

Cytochrome P450 Part 1: Multiplicity and Function

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ABSTRACT

While new cytochrome P450 (CYP450) enzymes continue to be identified, it is now possible to predict with some confidence the total number of human CYP450 enzymes. This review is an update of the CYP450 superfamily of drug metabolising enzymes. It comprises a brief history of CYP450 research, outlines the standard P450 nomenclature system, and describes CYP450 multiplicity, structure and function.

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INTRODUCTION

Cytochrome P450 (CYP450) is the generic name given to a large family of versatile enzymes that metabolise most drugs and a myriad of chemicals of toxicological importance (termed xenobiotic metabolism). Along with xenobiotic metabolism, many CYP450 enzymes play pivotal roles in diverse physiological processes including steroid and cholesterol biosynthesis, fatty acid metabolism (prostacyclin, thromboxane) and the maintenance of calcium homeostasis. Mutations in *CYP450* genes or deficiencies in CYP450 enzymes are clinically relevant in many instances, with the consequences dependent on the function of the compromised enzyme.

Advances in recombinant DNA technology have provided extensive insights into the multiplicity and function of CYP450 enzymes from many different species including humans. While new CYP450 enzymes continue to be identified, it is now possible to predict with some confidence the total number of human CYP450 enzymes.

This review comprises a brief history of the CYP450 enzymes, outlines the standard P450 nomenclature system, and describes CYP450 multiplicity, structure and function. Subsequent articles in this series will focus on the pharmacogenetics of these cytochromes and clinically important drug interactions involving CYP450 enzymes.

HISTORY

The term 'cytochrome P450' was coined in 1962 as a temporary name for a coloured substance in the cell.¹ This pigment, when reduced and bound with carbon monoxide, produced an unusual absorption peak at a wavelength of 450 nm. Cytochrome is a misnomer given that the CYP450s are enzymes rather than true cytochromes. Despite this, the name 'cytochrome P450' has stuck and is so widely accepted that any change would be impractical.

At first, CYP450 was believed to represent a single enzyme. Today it seems likely that humans and other mammals have approximately 50 distinct CYP450 enzymes. The total number may be higher in plants, possibly as high as several hundred. In the last 15 years of the 20th century, research was

largely concerned with defining CYP450 multiplicity in humans and a diverse range of other organisms. In recent years, with CYP450 multiplicity largely covered, CYP450 functional and structural studies have taken precedence.

NOMENCLATURE AND MULTIPLICITY

Initial evidence for CYP450 multiplicity came via enzyme purification but recombinant DNA technology enabled the recognition of the considerable enzyme multiplicity within the CYP450 system. As individual CYP450s were identified in various laboratories, several diverse CYP450 nomenclature systems emerged based on their molecular weights or preferences for substrates. The resultant plethora of names and accompanying confusion prompted prominent workers in the field to devise a standard nomenclature for the *CYP450* gene family based on amino acid sequence comparisons and the evolutionary relationships of the corresponding genes.²

First proposed in 1987, the nomenclature was devised with the premise that it would be updated as frequently as the identification of new CYP450 enzymes necessitated. Several updates of the *CYP450* gene superfamily have been published.³⁻⁶ In addition, an official web site has been established, based at the University of Memphis, to provide up-to-date information concerning CYP450 multiplicity in all species.⁷

Nomenclature

Recommendations for naming a CYP450 gene include:

- the root symbol *CYP* for cytochrome P450;
- an Arabic number for the CYP450 family;
- a letter for the subfamily; and
- an Arabic numeral for the individual gene.

When describing a *CYP450* gene, all letters and numerals are written in italics. The same nomenclature is recommended for the enzyme but it is written in nonitalicised form. Thus, the *CYP2D6* gene encodes the CYP2D6 enzyme.

Although the distinctions between CYP450 families and subfamilies are arbitrary, it is estimated that CYP450 families diverged from one another more than 1.2 billion years ago, so any enzyme in one CYP450 family is less than 40% similar, at the amino acid level, to a CYP450 from another family (e.g. CYP2D6 and CYP3A4 are less than 40% similar, CYP2D6 and CYP2C19 are more than 40% similar).²

The subfamilies are estimated to have diverged from one another approximately 400 million years ago. Any two CYP450 enzymes within a single mammalian CYP450 subfamily generally have more than 55% amino acid similarity (e.g. CYP2C9 and CYP2C19 are more than 55% similar).²

It must be stressed that the nomenclature system is based on evolutionary relationships between CYP450 enzymes and not on similarity in substrate profiles. Indeed some CYP450 enzymes from within a single family display markedly different substrate profiles and/or physiological function.

Nomenclature systems based on the substrate profiles of individual CYP450s are of little use given the overlapping substrate profiles of CYP450s and the capacity of multiple CYP450s to modify a single drug at different sites. The success of the CYP450 nomenclature based on evolutionary

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relationships has prompted the adoption of similar systems for other enzyme families involved in drug metabolism including the glucuronosyltransferases, flavin-containing monooxygenases and N-acetyltransferases.⁸⁻¹⁰

Multiplicity

CYP450 genes have been identified in animals, plants, yeast and bacteria. All *CYP450* genes are believed to be derived from a single ancestral gene which existed more than 2 billion years ago. The function of this 'original CYP450' is thought to have involved energy utilisation.

More than 6000 individual *CYP450* genes have been identified in all of the species studied. In humans, 18 families, 44 subfamilies, and 57 functional *CYP450* genes have been identified (Table 1).⁷ Only scant information is available regarding several of these enzymes, particularly about the function of the encoded enzyme.⁷

Due to recent advances in DNA sequencing technology and the extensive database of human DNA sequences resulting from the Human Genome Project, it is unlikely that many human *CYP450* enzymes remain to be identified.

FUNCTION

Functionally, *CYP450* enzymes may be broadly divided into three groups, those:

- involved in the metabolism of drugs and other foreign chemicals;
- functioning during steroidogenesis; and
- participating in other important endogenous functions.

This review will focus on the xenobiotic metabolism function of *CYP450* enzymes as this is clinically relevant to pharmacists. Although there are a number of enzyme families that are involved in xenobiotic metabolism, in general, the *CYP450* enzymes are the most important.

The overall effect of the chemical modifications associated with xenobiotic metabolism is to increase water solubility and/or size which facilitate urinary excretion by decreasing the ability of the chemical to be reabsorbed in the kidneys. The chemical modifications can also alter the chemical's pharmacological and/or toxicological effects. Commonly the pharmacological/toxicological effects are reduced, but there are a number of important cases in which the pharmacological or toxicological effect is increased.

Codeine is an example of a prodrug that is activated by the *CYP2D6* enzyme. In this case, the drug (codeine) administered has minimal pharmacological activity, and the metabolite (morphine) is responsible for the majority of the therapeutic effect. In the pharmacogenetics paper of this series, codeine will also be used as an example of the influence of genetic variability in *CYP450* enzymes on therapeutic effect. Depending on the activity of the *CYP2D6* enzyme in an individual, variable amounts of morphine will be produced and hence variable analgesia.

Paracetamol is an example of a drug that may be metabolised by a *CYP450* enzyme into a reactive metabolite. Under normal circumstances the reactive metabolite is quickly neutralised by a subsequent conjugation reaction. In overdose, however, the conjugation reaction may be insufficient leading to accumulation of the reactive metabolite and subsequent hepatotoxicity. Paracetamol is also a good example of competing pathways in drug metabolism and the problems that can ensue should the balance between the metabolism pathways be disturbed. There are a number of drugs that increase the activity of the *CYP450* enzymes involved in paracetamol metabolism. In a similar manner they may upset the balance between production and neutralisation of the reactive metabolite, thereby increasing the risk of hepatotoxicity.

Table 1. Human cytochrome P450 genes⁷

Human P450 families	Functional members	Main functions
CYP1 (3 subfamilies)	1A1, 1A2, 1B1	Drug/xenobiotic metabolism
CYP2 (13 subfamilies)	2A6, 2A7, 2A13, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 2F1, 2J2, 2R1, 2S1, 2U1, 2W1	Drug/xenobiotic and steroid metabolism
CYP3 (1 subfamily)	3A4, 3A5, 3A7, 3A43	Drug/xenobiotic metabolism
CYP4 (6 subfamilies)	4A11, 4A22, 4B1, 4F2, 4F3, 4F8, 4F11, 4F12, 4F22, 4V2, 4X1, 4Z1	Arachadonic acid and fatty acid metabolism
CYP5 (1 subfamily)	5A1	Thromboxane A ₂ synthesis
CYP7 (2 subfamilies)	7A1, 7B1	Rate-limiting step of bile acid biosynthesis (cholesterol elimination)
CYP8 (2 subfamilies)	8A1, 8B1	Prostacyclin and bile acid biosynthesis
CYP11 (2 subfamilies)	11A1, 11B1, 11B2	Key steps in steroid biosynthesis
CYP17 (1 subfamily)	17A1	Testosterone and oestrogen biosynthesis
CYP19 (1 subfamily)	19A1	Oestrogen biosynthesis (aromatase)
CYP20 (1 subfamily)	20A1	Unknown
CYP21 (1 subfamily)	21A2	Steroid biosynthesis
CYP24 (1 subfamily)	24A1	Vitamin D metabolism/inactivation
CYP26 (3 subfamilies)	26A1, 26B1, 26C1	Retinoic acid metabolism/inactivation
CYP27 (3 subfamilies)	27A1, 27B1, 27C1	Bile acid biosynthesis, vitamin D activation
CYP39 (1 subfamily)	39A1	Cholesterol metabolism
CYP46 (1 subfamily)	46A1	Cholesterol metabolism
CYP51 (1 subfamily)	51A1	Cholesterol biosynthesis

In addition to these functional human P450 genes, 58 pseudogenes have also been identified.⁷

Table 2. Major drug-metabolising cytochrome P450 enzymes (adapted from reference 11)

Human CYP450	Selected substrates	Drug classes*	Comments
CYP1A2	amitriptyline, caffeine, clozapine, imipramine, paracetamol, theophylline		Induced by cigarette smoking, dioxins, polycyclic aromatic hydrocarbons (e.g. overcooked meat), omeprazole
CYP2C9	diclofenac, naproxen, phenytoin, piroxicam, warfarin	Non-steroidal anti-inflammatory drugs	Genetically variable
CYP2C19	diazepam, omeprazole, propranolol	Proton pump inhibitors	Genetically variable
CYP2D6	amitriptyline, captopril, chlorpromazine, clomipramine, codeine, dextromethorphan, flecainide, fluoxetine, imipramine, metoprolol, paroxetine, perhexilene, thioridazine, venlafaxine	Beta-blockers	Genetically variable
CYP2E1	halothane, paracetamol		Induced by alcohol
CYP3A4	alprazolam, amiodarone, amitriptyline, carbamazepine, cisapride, clarithromycin, dexamethasone, erythromycin, ethinyl oestradiol, ketoconazole, midazolam, nifedipine, taxol, verapamil, warfarin	Statins, calcium channel blockers, immune modulators, macrolides, protease inhibitors, benzodiazepines	Induced by carbamazepine, phenytoin, rifampicin, steroids. Inhibited by grapefruit juice. Environment is the major source of interindividual variation in enzyme levels rather than genetic variation.

*A number of drugs in these drug classes are metabolised by the CYP450 isoform.

Predominant CYP450 Enzymes

Despite the large number of human CYP450 enzymes, the bulk of drug metabolism is catalysed by a relatively small number of enzymes found in families 1, 2 and 3. The six predominant drug-metabolising enzymes are CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 (Table 2). They are found predominantly in the liver, although other tissues may also be important for specific enzymes, such as CYP3A4 in the small intestine. Studies on genetic variability in drug metabolism and clinically relevant drug interactions, necessarily focus on these six drug-metabolising enzymes. Other CYP450 enzymes in families 1 through 4 may be important in the metabolism of individual drugs but their overall contribution to drug metabolism is generally considered minor.

These major xenobiotic metabolising enzymes have a number of interesting properties. A single CYP450 enzyme can often metabolise many different drugs with diverse structures. This is distinct from most other enzymes that metabolise a small number of chemicals of a very similar molecular structure. Multiple CYP450 enzymes may metabolise a single drug (e.g. amitriptyline). The different CYP450 isoforms may metabolise the drug at different sites or at the same site. The relative rates of metabolism by each CYP450 enzyme will determine which enzyme is of greatest clinical relevance in terms of drug clearance. In some cases, multiple CYP450 enzymes will metabolise the drug at a similar rate and hence multiple CYP450 enzymes will be quantitatively important in the drug's metabolism. In other cases, a single CYP450 enzyme will metabolise the drug at a much faster rate than the others and hence it will be the enzyme of clinical importance. It should be noted, that CYP450 enzymes that metabolise a drug relatively slowly can still be clinically significant if the metabolite is toxic.

FUTURE DIRECTIONS

Since the human CYP450 complement is essentially defined, future work on the xenobiotic metabolising CYP450 enzymes is likely to focus on the structural basis of individual CYP450 function and the clinical significance of inter-individual variability in CYP450-mediated metabolism.

From a drug discovery and development perspective, research is focused on the development of methods for fast and accurate prediction of CYP450 properties of drug candidates. The aim is to ensure that drug candidates with potential drug metabolism problems are discovered and dealt

with as early as possible in the drug development process thereby saving significant time and money. Technologies involved include structure-activity relationships, high-resolution 3D structures of the CYP450 protein structures and advanced pharmacokinetic models for *in vitro* to *in vivo* correlation.

From a clinical perspective the focus is on understanding and predicting inter-individual variability in the therapeutic and toxic effects of drugs. The utilisation of such predictions will improve the quality use of medicines by 'personalising' drug choice and dose in order to maximise therapeutic effect and minimise toxic effects. CYP450 enzymes are a common cause of inter-individual drug variability and the subsequent articles in this series, will further explore the intrinsic and extrinsic factors that are most important for a pharmacist to understand.

Competing interests: None declared

References

- Omura T, Sato R. A new cytochrome in liver microsomes. *J Biol Chem* 1962; 237: 1375-6.
- Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, et al. The P450 gene superfamily: recommended nomenclature. *DNA* 1987; 6: 1-11.
- Nebert DW, Nelson DR, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, et al. The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* 1989; 8: 1-13.
- Nebert DW, Nelson DR, Coon MJ, Estabrook RW, Feyereisen R, Fujii-Kuriyama Y, et al. The P450 superfamily: update on new sequences, gene mapping and recommended nomenclature. *DNA Cell Biol* 1991; 10: 1-14.
- Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, et al. The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol* 1993; 12: 1-51.
- Nelson DR, Koymans LO, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, et al. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 1996; 6: 1-42.
- Cytochrome P450 Standardised Nomenclature Committee. Available from <drnelson.utmem.edu/CytochromeP450.html>.
- Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Belanger A, et al. The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics* 1997; 7: 255-69.
- Lawton MP, Cashman JR, Cresteil T, Dolphin CT, Elfarra AA, Hines RN, et al. A nomenclature for the mammalian flavin-containing monooxygenase gene family based on amino acid sequence identities. *Arch Biochem Biophys* 1994; 308: 254-7.
- Vatsis KP, Weber WW, Bell DA, Dupret JM, Evans DA, Grant DM, et al. Nomenclature for N-acetyltransferases. *Pharmacogenetics* 1995; 5: 1-17.
- McKinnon RA, Wiese M. Factors influencing drug metabolism. In: Sansom LN, editor. Australian pharmaceutical formulary. 16th ed. Canberra: Pharmaceutical Society of Australia; 1997. p. 125-30.

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