Genetic Polymorphism and Toxicology—With Emphasis on Cytochrome P450

Inger Johansson and Magnus Ingelman-Sundberg

Section of Pharmacogenetics, Department of Physiology and Pharmacology, Karolinska Institutet, 171 77 Stockholm, Sweden

To whom correspondence should be addressed. Fax: +46-8337327. E-mail: magnus.ingelman-sundberg@ki.se.

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Individual susceptibility to environmental, chemical, and drug toxicity is to some extent determined by polymorphism in drug-metabolizing enzymes, in particular the cytochromes P450 (CYPs). This polymorphism is in particular translated into risk differences concerning drugs metabolized by the highly polymorphic enzymes CYP2C9, CYP2C19, and CYP2D6, whereas CYP enzymes active in procarcinogen activation are relatively well conserved without important functional polymorphisms. Examples of drug toxicities that can be predicted by P450 polymorphism include those exerted by codeine, tramadol, warfarin, acenocoumarol, and clopidogrel. The polymorphic CYP2A6 has a role in nicotine metabolism and smoking behavior. Besides this genetic variation, genome-wide association studies now allow for the identification of an increasing number of predictive genetic biomarkers among, e.g., human leukocyte antigens and to some extent drug transporters that provide useful information regarding the choice of the drug and drug dosage in order to avoid toxicity. The translation of this information into the clinical practice has been slow; however, an increasing number of pharmacogenomic drug labels are assigned, where the predictive genotyping before drug treatment can be mandatory, recommended, or only for informational purposes. In this review, we provide an update of the field with emphasis on CYP polymorphism.

Key Words: adverse drug reactions; nicotine; clopidogrel; CYP2D6; CYP2C9; CYP2C19; warfarin; codeine.

There are major interindividual differences in the capacity to metabolize and detoxify drugs and other xenobiotics. These are of genetic, epigenetic, environmental, and pathophysiological origin. Drug-drug interactions are of particular importance with respect to adverse effects. In fact, adverse drug reactions (ADRs) are one of the major causes of death, amounting to the fifth leading cause in the United States, encompassing 100,000 deaths annually. In addition, ADRs are also responsible for up to 7% of hospitalizations (Eichelbaum et al., 2006), a number that increases to > 30% in the elderly population (> 70 years of age) (Paul et al., 2008). The costs of ADRs are approximately the same as the cost of the drug treatment itself (Eichelbaum et al., 2006), and, unfortunately, lately the number of ADRs has increased immensely in comparison to the increase in drug prescription (Moore et al., 2007). A large part of these ADRs are because of metabolic activation of the parent drug causing reactive products or immune-mediated toxicity involving the innate immune system (Alfirevic and Pirmohamed, 2010). The ADRs mainly affect cardiac function or cause liver toxicity, and in fact, a major part of the drugs withdrawn from the market because of ADRs is because of their cause of cardiotoxicity or hepatotoxicity (Need et al., 2005). Besides drugs, other xenobiotics are subject to metabolic activation reactions; of particular importance are tobacco and tobacco smoke constituents. The individual susceptibility for toxicity after metabolism is, to some extent, dependent on the genetic polymorphism of the phase I and II enzymes, i.e., enzymes catalyzing oxidation and conjugation reactions, respectively. Such differences mainly translate into differences in susceptibility for drug toxicity. Increased knowledge of the mechanisms behind ADRs because of allelic cytochrome P450 (P450 or CYP) variants is essential for efficient and safe drug treatment and for successful drug development. In the current overview, we emphasize some recent findings in this area and summarize our view of the future challenges in this field.

CYTOCHROME P450

Many drugs and other chemicals in our environment as well as endogenous compounds are lipophilic and have to be metabolized to become more water soluble before excretion, mainly by the kidneys. CYPs in families 1–3 account for, approximately, 75% of all phase I metabolism of clinically used drugs (Bertz and Granneman, 1997; Evans and Relling, 1999) and are also involved in the metabolism of other chemicals. The
human genome contains 115 CYP genes, of which 57 are functional and the remaining are pseudogenes. Among CYP families 1–3, there are 22 different P450 isoforms, and among these, a high degree of polymorphism has been identified in contrast to the low degree of polymorphism among CYPs in families 4–51, mainly involved in biosynthesis and metabolism of endogenous compounds. The polymorphic xenobiotic-metabolizing CYP enzymes can be divided into two classes (Rodriguez-Antona and Ingelman-Sundberg, 2006) with respect to penetrance for interindividual susceptibility for xenobiotics:

- Class I: Composed of CYP1A1, CYP1A2, CYP2E1, and CYP3A4 without important functional polymorphism and active in metabolism of procarcinogens as well as drugs.
- Class II: Composed of CYP2A6, CYP2B6, CYP2C9, CYP2C19, and CYP2D6, which are highly polymorphic and are important for the metabolism of drugs, but not of procarcinogens.

CYP1B1 represents a special case with several rare defective alleles, which are associated with glaucoma (Libby et al., 2003).

Accordingly, the genetic variability of the class I P450s does not translate to different phenotypes making individuals more or less sensitive to, e.g., environmentally induced cancer. On the other hand, there are real possibilities of identifying P450-based genetic biomarkers that can predict the outcome of drug therapy, including ADRs as well as therapeutic failures because of nonresponsiveness. Thus, this review focuses mainly on the influence of genetic polymorphism on drug toxicity.

**GENETIC POLYMORPHISM OF CYP**

**Historical Aspects**

Interindividual variation in response to a xenobiotic was probably described first by Pythagoras in 510 BC when he noted that some individuals developed hemolytic anemia after ingestion of fava beans. In the beginning of the last century, Garrod and Oxon suggested that a genetic component was involved in biochemical processes where the cause of interindividual differences in adverse reactions was because of enzyme deficiencies (Garrod, 1902). Thirty years later, Snyder described the first population-based study to identify ethnic differences in a pharmacogenetic trait, the phenylthiocarbamate nontaster phenotype (Snyder, 1932). In 1953, Bonicke and Reif (1953) for the first time described interindividual differences in elimination of isoniazid, and in 1960, Price Evans presented evidence that the phenotypes “slow” and “rapid” acetylators of isoniazid were under genetic control (Evans et al., 1960). The ideas of Garrod and Oxon suggest that a relationship between a genetic defect and an abnormal drug response was refined in the late 1950’s (Motulsky, 1957), and the term pharmacogenetics was introduced by Vogel at this time (Vogel, 1959).

The first examples of CYP polymorphism were published in the mid-1970’s (Eichelbaum et al., 1975; Mahgoub et al., 1977). The data originated from in vivo phenotyping of subjects or from outliers identified in the clinics. Treatment with the antihypertensive drug, debrisoquine, or treatment with the antiarrhythmic drug, sparteine, resulted in a wide interindividual range of plasma concentrations. Individuals with very high plasma levels of debrisoquine experienced orthostatic hypotension, and high plasma concentrations of sparteine plasma were reported to cause severe side effects such as placental abruption and uterus contractions, which in some cases resulted in fetal death (cf. Ingelman-Sundberg, 2004b). Both of these two drugs are metabolized by CYP2D6. The molecular genetics behind the wide interindividual differences in capacity to metabolize debrisoquine and sparteine has been very well studied, and today, 85 different CYP2D6 allelic variants have been described (see www.cypalleles.ki.se).

Following these examples, an ultrarapid metabolizer (UM) phenotype was identified in 1993 (Johansson et al., 1993). The subjects with this phenotype carry more than two active gene copies. Such copy number variants have been shown to result in nonresponsiveness to antidepressant therapy (cf. Ingelman-Sundberg et al., 2007), an apparently higher incidence of suicides (Zackrisson et al., 2010), and toxicity following treatment with codeine (cf. Stamer et al., 2010). Other important P450 polymorphisms were discovered within the CYP2C subfamily in the middle of the 1990’s. The CYP2C9*2 and *3 alleles (Rettie et al., 1994; Sullivan-Klose et al., 1996) and CYP2C19*2 and *3 (De Morais et al., 1994a,b) alleles cause reduced drug metabolic capacity for therapeutics that are substrates for these two enzymes. A CYP2C19 allele (CYP2C19*17) causing increased expression of CYP2C19 enzyme was identified in 2006 (Sim et al., 2006). An overview of the most important polymorphic P450 alleles with respect to drug toxicity is provided in Table 1. With respect to interindividual differences in drug metabolism caused by polymorphic enzymes, CYP2C9, CYP2C19, and CYP2D6 are those enzymes, which account for the majority of Food and Drug Administration (FDA) drug label warnings relating to pharmacogenomic drug differences (Frueh et al., 2008).

**P450 Phenotypes**

As mentioned, almost all the xenobiotic-metabolizing P450s are polymorphic, and the allelic variants are summarized and described on the home page of the human CYP allele nomenclature committee (see www.cypalleles.ki.se). The occurrence of different gene variants translates into four major phenotypes:

- Poor metabolizers (PMs), carrying two defective alleles and, therefore, completely lack a certain enzyme activity,
Intermediate metabolizers, heterozygous for a defect allele or carrying two alleles resulting in enzyme with decreased activity.
Extensive metabolizers (EMs), carrying two functional alleles, and
Ultrarapid metabolizers, carrying > 2 active gene copies.

The mutations resulting in the lack of enzyme are splicing defects, amino acid substitutions, and gene deletions. There are many different mutations that cause reduced enzyme activity often by creating unstable enzymes or amino acid changes leading to reduced substrate binding. This is the basis for the CYP2C9*3 allele, whereas the mutations in the CYP2C9*2 allele cause mainly reduced interactions between the P450 reductase and P450 enzyme (Ingelman-Sundberg, 2004a). Ultrarapid metabolism is caused by gene duplications, where a proportionally increased amount of enzyme is expressed in relation to the number of gene copies since also the promoter region and regulatory elements controlling the gene transcription are duplicated.

Lack of or very low activity of a specific enzyme might result in the accumulation of certain drugs/chemicals and cause toxicity. On the other hand, ultrarapid enzyme activity can also cause drug toxicity when the metabolite is more active or reactive than the parent compound. Following, we provide important examples where CYP polymorphism has caused increased drug or xenobiotic toxicity in a subpopulation.

### TABLE 1
Importance of Polymorphic CYP for the Metabolism of Drugs and Carcinogens

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrates</th>
<th>Polymorphism frequency</th>
<th>Functional effects</th>
<th>Most important polymorphic variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Carcinogens</td>
<td>Relatively high</td>
<td>Unproven</td>
<td>No important functional variant alleles</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Drugs, carcinogens</td>
<td>High</td>
<td>Rare</td>
<td>CYP1A2<em>1F, CYP1A2</em>1K</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>Carcinogens, estrogens</td>
<td>Rare null alleles, frequent missense mutations</td>
<td>At least seven haplotypes with similar activity</td>
<td>CYP1B1*7</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Nicotine, drugs, carcinogens</td>
<td>High in Orientals, less frequent in Caucasians</td>
<td>Important for nicotine metabolism</td>
<td>CYP2A6<em>1B, CYP2A6</em>4, CYP2A6<em>9, CYP2A6</em>12</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Drugs</td>
<td>High</td>
<td>Reduced drug metabolism</td>
<td>CYP2B6<em>5, CYP2B6</em>6, CYP2B6*16</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Some drugs</td>
<td>High</td>
<td>Reduced drug metabolism</td>
<td>CYP2C8*3</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Drugs</td>
<td>Relatively rare in Caucasians</td>
<td>Very significant</td>
<td>CYP2C9<em>2, CYP2C9</em>3</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Drugs</td>
<td>High</td>
<td>Very significant</td>
<td>CYP2C19<em>2, CYP2C19</em>3, CYP2C19*17</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Drugs</td>
<td>Very high</td>
<td>Very significant</td>
<td>CYP2D6<em>2xn, CYP2D6</em>4, CYP2D6<em>5, CYP2D6</em>10, CYP2D6*17</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Carcinogens, solvents, few drugs</td>
<td>Low</td>
<td>No significant cases have been reported</td>
<td>No important functional variant alleles</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Drugs, carcinogens</td>
<td>Low</td>
<td>No or small</td>
<td>CYP3A4*1B</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>Drugs, carcinogens</td>
<td>High</td>
<td>Significant</td>
<td>CYP3A5<em>3, CYP3A5</em>6, CYP3A5*7</td>
</tr>
<tr>
<td>CYP3A7</td>
<td>Drugs, carcinogens</td>
<td>Low</td>
<td>Some</td>
<td>CYP3A7*2</td>
</tr>
<tr>
<td>Drug</td>
<td>Enzyme</td>
<td>Toxicity</td>
<td>Number of subjects and comments</td>
<td>References</td>
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<tr>
<td>Antiepileptic drugs</td>
<td></td>
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<tr>
<td>Phenytin</td>
<td>CYP2C9</td>
<td>Neurological signs of phenytin intoxication after 10 days of treatment of woman homozygous for CYP2C9*3</td>
<td>Case report</td>
<td>Brandolese et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case report: A female African-American developed phenytin toxicity (mental confusion, slurred speech, memory loss, and inability to stand) after 2 weeks of treatment. The patient was homozygous for the null allele CYP2C9*6.</td>
<td></td>
<td>Kidd et al. (2001)</td>
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<tr>
<td>Antiemetic drugs</td>
<td></td>
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<tr>
<td>Metoclopramide</td>
<td>CYP2D6</td>
<td>Metoclopramide-induced acute dystonic reaction in two CYP2D6 PMs.</td>
<td>Case report (two patients)</td>
<td>van der Padt et al. (2006)</td>
</tr>
<tr>
<td>Analgetics/anti-inflammatory drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codeine</td>
<td>CYP2D6</td>
<td>Quick onset of severe abdominal pain after codeine in an UM 72-year-old man, UM, developed life-threatening respiratory depression after 3 days treatment with codeine.</td>
<td>Case report</td>
<td>Dalen et al. (1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29-month-old child, UM, experienced apnea resulting in brain injury after a dose of acetaminophen and codeine.</td>
<td>Case report</td>
<td>Madadi et al. (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UMs 50% higher plasma concentrations of morphine and its glucuronides compared with EMs.</td>
<td></td>
<td>Voronov et al. (2007)</td>
</tr>
<tr>
<td>Tramadol</td>
<td>CYP2D6</td>
<td>CYP2D6 UMs developed postoperative respiratory depression while receiving the tramadol. (The metabolite O-desmethyltramadol is the analgesic opioid.)</td>
<td>Case report</td>
<td>Stamer et al. (2008)</td>
</tr>
<tr>
<td>Drug</td>
<td>Enzyme</td>
<td>Toxicity</td>
<td>Number of subjects and comments</td>
<td>References</td>
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<tr>
<td>UMs more sensitive to tramadol than EMs.</td>
<td></td>
<td>11 UMs, 11 EMs, 3 PMs Significant higher morphine plasma concentration and much higher frequency of nausea in UMs compared with EMs (90% cf. 9%).</td>
<td>Kirchheiner et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>CYP2B6</td>
<td>CYP2B6*6 carriers were found to have higher postmortem methadone concentrations in blood.</td>
<td>40 postmortem methadone cases.</td>
<td>Bunten et al. (2010)</td>
</tr>
<tr>
<td>NSAIDs in general</td>
<td>CYP2C9</td>
<td>Increased risk of gastrointestinal bleedings in *2 and *3 carriers.</td>
<td></td>
<td>Martinez et al. (2004) and Pilotto et al. (2007)</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>CYP2C9</td>
<td>Individuals homozygous for CYP2C9*3 allele have reduced capacity to metabolize S-warfarin and are, therefore, at risk for bleeding events at normal dosing.</td>
<td>58/127</td>
<td>Higashi et al. (2002)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>CYP2C9</td>
<td>CYP2C9*3 was strongly associated with supratherapeutic anticoagulation during the first month of treatment. There was a tentative, but nonsignificant, difference between CYP2C9 genotype in the incidence of bleeding.</td>
<td>1496 patients</td>
<td>Lindh et al. (2009)</td>
</tr>
<tr>
<td>Warfarin + celecoxib</td>
<td>CYP2C9</td>
<td>Case report: A woman who had been on warfarin treatment for a year was given celecoxib for joint pain. This resulted in bleeding complications and it was found that she was compound heterozygous for CYP2C9*2 and *3.</td>
<td>Case report</td>
<td>Malhi et al. (2004)</td>
</tr>
<tr>
<td>S-acenocoumarol</td>
<td>CYP2C9</td>
<td>Individuals with variant CYP2C9 alleles—risk for major bleeding events.</td>
<td></td>
<td>Visser et al. (2004)</td>
</tr>
</tbody>
</table>
Table 2—Continued

<table>
<thead>
<tr>
<th>Drug</th>
<th>Enzyme</th>
<th>Toxicity</th>
<th>Number of subjects and comments</th>
<th>References</th>
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<tbody>
<tr>
<td>Antiplatelet aggregation drug</td>
<td></td>
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</tr>
<tr>
<td>Clopidogrel</td>
<td>CYP2C19</td>
<td>Enhanced response to clopidogrel and increased risk of bleeding in CYP2C19<em>17 carriers. Improved protective effect of clopidogrel after myocardial infarction in patients carrying CYP2C19</em>17.</td>
<td>1524 patients, 76 homozygous for CYP2C19*17.</td>
<td>Sibbing et al. (2010)</td>
</tr>
<tr>
<td>Antianginal drug</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Perhexiline</td>
<td>CYP2D6</td>
<td>Significant overrepresentation of PM phenotype among perhexiline-induced neuropathy (50%). Three out of four patients with liver injury PM phenotype and 1 intermediate metabolizer. Four patients with liver injury.</td>
<td>20 patients with neuropathy and 14 without.</td>
<td>Shah et al. (1982)</td>
</tr>
<tr>
<td>Immunosuppressive drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tacrolimus</td>
<td>CYP3A5</td>
<td>CYP3A5 SNPs associated with development of nephrotoxicity in renal transplant recipients.</td>
<td></td>
<td>Kuypers et al. (2007)</td>
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<tr>
<td>HIV drugs</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Efavirenz</td>
<td>CYP2B6</td>
<td>Homozygous CYP2B6<em>6 correlates with high plasma concentration of efavirenz. Risk of side effects such as headache, dizziness, insomnia, and fatigue. CYP2B6 510TT (present in CYP2B6</em>6) significant association with higher plasma and intracellular exposure and neuropsychological toxicity in efavirenz-treated patients. CYP2B6*6—CNS side effects after 1 week of efavirenz treatment. Development of tolerance, no difference versus *1 after 24 weeks. Differences in plasma concentration both at 1 week and 24 weeks.</td>
<td>2/35 Japanese patients’ high plasma concentration, both CYP2D6*6 homozygous. 167 patients 152 patients</td>
<td>Tsuchiya et al. (2004)  Rotger et al. (2005)  Haas et al. (2004)</td>
</tr>
</tbody>
</table>
IMPORTANT EXAMPLES OF ADVERSE REACTIONS CAUSED BY P450 POLYMORPHISM

Central Nervous System–Active Drugs

A summary of examples of ADRs dependent on genetic polymorphism of P450s is presented in Table 2.

Morphine Toxicity—Ultrarapid Metabolism of Codeine

A tragic case was originally reported in a Canadian newspaper in May 2006. A 13-day-old baby died from morphine poisoning when the mother was prescribed codeine and acetaminophen during the postpartum period (Madadi et al., 2007). She was later identified as an UM of CYP2D6 substrates, carrying one extra copy of a functional CYP2D6 gene. The extra CYP2D6 enzyme resulted in increased O-demethylation of codeine to morphine, and consequently, very high concentrations of morphine were found in both the breast milk and in the blood from the child. Codeine itself has only mild analgesic and central nervous system (CNS)–depressant effects; however, increased CYP2D6 activity has been shown to result in serious morphine toxicity even with small doses of codeine. Respiratory depression has been reported after a small codeine dose in an adult patient (Gasche et al., 2004), and the UM genotype results in 50% higher plasma concentrations of morphine as compared with EMs (Kirchheiner et al., 2007). Since 2007, the FDA requires the manufacturers of prescription codeine products to include information in the “Precautions” section of the label to inform prescribing doctors about these risks and to help prevent morphine overdose in breastfed infants.

Tramadol Toxicity

Tramadol is another example of a drug that is metabolized by CYP2D6 to generate a pharmacologically active product, the analgesic opioid receptor agonist O-desmethyltramadol. Variability in response is, therefore, closely related to the CYP2D6 genotype (Stamer et al., 2007). Patients being CYP2D6 UM are at risk for toxic responses to tramadol treatment. Thus, a case report described a man with renal insufficiency and a CYP2D6 UM genotype who developed postoperative respiratory depression while receiving tramadol IV (Stamer et al., 2008). CYP2D6 UM patients have high plasma levels of O-desmethyltramadol and better pain control than EMs, but UMs also experience higher frequency of nausea (Kirchheiner et al., 2008). In conclusion, CYP2D6 UMs might be at greater risk for opiate-related adverse effects and might benefit from a lower dosage of prodrugs such as codeine and tramadol; special cautions must be taken for patients with renal insufficiency (Kosarac et al., 2009).
Methadone-Related Deaths

Methadone, an opioid μ-receptor agonist, is effective as a substitute for heroin addiction and is also used in the treatment of pain. Interindividual variation in blood methadone concentrations have been reported, and in addition, interindividual differences in sensitivity to methadone have been observed. Fatal poisonings occur typically at concentrations between 0.4 and 1.8 mg/ml. However, in some individuals, fatalities occur at much lower concentrations. In a recent publication, 40 postmortem cases were presented in which methadone had been implicated in the cause of death. The CYP2B6*6 alleles were significantly found to be associated with higher postmortem methadone concentration (Bunten et al., 2010). This allelic variant results in slow metabolism.

CYP2A6 Polymorphism and Nicotine Metabolism

Research, in particular by Tyndale and collaborators, has indicated an association between smoking behavior and CYP2A6 polymorphism (cf. Mwenifumbo and Tyndale, 2009). Specifically, a relation between the CYP2A6*4 allele and susceptibility for lung cancer has been published (Ariyoshi et al., 2002). The hypothesis explaining this has been the fact that CYP2A6 is a major catalyst of nicotine metabolism, and carriers of the defective CYP2A6*4 allele would require fewer cigarettes in order to achieve sufficient levels of nicotine in the blood. Such a relationship was recently confirmed in a genome-wide association study where the smoking behavior versus genetics in 87,000 individuals was analyzed where a very significant association to the CYP2A6 and CYP2B6 loci was found, whereas the association with lung cancer was less evident (Thorgeirsson et al., 2010).

Antidepressants—Cardiotoxicity

Mirtazapine is a tetracyclic antidepressant drug with few anticholinergic adverse effects. It has a dose-dependent sedative effect, which is therapeutically useful for depressed patients with sleep disorder. Interindividual variations in sedation and in cardiovascular effects have been described. Brockmoller et al. (2007) have shown differences in pharmacokinetics between the mirtazapine enantiomers. Only S(-) mirtazapine is efficiently hydroxylated by CYP2D6. Moreover, regarding the cardiovascular effect, there are indications for enantiomer differences, with a more pronounced effect on heart rate and blood pressure with the R(-) enantiomer. In CYP2D6 UMs, one might consider administration of higher doses in order to achieve an appropriate antidepressant and sedative effect; however, there will not be similar ultrarapid elimination of the cardiovascular-active R(-) mirtazapine, resulting in a high risk of cardiovascular adverse effects (Brockmoller et al., 2007).

Antiemetic Drug Metoclopramide—Acute Dystonic Reaction in a CYP2D6 PM

A case report from 2006 describes two patients who experienced acute dystonic reactions when receiving the antiemetic drug metoclopramide (van der Padt et al., 2006). Genetic analysis revealed that both patients were CYP2D6 PM. Metoclopramide is known to be both a substrate and an inhibitor of CYP2D6, and extrapyramidal syndrome (EPS) because of treatment with this drug has been observed in 1/500 patients. Drug-induced EPS is more frequently found in patients deficient in CYP2D6, and therefore, metoclopramide probably should be avoided in patients at risk of acute dystonic reactions (van der Padt et al., 2006).

Cardiovascular Drugs

Drugs Associated with Increased Risk of Bleeding Events

Several non-steroidal anti-inflammatory drugs (NSAIDs) are metabolized by CYP2C9, and variant alleles of CYP2C9 are more frequent in patients with NSAID-induced acute gastric bleedings. This effect is more pronounced for drugs metabolized mainly by CYP2C9 (Martinez et al., 2004).

Vitamin K Antagonists

The vitamin K antagonists warfarin, acenocoumarol, and phenprocoumon are used as oral anticoagulant agents. The S-enantiomers of these drugs are metabolized by CYP2C9, and for warfarin and phenprocoumon, the S-enantiomers are mainly responsible for the anticoagulant effect. Individuals carrying the variant CYP2C9 alleles *2 and *3 have a significant reduction of warfarin clearance and are more susceptible to adverse bleeding events (Kirchheiner and Brockmoller, 2005). Higher rates of bleeding complications during initiation warfarin treatment have been reported in patients carrying the *2 or *3 variants of CYP2C9 (Aithal et al., 1999; Takahashi and Echizen, 2001). From a large prospective study, including approximately 1500 patients, it was concluded that genotyping of CYP2C9 and VKORC1 predicted warfarin dose and patients predisposed to unstable anticoagulation during initiation of therapy. The overall dosing prediction by the CYP2C9 and VKORC1 polymorphism is about 35% whereas the influence of VKORC1 is much higher than that of CYP2C9 (Lenzini et al., 2010).

Interactions have been presented for the combination of acenocoumarol therapy and concomitant use of NSAID. Patients with variant CYP2C9 enzymes showed increased prothrombin time when NSAIDs were coadministered with celecoxib (40) or acenocoumarol (Beinema et al., 2007).

ADP P2Y12 Receptor Inhibitors

Clopidogrel is a prodrug, which is converted to its active metabolite in a CYP-catalyzed reaction. CYP2C19 is the main enzyme responsible for this activation. The active clopidogrel
metabolite inhibits the ADP P2Y12 receptor causing inhibition of platelet aggregation. Carriers of the CYP2C19*17 allele, an allelic variant associated with increased enzyme activity, are found to have an improved protective effect of clopidogrel treatment after acute myocardial infarction (Tiroch et al., 2010). Other studies have shown that the enhanced response to clopidogrel by CYP2C19*17 is also associated with an increased risk of bleeding (Sibbing et al., 2010). Associated with defective CYP2C19*2 and CYP2C19*3 alleles, reduced effectiveness of clopidogrel and an increased risk for stent thrombosis have been observed (Jin et al., 2010; Mega et al., 2010a; Rennings et al., 2010; Wallentin et al., 2010). Overall, during the last couple of months, about 20 studies have been published in this area, and in summary, defective alleles associate with decreased efficacy as reviewed in a recent meta-analysis (Mega et al., 2010b). Recently, prasugrel and ticagrelor have been developed against the same receptor, which are not subject to activation via the polymorphic enzyme CYP2C19, and in the future, the problems with the interindividual differences in the treatment because of the CYP2C19 polymorphism might be minimized.

Perhexiline-Induced Hepatotoxicity and Neuropathy

Perhexiline was developed as an antianginal drug in the 1970’s. Although, it was a drug with good efficacy, it was of limited use because of severe neurotoxicity and hepatotoxicity in a small subpopulation of patients. The toxicity was associated with high plasma concentrations in CYP2D6 PM patients during long-term treatment at standard doses (Morgan et al., 1984; Shah et al., 1982). CYP2D6 PMs were significantly overrepresented (50%) among patients with neurotoxicity and hepatitis, and no PMs were found among patients without neuropathy. The drug was withdrawn from the market in most countries in the late 1980’s but continued to be prescribed in some countries, e.g., Australia and New Zealand. The mechanism behind both the pharmacological effect and the toxicity of perhexiline has been elucidated. Perhexiline is a potent carnitine palmitoyltransferase (CPT)-1 and CPT-2 inhibitor, which might shift myocardial metabolism from fatty acids toward carbohydrate metabolism (Kennedy et al., 1996) resulting in increased myocardial energy production at lower oxygen cost (Essop and Opie, 2004). Dose-dependent toxic effects are suggested to be caused by profound CPT-1 inhibition and fatty acid metabolism inhibition (Ashrafian et al., 2007). Today, this highly potent antianginal drug is safely used in Australia based on careful monitoring of the plasma concentration in the treated patients. Genotyping service is offered in Australia, but not mandatory. In fact, the toxicity of perhexiline is a very rare problem subsequent to careful therapeutic drug monitoring.

Toxicity of Antiepileptic Drugs

In 2001, an interesting case with a woman treated with phenytoin was published (Brandolese et al., 2001). After 10 days of treatment, she developed manifest neurological signs of phenytoin intoxication and serum concentration determinations showed an elimination half-life of 103 h, which is almost five times longer than normal. Phenytoin metabolism is mainly catalyzed by CYP2C9 and to a lesser extent by CYP2C19. Genotyping analysis revealed that the patient was homozygous for CYP2C9*3 and heterozygous for CYP2C19*2, indicating mainly CYP2C9*3 as responsible for the drug overdose (Brandolese et al., 2001).

In the same year, another phenytoin intoxication case was published. This patient was a female African-American presented to the emergency department with mental confusion, slurred speech, memory loss, and inability to stand. The symptoms were caused by phenytoin toxicity, and the patient exhibited extremely poor clearance of phenytoin with an elimination half-life of 13 days, i.e., approximately 14 times longer than normal (Kidd et al., 2001). Genetic analysis revealed that she was homozygous for the first null allele identified for CYP2C9, the *6 variant. In African-Americans, the frequency of this allele is 0.6%, whereas Caucasians seem to lack this variant (Kidd et al., 2001). Besides these case reports, no important data have been obtained, and the area requires more research. However, monitoring of plasma concentrations of antiepileptic drugs is commonly used, resulting in relatively good control of dosage.

Adverse Reactions during Human Immunodeficiency Virus Treatment

The use of antiretroviral drugs has drastically improved the survival of human immunodeficiency virus (HIV)–infected patients. The choice of the treatment is critical because of the lifelong treatment and in some patients associated with toxicity.

Efavirenz is a nonnucleoside reverse transcriptase inhibitor and some patients develop CNS symptoms such as headache, dizziness, insomnia, and fatigue. High efavirenz plasma concentration is reported to increase the risk of these adverse symptoms. The CYP2B6*6 allele, which results in an enzyme with relatively slow metabolic capacity, has been found to be associated with high efavirenz plasma concentrations. In a Japanese study with 60 HIV patients, significantly two subjects homozygous for the CYP2B6*6 allele had the highest plasma concentration of efavirenz (Tsuchiya et al., 2004). In addition, another nine samples were selected, six having normal concentration and three with elevated efavirenz concentration, and all these three were from individuals homozygous for CYP2B6*6. Overall, there was a significant correlation between the CYP2B6*6/*6 genotype and high plasma concentration of efavirenz, and treatment with lower doses would decrease the incidence of toxicity in these
patients. Furthermore, in a Swiss study on 167 efavirenz-treated patients, a significant association was observed between CYP2B6 516TT (one of the two single nucleotide polymorphisms [SNPs] in CYP2B6*6) and sleep disorder, as well as fatigue (Rotger et al., 2005).

Abacavir is associated with a severe multiorgan hypersensitivity syndrome in 5–8% of Caucasian HIV patients and 3% of African-American patients. In Caucasians, this reaction only occurs in patients with HLA-B genotype *5701, and as many as 50% of patients with this specific genotype develop this severe adverse reaction during abacavir treatment. A large multicenter study has clearly shown that prescreening for this genotype in order to avoid abacavir treatment in HLA-B*5701-positive patients has significantly reduced the risk of the severe hypersensitivity reaction because of this drug and increased the safety of the therapy (Mallal et al., 2008; Phillips and Mallal, 2010).

Other Genetic Determinants of Drug Toxicity

The area of pharmacogenomic biomarkers with a predictive value for ADRs is expanding. Besides the case of abacavir, variant human leukocyte antigen (HLA) class I and II alleles have been found to predict the toxicity of many drugs. Many of these have been identified through genome-wide association analyses of large cohorts of patients and controls. Similarly, hypersensitivity reactions to the antiepileptic drug carbamazepine is fully linked to the HLA-B*1502 allele in Asians, but not in Caucasians (Chung et al., 2004). In addition, allopurinol, an uric acid lowering drug prescribed to patients with hyperuricemia and gouty arthritis, causes Stevens-Johnson syndrome and toxic epidermal necrolysis in certain subjects, and the toxicity is highly associated to the HLA-B*5801 allele (Hung et al., 2005).

Interestingly, it has been described that subjects with the HLA-B*5701 genotype have an increased risk of experiencing flucloxacillin-induced hepatotoxicity, although at lower frequency. One in 500 patients with the HLA-B*5701 genotype has been reported to develop liver injury from flucloxacillin (Daly et al., 2009). Specifically, the odds ratio was as high as 30, and 65% of the cases could be identified as carriers of this allele. The low frequency of the drug-induced liver injury in question would, however, not warrant population screening for people undergoing the therapy. In addition, it has been proposed that flucloxacillin is a ligand of the nuclear receptor, pregnane X receptor, and that genetic polymorphism in this gene might contribute to the hepatotoxic effect of the antibiotic flucloxacillin (Andrews et al., 2010).

Additional linkages between the human leukocyte antigens and ADRs have been noted during treatment with co-amoxiclav where the drug-induced liver injury correlates to HLA-DRB1*1501, although the association is much weaker than noted for HLA-B*5701 and flucloxacillin-induced toxicity (cf. Daly, 2010; Daly and Day, 2009). Similar HLA antigens also predict the toxicity exerted by the cyclooxygenas-2 inhibitor lumiracoxib, previously withdrawn from the market because of drug-induced liver injury reactions, where a high predictability of toxicity was observed for, e.g., HLA-DQA1*0102 allele (Singer et al., 2010). In fact, this predictability is so high that the company has now applied for reentering the market for treatment of genetically defined populations.

POLYMORPHIC ALLELES IN CANCER THERAPY

Polymorphism in gene encoding enzymes, active in the metabolism of anticancer drugs, is of special importance because of the narrow therapeutic range of these drugs and their use at near cytotoxic doses to nontransformed cells. With respect to influence on toxicity, the UGT1A1*28 polymorphism is of great importance for the treatment of colon cancer with irinotecan where the active metabolite SN58 is metabolized by the UGT1A1 enzyme. Subjects carrying the common UGT1A1*28 allele, having an extra TA repeat in the promoter region causing lower enzyme expression, are at higher risk for suffering ADRs (Iyer et al., 2002). Interestingly, the transgenic expression of this variant in mice causes higher bilirubin levels and Steven Johnson like symptoms, as compared with mice transgenic for the wild-type UGT1A1 allele (Cai et al., 2010). The FDA recommends dose adjustments in relation to the UGT1A1 polymorphism.

Furthermore, treatment with thiopurines is a clear case of polyphonically important susceptibility for toxicity, in particular leukopenia, where subjects defective for the thiopurine S-methyltransferase enzyme are unable to metabolize the thiopurines. This is of concern, especially with respect to azathioprine and 6-mercaptopurine, which are used in the treatment of leukemia and autoimmune diseases, e.g., inflammatory bowel disease. The frequency of subjects defective in this enzyme is rather low (about 1% in Caucasian populations), but the therapy in these particular patients is severely improved by predictive geno/phenotyping (Evans, 2004). An individualized dosing of these anticancer agents reduces the occurrence of secondary tumors, e.g., in the brain (Pui et al., 2005).

CONCLUSION

The data at hand indicate that the susceptibility for cancer and the metabolic activation of procarcinogens in humans are not influenced to a great extent by CYP polymorphism. By contrast, there are a growing number of reports where the polymorphism influences the drug treatment outcome mainly in relation to the highly polymorphic CYP2B6, CYP2C9, CYP2C19, and CYP2D6 enzymes. The most striking influence seen at present is the effects of the defective and rapid CYP2C19 alleles on the outcome of clopidogrel treatment; the
world’s second most sold drug today as well as the severe adverse reaction in CYP2D6 UMs seen after codeine therapy. Another important indirect effect is observed in the cases of nonresponsiveness to antidepressant therapy and suicides reported in subjects being UMs for CYP2D6. Further important CYP polymorphism influenced drug toxicities include bleeding, as a result of overdosing of patients carrying defective CYP2C9 alleles. In addition, the CYP2A6 polymorphic influence of nicotine metabolism and smoking behavior is important. The rapid development of the next generation sequencing methods and array techniques for analyses of copy number variations and SNPs results in an increasing rate of observations where genetic variants, in particular of human leukocyte antigens, are found to be associated with specific drug toxicity. It is our expectation that this research field will provide both the industry and the clinicians with useful pharmacogenomic biomarkers that can aid in procedures for drug development as well as for the specific drug treatment in order to optimize the results and improve human health. The process is slow, and for a relevant basis for decisions of mandatory biomarkers to be used, further prospective large clinical studies are required. One fruitful manner in which this can be achieved is a closer collaboration between industry and academics, and the results of, e.g., the industrial consortia supporting research in the area of drug-induced liver injury are promising in this respect.

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