Pharmacogenetics of drug-metabolizing enzymes: implications for a safer and more effective drug therapy

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The majority of phase I- and phase II-dependent drug metabolism is carried out by polymorphic enzymes which can cause abolished, quantitatively or qualitatively decreased or enhanced drug metabolism. Several examples exist where subjects carrying certain alleles do not benefit from drug therapy due to ultrarapid metabolism caused by multiple genes or by induction of gene expression or, alternatively, suffer from adverse effects of the drug treatment due to the presence of defective alleles. It is likely that future predictive genotyping for such enzymes might benefit 15–25% of drug treatments, and thereby allow prevention of adverse drug reactions and casualties, and thus improve the health of a significant fraction of the patients. However, it will take time before this will be a reality within the clinic. We describe some important aspects in the field with emphasis on cytochrome P450 and discuss also polymorphic aspects of foetal expression of *CYP3A5* and *CYP3A7*.

Keywords: ultrarapid metabolizers; poor metabolizers; adverse drug reactions; genetic polymorphism

1. INTRODUCTION

Pharmacogenetics is a rapidly growing field of interest encompassing genetic variation in genes encoding drug transporters, drug-metabolizing enzymes and drug targets, as well as genes related to the action of drugs. The interest is based on the fact that only 30–60% of common drug therapy is successful and that adverse drug reactions cause 7% of all hospital admissions, 4% withdrawal of new medical entities, and cost society an amount equal to the cost of drug treatment *per se* (see Ingelman-Sundberg 2004 for references).

The rationale behind pharmacogenetics is to find genetic polymorphisms in the genes encoding proteins and enzymes involved in drug transport, metabolism and action that can predict the usefulness of a particular drug, increasing the number of responders and decreasing the number of subjects affected by adverse drug reactions. With respect to the relative roles of genes encoding transporters, metabolizing enzymes and targets, it is evident that the polymorphism of drug-metabolizing genes by far has the highest impact for interindividual differences in drug response. However, one should keep in mind that genetics only contributes to a fraction of the explanations behind interindividual differences in drug metabolism. Inhibition and induction of drug metabolism has also a major impact for such differences, and in an illustrating study Backman *et al.* showed, for example, that the area under the curve after a single dose of midazolam differed 400-fold between subjects taking concomitant

itraconazole as compared to subjects being treated with the *CYP3A4* inducer rifampicin (Backman *et al.* 1998).

In the case of applications in pharmacogenetics, it is clear that the data we have today is substantial and provides clinicians with information that could facilitate an individualized therapy of patients, both with respect to the choice of the drug, and also with respect to the dose of the specific drug. Pharmacogenetics is, however, still in the beginning; knowledge about genetic variation at the level of drug metabolism is extensive, whereas the knowledge about interindividual differences in the function of drug transporters and drug targets is scarcer. In addition, some of the results in this field are incomplete, conflicting and sometimes difficult to interpret. But the field as a whole is very promising and, based on rapidly developing techniques, increased understanding of the human genome, its function and regulation, and the establishment of many novel and useful databases, pharmacogenetics has a great future. Here we consider some important achievements with emphasis on the pharmacogenetics of drug-metabolizing enzymes. Some recent previous reviews in this field include those of Weinshilboum (2003), Evans & Relling (2004), Ingelman-Sundberg (2004) and Meyer (2004).

2. VALUABLE DATABASES

On the basis of the Human Genome Project and now the HapMap Project, it is evident that we can very rapidly obtain an increasing amount of information about polymorphisms influencing gene products of importance for drug action. On the site locus-specific mutation databases at http://archive.uwcm.ac.uk/uwcm/mg/docs/oth_mut.html, links to most relevant databases summarizing novel variant alleles in genes of

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importance are present. This includes databases covering various drug receptors, drug transporters and drug-metabolizing enzymes. The transporter database at <http://www.ilab.rise.waseda.ac.jp/tp-search/> is a specific database for drug transporters. The HapMap Project at <http://www.hapmap.org/index.html.en> is of particular use in order to find characterized haplotypes, specific single nucleotide polymorphisms (SNPs) and allele frequencies in various ethnic groups. The International HapMap Project studies the common patterns of human genetic variation in samples from populations with African, Asian and European ancestry (<http://www.hapmap.org>). The project is freely available in the public domain. The basis of the project is the strong associations between SNPs, which leads to the selection of 'tag' SNPs that represent the common haplotypes in a region. The genotyping of the tag SNPs provides enough information to predict much of the remainder of the frequent SNPs present in that region. The goal of the project is not to identify disease-related genes directly, rather, to identify haplotypes that can be used in association studies. Therefore, the HapMap Project will facilitate future studies that relate genetic variation to health and disease (The International HapMap Consortium 2003). The accuracy of the HapMap data for higher-frequency SNPs is high, but the performance of tag SNPs in representing rare variation is poor (Ahmadi *et al.* 2005). For this reason, this project is especially useful for genetic variants that are relatively common in populations, such as those of drug-metabolizing enzymes and, therefore, drug response.

3. DRUG TRANSPORTERS

Transporters of drugs that are expected to influence drug therapy are present in the intestine, blood brain barrier, intracellularly in the CNS and liver, and in addition, in the kidney membranes. With respect to established polymorphism affecting drug disposition, it is clear that three different research groups identified the *OATP-C (SLC21A6)* polymorphism to be of considerable importance for the pharmacokinetics of pravastatin (Nishizato *et al.* 2003; Mwinyi *et al.* 2004; Niemi *et al.* 2004). However, in many other cases regarding polymorphism, for example, the *MRP* and *MDR* genes, there is currently no consensus as to how to identify clear haplotypes of established roles in drug metabolism and disposition. One of the reasons is the presence of multiple haplotypes of the rather big transporters making true functional association studies difficult. The *MDR1* gene exists in at least 64 different haplotypes, many with mutations creating amino acid changes (Kroetz *et al.* 2003). Another reason for the difficulties of these studies might be the partly overlapping substrate specificities between different transporters and their general less penetrance in influencing drug disposition. The research in this area is intensive and indeed we will observe more examples of functionally polymorphic drug transporters in the near future.

4. CYTOCHROMES P450

There are 57 known active *P450* genes in the human genome and 58 pseudogenes (<http://drnelson.utmem>.

Figure 1. Active *P450* genes in the human genome. The different families of *P450* in humans are represented by vertical lines, those of families 1–3 are enzymes involved in xenobiotic metabolism, while those in higher families metabolize endogenous substrates. The subfamilies are represented by a letter and isoforms are followed by an Arabic number.

[edu/CytochromeP450.html](http://www.imm.ki.se/cypalleles); Nelson *et al.* 2004). This is less than the 102 active genes in mice, 249 in thale cress and 343 in rice, but similar to the 54 genes which are found in dogs.

The cytochrome *P450* enzyme in families 1–3 are generally polymorphic and responsible for 70–80% of all phase I-dependent metabolism of clinically used drugs (Bertz & Granneman 1997; Evans & Relling 1999), and they participate in the metabolism of a huge number of xenobiotics (figure 1). The polymorphic forms of *P450*s are many times responsible for the development of adverse drug reactions (Phillips *et al.* 2001). The mutations in the *CYP* genes can produce enzyme products with abolished, reduced, altered or increased enzyme activity. This forms four major phenotypes: poor metabolizers (PMs) lacking functional enzyme, intermediate metabolizers (IMs) being heterozygous for a defect allele, efficient metabolizers (EMs) carrying two functional gene copies and ultrarapid metabolizers (UMs) carrying more than two functional gene copies (Ingelman-Sundberg 2004). 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 can cause the synthesis of enzymes with altered substrate specificity.

The functional importance of the variant alleles varies and the frequencies of their distribution in different ethnic groups is tremendously different. On the human *CYP* allele nomenclature Web site in our server at Karolinska Institutet (<http://www.imm.ki.se/cypalleles>) with Sarah C. Sim as Webmaster, updated information in this area is obtained. The rapid development in the field required the establishment of a website which was formed after discussions with several investigators in the field, e.g. Ann Daly and Dan Nebert. It contains recommended nomenclature regarding the various allelic variants of human *P450*s.

The aim is to avoid 'home-made' allelic designations that can confuse the nomenclature system and thus to encourage scientists worldwide to speak the same language. In addition, a rapid publication and summary of alleles would prevent unnecessary doubling of research efforts aimed at characterizing alleles already described. Currently, the website covers the nomenclature for polymorphic alleles of *CYP1A1*, *CYP1A2*, *CYP1B1*, *CYP2A6*, *CYP2A13*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1*, *CYP2F2*, *CYP2R1*, *CYP2S1*, *CYP3A4*, *CYP3A5*, *CYP3A7*, *CYP3A43*, *CYP4B1*, *CYP5A1*, *CYP8A1* and *CYP21A2*. Each isoform has its own page that lists the alleles with their nucleotide changes, their functional consequences and links to publications identifying or characterizing the alleles. Submissions to the website are peer reviewed before publication. Usually a full manuscript/paper is required as a basis for inclusion of a new allele into the website. Emphasis is given to publish alleles that alter enzyme expression and/or function. In recent times, many different haplotype variants of the *CYP* genes with mutations in remote flanking regions or in introns have been submitted, and in due time a system for haplotype publication has to be developed.

A reasonable estimate of the penetrance of polymorphism in the genes encoding drug-metabolizing enzymes as to significantly affect the outcome of drug treatment is that such genetic variability might be of importance in 20–30% of all drug therapy (table 1). The major enzymes contributing to this variability except for P450s are in particular UDP-glucuronosyl-transferase, *N*-acetyltransferase-2, dihydropyrimidine dehydrogenase and thiopurine methyltransferase. The quantitative estimate given above is based to a large extent on the fact that the major polymorphic human cytochrome P450 enzymes, CYP2C9, CYP2C19 and CYP2D6 metabolize about 40–45% of all drugs on the market and that estimates by Kirchheiner *et al.* (Kirchheiner *et al.* 2004; Kirchheiner & Brockmoller 2005) indicate that about 50% of the metabolism of the drugs that are substrates for these enzymes is significantly affected by the polymorphism. Furthermore, it is likely that polymorphism in other P450 genes such as *CYP2B6* and *CYP3A4* might be significant for interindividual differences in drug therapy. The definite answer has to await further studies *in vivo*, showing the importance of different *CYP2B6* alleles and the identification of genetic elements responsible for interindividual differences in the expression of *CYP3A4*, that from *in vivo* studies have been postulated to be regulated to a great extent by inheritance (Ozdemir *et al.* 2000).

As mentioned, the most important polymorphic P450 enzymes are CYP2C9, CYP2C19 and CYP2D6. CYP2D6 metabolizes about 25% of the clinically used drugs and the dosing required in order to achieve the same plasma levels of a drug mainly metabolized by CYP2D6 can differ 10 to 30-fold between individuals. In Western Europe one can calculate, based on the individual studies in the various countries, that 5.5% of the population carry more than two functional gene copies of *CYP2D6* and are UMs for CYP2D6 substrates (Ingelman-Sundberg 2005). In these

subjects, no response is often to be expected because of too rapid metabolism. Seven per cent of the European population are PMs, where too-high plasma levels of the drugs are to be expected at ordinary dosage and a higher frequency of adverse drug reactions are seen. In contrast to the extensive span in metabolic capacity, dosing today is, however, principally based only on the population average. By predictive genotyping for CYP2D6 PMs and UMs, a more appropriate initial dosing could be achieved in 50–60 million people only within Western Europe, which would be relevant for approximately 10–12% of all drugs on the market. By contrast, the fraction of populations lacking CYP2C9 and CYP2C19 is much lower and amounts to about 2 and 3%, respectively. However, the recent review by Kirchheiner & Brockmoller (2005) indeed indicates that dosing of anticoagulants, antidiabetics and antiepileptics are much dependent on the *CYP2C9* genotype. Also, the fraction of PMs for CYP2C19 is 20–25% among the Asian population (Ingelman-Sundberg *et al.* 1999). In addition, in our laboratory a common *CYP2C19* allele causing a higher *CYP2C19* expression has recently been characterized, indicating the possibility of underdosing in a large fraction of Caucasians and Africans (Sim *et al.* submitted).

5. CLINICAL RELEVANCE OF CYTOCHROME P450 POLYMORPHISM

As mentioned, one could estimate that 15–20% of all drug treatment is influenced by the polymorphism of the cytochrome P450 genes. For treatment of depression, both the polymorphism of CYP2D6 and CYP2C19 influence the outcome. Tricyclic antidepressants are almost entirely metabolized by CYP2D6, and dosage is much related to the *CYP2D6* phenotype, whereas several of the serotonergic specific recapture inhibitors (SSRIs) are metabolized by the polymorphic CYP2C19. Dosage of the tricyclic antidepressant nortriptyline is much dependent on the number of active *CYP2D6* genes and PMs lacking functional enzyme would need 30–50 mg as compared to UMs that would require 500 mg in order to achieve the same plasma levels. Since standard dosing is 150 mg, one would expect that no appropriate effects would be seen in the group of UMs. Indeed, two recent different studies have now shown that subjects of the UM phenotype are highly overrepresented in patients that were classified as non-responders of antidepressant therapy in the psychiatric clinic (Kawanishi *et al.* 2004; Rau *et al.* 2004).

The pharmacokinetics of citalopram are influenced by the *CYP2C19* polymorphism. Actually, the area under the curve obtained is much different between PMs and EMs (Herrlin *et al.* 2003). The clinical impact of this difference in pharmacokinetics is, however, not documented, since there are no clear dose effect relationships for this kind of drug treatment. Treatment with the SSRI drug sertraline, a CYP2C19 substrate, has been found to display adverse drug reactions such as nausea and dizziness, an effect possibly caused by toxic concentrations of the accumulated drug in CYP2C19 PMs. In addition, the metabolism of valproate, commonly used in bipolar

disorders, has been found to be much influenced by the *CYP2C9* polymorphism (Ingelman-Sundberg 2004).

Many antipsychotic drugs are metabolized by *CYP2D6*. Parkinsonian side effects are seen at higher frequencies in PMs as evident from several prospective and retrospective studies (see Kirchheiner *et al.* 2004 for references). Oversedation is often seen in PMs for *CYP2D6* after treatment with perphenazine, thioridazine and other antipsychotics. By contrast, no significant relationship between *CYP2D6* polymorphism has been described for tardive dyskinesia, acute dystonia or akathisia (Dahl 2002).

CYP2C19 metabolizes several different proton pump inhibitors like omeprazole, lansoprazole and pantoprazole. Using a 20 mg dosage of omeprazole, healing of ulcers has been found to be much higher in subjects lacking one or two functional copies of the *CYP2C19* gene (Furuta *et al.* 1998). Bertilsson and collaborators have found that changes in gut pH after treatment by e.g. omeprazole are more pronounced in *CYP2C19* PMs (Sagar *et al.* 2000). Long-term treatment of proton pump inhibitors leads to a more extensive gastrin release in PMs as compared to EMs (Sagar *et al.* 2000).

The anticoagulants warfarin, and coumarols such as acenocoumarol, are metabolized by *CYP2C9*. The maintenance dose of warfarin has been found to differ in many different studies between individuals carrying 2, 1 or 0 functionally correct *CYP2C9* alleles (Kirchheiner & Brockmoller 2005). *CYP2C9* genotyping to predict a safer and individually based warfarin treatment with less side effects might be valuable. A similar relationship would exist for coumarols.

No efficacy is seen in *CYP2D6* PMs after treatment by the pain relieving drugs codeine and tramadol, which are required to be metabolized by *CYP2D6* to their active metabolites (Sindrup & Brosen 1995; Sindrup *et al.* 1999). Adverse drug reactions of codeine treatment have been described in *CYP2D6* UM when treated with ethylmorphine, oxycodone and hydrocodone, probably because of extensive formation of morphine (Dalen *et al.* 1997; Gasche *et al.* 2004).

The *CYP2C9* polymorphism influences the pharmacokinetics of phenytoin, and several examples of adverse drug reactions have been described in patients with defective *CYP2C9* alleles upon phenytoin treatment, such as CNS intoxication that includes ataxia, diplopia and other neurological symptoms (Kirchheiner & Brockmoller 2005).

6. PHARMACOGENETICS OF CANCER TREATMENT

Treatment of cancer patients with cancer drugs is of special relevance because of the toxic action of the drugs used. Overdosing and underdosing have particular importance, and variation in drug-metabolizing genes might affect the therapeutic outcome to a great extent. As reviewed in Relling & Dervieux (2001) the polymorphism of dihydropyrimidine dehydrogenase, thiopurine methyltransferase, glutathione transferases, glucuronosyl transferase 1A1 and methylene tetrahydrofolate reductase affect the outcome of treatment with different cancer drugs. A particular treatment with

irinotecan against colon cancer is dependent on the TA repeat polymorphism in the *UGT1A1* gene (Marsh & McLeod 2004), and the FDA has labelled the drug for pharmacogenetic testing before dosing. Homozygous *UGT1A1**28 genotypes (7/7 genotype) are at significantly greater risk for developing neutropenia, and acute and delayed diarrhoea.

Targeted therapy of specific cancers is an interesting area that is developing rapidly. This includes use of imatinib mesylate (Gleevec) for patients with bcr/abl-positive chronic myelogenous leukaemia and cetuximab (Erbix) for patients with epidermal growth factor receptor overexpression in metastatic colorectal cancer (see Ross *et al.* 2004). An interesting novel-targeted therapy of breast cancer is Herceptin (trastuzumab), an antibody against the HER-2/neu receptor, where efficacy is severely increased in tumours overexpressing the receptor (Altundag *et al.* 2005).

Among the cytochrome P450s, *CYP2B6* metabolizes several anticancer drugs, including cyclophosphamide, and preliminary indications exist for an increased metabolism among subjects carrying a variant allele, *CYP2B6**6 (Xie *et al.* 2003). Tamoxifen is metabolized to its active metabolite by *CYP2D6*, and a smaller therapeutic effect has been observed in PMs for *CYP2D6* and predictive pheno/genotyping could be relevant before entering the treatment (Jin *et al.* 2005). The effect of anti-emetic drug treatment of cancer patients with drugs like the 5-hydroxytryptamine type 3 receptor antagonists tropisetron and ondansetron has been found to be related to the *CYP2D6* phenotype (Kaiser *et al.* 2002). Lower plasma levels and higher frequency and intensity of vomiting were seen in subjects carrying more active gene copies of *CYP2D6*.

7. POLYMORPHIC FOETAL DRUG METABOLISM

In the adult liver, multiple drug-metabolizing P450s are expressed to facilitate the metabolism and elimination of a wide range of compounds. In contrast, the foetal liver has only a few drug-metabolizing P450s, which have to overcome drug exposures occurring at the foetal stage. *CYP3A7* expression is maximum in foetal life and declines after birth, paralleled by an increase in *CYP3A4* levels (Lacroix *et al.* 1997; Tateishi *et al.* 1997; Stevens *et al.* 2003). The large variation in *CYP3A4* and *CYP3A7* expression during development has an impact on total hepatic CYP3A content which, together with the different substrate specificities of *CYP3A4* and *CYP3A7* (Williams *et al.* 2004), result in differences in pharmacokinetics and efficacy and toxicity of drugs between adults and foetal/early infancy.

The important role of *CYP3A7* in the metabolism of endogenous substrates such as steroids and retinoic acid, in addition to xenobiotics reaching the foetus, has probably resulted in a well-conserved enzyme. To date, only one polymorphic variant, *CYP3A7**2, where Thr⁴⁰⁹ is changed to Arg, has been described. Using heterologous expression systems, it was shown in our laboratory that *CYP3A7**2 was an active enzyme with a moderately higher specific activity than *CYP3A7**1 using dehydroepiandrosterone (DHEA),

Table 1. Relative importance of polymorphisms in human P450s involved in drug metabolism. (The number of + illustrates the relative importance between the different forms of P450.)

enzyme	estimated fraction of drug metabolism ^a (%)	substrates	clinical effects of the polymorphism	significance of polymorphism ^b
CYP1A2	5	drugs carcinogens	not documented	+
CYP2A6	2	nicotine, drugs, carcinogens	nicotine metabolism	+
CYP2B6	2–4	drugs	efavirenz and cyclophosphamide metabolism	+
CYP2C8	1	drugs	taxol metabolism	+
CYP2C9	10	drugs	effects of warfarin, coumarols, anti-diabetic drugs, phenytoin	+++
CYP2C19	5	drugs	effects of proton pump inhibitors, citalopram	+++
CYP2D6	20–30	drugs	effects on treatment with tricyclic antidepressants, antipsychotics, codeine	+++
CYP2E1	2–4	carcinogens, solvents, drugs	not shown	—
CYP3A4	40–45	drugs, carcinogens	not shown	—
CYP3A5	<1	drugs	not shown	—

^a The estimated fraction of responsibility for drug metabolism in phase 1 reactions. Data from Bertz & Granneman (1997), Evans & Relling (1999) and from the author.

^b The significance of the polymorphism is based on the number of reports showing impact of the P450 polymorphism on the pharmacokinetics of drugs that are substrates for the enzyme in question.

and in mammalian expression systems it was shown that the protein stability was similar to that of CYP3A7*1 (Rodríguez-Antona *et al.* 2005b). In a recent paper by Leeder *et al.* using foetal liver microsomes, no significant differences in DHEA metabolism were found between CYP3A7*1 and CYP3A7*2 (Leeder *et al.* in press).

A polymorphic variant of CYP3A7 exists where the proximal promoter has been exchanged by the corresponding region of CYP3A4 containing the pregnane X receptor (PXR) element (CYP3A7*1C; $q=0.03$ Caucasians, $q=0.06$ African Americans). Individuals of this genotype express more CYP3A7 mRNA (Burk *et al.* 2002) and CYP3A7 protein, although significant CYP3A7 protein is seen also in livers of other CYP3A7 genotypes (Sim *et al.* submitted). However, adult liver contain high levels of CYP3A4 protein. In adult Caucasian livers, approximately 180 pmol mg^{-1} CYP3A is expressed, of which 2.2% would be composed of CYP3A7 (Sim *et al.* submitted).

CYP3A5 is expressed in foetal stages in a polymorphic manner due to frequent SNPs in intronic sequences that result in alternative splicing (Kuehl *et al.* 2001). Mainly individuals with at least one CYP3A5*1 allele have functional protein. The contribution of CYP3A5 to total CYP3A metabolism in adult liver is mainly determined by its expression relative to CYP3A4 and their specific activities. In most drug biotransformations, CYP3A4 is a more efficient enzyme than CYP3A5 and because CYP3A4 expression levels seem to be higher or similar to those of CYP3A5, the impact of CYP3A5 in adult liver drug metabolism is expected to be modest (Westlind-Johnsson *et al.* 2003; Williams *et al.* 2004). On the contrary, CYP3A5 catalyses the metabolism of several drugs more efficiently than CYP3A7 (Williams *et al.* 2004); therefore, CYP3A5 polymorphisms can relatively have a higher impact on foetal drug metabolism/toxicity (figure 2a).

CYP3A7.1L is a novel active enzyme derived from alternative splicing of CYP3A7 with the pseudogene CYP3AP1 (Finta & Zaphiropoulos 2000; Rodríguez-Antona *et al.* 2005a). This alternative transcript can be detected in several adult and foetus tissues and its expression is regulated in a developmental/tissue specific manner. A SNP at –6 of the first splicing site of CYP3AP1 (CYP3A7_39256 T>A; figure 2b), with pronounced interethnic differences, has been shown to abolish the alternative splicing (Rodríguez-Antona *et al.* 2005a). The contribution of this enzyme to drug metabolism in specific tissues is unknown, but the strong linkage disequilibrium between CYP3A7*2, CYP3A5*1 and CYP3A7_39256 A results in two frequent haplotypes with large interethnic differences that could have an important role in foetal stage and early infancy toxicology (figure 3).

8. FUTURE ASPECTS

Most important drug-metabolizing enzymes have now been identified and their polymorphic variants characterized with respect to regulation and function. In general, the impact of these polymorphisms is higher than that of drug transporters and drug receptors, and in many cases influences the outcome of drug treatment extensively. Indeed most pharmaceutical industries now screen drug candidates for interactions with polymorphic phase I enzymes early in development. The development of methods able to detect tight interactions with one of the highly polymorphic enzymes, and the existence of alternative candidates with similar pharmacological potency makes for withdrawal early in development of candidates interacting with polymorphic enzymes. It is, therefore, likely that the number of drugs which are more or less selectively metabolized by polymorphic enzymes will decrease in the future. With respect to the role of pharmacogenetics for marketing new drugs, it is likely that genetically defined populations in the future will be

Figure 2. (a) Rate of alprazolam 4- and 1-hydroxylation by human foetal liver microsomes homozygous for *CYP3A7*2/CYP3A5*1* when compared to *CYP3A7*1/CYP3A5*3* foetal liver. 4OH-alprazolam is produced by CYP3A7 and CYP3A5, alprazolam 1-hydroxylation is mainly catalysed by CYP3A5. Data from Rodriguez-Antona *et al.* 2005b. FLM, foetal liver microsomes; 4OH-Alpr, 4-hydroxy alprazolam; 1OH-Alpr, 1-hydroxy alprazolam. (b) Structure of CYP3A7-3AP1 mRNA and CYP3A7.1L protein. The rectangles represent part of the coding region of CYP3A7-3AP1, specifically, the last 3 exons from the 15 exons full transcript encoding the novel carboxy-terminal sequence of CYP3A7.1L are shown. The arrows indicate the splicing junctions. Intronic sequences are shown in lower case, and the polymorphism abolishing the pseudogene splicing is

Figure 3. (a) Human cytochrome P450 family 3. The human *CYP3A* locus is located on chromosome 7q21-q22.1 and contains four genes: *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43*, and three pseudogenes: *CYP3AP1*, *CYP3AP2* and *CYP3AP3*. The linkage disequilibrium between functional SNPs in this region results in different common CYP3A haplotypes that have pronounced interethnic differences. The developmental regulation of the *CYP3A* genes in combination with the different alleles result in the expression of different sets of CYP3A enzymes. (b) Common CYP3A haplotypes. The two most frequent haplotypes that result in expression of different sets of CYP3A enzymes are depicted. The frequencies estimated in Caucasians and Africans are shown. In Caucasians the most common allele combination is: *CYP3A7*1/CYP3A7_39256 T/CYP3A5*3* which results in the expression of CYP3A7.1 and CYP3A7.1L proteins. In Africans the most frequent allele combination is: *CYP3A7*2/CYP3A7_39256 A/CYP3A5*1* resulting in the expression of CYP3A7.2 and CYP3A5 proteins.

the target for specific drugs and also used in clinical trials, although since the market is restricted, the cost-benefit analysis would most probably cause a decrease in the number of such drugs.

A more extensive problem concerns the value of pharmacogenetics for the use of drugs already established on the market. To make reliable estimates of the value of predictive genotyping, large prospective clinical trials of uniformly treated and systematically characterized patients, coupled with high throughput genomic methods and sophisticated bioinformatics analyses are required. At present it is indeed severely difficult to obtain funding for such studies both from industry and from governmental institutions and, therefore, it would be expected that only a few of these will be seen in the near future. An implementation of pharmacogenetics in the clinic has apparently not yet been the case (Gardiner & Begg 2005). Despite this, it can be considered unethical not to make such analyses before initiating drug regimens if it is known that a large portion of the population indeed will never respond to a standard dose of the drug, as exemplified with antidepressant therapy of CYP2D6 UMs above. Maybe the only manner by which pharmacogenetics could be translated into routine clinical practice is by the establishment of guidelines from regulatory agencies, identifying the specific drugs where predictive genotyping should be taken into consideration before initiation of the drug therapy.

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