

# Cytochrome P450 3A pharmacogenetics: the road that needs traveled

DA Flockhart<sup>1</sup> and JM Rae<sup>2</sup>

<sup>1</sup>Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, IN, USA; <sup>2</sup>Division of Hematology and Oncology, University of Michigan Medical Center, Ann Arbor, MI, USA

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'The woods are lovely, dark and deep  
But I have promises to keep  
And miles to go before I sleep  
And miles to go before I sleep'

Robert Frost

No one should debate the importance of cytochrome P450 3A to inter-individual differences in the pharmacokinetics of drugs. The enzyme is prominent in the liver<sup>1</sup> and GI tract,<sup>2</sup> is notoriously prolific and promiscuous in its xenobiotic and medicinal substrate selection, and famously unpredictable, having a five- to 20-fold variability in its ability to clear a drug between individuals,<sup>3</sup> resulting in untold difficulties for the prescribers of up to 50% of all medicines.<sup>4</sup>

Despite this, study of the pharmacogenetics of the cytochrome P450 3A family of enzymes has evoked the dark and the deep, and has until recently been a moribund pursuit, a graveyard of many noble but unsuccessful attempts to demonstrate a genetic effect on phenotype.<sup>5–7</sup> Early studies on this family of enzymes focused on the CYP3A4 isoform since it appeared to be the predominant isoform expressed in human liver, but it is now clear that there are four enzymes that are part of this system, the cytochromes P450 3A4, 3A5, 3A7 and most

recently 3A43,<sup>8</sup> and that all may be relevant to drug disposition in adults. The high homogeneity between these genes themselves and the pseudogenes CYP3AP1 and CYP3AP2<sup>9</sup> has complicated the efforts of genetic researchers, who have to date been unable to demonstrate the presence of any genetic variant in the coding region of these proteins that results in a clinically important change in activity.<sup>10</sup> Not to be deterred, and perhaps because of data that suggest that the genetic component of the inter-individual variability in CYP3A activity is between 60 and 90%,<sup>11</sup> their attention has therefore switched to noncoding regions, and this has yielded results that may have important implications, and which make clear that to ignore noncoding genetic variants is only to perpetuate our ignorance. Specifically, the recent description of genetic variants in noncoding regions of CYP3A4,<sup>6</sup> CYP3A5<sup>12</sup> and CYP3A7<sup>13</sup> may have important biological and clinical implications, but there remain important gaps in our knowledge of these enzymes that suggest a number of immediate research priorities.

First to cytochrome P450 3A4 itself. In 1998, reports that a single SNP in the promoter of CYP3A4 resulted in higher clinical stage and grade prostate tumors<sup>14</sup> and treatment-related leukemia<sup>15</sup> set off a rush to confirmation

that has not been consummated, although independent data have indicated that this SNP could result in a small change in hepatic clearance when erythromycin or nifedipine were used as probes.<sup>6</sup> There is at best, a 30% change in hepatic clearance that results, so that in rare circumstances a prescribing physician would have to change the daily dose of drug from 100 to 70 mg to maintain the same steady-state concentration: a debatable priority in a busy clinical environment.

CYP3A5 has been considered less important than CYP3A4 because it appeared in early studies to be expressed in only a third of all livers, at a much lower level and to have a narrower substrate range than CYP3A4.<sup>16</sup> In 2001, Kuehl *et al*<sup>12</sup> published data that contradicted this teaching. Although their results confirmed the expression of CYP3A5 in approximately a third of Caucasians, 50% of African-Americans expressed the enzyme. More importantly, their results showed that the level of CYP3A5 expression is comparable to that of CYP3A4 in those individuals who express CYP3A5. These results suggested that CYP3A5 might play a significant role in aggregate CYP3A activity. Using large-scale sequencing technologies, Kuehl *et al* were able to identify a common CYP3A5 genotype that correlated with activity: a single-nucleotide polymorphism in the third intron that creates an aberrant splice site and results in a truncated non-functional protein. Using analysis of genomic DNA from a large number of patients with known drug metabolism phenotypes, the authors found that in Caucasians and African-Americans who carry the CYP3A5\*1 allele, CYP3A5 accounts for at least 50% of the total CYP3A protein. Analysis of human liver CYP3A5 cDNA revealed that only those people with a CYP3A5\*1 allele produce high levels of full-length CYP3A5 mRNA and express CYP3A5, whereas individuals with variant alleles have no functional

CYP3A5 enzyme.<sup>12</sup> It follows that genetic testing may be able to identify individual patients with null or high CYP3A5 activity.

Research on cytochrome P450 3A7 has also been resurrected. This isoform was thought at one point to exist only in fetal liver,<sup>17</sup> with a critical switch from CYP3A7 to CYP3A4 being made in the first few weeks of life. While it has been clear for some time that CYP3A7 RNA is expressed in adult liver,<sup>18</sup> new data presented by Burk *et al*<sup>13</sup> indicate that CYP3A7 is found in all intestinal tissue tested, and that a genetic variant that involves a unique transposition of a PXR-sensitizing ER6 element into the CYP3A7 promoter results in increased expression in both tissues, with ~11% of livers carrying a high-expression phenotype. Coupled with data indicating that CYP3A7 may be up to 20 times more active as a catalyst for substrates such as retinoic acid,<sup>19</sup> these results suggest that genetic variation in CYP3A7 may also have a future as a subject of research.

While these new findings are important, some perspective from consideration of other genetically polymorphic cytochrome P450 enzymes is merited. The known SNPs that change activity of CYP2D6 resulted in at least a 10-fold decrease in the clearance of codeine to morphine,<sup>20</sup> a seven-fold decrease in the clearance of desipramine,<sup>21</sup> and as high as a 17-fold increase in the small number of patients with 13 alleles<sup>22</sup> that resulted in notable, if subjective, experiences of dry mouth and hypotension on the part of patients. For CYP2C19, the clearance of omeprazole to lansoprazole is altered five- to 10-fold by the CYP2C19\*2 or \*3 alleles,<sup>23,24</sup> and this may result in a clinically important improvement in the efficacy of omeprazole to treat *Helicobacter Pylori* infection.<sup>25</sup> In the case of CYP2C9, the poor metabolizer genotype results in a clinically effective dose of warfarin (0.5 mg) that is one-tenth of the routine dose.<sup>26</sup> Despite these relatively large changes in metabolic activity and the attendant clinical consequences,

we do not yet routinely test for the genotype of *CYP2C9*, *CYP2C19* or *CYP2D6* in clinical practice. In an economic and scientific environment where we have been unsuccessful in making the case for genetic testing of these enzymes, the case for testing CYP3A4 or CYP3A5 genotype, which one would predict to change clearance by less than 50%, is at best unclear.

On the other hand, this may be a narrow view for two reasons. First, it is quite possible that the use of multiple genotype testing may enhance the collective predictive potential, and that CYP3A5 and CYP3A7 genotyping may importantly improve the positive predictive value of other genetic tests. Second, there may be important genetic effects of CYP3A5 genotype in local tissues, such as the lung and kidney despite a small effect on the aggregate of systemic drug metabolism. Recently, Givens *et al.* have shown a notable correlation between CYP3A5 genotype and systolic blood pressure<sup>27</sup>, and this observation heralds an important area of future research: improved understanding of the effects of CYP3A genotype on local metabolism of endogenous mediators that may have important clinical effects. In the context of drug metabolism, it is clear that many questions remain unanswered. What meaningful pharmacokinetic effects are brought about by the genetic variants in intron 3 of CYP3A5 and in the promoter of CYP3A7? It remains the case that we have not identified a clinically used drug that can distinguish between these isoforms, so changes in the activity of one isoform may be diluted by a lack of change in the rate of others that metabolize the same drug. It follows that research should focus at first on finding pharmacokinetic changes for the drugs that are likely to matter: cyclosporine, tacrolimus, the HIV protease inhibitors and other drugs of relatively narrow therapeutic range. DNA should be assiduously collected in trials involving these agents, and in every possible context, measures

of toxicity and of efficacy should supplement pharmacokinetic measures. When changes in efficacy or toxicity are found with consistency in such clinical trials, they should be extended so that objective measures of real clinical outcome such as cost, morbidity that can be objectively assessed or mortality can be documented. We have seen the start of genetic research on this most important of drug-metabolizing enzymes, and pharmacogenomics does have great promise, but we have miles to go before we sleep. Miles.

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#### Correspondence should be sent to

DA Flockhart, MD, PhD,  
Division of Clinical Pharmacology,  
Indiana University School of Medicine,  
Wishard Hospital, 1001 West 10th Street,  
Indianapolis, IN 46202, USA  
Tel: +1 202 687 2882  
Fax: +1 202 687 7894  
E-mail: dlockha@iupui.edu

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## Polymorphic CYP2A6 and its clinical and toxicological significance

A Rautio

Department of Pharmacology and Toxicology, University of Oulu, Finland

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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 are primarily cleared from the body through the action of CYP enzymes mainly in the liver.<sup>1</sup> About 40% of human CYP-dependent drug metabolism is carried out by polymorphic enzymes, which can result in therapeutic failures and adverse effects from ensuing inter-individual variability in drug concentrations. Several examples exist where subjects carrying certain variant alleles suffer from adverse effects from drug treatment due to the presence of defective alleles.<sup>2</sup> The well-known polymorphic CYP genes are CYP2C9, CYP2C19 and CYP2D6. 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.<sup>3</sup>

CYP2A6 represents up to 10% of human microsomal P450 proteins. CYP2A6 is the major enzyme catalysing the oxidative metabolism of nicotine and cotinine and it contributes to the metabolism of some pharmaceuticals (eg fadrozole, tegafur, SM-12502), nitrosamines, other carcinogens (eg aflatoxin B1) and a number of coumarin-type alkaloids.<sup>4–6</sup> CYP2A6 may be inducible by antiepileptic drugs and it is decreased in alcohol-induced severe cirrhosis.<sup>4</sup>

The members in human CYP2A gene subfamily are CYP2A6, CYP2A7 and CYP2A13.<sup>7</sup> CYP2A6 is expressed in the liver whereas CYP2A13 is an extrahepatic enzyme; the CYP2A7 gene encodes an unstable and inactive protein.<sup>5</sup> The organization and structure of the CYP2A gene cluster and several polymorphic alleles of the CYP2A6 gene have now been characterized (see <http://www.imm.ki.se/CYPalleles>). There are three gene deletion alleles (CYP2A\*4A, \*4B and \*4D) and numerous SNPs. Recently, in

Pharmacogenetics journal a paper by Ariyoshi *et al.*<sup>8</sup> was published concerning the gene deletion allele CYP2A6\*4B in Japanese. During their previous studies<sup>9</sup> the group had already identified two genotypes of the CYP2A6 gene (D-type and E-type or CYP2A6\*4A; see <http://www.imm.ki.se/CYPalleles>). These earlier results could be interpreted as the D-type deletion being a partial CYP2A6 gene-deleted allele. However, studies on the CYP2A6 gene have been rather problematic, because the highly (94%) homologous CYP2A7 gene is located just 25 kb upstream of the CYP2A6 gene. For example, the allele termed CYP2A6\*3, which was one of the earliest variants found, is most probably an artefact based on the presence of CYP2A7-derived 3'-sequences in the CYP2A6\*1B allele and the consequent amplification of CYP2A7 sequences by the original PCR-based genotyping assay for CYP2A6\*1, CYP2A6\*2 and CYP2A6\*3 alleles.<sup>10</sup>

Ariyoshi *et al.* showed in their paper that by combining different types of methods they could resolve the discrepant results in PCR, PCR-RFLP and Southern blot analyses when genotyping the samples of the same subjects. The published study showed that the genotype previously named as 'D-type' is actually composed of CYP2A6\*4A and an entire CYP2A6 gene-deleted allele, CYP2A6\*4B. They estimated that the allele frequency of the CYP2A6\*4B in Japanese is 0.60% and that of the CYP2A6\*4A 19.0%.

It is of interest that a significant proportion of the Japanese lack the CYP2A6 protein completely due to the relatively high incidence of CYP2A6 gene deletion alleles in Japanese and Chinese populations.<sup>9,11</sup> The frequency of poor metabolizers (PMs) in European and Middle East populations is about 1% or less, whereas it is much higher in the Asian populations (up to 20%). Also some alleles which decrease the ability of CYP2A6 to metabolize nicotine and coumarin have been found in higher frequencies in Japanese and Chinese in comparison with Caucasians.<sup>12,13</sup>

Why is it of importance to elucidate the exact alleles and genotypes of CYP2A6? The main reasons to do this are the marked role of CYP2A6 in the metabolism of various substrates, especially pharmacologically and toxicologically relevant compounds, and the importance of complete and unequivocal elucidation of all functional alleles for global genotyping studies.

Interest in CYP2A6 has risen considerably after nicotine and some tobacco-specific nitrosamines were established as high-affinity substrates for this enzyme. Rao *et al.*<sup>14</sup> reported that the individuals who are nicotine-dependent and have defective CYP2A6 alleles smoked fewer cigarettes and it is assumed that the deletion alleles may be protective regarding cancer by the decreased metabolic activation of procarcinogens found in tobacco smoke. Several case-control studies have addressed the relationship between CYP2A6 status and smoking habits as well as the role of CYP2A6 polymorphisms in lung cancer risk, but the results thus far have been inconclusive.<sup>5</sup> However, one reason for these inconclusive results may have been that only a few alleles have been assayed.

There are now more than 10 different allelic variants known to cause abolished or decreased enzyme activity. The inability to take into account all the functional variant alleles in addition to some methodological problems as shown by the article of Arioyshi *et al.* are almost

certainly behind the inconclusive and discrepant results in published studies. It is worth pointing out the importance of identifying the correct genotype and its functional consequences unequivocally before a relationship between CYP2A6 status and various cancer risks is studied.

What is the toxicological and clinical significance of polymorphisms of CYP2A6? CYP2A6 deletion alleles (CYP2A6\*4A, CYP2A6\*4B and CYP2A6\*4D) are of great importance in studies aimed at correlating smoking behaviour, pre-carcinogen activation or drug metabolism with the CYP2A6 genotype, especially in Oriental populations. It is important to know if the different alleles are producing active enzyme able to metabolize drugs and other perhaps toxic or carcinogenic chemicals. In molecular epidemiological studies or in clinical trials a need to measure the genotype status of all functionally relevant alleles of important CYP genes has become increasingly more acute.

It is possible that in the future, when physicians prescribe medication to their patients, they will have information on the patient's CYP status. Will the CYP2A6 gene be tested together with the CYP2C9, CYP2C19 and CYP2D6 genes? It seems quite likely that CYP2A6 is included occasionally, if it is a rate limiting metabolizing enzyme for a drug that has 'dangerous' effects. There are already a couple of examples in which CYP2A6 pharmacogenetics might be of importance to know. Daigo *et al.*<sup>6</sup> found that when giving tegafur (an anticancer drug) to gastric cancer patients, one patient had four-fold higher plasma tegafur concentrations than the others. Tegafur is metabolized via CYP2A6 to 5-fluorouracil, which is the active drug. Those persons with a CYP2A6 poor metabolizer status could not produce high enough concentrations of the active metabolite to have a beneficial effect of the drug treatment. Another example is a platelet-activating factor receptor antagonist, SM-12502, which affects blood coagulation. In this case, a CYP2A6 PM patient might be very sensitive to the drug.

Also single nucleotide polymorphisms (SNPs) can be of importance in addition to deletions, because they can change the substrate selectivity and turnover of the CYP2A6 enzyme.<sup>13</sup> In drug development, ethnic differences regarding CYP2A6 must be taken into consideration if the new chemical entity is significantly metabolized via this enzyme. However, CYP2A6 could be even more significant in tobacco-related behaviours and diseases, because of its role in nicotine and nitrosamine metabolism. Thus far the elucidation of the role of CYP2A6 polymorphisms in smoking habits and ill effects has led to inconclusive findings, but it is hoped that the more complete and functionally relevant analysis of CYP2A6 polymorphisms and variant alleles, such as the one in Arioyshi's paper, would give more definite answers in the future.

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#### Correspondence should be sent to

A Rautio, Department of Pharmacology and Toxicology, University of Oulu, PO Box 5000, University of Oulu, FIN-90014, Finland,  
Tel: +358 8 5375250  
Fax: +358 8 5375247  
E-mail: arja.rautio@oulu.fi

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