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REVIEW

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Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms

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Abstract The cytochrome P450s are responsible for about 75% of phase I dependent drug metabolism and for the metabolism of a huge amount of dietary constituents and endogenous chemicals. The human has 59 active genes, and 6 of those encode important drug metabolising enzymes. About 40% of cytochrome P450 dependent drug metabolism is catalysed by polymorphic enzymes and such drug P450 interactions are frequently seen in adverse drug reaction reports. In this contribution an update of human cytochrome P450 enzymology and pharmacogenetics is given with particular emphasis on CYP1B1, CYP2B6, CYP2E1 and CYP3As.

Keywords Poor metabolizers · Adverse drug reactions · Genetic polymorphism · Haplotypes · Cancer drugs · Ultrarapid metabolizers

Introduction

The cytochromes P450 are responsible for the metabolism of endogenous as well as exogenous compounds. The human P450 forms can be divided into three major groups:

1. Those in CYP families 5–51 of endogenous importance having usually high affinity for the substrates and being relatively well conserved during evolution
2. Those in CYP families 1–3, with usually less affinity for their substrates, being less conserved evolutionary and which exhibit important genetic polymorphisms and those in family 4 with roles in both fatty acid and related substrate metabolism and metabolism of some xenobiotics

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The cytochromes P450 in families 1–3 are responsible for 70–80% of all phase I dependent metabolism of clinically used drugs (Bertz and Granneman 1997; Evans and Relling 1999) and participate in the metabolism of a huge number of xenobiotic chemicals. This can lead to the metabolic activation of pre-carcinogens and drugs, which might exert toxic or carcinogenic effects. Indeed adverse drug reactions (ADRs) are more of a problem in drug treatment and drug development than previously thought. It has been estimated that adverse drug reactions cost the US society about US$ 100 billion, that they cause more than 100,000 deaths annually in the US, up to 7% of all hospital admissions in the UK and 13% of all admissions to internal medicine clinics in Sweden (see Ingelman-Sundberg 2001 for references). A recent analysis revealed that 59% of drugs cited in ADR-studies are metabolised by polymorphic phase 1 enzymes and that P450s account for 86% of those. By contrast, only 20% of drugs that were substrates for non-polymorphic enzymes were in the ADR reports (Philipps et al. 2002).

Knowledge about the cytochrome P450 system, taking a central place in the cause for ADRs by constituting an important factor for interindividual differences in drug clearance due to genetic polymorphism and basis for drug interactions, is fundamental both for drug therapy and for drug development. Furthermore, by knowing the P450 genetics and biology, a lot can be understood with respect to basis for metabolic activation of carcinogens and the relative risk for toxic or carcinogenic effects of other xenobiotics. This is true for the difference between ethnic groups and individuals with respect to exposure to carcinogens in relation to:

1. Dietary habits
2. Environmental exposure
3. Expression of particular isoforms of cytochrome P450

In this contribution an overview about the P450s participating in xenobiotics metabolism is given with focus on those like CYP1B1, CYP2B6, CYP2E1, and CYP3As that are not covered in other contributions to this series.

Cytochromes P450

The nomenclature for P450 s following the recommendations given (Nelson et al. 1996; http://drnelson.utmem.edu/CytochromeP450.html) is based on naming cytochromes P450 with CYP followed by a number indicating the gene family (>40% identity on amino acid sequence level is required for being in the same family), a letter indicating the subfamily (>55% amino acid sequence identity) and a num-

Table 1 Human cytochrome P450 genes and their polymorphisms. The table is an update of the human P450 genes identified in July 2002 and the data regarding the gene names and localisation are from (http://drnelson.utmem.edu/CytochromeP450.html). PG denotes pseudogene (in italic) and MP denotes that missense mutations have been described. Those marked *MP* in italic are present on the Human CYP allele nomenclature Committee web page (http://www.imm.ki.se/CYPalleles/). *nd* not described

CYP gene	Chromosome	Polymorphism missense (MP)/ pseudogene (PG)	In vivo importance of the polymorphism[a]	CYP gene	Chromosome	Polymorphism missense MP/ pseudogene PG	In vivo importance of the polymorphism[a]
1A1	15	*MP*		*4F3*	19	nd	
1A2	15	*MP*	Yes	*4F8*	19	nd	
1B1	2	*MP*	Perhaps	*4F9P*	*19*	*PG*	
1P	9	PG		*4F10P*	*19*	*PG*	
2A6	19	*MP*	Yes	*4F11*	19	nd	
2A7	19	PG?		*4F12*	19	nd	
2A7PT	*19*	*PG*		*4F22*	19	nd	
2A7PC	*19*	*PG*		*4F23P*	*19*	*PG*	
2A13	19	nd		*4F24P*	*19*	*PG*	
2A18P	*19*	*PG*		*4F25P*	*15*	*PG*	
2B6	19	*MP*	Perhaps	*4F26P*	*9*	*PG*	
2B7P1	*19*	*PG*		*4F27P*	*2*	*PG*	
2B7P2	*19*	*PG*		*4F28*	21	nd	
2B7P3	*19*	*PG*		*4V2*	4	nd	
2C8	10	*MP*	Yes	*4X1*	1	nd	
2C9	10	*MP*	Yes	*5A1*	7	*MP*	Yes
2C18	10	*MP*		*7A1*	8	nd	
2C19	10	*MP*	Yes	*7B1*	8	nd	
2CP	10	PG		*8A1*	20	*MP*	Yes
2D6	22	*MP*	Yes	*8B1*	3	nd	
2D7P	*22*	*PG*		*11A1*	8?	nd	
2D8P	*22*	*PG*		*11B1*	8	nd	
2E1	10	*MP*		*11B2*		nd	
2F1	19	MP?		*17*	10	MP	Yes
2F1P	*19*	*PG*		*19*	15	MP	Perhaps
2G1P	*19*	*PG*		*21A2*	6	*MP*	Yes
2G2P	*19*	*PG*		*24*	20	nd	
2J2	8	*MP*		*26A1*	10	nd	
2R1	11	nd		*26B1*	2	nd	
2S1	19	nd		*26C1*	10	nd	
2T2P	*19*	*PG*		*27A1*	2	MP	Yes
2T3P	*19*	*PG*		*27B1*	2	nd	
2U1	4	nd		*27C1*	2	nd	
2W1	7	nd		*39A1*	6	nd	
3A4	7	*MP*		*46*	1	nd	
3A5	7	*MP*	Perhaps	*46P*	1	PG	
3A5P1	*7*	*PG*		*51*	7	nd	
3A5P2	*7*	*PG*		*51P1*	*3*	*PG*	
3A7	7	*MP*		*51P2*	*13*	*PG*	
3A43	7	nd	Functional?				
4A11	1	nd					
4A20	1	nd					
4A20P	1	PG					
4B1	1	nd					
4F2	19	nd					

[a]This column denotes polymorphism of importance for the in vivo function of the enzyme in question

ber for the gene. In order to have the same gene number the genes must have the same function and exhibit high conservation.

The completion of the sequence of the human genome revealed the presence of about 107 human P450 genes: 59 active and about 48 pseudogenes (http://drnelson.utmem.edu/CytochromeP450.html). All the currently known different active human cytochrome P450 genes are listed in Table 1 as well as the major part of the pseudogenes. The total number of P450 genes might be considered as surprisingly low in relation to the number in lower organisms like *Arabidopsis* with 239 P450 genes and rice with 458 P450 genes. The cytochrome P450 based detoxification potential appears to be higher in rodents and the recently completed mouse genome revealed 108 functional CYP genes. The background might be inherent in a higher need for metabolism of dietary components.

As evident from Table 1 it is apparent that there is a difference between the characteristics of genes encoding xenobiotics metabolism (CYP1–3) and those of importance for the metabolism of endogenous compounds. The number of pseudogenes are by far most apparent in the CYP2

Table 2 General characteristics of human cytochrome P450 enzymes in families 1–3. Only those where full length clones have been described and the protein expressed are considered

Enzyme	Marker substrate reaction	Substrate specificity	Main tissue localization	Comments
CYP1A1	Ethoxyresorufin O-deethylation	Pre-carcinogens, PAHs	Extrahepatic	Inducible by PAHs, endogenous role in cell growth?
CYP1A2	Phenacetin O-deethylation. ethoxyresorufin O-deethylation	Aromatic, amines, PAHs	Liver	Inducible by PAHs and some drugs
CYP1B1	Estradiol-4-hydroxylation	DMBA, oestradiol	Extrahepatic	Inducible by PAHs, high affinity for some PAHs
CYP2A6	Coumarin 7-hydroxylation	Nicotine	Liver	The major nicotine oxidase, active on some drugs and carcinogens
CYP2A13	Coumarin 7-hydroxylation		Olfactory mucosa	
CYP2C8	Taxol hydroxylation		Liver	Might have a role in hepatic drug metabolism
CYP2C9	Tolbutamide methylhydroxylation, losartan hydroxylation, S-warfarin 7-hydroxylation	Drugs	Liver	Very important for drug metabolism
CYP2C18	?	Some drugs	Extrahepatic	Highly polymorphic
CYP2C19	S-mephenytoin 4-hydroxylation, omeprazole 5-hydroxylation	Drugs	Liver	Highly polymorphic, very important for drug metabolism
CYP2D6	Dextromethorphan O-deethylation, bufuralol 1'-hydroxylation, debrisoquine 4-hydrohylation	Drugs	Liver	Very important for drug metabolism
CYP2E1	Chlorzoxazone 6-hydroxylation	Solvents, drugs, pre-carcinogens	Liver	The main enzyme which metabolises organic solvents, highly inducible
CYP2F1			Lung	Active?
CYP2J2	Arachidonic acid hydroxylation	Fatty acids	Extrahepatic	
CYP2R1	Vitamin D25 hydroxylase	Vitamin D	Extrahepatic	The function recently revealed
CYP2S1	Trans-retinol oxidation	Small aromatic hydrocarbons	Extrahepatic	Inducible by UV-light in skin
CYP3A4	Testosterone 6β-hydroxylation, midazolam 1'-hydroxylation, erythromycin *N*-demethylase	Drugs, pre-carcinogens, dietary components	Liver, intestine	The most important P450 in drug metabolism
CYP3A5	Same as CYP3A4	Similar as CYP3A4	Liver, intestine	Expressed in some individuals
CYP3A7	Same as CYP3A4	Similar as CYP3A4	Liver, intestine	Mainly expressed in fetal liver
CYP3A43	Not known	Not known	Liver	Tiny expression, active?

family (20), followed by CYP4 (18) and CYP3 (5). Among genes encoding P450s metabolising endogenous substrates only 4 pseudogenes have been found. This indicates a high extent of gene inactivation particularly in these genes encoding xenobiotics metabolism, a result of adaptation to the environment. Also, amongst the families 1–3 all P450 genes are functionally polymorphic, with exception for CYP1A1, CYP2E1, and CYP3A4, which are relatively well conserved. The reason for this conservation might be the endogenous importance of the corresponding enzymes. By contrast, only 6 out of 20 CYPs of importance for metabolism of endogenous compounds have at present been found to exhibit genetic polymorphism. The reason for this difference is inherent in the higher genetic variation of genes whose gene products are active in the metabolism of environmental agents. What is somewhat surprising is how many pseudogenes that this variation has created that still are present in the human genome.

Concerning the P450s in families 1–3, their main function and properties are summarised in Table 2. The major forms of importance for drug metabolism are CYP2C9, CYP2C19, CYP2D6 and CYP3A4, whereas CYP1A1, CYP1A2, CYP1B1, CYP2E1 and CYP3A4 are the most important isoforms responsible for metabolic activation of pre-carcinogens (see Table 3). Amongst the genes in families 2–3 some new ones like CYP2R1, CYP2S1, CYP2U1 and CYP3A43 have appeared as a consequence of the initiatives of the human genome project. The function and importance of those are still incompletely known. CYP2S1 is an extrahepatic enzyme expressed in lung and in the intestinal tract (Rylander et al. 2001), inducible by dioxin (Rivera et al. 2002) and able to metabolise trans-retinol (Smith et al. 2003) as well as naphthalene (S.I. Miura, M. Ingelman-Sundberg, unpublished observations) whereas CYP3A43 appears to be a pseudoprotein (see below).

Some relevant internet-sites about cytochromes P450 are given in Table 4.

Table 3 Participation of various human cytochrome P450s in the metabolic activation of pre-carcinogens. *MeIQ* 2-amino-3,4-dimethylimidazo[4,5-f]quinoxaline, *DMBA* dimethylbenzanthrazene, *IQ* 2-amino-3-methylimidazo[4,5-f]quinoxaline, *PhIP* 2-amino-1-methyl-6 phenylimidazo[4,5-b]pyridine, *Glu* P-1, 2-amino-6,-methyldipyrido-[1,2-a:3',2'-d]imidazole, *Trp* P-2, 2-aminodipyrido[1,2-a:3',2'-d]imidazole, *DEN* 3-amino-1-methyl-5H-pyrido[4,3-b]indole, *DMN* N-nitrosodimethylamine, *MOCA* 4,4'-methylene-bis(2-chloroaniline), *NNK* 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone

CYP1A1	CYP1A2	CYP1B1	CYP2A6	CYP2E1	CYP3A4
Benzo(a)pyrene	PhIP	DMBA	DEN	DEN	Aflatoxin B1
	Glu P-1	Benzo(a)pyrene	Aflatoxin B1	N-nitroso-dimethylamine	Aflatoxin G1
	N-nitroso-diethylamine	Oestradiol	MOCA	NNK	Stergmato-cystine
	IQ	Benzanthracene	IQ	N-nitroso-nicotine	Benzo(a)pyrene
	MeIQ	3-Methyl-cholantrene	MeIQ	Styrene	Oestradiol
	MeIQx	1-Ethynyl-pyrene	NNK	Vinyl chloride	6-Amino-chrysene
	Trp P-2			Vinyl bromide	1-Nitropyrene
	4-Amino-biphenyl			Ethyl carbamate	Senecionine
	2-Acetyl-aminofluorene			Methylene chloride	
	NNK			Chloroform	
				Benzene	

Table 4 Useful internet sites in cytochrome P450 research (assessed 6 July 2003)

Site	Address	Information
P450 substrate	http://medicine.iupui.edu/flockhart/	Current update about P450 substrates, inducers and inhibitors
Nelsons home page	http://drnelson.utmem.edu/CytochromeP450.html	All P450 forms in human and other species
Human CYP allele nomenclature home page	http://www.imm.ki.se/CYPalleles/	Current update about new allelic forms of human cytochromes P450s with links to relevant references
Cytochrome P450 database	http://cpd.ibmh.msk.su/	Important collection of P450 nomenclature, structure and properties
Kirill N. Degtyarenko's Directory of P450-containing Systems	http://www.icgeb.trieste.it/~p450srv/	Important information of P450 redoxpartners and ligands
GENTEST's human P450 metabolism database	http://www.gentest.com/human_p450_database/index.html	Human cytochrome P450 metabolism organised according to enzyme, therapeutic area, chemical substance, reaction, and type (substrate, inhibitor, activator, or inducer)

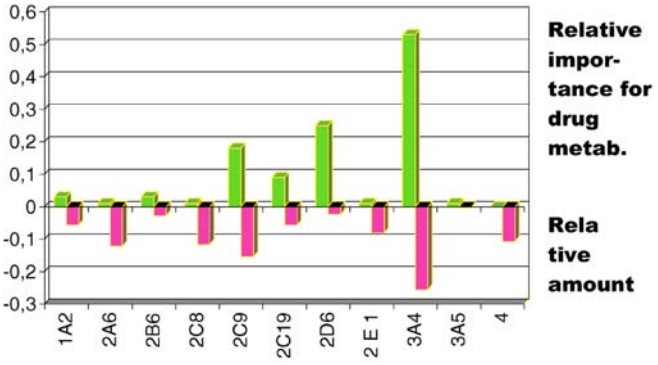

Fig. 1 Relative amount of the hepatic human cytochrome P450 forms in liver and relative importance for metabolism of clinically used drugs. The data regarding the relative importance for drug metabolism are based on the clearance of 315 drugs, 56% primarily cleared by P450, 26% by unknown P450, (Bertz and Granneman 1997). Data about the relative amount of the various forms of P450 are from Gentest Inc. (http://www.gentest.com/prod_inserts/H161_2.htm#immunoquantitation). The absolute values are in pmol/mg (% of total P450): total P450, 534 (100); CYP1A1, 0 (0); CYP1A2, 45 (8); CYP2A6, 68 (13); CYP2B6, 39 (7), CYP2C8, 64 (12), CYP2C9, 96 (18), CYP2C18, 0 (0); CYP2C19, 19 (4), CYP2D6, 10 (2); CYP2E1, 49 (9); CYP3A4, 108 (20), CYP3A5, 1.0 (0.2), 3A7, 0 (0); others 35 (6.8)

Relative amounts and importance of different hepatic P450s

The hepatic expression of different forms cytochromes P450 has been determined using specific antibodies by two different laboratories. The data presented by Shimada et al. (1994) are different in many respects to those provided by Gentest Inc. on their homepage (http://www.gentest.com/prod_inserts/H161_2.htm#immunoquantitation; see Fig. 1). Both labs identify CYP2C and CYP3A as the major forms of P450s in the liver, whereas the values for CYP2A6 and CYP2E1 are more discrepant. The methods used by Gentest Inc. makes quantification of more forms of P450 possible due to enzyme specific assays for the different isoforms in the CYP2C and CYP3A family. The amount of CYP2D6 might be underestimated in relation to an investigation by Zanger et al. (2001) where the amount of CYP2D6 based on the expression in 79 different livers is 3.9% of all hepatic P450.

There is no apparent relationship between the amount of hepatic CYPs and their relative importance of the enzymes in drug metabolism as illustrated in Fig. 1. This might indicate a prominent role for metabolism of e.g. dietary components among those P450s expressed at a high level but being relatively inactive in drug metabolism.

Most cytochrome P450 enzymes are preferentially expressed in the centrilobular area of the liver (Oinonen and Lindros 1998). The basis for this heterogeneous expression is unknown but inherent probably in the phenotype influenced by environmental factors and neighbouring cells. This has toxicological consequences and in general the centrilobular area is more sensitive to damage by drugs and ethanol that are activated by the action of P450.

Knowledge about the cytochrome P450 structure

The xenobiotics metabolising cytochromes P450 are membrane bound, hydrophobic in their character and therefore very difficult to crystallise. At present we have the knowledge about the three-dimensional structure of 7 different bacterial P450s and 4 mammalian membrane bound, namely rabbit liver CYP2B4 and CYP2C5, human CYP2C8 and CYP2C9. Enzyme products being able to crystallise were obtained after truncation of the NH_2-terminal part and mutations of some critical amino acids e.g. in the G-F loop (cf. Williams et al. 2000; Cosme and Johnson 2000). These enzymes can be used as templates in modelling thus achieving reasonable good models for the drug metabolising P450s. However, as yet different models for the human P450s are not at a stage where one can predict substrates and inhibitors at satisfactory resolution among the members of the subfamilies not yet crystallised. The CYP2C5 and CYP2C9 structures can to a great extent predict the structure of the human CYP2C19 enzyme (cf. Afzelius et al. 2001), whereas other templates are necessary in order to be able to make good predictions for the structures of e.g. CYP1A2, CYP2E1 and CYP3A4. The recommendations at present must be to use the current P450 templates with caution for predicting the structures of the human CYP1A, CYP1B, CYP2A, CYP2B, CYP2D, CYP2E and CYP3A enzymes.

Substrate recognition sites

In the P450 structure of the CYP2 family, Gotoh (1992) originally proposed the presence of six different substrate recognition sites (SRS). Experiments with mutagenesis validation in general have supported the existence of such structures (see Fig. 2). In the 3-D models, these SRS are concentrated into the active sites of the enzymes. In the CYP2D6 enzyme the positions of the SRS are given in Fig. 2 and mutagenesis experiments reveal that many of these amino acids are of importance for substrate binding. An analysis of the CYP3A active sites through pharmacophore modelling has also been carried out (Ekins et al. 2003).

Regulation of the cytochromes P450

Most of the xenobiotics metabolising cytochromes P450 are inducible. One exception is CYP2D6, where instead

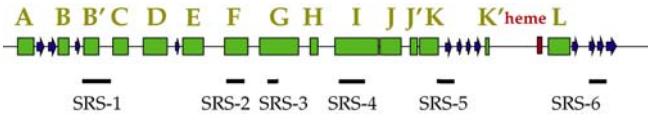

Fig. 2 Cytochrome P450 secondary structure and substrate recognition sites The different helices are marked as the six different substrate recognition sequences (*SRS*). In CYP2D6 the SRS correspond to the following amino acids: SRS1:100–125; SRS2:213–219; SRS3:239–246; SRS4: 294–312; SRS5:369–375; SRS6:480–487

selection for alleles containing multiple gene copies has been a manner to increase the detoxifying potential of the enzyme (Ingelman-Sundberg et al. 1999). The induction process is believed to be an important manner for increasing the defence against environmental toxins in the past. Survival of certain species of *Drosophila* is thus dependent on the ability to induce certain forms of P450 (Danielson et al. 1997). Relatively scarce information exists about the factors of importance for the constitutive expression whereas our knowledge regarding the transcriptional factors governing the inducible expression has increased in recent years. In general control of P450 expression can be exerted at the transcriptional, mRNA, translational and post-translational level. Posttranslational regulation has been described for CYP1A1 (Werlinder et al. 2001), CYP1A2, CYP2E1 and CYP3A4. The transcriptional control is of highest importance and three crucial cytosolic receptors sense the concentration of the environmental xenobiotics, namely the pregnane X-receptor (PXR), constitutive androgen receptor (CAR) and the Ah-receptor (AHR). They regulate CYP1A1, CYP1A2 and CYP2S1 (AHR), CYP2C9, CYP3A4 (PXR) and CYP2B6, CYP2C9, CYP3A4 (CAR). Increasing cellular amounts of environmental xenobiotics leads to activation of several P450s as well as phase II enzymes causing more enzymes to be expressed and in turn to lowering amounts of the xenobiotics (Fig. 3). It is now evident that these transcriptional factors are involved in the control of most human drug metabolising P450s although more information is needed regarding their action in detail and the synergism between the receptors and with other transcriptional factors including hormone receptors as well as activators and repressors. An interesting modulation of oestrogen receptor function by AhR was recently shown (Ohtake et al. 2003). The receptors seem to be rather promiscuous and all the receptors have been shown to bind several different chemicals of diverse structures. In addition, there seems to be a manner for the xenobiotics to activate these cytosolic receptors through binding to extracellular receptors as evident from experiments using protein kinase and phosphatase inhibitors. Although the details are far from being elucidated, experiments with the protein kinase inhibitors and phosphatase inhibitors indicate the existence of signal transduction chains leading to the activation of these receptors in the absence of ligands (see Fig. 3; cf. Backlund et al. 1997). In this manner the number of cellular structures being able to sense the xenobiotics would be much higher creating a higher detoxification sensing potential.

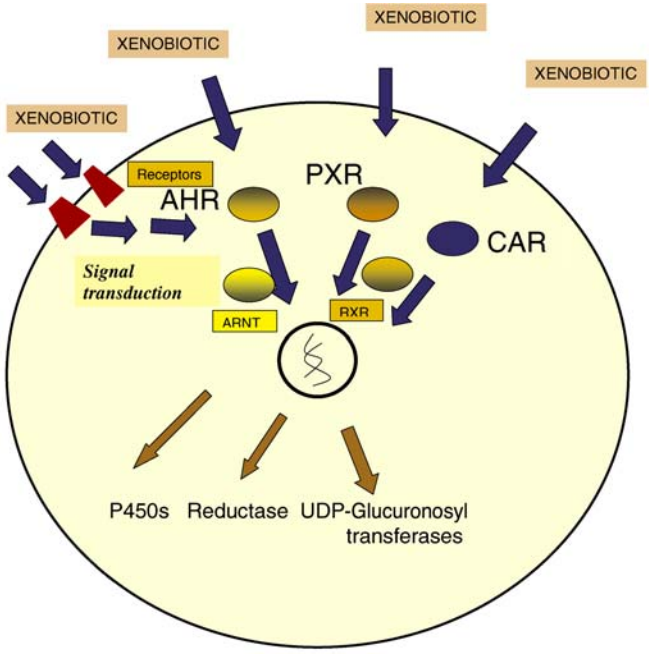

Fig. 3 Sensing mechanisms for the regulation of expression of cytochrome P450s and other xenobiotic metabolising enzymes. The level of the xenobiotic is sensed by receptors in the cytosol. In addition, in some cases extracellular receptors can activate some of these receptors by signal transduction pathways

How to identify the contribution of a specific P450 to a reaction

Characterisation of the contribution to a specific reaction by a certain form of P450 is important with respect to the identification of a given isoform responsible for the metabolism of e.g. a new drug candidate. Not only is it important to identify the enzyme responsible for its clearance in vivo because of possible complications with respect to the action of polymorphic forms of P450, but also the knowledge of possible drug-drug interactions at the site of metabolism is of utmost importance for recommendations for use in the clinic. Also one would like to use systems that allow an estimation of the in vivo clearance of a drug based on the catalytic properties. For this purpose one might use recombinant P450 enzymes, isolated human microsomes, computer models, human hepatocytes, tissue slices, in combination with specific inhibitors. An internet site, maintained by D.A. Flockart updates lists of substrates, inhibitors and inducers of the different P450s (see http://medicine.iupui.edu/flockhart/, assessed 6 July 2003). This list provides links to the specific references in the literature. However, using substrates and inhibitors might be problematic in view of their specificity, see (Eagling et al. 1998). See Rendic and Di Carlo (1997) and Pelkonen et al. (1998) for reviews in this field. In general the identification of a specific form of P450 participating in the metabolism of a candidate drug would in optimal cases involve the use of recombinant enzymes and calculation of the intrinsic clearance of the drug by the isoforms involved. Extrapolation to the in vivo situation can then be done. The Km and Vmax values are determined and used to estimate the intrinsic clearance (Cl_{int}) according to $Cl_{int}= V_o/C=V_{max}/K_m+C$ where V_0 is the initial metabolic rate and C the unbound drug concentration. At substrate concentrations considerably lower than K_m, $Cl_{int}=Vmax/K_m$ (Ito et al. 1998). Cl_{int} in vivo can be calculated from Cl_{int} in vitro using a factor of 45 mg microsomal protein/g liver (Houston 1994) and a liver mass of 1,500 g. The contribu-

tion of each form of P450 can be determined from the concentrations given in Fig. 1, see (http://www.gentest.com/prod_inserts/H161_2.htm#immunoquantitation). To calculate the hepatic clearance, Cl_H, the well-stirred model excluding protein binding is applied: $Cl_H=Q_H *Cl_{int}/Q_H+Cl_{int}$ with the hepatic blood flow, Q_H=1,450 ml/min (Davies and Morris 1993).

Furthermore, interaction studies in human liver microsomes using the P450 specific inhibitors give important information to the contribution of different P450 enzymes. The experience from the EU-supported EUROCYP project is that these in vitro systems to a great extent can predict the enzyme specificity and drug clearance provided that the candidate drug is not metabolised in phase II reactions (Andersson et al. 2001; Pelkonen et al. 2001b; Hidestrand et al. 2001). In such cases liver slices and hepatocytes offer good systems, although the clearance is somewhat underestimated here.

Regarding the physiological and toxicological roles of different CYPs, the mouse knock out and transgenic models offer tremendous advantages (Gonzalez and Kimura 2003).

CYP1B1, CYP2B6, CYP2E1 and CYP3As

CYP1B1

Human CYP1B1 has been shown to be an important enzyme in the activation of diverse procarcinogens such as arylarenes, nitroarenes, polycyclic aromatic hydrocarbons and arylamines to reactive metabolites that cause DNA damage (Shimada et al. 1996). See Murray et al. (2001) for a review about the enzyme. CYP1B1 is constitutively expressed in steroidogenic tissues such as uterus, breast, ovary, testis, prostate and the adrenal gland, and is active in the metabolism of oestradiol (Hayes et al. 1996) as well as of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene, known procarcinogens (Crespi et al. 1997). The enzyme is also present in many other extrahepatic tissues including kidney, thymus, spleen, brain, heart, lung, colon and intestine (Shimada et al. 1996) A nuclear localisation of the enzyme has been proposed based upon immunohistochemistry experiments (Muskhelishvili et al. 2001). CYP1B1 is expressed at a higher level in a wide range of human cancers including cancers of the skin, brain, testis (Murray et al. 1997) and breast cancer (McKay et al. 1995) as compared to the non-transformed tissue. This might have implications for tumour development and progression and the development of anticancer drugs specifically activated by CYP1B1. There are considerable species differences regarding the regulation, metabolic specificity, and tissue-specific expression of this P450 (see Murray et al. 2001).

CYP2B6

CYP2B6 is yet not well characterised. The immunochemically measured amount in liver varies a lot, between 0.7 to 70 pmol/mg microsomal protein (Ekins et al. 1997, Ekins and Wrighton 1999) and it has been shown that CYP2B6 shows genetic polymorphism (Jinno et al. 2003; Zanger et al. 2002). More than nine different alleles have been found (http://www.imm.ki.se/CYPalleles/cyp2b6.htm). The haplotypes have yet not been fully elucidated, but it seems that there are several different rather rare haplotypes of CYP2B6 in the populations. By using more sensitive methods it seems that a large majority of the individuals express both CYP2B6 mRNA and protein (Hakkola et al. 1994).

S-mephenytoin N-demethylation to nirvanol has been suggested to be a CYP2B6-specific probe activity (Ekins et al. 1998). CYP2B6 seems to be involved in cyclophosphamide hydroxylation (Roy et al. 1999) an important step in its conversion to a cytostatic agent. Buproprion hydroxylation, an antidepressant also used as an antismoking agent, has turned out to be a fairly selective reaction for 2B6 when measuring activities in vitro (Faucette et al. 2001, 2000). Triethylenethiophosphoramide has been found to be a specific inhibitor (Rae et al. 2002).

CYP2B6 is induced by e.g. phenobarbital and cyclophosphamide (Gervot et al. 1999). Both PXR and CAR have been shown to be involved in the regulation of this protein in response to phenobarbital (Sueyoshi et al. 1999) and other xenobiotics (Goodwin et al. 2001). CYP2B6 has a more important role than previously thought and the enzyme has recently been suggested to be of major importance for clearance of besides buproprion, selegiline (Hidestrand et al. 2001), ketamine (Yanagihara et al. 2001), propofol (Oda et al. 2001), artemisinin (Simonsson et al. 2003) and chlormethiazole (Y. Terelius, unpublished observations). Modelling of CYP2B6 might now be much facilitated after the presentation of the CYP2B4 crystal structure.

CYP2E1

The CYP2E subfamily has only been described in mammals and there is only one gene in the CYP2E locus in humans (see Ronis et al. 1996). CYP2E1 is constitutively expressed in the liver, where the highest concentration (almost 100 µM after induction with e.g. acetone combined with starvation) is found in the centrilobular region particularly in the 4–5 layers of hepatocytes surrounding the terminal hepatic venules (Ingelman-Sundberg et al. 1988).

As mentioned CYP2E1 is one of the few drug metabolising enzymes that is reasonably conserved between mammalian species. This might be due to important endogenous roles and the endogenous regulation of CYP2E1 is similar to that of other gluconeogenetic enzymes, which includes repression of enzyme expression during well fed conditions and increased expression during starvation and diabetes. It physiological substrates appears to be the gluconeogenetic precursors acetone and acetol (see Ronis et al. 1996) as well as fatty acids (Leclercq et al. 2000). The control of CYP2E1 expression is very complex and involves the transcriptional, mRNA, translational and post-

translational levels. Studies in rats have demonstrated increases of CYP2E1 either at transcriptional or post-transcriptional level in chemically induced diabetes and starvation (Johansson et al. 1990; see Ronis et al. 1996). Insulin treatment causes a decrease of the CYP2E1 protein in chemically induced diabetes, by increasing the rate of mRNA turnover (De Waziers et al. 1995). The expression of CYP2E1 is also regulated by different cytokines. Similar to other P450s such as CYP1A2, CYP2C, and CYP3A, the level of CYP2E1 is decreased by e.g. IL-1, IL-6 and TNF-α in primary culture of human hepatocytes (Abdel-Razzak et al. 1993). By contrast, Il-4 induces the expression of CYP2E1at least in primary hepatocytes and human hepatoma cells B16A2 (LeJossic et al. 1996).

Besides the regulation of expression, also the intracellular transport of CYP2E1 is regulated in a complex manner and substrates and phosphorylation reactions can determine the fate of the enzyme. Thus experiments in vitro using hepatocyte culture system (Eliasson et al. 1988) and determination of CYP2E1 turnover in vivo in rats chronically treated with acetone (Song et al. 1989) have shown that CYP2E1 inducers increase the CYP2E1 level by protein stabilisation. In the absence of inducers, CYP2E1 is degraded in a bi-phasic fashion with a short half-life of 7 h and a longer half-life of 37 h. However, after chronic acetone administration, the rapid phase of degradation is abolished and the degradation follows the slow autophagosomal lysosomal pathway for protein degradation (see Ronis et al. 1996). The substrate binding protects CYP2E1 from phosphorylation at Ser-129, and the subsequent intracellular processing (Eliasson et al. 1990; Roberts et al. 1995; Robin et al. 2002) which involves either transport to the mitochondria or ubiquitination and degradation by the proteaosome complex. The rapid degradation of CYP2E1 might be partly due to covalent modifications by the radicals produced by CYP2E1 or to other changes during catalytic cycling. Inhibition of electron supply from NADPH-cytochrome P450 stabilises the protein (Zhukov et al. 1999).

CYP2E1 can thus be transported into the mitochondria, apparently in its intact form (Robin et al. 2001) or after cleavage of the membrane anchor sequence (Neve and Ingelman-Sundberg 2001). In the latter case, a mitochondrial import signal in the protein is responsible for its import (Neve and Ingelman-Sundberg 2000) whereafter it is cleaved by mitochondrial proteases to yield a soluble form that is catalytically active (Neve and Ingelman-Sundberg 2000, 2001). The amount of soluble mitochondrial CYP2E1 in intact liver is however very small. In addition, the amount of membrane bound mitochondrial CYP2E1 is not detectable in uninduced animals (E. Neve, unpublished observations).

CYP2E1 has very broad substrate specificity. More than 70 different chemicals with diverse structures have been identified to be metabolised by CYP2E1 (Ronis et al. 1996). In general most CYP2E1 substrates are small and hydrophobic in character. Many of the substrates exert a high affinity to the enzyme. Among the substrates are alcohols/ketones/aldehydes, aromatic compounds, halogenated alkanes or alkanes, anaesthetics, drugs and carcinogens (nitrosamines and azo carcinogens). Many of the CYP2E1 substrates are also inducers of the enzyme. The inducers commonly used in experimental animals are ethanol, acetone, isoniazid, pyridine and pyrazole. Among these, pyridine, acetone and isoniazid have been described to increase the translation efficiency of the CYP2E1 mRNA (see Ronis et al. 1996).

Chlorzoxazone 6-hydroxylation is the preferred method to measure CYP2E1 activity in liver microsomes and in vivo (Peter et al. 1990). Also CYP1A1 and CYP1A2 catalyse the reaction, but the Km values are higher and turnover numbers lower than with CYP2E1.

Many different types of inhibitors have been used as previously reviewed in Ronis et al. 1996). Both chlormethiazole (CMZ); diallysulfide (DAS); phenethyl isothiocyanate (PET) and dihydrocapsacin (DHC) are able to depress the concentration of CYP2E1 in vivo in rats (Badger et al. 1995). Chlormethiazole is the most efficient and specific inhibitor and acts both at the transcriptional and post transcriptional level (Simi and Ingelman-Sundberg 1999; Gebhardt et al. 1997). In humans chlormethiazole is very efficient inhibitor and abolish the CYP2E1 dependent activity after treatment (Gebhardt et al. 1997), a way to make a human knock down.

CYP3A4

CYP3A4 is the major form of human P450 both with respect to the amount (about 25% of all hepatic P450 (Fig. 1) and the importance in drug oxidations (Guengerich 1995; Shimada et al. 1994), responsible for about 50% of the metabolism of clinically used drugs (Fig. 1; Bertz and Granneman 1997). CYP3A4 is highly inducible by a range of drugs and other chemicals. There is very large interindividual variability in expression, with hepatic microsomal apoprotein content varying by 40- to 50-fold.

CYP3A4 is extensively expressed in the intestine, indeed at high levels, 50% of hepatic content and 70% of total P450 present (McKinnon et al. 1995). Apoprotein content increases slightly from the duodenum to the jejunum and then decreases considerably towards the ileum (Zhang et al. 1999). The enzyme has a substrate specificity and inducibility that is similar to the drug transporter P-glycoprotein (P-gP) in the intestine. The genes are on the same position on chromosome 7q21.1 and apparently use similar DR4 regulatory elements binding PXR (Geick et al. 2001). The broad substrate specificity and capacity of CYP3A4 in the metabolism of xenobiotics compounds and the coregulation with the drug transporter P-gP both present at high concentrations in the intestine, makes these two components maybe the most important unit for elimination of xenobiotics in the body. Thus, CYP3A4 metabolises a very large number of drugs, other xenobiotic chemicals and endogenous compounds. The list of the known substrates which amount to over 80 are present on the CYP interaction home page http://medicine.iupui.edu/flockhart/ and in a review by Guengerich (1999).

The active site of CYP3A4 may accommodate two molecules (of either the same or different compounds) and that

these influence the kinetics of the other, some compounds causing positive cooperativity, homotropic and heterotropic cooperativity, respectively (Harlow and Halpert 1998). This complicates inhibitory and interaction studies and it is recommended to use several different CYP3A4 substrates in three or four different groups of experiments (Kenworthy et al. 1999). The active site might harbour very small molecules like some small benzimidazoles but also large substrates like the peptide-like cyclosporin with Mr>5000. One might expect that the site is somewhat flexible in its nature and adapts partly to the substrate in question. CYP3A4 has recently been crystallised by Astex Technology and the structure is to be released soon.

Probe substrates for CYP3A4 include midazolam (1'-hydroxylation), erythromycin (N-demethylation), cortisol (6β-hydroxylation; in vivo) and testosterone (6β-hydroxylation; in vitro; see Pelkonen et al. 2001a). In vitro an up to 50-fold variation in activities are seen (Westlind et al. 1999) whereas in in vivo studies the variation is less (Kinirons et al. 1999; Floyd et al. 2003). The reasons for this discrepancy might be the contribution of non-CYP3A4 enzymes to the metabolism of the CYP3A4 substrates used. Its importance is manifested in that the enzyme to a great extent determines the duration and intensity of action of such drugs as cyclosporin A, HIV protease inhibitors, macrolide antibiotics, dihydropyridine calcium antagonists, HMGCoA reductase inhibitors, non-sedative antihistamines, benzodiazepines, and the anti-convulsant, carbamazepine (see Pelkonen et al. 1998, 2001a). CYP3A4 is also very important for the metabolism of dietary and environmental chemicals, e.g. of flavanoids, mycotoxins such as aflatoxin B_1, pesticides and a number of food additives and possibly also endogenous peptides (see Guengerich 1999).

CYP3A4 can be inhibited by a number of drugs and other exogenous chemicals (Thummel and Wilkinson 1998; Pelkonen et al. 1998). Amongst potent, relatively selective inhibitors of this enzyme are azole antifungal agents such as ketoconazole, macrolide antibiotics such as troleandomycin, HIV protease inhibitors such as saquinivir, antidepressants such as fluoxetine, and the furanocoumarin, 6',7'-dihydroxybergamottin, found in grapefruit juice (see Guengerich 1999). Many of these agents form tightly-bound complexes with the enzyme or serve as mechanism-based inhibitors (Thummel and Wilkinson 1998).

CYP3A4 is inducible by several different classes of compounds, both therapeutic agents and dietary chemicals. Amongst drugs that can induce CYP3A4 are certain macrolide antibiotics, rifamycins, anticonvulsants such as carbamazepine and glucocorticoids, although these appear to be only weak inducers in humans (see Pelkonen et al. 2001a for references). Also components in the natural plant remedy St John's Wort, in particular hyperforin, are potent inducers of CYP3A4, a fact that is of importance for interactions during drug therapy (Moore et al. 2000).

Induction of CYP3A4 is mediated by the pregnane X receptor (PXR; Lehman et al. 1998). On binding of an inducer ligand, the inducer-receptor dimerises with the retinoic acid X receptor (RXR) and the dimer binds to a cognate 5'-upstream response element on the CYP3A4 gene, resulting in transcriptional activation (Honkakoski and Negishi 2000; Kliewer et al. 1999). Examination of the upstream region of the CYP3A4 gene has revealed binding sites for a number of transcription factors including AP-3, hepatocyte nuclear factor-4 and -5 and a glucocorticoid response element (GRE; Hashimoto et al. 1993). HNF-4 plays a role in the inducible (Iwahori et al. 2003) and constitutive expression of CYP3A4 (Jover et al. 2001), the former through modulation of PXR expression.

CYP3A5

CYP3A5 is expressed at much lower levels than CYP3A4 in liver, but has a wider tissue distribution. Some scientists have proposed a high expression constituting perhaps the dominating drug metabolising enzyme in some liver (Kuehl et al. 2001), whereas other investigators find only low levels of CYP3A5 mRNA expression (Koch et al. 2002) and by using peptide specific antibodies and peptide linked carrier proteins for quantification others have concluded that CYP3A5 only accounts for 2% of the total CYP3A protein in Caucasian livers (Westlind-Johnsson et al. 2003). The expression of CYP3A5 is generally higher among African-Americans as compared to Caucasians (Kuehl et al. 2001) The variability in CYP3A5 expression is caused by single-nucleotide polymorphisms (SNPs) in *CYP3A5*3* and *CYP3A5*6* that cause alternative splicing and protein truncation result in the absence of CYP3A5 from tissues of some people (Kuehl et al. 2001). The substrate specificity of CYP3A5 is similar to that of CYP3A4, but generally it is less active (Ohmori et al. 1998). No form-specific substrates for CYP3A5 are known (see Guengerich 1999). Thus CYP3A5 appears to metabolise most of the substrates metabolised by CYP3A4, but there are some exceptions (Guengerich 1999).

CYP3A5 appears to be inhibited by many of the compounds that inhibit CYP3A4, although detailed information is lacking. Ketoconazole, fluconazole and itraconazole are less potent inhibitors of CYP3A5 than CYP3A4 (Gibbs et al. 1999) whereas triacetyloleandomycin is as effective (Ohmori et al. 1998).

In vivo phenotyping with low amounts of midazolam reveals that CYP3A5 does not contribute significantly to midazolam oxidation (Floyd et al. 2003).

CYP3A7

The highest levels of CYP3A7 protein are found in fetal liver, where it accounts for 30 –50% of the total P450 present (Wrighton and Vandenbranden 1989). Expression is detectable very early on, in the embryonic stage. The levels vary with gestational age, and are greatest during the first week after birth whereafter the expression declines to very low to undetectable in adult liver (de Wildt et al. 1999). The decline in CYP3A7 after birth is paralleled by an increase in CYP3A4 expression such that the total CYP3A

content of the liver remains relatively constant (de Wildt et al. 1999). In adult liver there seems to be undetectable levels of CYP3A7 (Fig. 1)

CYP3A7 metabolises both endogenous and exogenous compounds. CYP3A7 metabolises many of the substrates metabolised by CYP3A4, albeit with significant differences in activity (Gillam et al. 1997). Interestingly, CYP3A7 catalyses the 16α-hydroxylation of dehydroepiandrosterone and of its 3-sulphate much more actively than either CYP3A4 or CYP3A5 (Ohmori et al. 1998).

CYP3A7 is inhibited by some of the compounds that inhibit CYP3A4 and CYP3A5, although there are differences in the specificity of inhibition. For example, triazolam inhibits 6β-hydroxylation of testosterone catalysed by CYP3A7 but not by CYP3A4 (Ohmori et al. 1998). Triacetyloleandomycin, a diagnostic inhibitor of CYP3A4, inhibits the activity of CYP3A7.

The 5'-upstream region of the *CYP3A7* gene possesses a similar PXR responsive promoter to CYP3A4, and is inducible by rifampicin and clotrimazole (Pascussi et al. 1999).

CYP3A43

This gene was identified as a result of the human genome project. It has 71–76% amino acid homology to the other members of the CYP3A locus, but in comparison to them non conserved residues are found also at other positions in the sequence (Westlind et al. 2001). The cDNA was cloned by three different laboratories and was found to be inducible by rifampicin (Gellner et al. 2001). Expression in bacteria revealed hardly any significant activity using testosterone as a substrate (Domanski et al. 2001), whereas expression in either yeast, mammalian COS I cell, HEK293 cells or HepG2 cells failed to yield any properly folded enzyme (Westlind et al. 2001). Expression analysis in different tissues revealed highest mRNA expression in liver and some in extrahepatic tissues, e.g. the prostate, (Domanski et al. 2001; Westlind et al. 2001; Gellner et al. 2001) but the amount of CYP3A43 mRNA in liver was found to be only 0.1% of that of CYP3A4 (Westlind et al. 2001). It might be concluded that CYP3A43 does not play any significant role for drug metabolism and it is likely that the enzyme is actually a pseudoprotein without any function in mammalian cells.

Polymorphism amongst the cytochromes P450

As mentioned all hepatic drug metabolising P450s are polymorphic as summarised in Table 1. The clinically most important polymorphism is seen with CYP2C9, CYP2C19 and CYP2D6. The functional importance of the polymorphisms of the xenobiotics metabolising P450s is summarised in Table 5. The mutations in the CYP genes can cause enzyme products with abolished, reduced, altered or increased enzyme activity. Abolished enzyme activity is commonly seen where the whole gene has been deleted, but has also its origin in mutations causing altered splicing, stop codons, abolished transcriptional start sites and deleterious amino acid changes. Mutations in substrate recognition sites (SRS) can cause the synthesis of enzymes with an altered substrate specificity as seen with *CYP2D6*17* (Oscarson et al. 1997) found entirely in black African populations. Furthermore, mutations in the folding region might lead to an altered protein folding and different substrate specificity as seen with *CYP2D6*10* (Fukuda et al. 2000). Increased activity is seen in subjects carrying multiple copies of an active P450 gene as described for CYP2D6 (Johansson et al. 1993) and CYP2A6 (Rao et al. 2000).

A web page for human cytochrome P450 alleles

In recent years a lot of research has been focused on the identification and characterisation of polymorphic human

Table 5 Functional importance of polymorphism in human P450s involved in xenobiotics metabolism

Enzyme	Substrates	Frequency of variant alleles	Functional effects	Clinical effects
CYP1A1	Carcinogens	Relatively high	Unproven	No
CYP1A2	Drugs	High	Polymorphic induction	Yes
	Carcinogens		Rare alleles yielding less expr	
CYP1B1	Carcinogens	Rare null alleles	7 haplotypes with similar activity	Yes, glaucoma
	Oestrogens	Frequent missense mutations		
CYP2A6	Nicotine, drugs, high in orientals	Important for	Perhaps	
	Carcinogens	Less frequent in Caucasians	Nicotine metabolism	
CYP2B6	Drugs	Relatively low	Reduced drug metabolism	Yes
CYP2C8	Some drugs	High	Taxol metabolism	(Yes)
CYP2C9	Drugs	Relatively low	Very significant	Yes
CYP2C19	Drugs	High	Very significant	Yes
CYP2D6	Drugs	Very significant	Yes	
CYP2E1	Carcinogens, solvents, some drugs	High	Not shown	No
CYP3A4	Drugs, carcinogens	Low	No importance of polymorphism	No
CYP3A5	Drugs	High	No expression	No

P450 genes. The rapid development in the field required the establishment of a web page http://www.imm.ki.se/CYPalleles/ with continuously updated information and recommended nomenclature regarding the various allelic forms of P450s. The aims were to encourage scientists worldwide to speak the same language and to avoid "home-made" allelic designations that can confuse the nomenclature system and the scientific literature. In addition, a rapid publication of new alleles would prevent unnecessary doubling of research efforts aimed at characterising alleles already described. Currently it covers the nomenclature for polymorphic alleles of *CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP3A7, CYP4B1, CYP5A1, CYP8A1* and *CYP21*, although in the nearest future nomenclature systems for all polymorphic CYPs are going to be incorporated. Letters have been published in *Pharmacogenetics* and *Cancer Epidemiology, Biomarkers and Prevention* describing some general decisions taken with respect to the nomenclature system and features of the page (Ingelman-Sundberg et al. 2000, 2001).

The important polymorphisms of CYP1A2, CYP2A6, CYP2Cs, CYP2D6 are covered in other contributions in this series whereas here I focus on the polymorphism of P450s 1B1, 2B6, 2E1, 3A4 and 3A5.

CYP1B1

The human CYP1B1 gene encompasses three exons with the coding region starting in exon 2 (Tang et al. 1996). Six different single nucleotide polymorphic sites (SNPs) have previously been reported in the human CYP1B1 gene (Stoilov et al. 1998) of which four cause amino acid substitutions as well as many rare alleles with deleterious mutations causing an inactive enzyme. Because of the tight linkage between the occurrence of these detrimental mutations and congenital glaucoma, Stoilov et al. (1997) have implicated CYP1B1 to be a candidate gene for congenital glaucoma associated with the GLC3A locus on chromosome 2. Thus, CYP1B1 may have a role in the metabolism of oxidised growth-effector molecules. Knock out mice for Cyp1b1 indeed develop a form of glaucoma.

Seven common CYP1B1 alleles have been identified so far. Initially *CYP1B1*2* with two linked polymorphisms in exon 2, resulting in the Arg48Gly and Ala119Ser amino acid substitutions, respectively, *CYP1B1*3*, with Leu432Val exchange, and *CYP1B1*4* with Asn453Ser were described (see http://www.imm.ki.se/CYPalleles/cyp1b1.htm). The role of these allelic variants for the metabolism of oestradiol and polyaromatic hydrocarbons has been investigated with variable results (Shimada et al. 1999; McLellan et al. 2000) and their linkage to different forms of cancers, in particular breast cancer, been analysed. One might conclude that none of these alleles would be of any major importance with respect to altered function in vivo or in vitro of the CYP1B1 enzyme.

One fundamental question is which apparent CYP1B1 haplotypes that are present since mutations in combination might affect the function of the enzyme, as seen e.g. with the CYP2D6.17 enzyme, where two mutations have to be present together in order to cause the altered function of the enzyme seen in vivo or in vitro. Aklilu et al. (2002) screened mutations in 150 Ethiopians and found one additional missense mutation (Ala435Thr) yielding in total 32 different possible haplotypes. The development of a haplotype analysis revealed that 7 of them existed in the population (see Table 6) and that two of them, CYP1B1.6 (having Arg48Gly, Ala119Ser and Leu432Val) as well as CYP1B1.7 (with Arg48Gly, Ala119Ser, Leu432Val and Ala435Thr) caused an enzyme with impaired intrinsic clearance for oestradiol to 20% of the wild type enzyme capacity. These results emphasise the necessity to analyse function and e.g. cancer risk in relation to enzyme haplotype instead of to single SNPs. The distribution of these haplotypes in the population turned out to be very complex and indeed only one individual out of 150 was homozygous for the wild type allele (*CYP1B1*1*). The complexity of the haplotype distribution and the relatively small effects seen on function indicates that the polymorphism of CYP1B1 might not be strongly related to altered risk for cancer in relation to either smoking and exposure for polyaromatic hydrocarbons or oestradiol in a life long manner.

CYP2B6

The hepatic level of CYP2B6 is known to vary considerable between individuals and in many studies apparently no expression of the protein was seen. However, by using better antibodies and higher sensitivity for detection on Western-blotting analyses it has been apparent that most

Table 6 Common variants of the human *CYP1B1* gene

Allele	Nucleotide change	Amino acid substitutions	Trivial name
*CYP1B1*1*	None		wt
*CYP1B1*2*	142C>G; 355G>T	R48G+ A119S	m1+m2
*CYP1B1*3*	4326C>G	L432V	m3
*CYP1B1*4*	4390A>G	N453S	m4
*CYP1B1*5*	142C>G; 4326C>G	R48G+L432V	m1+m3
*CYP1B1*6*	142C>G; 355G>T; 4326C>G	R48G+A119S+L432V	m1+m2+m3
*CYP1B1*7*	142C>G; 355G>T; 4326C>G; 4360C>G	R48G+A119S+L432V+A443G	m1+m2+m3+m5

livers express some protein. The basis for an important part of the genetics behind the interindividual differences in expression was recently worked out by Zanger and collaborators (Lang et al. 2001). They found six new variant alleles having 5 different missense mutations in combinations. The three most common mutations Gln172His, Lys262Arg and Arg487Cys were present at frequencies of 28, 32 and 14%, respectively among 215 individuals. Haplotype analysis revealed that these could exist in combinations yielding four different alleles (*CYP2B6*4*, **5*, *6* and *7*). In addition, *CYP2B6*2* (with Arg22Cys) and CYP2B6*3 (with Ser 259Arg) existed with allele frequencies of 5 and 0.5%, respectively. Analysis of hepatic expression showed that the Arg487Cys mutation caused lower expression, whereas the other haplotypes were not associated with lower expression. The important interindividual variations in *CYP2B6* seen independent on these polymorphic sites might indicate the presence of additional genetic variants of *CYP2B6* to be identified.

CYP2E1

Initially, several polymorphisms in human *CYP2E1* gene were detected by RFLP by various restriction enzymes (see http://www.imm.ki.se/cypalleles/cyp2e1.htm for references). In these polymorphisms (currently termed *CYP2E1*1B*, *CYP2E1*5B*, and *CYP2E1*6*) the mutations have been shown to locate in either introns or in the 5'-flanking region. There are contradictory results as to whether these polymorphisms correlate to the in vitro transcriptional rate. The incidence of these polymorphisms has been investigated in relation to different type of cancer, alcoholic liver disease and to alcoholism. However the results are controversial and no firm conclusions can be drawn.

A polymorphic 100 bp insertion was found in the 5'-flanking region of the *CYP2E1* gene in some individuals by using RFLP analysis with different restriction enzymes. The region was limited to a 600 bp region, –2,270 to –1,672 (McCarver et al. 1998). The insertion was associated with increased chlorzoxazone hydroxylation in obese patients and subjects drinking ethanol in a relatively small number of subjects. The basis for the insertion polymorphism was shown to be repeat sequence present in 5, 6 or 8 copies (Hu et al. 1999). It is unlikely that this polymorphism is of significance with respect to altered levels of expression of CYP2E1 carrying various numbers of repeats.

SSCP screening of 200 individuals of Chinese and Caucasian origin revealed very few variant alleles with functional mutations. Indeed only three subjects exhibited a CYP2E1 gene with an altered open reading frame and two variant alleles, *CYP2E1*2* with Arg76His and *CYP2E1*3* with Val389Ile was found. The *CYP2E1*2* allele was found to cause only 40% of the activity of the *CYP2E1*1* allele, when expressed in COS 1 cells (Hu et al. 1997).

Several mutations in the *CYP2E1* gene have also been identified recently, at positions –316A>G, –297T>A, –35G>T, 1107G>C in intron 1, 4804G>A creating Val179Ile in exon 4 and 10157C>T in exon 8 (Fairbrother et al. 1998). The allele frequencies were low (0.013 to 0.052), with the exception of –297T>A) which had an allele frequency of 0.20. A reporter gene containing both G(–35)T/T(–297)A mutations had a 1.8-fold and 2.5-fold higher luciferase activity compared to the wild-type and –297T>A only, respectively, whereas the rest of mutations have no functional significance for CYP2E1 expression (Fairbrother et al. 1998). In conclusion, it appears that CYP2E1 is well conserved, possibly due to high endogenous importance in gluconeogenesis during conditions of severe fasting.

CYP3A4

In general there is considerable interindividual variation in the expression and activity of CYP3A4 in human liver. Investigations have suggested that the majority (90%) of this variation has its origin in genetic factors (Ozdemir et al. 2000). A lot of efforts have been to sequence the gene from many hundreds of individuals, but generally only very rare polymorphic sites have been identified. Today 19 different alleles with missense mutations have been described (http://www.imm.ki.se/CYPalleles/cyp3a4.htm) and some alleles with mutations in the 5'-upstream regulatory region of unclear importance. Only a few of them have been shown to alter the properties of the enzyme. *CYP3A4*2* with S222P and *CYP3A4*12* with L373F yields an enzyme with slightly altered substrate specificity, *CYP3A4*8* having R130Q and *CYP3A4*13* with P416L cause no expression of P450 holoprotein in bacteria and one *CYP3A4*11* with T363 M is expressed at lower levels, whereas many of the allelic variants did not cause any altered function in vitro of the enzyme (Eiselt et al. 2001). Furthermore, recent screening for the presence of these variants have revealed that in a population of 500 Caucasians only the *CYP3A4*3* allele was found at a significant frequency (1%) whereas all other forms were essentially absent including *CYP3A4*2* which appears to be a sequencing artefact (van Schaik et al. 2003). Thus, the enzyme is extremely well preserved. The reason behind this appears to be the major role of this enzyme in the metabolism of dietary and other environmental factors. It can be assumed that in the past a selection pressure has been present not allowing survival of subjects homozygous for defect *CYP3A4* alleles. There is a need to look for genetic variations in genes encoding proteins participating in the regulation of CYP3A4 transcription, e.g. of PXR (Hustert et al. 2001) in addition to genes determining the control the posttranslational regulation of the protein, in order to find any possible genetic basis for the very high interindividual variability in the amount and inducibility of CYP3A4 among humans.

CYP3A5

As mentioned the polymorphism of the *CYP3A5* gene has been claimed to be relevant with respect to the interindi-

vidual variability seen in the expression of the enzyme (Kuehl et al. 2001). Genotyping analysis for the mutations in the introns creating the splicing defects (*CYP3A5*3* and *CYP3A5*6*) would predict the expression to a great extent. However Koue and collaborators have also recently described some additional defect variants (see http://www.imm.ki.se/CYPalleles/cyp3a5.htm). By contrast, other investigator have found no influence of the presence or the absence of CYP3A5 on the clearance of midazolam (Floyd et al. 2003) and found only a contribution of 2% of CYP3A5 to the overall content of CYP3A in Caucasian livers (Westlind-Johnsson et al. 2003).

Conclusions on CYP polymorphism

The polymorphisms of CYP2C9, CYP2C19 and CYP2D6 are highly relevant for the metabolism of clinically important drugs and influence responsiveness and adverse drug reactions (see e.g. Ingelman-Sundberg 2001; Weinshilboum 2003). By contrast, the genetic polymorphism of the enzymes here covered with some exception for CYP2B6, are much less penetrant as to explain any important variation in drug metabolism or drug toxicity caused by inheritance. In most cases this is a reflection of the high importance of these enzymes for the human subjects.

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