt3a* gene (*Drosophila* homologue in mouse) | encodes Wingless-Int proteins involved in determination of left-right and dorsal-ventral axes |