Polymorphisms of CYP2A6 and its practical consequences

Hannu Raunio¹, Arja Rautio², Harriet Gullstén² & Olavi Pelkonen²

¹Department of Pharmacology and Toxicology, University of Kuopio, Box 1627, 70211 Kuopio and ²Department of Pharmacology and Toxicology, University of Oulu, Box 5000, 90014 Oulu, Finland

CYP2A6 is an hepatic enzyme predominantly with some expression in specialized extrahepatic cell types. The CYP2A6 enzyme has a somewhat restricted active site, accepting only a few xenobiotics as substrates. Interest in CYP2A6 has risen considerably after nicotine and some tobacco specific nitrosamines were established as high-affinity substrates for this enzyme. Recently, the organization and structures of the *CYP2A* gene cluster and several polymorphic alleles of the *CYP2A6* gene have been characterized. Two alleles with a point mutation and at least three different types of gene deletion, all leading to deficient gene function, have been found. The frequencies of these alleles vary considerably among different ethnic populations, the deletion alleles being most common in Orientals (up to 20%). The frequency of point mutations are low in all populations studied thus far (<3%). Several case-control studies have addressed the relationship between CYP2A6 status and smoking habits as well as the role of CYP2A6 polymorphism in lung cancer risk. Studies in Japanese suggest that CYP2A6 poor metabolizer genotypes result in altered nicotine kinetics and may lower cigarette smoking elicited lung cancer risk, whereas similar studies in Caucasian populations have not revealed any clear associations between variant CYP2A6 genotypes and smoking behaviour or lung cancer predisposition.

Keywords: coumarin, lung cancer, nicotine, tobacco smoke

Introduction

The cytochrome P450 (CYP) enzymes play a crucial role in the metabolism of exogenous compounds including pharmaceutical agents. It is estimated that about one half of drugs in common use are primarily cleared from the body through the action of CYP enzymes [1]. About 40% of human CYP-dependent drug metabolism is carried out by polymorphic enzymes, which can result in altered drug pharmacokinetics. Several examples exist where subjects carrying certain variant alleles suffer from adverse effects from drug treatment due to the presence of defective alleles [2]. The most thoroughly characterized CYP polymorphic genes are *CYP2C9*, *CYP2C19*, and *CYP2D6*. Recently, a functional mutation has also been found in the *CYP3A4* gene [3], which is a key CYP enzyme in drug metabolism due to its abundance in human liver and the large number of substrate drugs [4]. Many of the

polymorphic CYP enzymes also metabolize numerous toxicologically significant compounds, such as food additives, pollutants, solvents, and recreational drugs, many of which cause acute or delayed toxic effects [5]. During the last few years a substantial amount of new information on the genetic regulation of CYP2A6 has been uncovered. This review will summarize the key features of this polymorphism and its practical consequences.

CYP2A6 substrates and inhibitors

The CYP2A6 enzyme participates in the metabolism of several compounds that for practical purposes can be categorized as pharmaceuticals and toxic compounds. Some of the most relevant CYP2A6 substrates are summarized in Table 1. Compared with CYP2D6 and especially CYP3A4, relatively few clinically used pharmaceuticals are metabolized to a significant degree by the CYP2A6 enzyme. Of the substrates listed in Table 1, the most selective for CYP2A6 are coumarin, a compound present in some plants, SM-12502, a novel platelet-activating factor receptor antagonist, and nicotine. Most of the other

Correspondence: Hannu Raunio, Department of Pharmacology and Toxicology, University of Kuopio, Box 1627, 70211 Kuopio, Finland. Tel.: +358 17 162406; Fax: +358 17 162424; E-mail: hannu.raunio@uku.fi

Received 20 July 2000, accepted 12 July 2001.

Table 1 CYP2A6 substrates.

Drugs	Assay/end-point	Toxic agents	Assay/end-point
Coumarin	7-hydroxylation	Nicotine	N-1'-oxidation
Methoxyflurane	Dehalogenation	Cotinine	3'-hydroxylation
Halothane	Reduction	NNK	Mutagenicity
SM-12502	S-oxidation	NDEA	Mutagenicity
Losigamone	Oxidation	AFB ₁	Mutagenicity
Valproic acid	Oxidation	MOCA	N-oxidation
Letrozole	Oxidation	1,3-butadiene	Monoxide formation
Disulfiram	Sulfoxidation	Quinoline	1-oxidation
		DCBN	Protein adduct formation
		MTBE	O-demethylation

SM-12502, 3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride; NNK, 4-methylnitrosamino-1-(3-pyridyl)-1-butanone; NDEA, N-nitrosodiethylamine; AFB₁, aflatoxin B₁; MOCA, 4,4'-methylenebis(2-chloroaniline); DCBN, 2,6-dichlorobenzonitrile; MTBE, methyl tert-butyl ether. References [6, 7, 56–59].

xenobiotics listed in Table 1 are only partially metabolized by CYP2A6, and in most cases the kinetics of metabolism have not been worked out thoroughly [6, 7]. For example, halothane oxidation is catalysed by CYP2E1 and CYP2A6 as the low and high K_m enzymes, respectively, with no role for CYP3A4. In contrast, reduction of halothane is mediated by CYP2A6 and CYP3A4 as the low and high K_m enzymes, respectively, with no role for CYP2E1 [8].

Coumarin is a naturally occurring compound which has been extensively used in some European countries as a natural remedy for various conditions including lymphoedema. Very high doses of coumarin have also been used as adjuvant therapy in some forms of cancer [9]. Both *in vitro* and *in vivo* studies have shown that coumarin is very specifically metabolized by CYP2A6, and coumarin is thus used as a preferred *in vivo* probe drug for phenotyping for CYP2A6 [10, 11, 12].

CYP2A6 is the high-affinity metabolizer of both nicotine and its oxidized metabolite cotinine. Studies with heterologously expressed CYP enzymes and human liver microsomes *in vitro* have revealed the key role of CYP2A6 in the catabolism of nicotine (Figure 1). In this two-step reaction, nicotine is first oxidized to nicotine iminium ion and subsequently to cotinine by cytosolic aldehyde oxidase. The CYP2A6-mediated formation of nicotine iminium ion from nicotine is the rate limiting step in this reaction, and up to 80% of nicotine is metabolized to cotinine [13–17]. Further downstream of this metabolic cascade, cotinine is oxidized to several metabolites by CYP enzymes, including possibly CYP2A6 [18, 19]. CYP2A6 appears to be the sole catalyst of nicotine catabolism at low, physiologically relevant nicotine concentrations (50 μM). At high nicotine concentrations (500 μM),

Figure 1 Participation of CYP2A6 in the oxidative metabolism of nicotine and cotinine.

other CYP isoforms, such as CYP2B6, also participate in nicotine oxidation [20].

Several compounds have been tested for their inhibitory effects on the CYP2A6 enzyme *in vitro*. Compounds that have been found to be relatively potent, although not necessarily highly selective CYP2A6 inhibitors include methoxsalen (8-methoxypsoralen) [21–23], menthofuran [24], pilocarpine [25] and the monoamine oxidase inhibitor tranylcypromine [Taavitsainen *et al.* unpublished]. Of these inhibitors, methoxsalen can be used to suppress CYP2A6 function *in vivo* [26]. The reduction in CYP2A6 activity *in vivo* caused by a single 50 mg dose of methoxsalen is, however, rather weak and transient [27].

CYP2A gene cluster and expression

The *CYP2A6* gene was mapped to chromosome 19 where it is located within a 350-bp gene cluster together with the *CYP2A7* and *CYP2A13* genes and genes in the *CYP2B* and *CYP2F* subfamilies [28]. Figure 2 depicts the general organization of this gene cluster, which contains the known functional *CYP2A6*, *CYP2B6* and *CYP2F1* genes, the known nonfunctional genes *CYP2A7* and *CYP2B7*, as well as several pseudogenes. The homology between *CYP2A6* and *CYP2A7* genes is 94%, but the *CYP2A7* protein lacks one of the heme-binding residues which results in defective incorporation of heme and an inactive enzyme [29, 30]. Full-length *CYP2A13* cDNA has recently been isolated; the encoded protein efficiently catalyses the metabolic activation of the tobacco specific procarcinogen 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK) [31].

CYP2A6 is mainly expressed in the liver, where it represents 1–10% of the total liver CYP content [7]. Minor

Figure 2 Scheme of the *CYP2A-2B-2F* gene cluster. A cluster spanning 350 kb is shown. The directions of transcription are indicated by arrows. P denotes a pseudogene. Redrawn from [28, 39].

Table 2 *CYP2A6* allele nomenclature.

Allele	Nucleotide change	Trivial name	Effect	Enzyme activity
CYP2A6*1A	none			normal
CYP2A6*1B			gene conversion in 3' flanking region	normal
CYP2A6*2	479T>A	v1	L160H	absent
CYP2A6*3	2A6/2A7 hybrid	v2	?	?
CYP2A6*4A	gene deletion	CYP2A6del	deletion	absent
CYP2A6*4B	gene deletion	D-type	deletion	absent
CYP2A6*4C	gene deletion	E-type	deletion	absent
CYP2A6*4D	gene deletion		deletion	absent
CYP2A6*5	1436G>T		G479V	absent

Source: <http://www.imm.ki.se/CYPalleles>.

amounts of CYP2A6 are also found in the nasal mucosa [32, 33] and bronchial epithelial cells [34]. CYP enzymes orthologous to CYP2A6 have been detected at relatively high concentrations in the olfactory tissues of several animal species, indicating that these enzymes could participate in the metabolism of odourants and the processing of olfactory signals [35, 36]. Interestingly, a recent report shows that both CYP2A6 and CYP2A13 are present in the olfactory mucosa of human fetuses [37].

CYP2A6 polymorphism

Phenotyping and *in vitro* studies carried out before knowledge of the underlying genetic mechanisms indicated that the prevalence of CYP2A6 poor metabolizers in the Caucasian population is $\leq 1\%$, and that the prevalence is much more common in Orientals, up to 20% of the population [11, 38]. Several variant alleles of the *CYP2A6* gene have been characterized, beginning about 10 years ago when the sequences of the wild-type and one variant allele were reported [29]. The first variant allele characterized was a point mutation resulting in leucine to histidine conversion in codon 160. This allele, later designated CYP2A6*2, encodes a protein devoid of catalytic

Figure 3 Scheme for the *CYP2A6* alleles. The shaded and un-shaded parts denote *CYP2A7* and *CYP2A6* sequences, respectively. The two alleles with point mutations are CYP2A6*2 and CYP2A6*5. As a result of various cross-over events, the whole *CYP2A6* gene is deleted (CYP2A6*4A). The CYP2A6*1B and CYP2A6*5 alleles contain *CYP2A7* sequences at the 3' ends of the gene. Adapted from [40, 43].

activity [29]. A few years later, another allele (CYP2A6*3) was reported, and genotyping methods were described for detecting both CYP2A6*2 and CYP2A6*3 alleles [39]. Very recently, improvements have been made to the original genotyping methods and new variant alleles of CYP2A6 have been found. The current status of CYP2A6 alleles is summarized in Table 2 and Figure 3.

Table 3 Interethnic distribution of CYP2A6 alleles.

Population	CYP2A6*1A/B ¹	CYP2A6*2	CYP2A6*4 ²	CYP2A6*5
Caucasian ³	96.5–98.9%	2.3–3.0%	0.5–1.0%	0%
Chinese	83.8%	0–0.7%	15.1%	1.0%
Japanese ⁴	about 80%	ND	about 20%	ND

¹Combined CYP2A6*1A and CYP2A6*1B.²Combined CYP2A6*4A–D.³Finnish, Spanish, Swedish, American.⁴The data on Japanese is less complete than in Caucasian and Chinese populations.

ND, not determined.

Data compiled from [12, 40, 41, 43, 46].

CYP2A6*3, originally thought to be a true variant allele, has later been shown to be lacking in different populations [40–42]. One reason for the original misclassification turned out to be due to the very common CYP2A6*1B allele exhibiting a gene conversion between 3' flanking regions of the CYP2A6 and CYP2A7 genes [43]. As a result of this, CYP2A7 sequences are also amplified when the original genotyping method [39] is used, resulting in misclassification of this allele. Several of the CYP2A6 alleles have been created by unequal crossover events. For example, the CYP2A6*4A deletion allele is apparently generated in an unequal crossover between the 3'-flanking regions of the CYP2A6 and CYP2A7 genes. In the CYP2A6*4D allele, the crossover region is located in intron 8 or exon 9 [40]. It is likely that some of the four deletion alleles listed as being separate entities in Table 2 are actually the same.

The two different point mutations found (CYP2A6*2 and CYP2A6*5) abolish the catalytic activity of the encoded enzyme, as do the gene deletions [12, 43]. When phenotyped with the coumarin test, individuals homozygous for the CYP2A6*2 allele totally lack coumarin 7-hydroxylation capacity, and heterozygosity for the CYP2A6*2 allele confers an intermediate metabolizer phenotype [12, 44]. As expected, individuals homozygous for the CYP2A6*4 deletion allele do not have coumarin 7-hydroxylation capacity in the phenotyping test [40].

The frequencies of the major CYP2A6 alleles in different ethnic populations are listed in Table 3. The allelic frequencies of CYP2A6*2 are rather low (<3%) in Caucasian and Chinese populations [12], whereas the CYP2A6*4A allele is fairly common in Chinese and Japanese [40, 45]. The CYP2A6*5 allele is rare, with a frequency of 0–1% in Caucasian and Chinese populations [43]. Overall, the frequencies of all the major variant alleles of CYP2A6 are rather uncommon in Caucasians, while some of these alleles are highly prevalent in Oriental populations.

Consequences of CYP2A6 polymorphisms

Due to the limited number of CYP2A6 substrates that are used as drugs, CYP2A6 polymorphisms are not significant factors in drug therapy. There are, however, striking examples of aberrant pharmacokinetics of CYP2A6 substrate drugs in poor metabolizers. *In vitro* studies have shown that the drug SM-12502 is metabolised very slowly in liver microsomes obtained from individuals homozygous for the CYP2A6*4B allele [45]. An *in vivo* study in Japanese individuals revealed that three out of the 28 study subjects were poor metabolizers of SM-12502, and that all three individuals were homozygous for the CYP2A6*4C deletion allele [46].

Greater emphasis is now placed on avoiding the unwanted consequences of altered drug kinetics resulting from metabolism via polymorphic CYP enzymes. The pharmaceutical industry therefore screens for CYP mediated metabolic patterns of their drug candidates, and compounds that are extensively metabolized through polymorphic CYPs are looked at more critically than previously [47]. As illustrated by CYP2A6, CYP2C19, and CYP2D6 polymorphisms, there are very significant interethnic variations in the frequencies of variant drug metabolizer phenotypes [2]. Therefore, regulatory agencies may soon require clinical drug studies to be performed in more than one ethnic population before approval for world-wide use.

The ability of CYP2A6 to metabolize nicotine and several procarcinogens present in cigarette smoke makes this polymorphism particularly interesting. Recent studies have shown that the kinetics of nicotine metabolism are altered in individuals carrying the variant CYP2A6 alleles. *In vitro* studies with human liver microsomes have indicated that the nicotine C-oxidation $V_{\max}$ to K_m ratios are clearly lowered by the CYP2A6*1/CYP2A6*2 genotype [48]. Japanese individuals homozygous for a CYP2A6 gene deletion had only 15% of the levels of cotinine in urine when compared with subjects having at least one active gene, after smoking an equal number of cigarettes [42]. Another study showed that an individual carrying a homozygous deletion of the CYP2A6 gene showed no detectable levels of cotinine in plasma after smoking [49].

Soon after the discovery of CYP2A6 polymorphism, it was hypothesized that CYP2A6 poor metabolizers could differ in their smoking habits from extensive metabolizers, and that this polymorphism could be associated with increased risk of acquiring cigarette smoke induced lung cancer [50, 51]. Initial studies [50, 51] testing this hypothesis were carried out using the first published genotyping method [39], which gives an overestimate of CYP2A6*2 allele frequencies and also detects the (probably) non-existent CYP2A6*3 allele. Thus, the genotyping results obtained in these studies [50, 51] did not reflect the actual

situation and the results need to be interpreted with caution.

Several studies are being currently carried out with improved genotyping methods to test whether there is an association between CYP2A6 poor metabolizer genotypes and smoking habits. Initial results of some of these studies are now available, and they do not demonstrate any clear association between cigarette consumption and CYP2A6 defective alleles [unpublished observations]. There is also an interest in modulating smoking behaviour by inhibitors of CYP2A6, since pilot studies have shown that CYP2A6 inhibition alone or combined with oral nicotine could decrease smoking [52].

Another intriguing facet of CYP2A6 is its capacity to activate several procarcinogens present in tobacco smoke and diet, such as nitrosamines and aflatoxins. For example, the potent pulmonary carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is metabolically activated by this enzyme [53]. Theoretically, CYP2A6 poor metabolizers could be protected from developing cancer at the target sites of these carcinogens, mainly lung and liver. A study in Spanish patients with liver cancer and cirrhosis showed a protective effect of the CYP2A6 poor metabolizer genotype on liver cirrhosis but not cancer [54]. Since this study was conducted using the original genotyping method [39], the result needs to be verified.

There is thus a biologically plausible link between the CYP2A6 polymorphism and lung cancer predisposition. On the one hand, the CYP2A6 poor metabolizer phenotype could influence cigarette smoking habits, and on the other, an increased rate of procarcinogen activation could occur in CYP2A6 extensive metabolizers. Consistent with this hypothesis, a pilot case-control study [55] in a Japanese population showed that the frequency of subjects homozygous for the CYP2A6 gene deletion was lower in lung cancer patients ($n=492$) than in healthy control subjects ($n=402$), with an odds ratio of 0.25 (95% CI 0.08–0.83). In an allele based analysis, there was also a significant decrease in the odds ratio for the deletion allele [55]. Several on-going studies in various ethnic groups are addressing the putative link between CYP2A6 polymorphisms and lung cancer. Thus far, case-control studies in Finnish, French and Chinese populations have revealed no associations between lung cancer incidence and CYP2A6 status [unpublished observations].

Since there are major differences in the frequencies of the CYP2A6 variant alleles between various ethnic groups, the practical impacts of this polymorphism will probably be different in different populations. Due to the paucity of CYP2A6 poor metabolizers in the Caucasian populations, it is highly unlikely that this polymorphism will have any major effect on disease susceptibility at the population level. The situation is totally different in the Orientals,

where a much larger proportion of the population are CYP2A6 poor metabolizers.

In conclusion, several polymorphisms of the CYP2A6 gene have been characterized. It is likely that most of the CYP2A6 alleles leading to the poor metabolizer phenotype have already been identified. Nevertheless, several more silent mutations and alleles encoding functionally altered enzyme activities are probably yet uncovered. The relationship between CYP2A6 polymorphisms and smoking behaviour and chemically induced diseases, especially tobacco smoke elicited cancers, is a field of on-going research. Since many studies addressing these issues are being carried out, we will soon have more definitive answers to these interesting questions.

Studies in the laboratory of the authors have been supported by the Finnish Academy of Sciences. We thank Drs Mikael Oscarson and Magnus Ingelman-Sundberg for comments on the manuscript.

References

- 1 *Handbook of Drug Metabolism*, ed. Woolf TF. Marcel Dekker, 1999: New York.
- 2 Ingelman-Sundberg M, Oscarson M, McLellan RA. Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. *Trends Pharmacol Sci* 1999; **20**: 342–349.
- 3 Sata F, Sapone A, Elizondo G, *et al.* CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. *Clin Pharmacol Ther* 2000; **67**: 48–56.
- 4 Guengerich FP. Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol* 1999; **39**: 1–17.
- 5 Nebert DW, Ingelman-Sundberg M, Daly AK. Genetic epidemiology of environmental toxicity and cancer susceptibility: human allelic polymorphisms in drug-metabolizing enzyme genes, their functional importance, and nomenclature issues. *Drug Metab Rev* 1999; **31**: 467–487.
- 6 Chang TKH, Waxman DJ. The CYP2A subfamily. In *Cytochromes P450. Metabolic and Toxicological Aspects*, ed. Ioannides C. Boca Raton: CRC Press, 1996: 99–134.
- 7 Pelkonen O, Rautio A, Raunio H, Pasanen M. CYP2A6: a human coumarin 7-hydroxylase. *Toxicology* 2000; **144**: 139–147.
- 8 Spracklin DK, Kharasch ED. Human halothane reduction *in vitro* by cytochrome P450 2A6 and 3A4: identification of low and high K_m isoforms. *Drug Metab Dispos* 1998; **26**: 605–607.
- 9 *Coumarins. Biology, Applications, Mode of Action*, eds O'Kennedy R, Thornes RD. John Wiley & Sons, 1997: Chichester.
- 10 Cholerton S, Idle ME, Vas A, Gonzalez FJ, Idle JR. Comparison of a novel thin-layer chromatographic-fluorescence detection method with a spectrofluorometric method for the determination of 7-hydroxycoumarin in human urine. *J Chromatogr* 1992; **575**: 325–330.
- 11 Rautio A, Kraul H, Kojo A, Salmela E, Pelkonen O. Interindividual variability of coumarin 7-hydroxylation in healthy individuals. *Pharmacogenetics* 1992; **2**: 227–233.

- 12 Oscarson M, Gullstén H, Rautio A, *et al.* Genotyping of human cytochrome P450 2A6 (CYP2A6), a nicotine C-oxidase. *FEBS Lett* 1998; **438**: 201–205.
- 13 Cashman JR, Park SB, Yang Z-C, *et al.* Metabolism of nicotine by human liver microsomes: stereoselective formation of *trans*-nicotine *N'*-oxide. *Chem Res Toxicol* 1992; **5**: 639–646.
- 14 McCracken NW, Cholerton S, Idle J. Cotinine formation by cDNA-expressed human cytochromes P450. *Med Sci Res* 1992; **20**: 877–878.
- 15 Berkman CE, Park SB, Wrighton SA, Cashman JR. *In vitro*–*in vivo* correlations of human (S)-nicotine metabolism. *Biochem Pharmacol* 1995; **50**: 565–570.
- 16 Nakajima M, Yamamoto T, Nunoya K-I, *et al.* Role of human cytochrome P4502A6 in C-oxidation of nicotine. *Drug Metab Dispos* 1996; **24**: 1212–1217.
- 17 Messina ES, Tyndale RF, Sellers EM. A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. *J Pharmacol Exp Ther* 1997; **282**: 1608–1614.
- 18 Nakajima M, Yamamoto T, Nunoya K-I, *et al.* Characterization of CYP2A6 involved in 3'-hydroxylation of cotinine in human liver microsomes. *J Pharmacol Exp Ther* 1996; **277**: 1010–1015.
- 19 Murphy SE, Johnson LM, Pullo DA. Characterization of multiple products of cytochrome P450 2A6-catalyzed cotinine metabolism. *Chem Res Toxicol* 1999; **12**: 639–645.
- 20 Yamazaki H, Inoue K, Hashimoto M, Shimada T. Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. *Arch Toxicol* 1999; **73**: 65–70.
- 21 Mäenpää J, Sigusch H, Raunio H, *et al.* Differential inhibition of coumarin 7-hydroxylase activity in mouse and human liver microsomes. *Biochem Pharmacol* 1993; **45**: 1035–1042.
- 22 Draper AJ, Madan A, Parkinson A. Inhibition of coumarin 7-hydroxylase activity in human liver microsomes. *Arch Biochem Biophys* 1997; **341**: 47–61.
- 23 Koenings LL, Trager WF. Mechanism-based inactivation of P450 2A6 by furanocoumarins. *Biochemistry* 1998; **37**: 10 047–10 052.
- 24 Khojasteh-Bakht SC, Koenings LL, Peter RM, Trager WF, Nelson SD. (R)-(+)-Menthofuran is a potent, mechanism-based inactivator of human liver cytochrome P450 2A6. *Drug Metab Dispos* 1998; **26**: 701–704.
- 25 Kimonen T, Juvonen RO, Alhava E, Pasanen M. The inhibition of CYP enzymes in mouse and human liver by pilocarpine. *Br J Pharmacol* 1995; **114**: 832–836.
- 26 Mäenpää J, Juvonen R, Raunio H, Rautio A, Pelkonen O. Methoxsalen and coumarin interactions in man and mouse. *Biochem Pharmacol* 1994; **48**: 1363–1369.
- 27 Kharasch ED, Hankins DC, Taraday JK. Single-dose methoxsalen effects on human cytochrome P-450 2A6 activity. *Drug Metab Dispos* 2000; **28**: 28–33.
- 28 Hoffinan SMG, Fernandez-Salguero P, Gonzalez FJ, Mohrenweiser HW. Organization and evolution of the cytochrome P450 CYP2A–2B–2F subfamily gene cluster on human chromosome 19. *J Molec Evolut* 1995; **41**: 894–900.
- 29 Yamano S, Tatsuno J, Gonzalez FJ. The CYP2A3 gene product catalyzes coumarin 7-hydroxylation in human liver microsomes. *Biochemistry* 1990; **29**: 1322–1329.
- 30 Ding S, Lake BG, Friedberg T, Wolf CR. Expression and alternative splicing of the cytochrome P-450 CYP2A7. *Biochem J* 1995; **306**: 161–166.
- 31 Hong J-Y, Ding X, Su T, Smith TJ, Bao Z. Metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by human cytochrome P4502A13. *Proc Am Assoc for Cancer Res* 2000; **41**: 840.
- 32 Su T, Sheng JJ, Lipinskas TW, Ding X. Expression of CYP2A genes in rodent and human nasal mucosa. *Drug Metab Dispos* 1996; **24**: 884–890.
- 33 Koskela S, Hakkola J, Hukkanen J, *et al.* Expression of CYP2A genes in human liver and extrahepatic tissues. *Biochem Pharmacol* 1999; **57**: 1407–1413.
- 34 Crawford EL, Weaver DA, DeMuth JP, *et al.* Measurement of cytochrome P450 2A6 and 2E1 gene expression in primary human bronchial epithelial cells. *Carcinogenesis* 1998; **19**: 1867–1871.
- 35 Brittebo EB. Metabolism-dependent activation and toxicity of chemicals in nasal glands. *Mutat Res* 1997; **380**: 61–75.
- 36 Thornton-Manning JR, Dahl AR. Metabolic capacity of nasal tissue interspecies comparisons of xenobiotic-metabolizing enzymes. *Mutat Res* 1997; **380**: 43–59.
- 37 Gu J, Su T, Chen Y, Zhang QY, Ding X. Expression of biotransformation enzymes in human fetal olfactory mucosa: potential roles in developmental toxicity. *Toxicol Appl Pharmacol* 2000; **165**: 158–162.
- 38 Shimada T, Yamazaki H, Guengerich FP. Ethnic-related differences in coumarin 7-hydroxylation activity catalyzed by cytochrome P2402A6 in liver microsomes of Japanese and Caucasians populations. *Xenobiotica* 1996; **26**: 395–403.
- 39 Fernandez-Salguero P, Hoffinan SMG, Cholerton S, *et al.* A genetic polymorphism in coumarin 7-hydroxylation: sequence of the human CYP2A genes and identification of variant CYP2A6 alleles. *Am J Hum Genet* 1995; **57**: 651–660.
- 40 Oscarson M, McLellan RA, Gullstén H, *et al.* Characterisation and PCR-based detection of a CYP2A6 gene deletion found at a high frequency in a Chinese population. *FEBS Lett* 1999; **448**: 105–110.
- 41 Chen G-F, Tang YM, Green B, *et al.* Low frequency of CYP2A6 gene polymorphism as revealed by a one-step polymerase chain reaction method. *Pharmacogenetics* 1999; **9**: 327–332.
- 42 Kitagawa K, Kunugita N, Katoh T, Yang M, Kawamoto T. The significance of the homozygous CYP2A6 deletion on nicotine metabolism: a new genotyping method of CYP2A6 using a single PCR-RFLP. *Biochem Biophys Res Commun* 1999; **262**: 146–151.
- 43 Oscarson M, McLellan RA, Gullstén H, *et al.* Identification and characterisation of novel polymorphisms in the CYP2A locus: implications for nicotine metabolism. *FEBS Lett* 1999; **460**: 321–327.
- 44 Hadidi H, Zahlsen K, Idle JR, Cholerton S. A single amino acid substitution (Leu160His) in cytochrome P450 CYP2A6 causes switching from 7-hydroxylation to 3-hydroxylation of coumarin. *Fd Chem Toxicol* 1997; **35**: 903–907.
- 45 Nunoya K-I, Yokoi T, Kimura K, *et al.* A new deleted allele in the human cytochrome P450 2A6 (CYP2A6) gene found in individuals showing poor metabolic capacity to coumarin and (+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride (SM-12502). *Pharmacogenetics* 1998; **8**: 239–249.
- 46 Nunoya K-I, Yokoi T, Kimura K, *et al.* A new CYP2A6 gene deletion responsible for the *in vivo* polymorphic metabolism of

- (+)-cis-3,5-dimethyl-2-(3-pyridyl) thiazolidin-4-one hydrochloride in humans. *J Pharmacol Exp Ther* 1999; **289**: 437–442.
- 47 Kurth JH. Pharmacogenomics: future promise of a tool for identifying patients at risk. *Drug Inform J* 2000; **34**: 223–227.
 - 48 Inoue K, Yamazaki H, Shimada T. CYP2A6 genetic polymorphisms and liver microsomal coumarin and nicotine oxidation activities in Japanese and Caucasians. *Arch Toxicol* 2000; **73**: 532–539.
 - 49 Nakajima M, Yamagishi S, Yamamoto H, *et al.* Deficient cotinine formation from nicotine is attributed to the whole deletion of the CYP2A6 gene in humans. *Clin Pharmacol Ther* 2000; **67**: 57–69.
 - 50 Pianezza ML, Sellers EM, Tyndale RF. Nicotine metabolism defect reduces smoking. *Nature* 1998; **393**: 750.
 - 51 London S, Idle JR, Daly AK, Coetzee GA. Genetic variation of CYP2A6, smoking, and risk of cancer. *Lancet* 1999; **353**: 898–899.
 - 52 Sellers EM, Kaplan HL, Romach MK, Tyndale RF. Inhibition of CYP2A6 decreases smoking. *Clin Pharmacol Ther* 2000; **63**: 153.
 - 53 Gonzalez FJ, Gelboin HV. Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. *Drug Metab Rev* 1994; **26**: 165–183.
 - 54 Gullstén H, Agundez JAG, Benitez J, *et al.* CYP2A6 gene polymorphism and risk of liver cancer and cirrhosis. *Pharmacogenetics* 1997; **7**: 247–250.
 - 55 Miyamoto M, Umetsu Y, Dosaka-Akita H, *et al.* CYP2A6 gene deletion reduces susceptibility to lung cancer. *Biochem Biophys Res Commun* 1999; **261**: 658–660.
 - 56 Raunio H, Rautio A, Pelkonen O. CYP2A6: function, expression and genetic polymorphism. In *Metabolic Polymorphisms and Cancer*, eds Boffetta P, Caporaso N, Cuzick J, Lang M, Vineis P. IARC, 1997: Lyon.
 - 57 Madan A, Parkinson A, Faiman MD. Identification of the human and rat P450 enzymes responsible for the sulfoxidation of S-methyl N,N-diethylthiolcarbamate (DETC-ME). The terminal step in the bioactivation of disulfiram. *Drug Metab Dispos* 1995; **23**: 1153.
 - 58 Sadeque AJM, Fisher MB, Korzekwa KR, Gonzalez FJ, Rettie AE. Human CYP2C9 and CYP2A6 mediate formation of the hepatotoxin 4-ene-valproic acid. *J Pharmacol Exp Ther* 1997; **283**: 698–703.
 - 59 Hong J-Y, Wang YY, Bondoc FY, *et al.* Metabolism of methyl tert-butyl ether and other gasoline ethers by human liver microsomes and heterologously expressed human cytochromes P450: identification of CYP2A6 as a major catalyst. *Toxicol Appl Pharmacol* 1999; **160**: 43–48.