Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future

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The field of cytochrome P450 pharmacogenetics has progressed rapidly during the past 25 years. All the major human drug-metabolizing P450 enzymes have been identified and cloned, and the major gene variants that cause inter-individual variability in drug response and are related to adverse drug reactions have been identified. This information now provides the basis for the use of predictive pharmacogenetics to yield drug therapies that are more efficient and safer. Today, we understand which drugs warrant dosing based on pharmacogenetics to improve drug treatment. It is anticipated that, in the future, genotyping could be used to personalize drug treatment for vast numbers of subjects, decreasing the cost of drug treatment and increasing the efficacy of drugs and health in general. I estimate that such personalized P450 gene-based treatment would be relevant for 10–20% of all drug therapy.

The cytochrome P450 enzymes in families 1–3 mediate 70–80% of all phase I-dependent metabolism of clinically used drugs [1,2] and participate in the metabolism of a huge number of xenobiotic chemicals. The polymorphic forms of P450s are responsible for the development of a significant number of adverse drug reactions (ADRs). According to Phillips *et al.* [3], 56% of drugs that are cited in ADR studies are metabolized by polymorphic phase I enzymes, of which 86% are P450s. Only 20% of drugs that are substrates for non-polymorphic enzymes are in the ADR reports. It has been estimated that: (i) ADRs cost the US society ∼US$100 billion; (ii) ADRs cause >100 000 deaths annually in the USA; and (iii) up to 7% of all hospital admissions in the UK and Sweden are due to ADRs (see [4] for references). In addition, the costs of treating patients who possess polymorphic forms of P450s are much higher than those required to treat patients who possess non-polymorphic alleles. Furthermore, the number of non-responders to drug therapy is high and represents 30–60% of subjects treated with drugs [5]. Thus, knowledge about the P450 system is fundamental both for drug therapy and for drug development [6,7]. In this article, progress in the study of polymorphic P450s that are important for drug metabolism is presented with special emphasis on the clinical relevance of this research and the history of the discoveries.

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Cytochrome P450 25 years ago

When *TiPS* was first published in 1979 the debate regarding whether one or more forms of P450 existed had recently been resolved. In 1978, the first P450 was purified to homogeneity, now termed CYP2B4, and the first P450 (CYP2B1 from rat) was cloned three years later. Attendees of the 5th Microsomes and Drug Oxidation (MDO) meeting in Tokyo were surprised and silent when the first cDNA sequence was presented by Fujii-Kuriyama in the summer of 1981. At that time it was difficult to foresee the rapid development that has since been achieved during the subsequent 23 years, including the identification of the role of P450s in drug metabolism and the major genetic variants of P450s that cause both inter-individual variability in drug response and ADRs.

Human cytochrome P450 genes

The milestone of the completion of the sequence of the human genome allowed David Nelson to provide evidence for the presence of 57 different active genes encoding P450 enzymes (http://drnelson.utmem.edu/CytochromeP450.html) and a similar number (58) of pseudogenes [8]. This number of active genes is much smaller than that observed in rice (323 genes), Thale cress (249 genes) or the mouse (102 genes) but is similar to that observed in the dog (54 genes). The major P450 forms that are important in human drug metabolism are shown, together with their properties and polymorphisms, in Table 1.

Polymorphism of P450 genes

All genes encoding P450 enzymes in families 1–3 are polymorphic. The functional importance of the variant alleles, however, differs and the frequencies of their distribution in different ethnic groups also differ. Updated information can be found on the Human CYPallele Nomenclature Website (http://www.imm.ki.se/cypalleles). Polymorphic enzymes (in particular CYP2C9, CYP2C19 and CYP2D6) mediate ∼40% of P450-mediated drug metabolism, which makes drug dosing problematic. In general, four phenotypes can be identified: poor metabolizers (PMs), who lack the functional enzyme; intermediary metabolizers (IMs), who are heterozygous for one deficient allele or carry two alleles that cause reduced activity; extensive metabolizers (EMs), who have two normal alleles; and ultrarapid metabolizers (UMs), who

Table 1. Relative importance of polymorphisms in human cytochrome P450 enzymes involved in drug metabolism

Enzyme	Fraction of drug metabolism (%)[a]	Substrates	Major allelic variants[b]	Clinical effects of the polymorphism	Significance of the polymorphism[c]
CYP1A2	5	Drugs, carcinogens	CYP1A2*1K	Less enzyme expression and inducibility	+
CYP2A6	2	Nicotine, drugs, carcinogens	CYP2A6*4, CYP2A6*9	Altered nicotine metabolism	+
CYP2B6	2–4	Drugs	–	Significant for the metabolism of cancer drugs	+
CYP2C8	1	Drugs	CYP2C8*3	Altered taxol metabolism	+
CYP2C9	10	Drugs	CYP2C9*2, CYP2C9*3	Drug dosage[d]	+++
CYP2C19	5	Drugs	CYP2C19*2, CYP2C19*3	Drug dosage[d], drug efficacy	+++
CYP2D6	20–30	Drugs	CYP2D6*2xn	No response	+++
			CYP2D6*4	Drug dosage[d]	+++
			CYP2D6*10	Drug dosage[d]	+
			CYP2D6*17	Drug dosage[d]?	+
			CYP2D6*41	Drug dosage[d]?	+
CYP2E1	2–4	Carcinogens, solvents, drugs	–	No conclusive studies	–
CYP3A4	40–45	Drugs, carcinogens	Rare	No conclusive studies	–
CYP3A5	<1	Drugs	CYP3A5*3	No conclusive studies	–

[a]The estimated fraction of responsibility of the respective enzyme for drug metabolism in phase 1 reactions as a percentage of drugs metabolized by all cytochrome P450 enzymes. Data are from Bertz and Granneman [1], Relling and Evans [2] and M. Ingelman-Sundberg, unpublished.
[b]A description of the alleles can be found on the human cytochrome P450 allele nomenclature committee home page (http://www.imm.ki.se/CYPalleles/).
[c]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. Increasing numbers of ' + ' illustrate the increasing importance of the polymorphism relative to the other forms of P450.
[d]The dose of the drug is advantageously adjusted depending on the genotype with respect to the individual enzyme.

have multiple gene copies, a trait that is dominantly inherited. As illustrated in Figure 1 with CYP2D6-dependent metabolism as an example, the rate of metabolism for a certain drug can differ 1000-fold between phenotypes. Thus, the dosing required to achieve the same plasma levels of a drug metabolized mainly by CYP2D6, such as nortriptyline, differs 10–20-fold among individuals. Despite this extensive variation in metabolic capacity among individuals, dosing is, at present, principally population based (i.e. doses are based on the plasma levels of the drug obtained on average in the population at a certain dosage). Accordingly, in the European population, determination of appropriate doses of drugs that are substrates for CYP2D6 is not routinely carried out for: (i) 5.5% of the population who are UMs (taking into account the different frequencies of UMs in different countries) and are not expected to exhibit a response as a result of too rapid metabolism of the drug; and (ii) 7% of the population who are PMs, in which ADRs as a result of too high plasma levels of the drug are likely (Figure 2). That is, 35–50 million people in Europe could be considered as 'forgotten' with respect to the 20–30% of all drugs that are metabolized by CYP2D6 because they do not receive dosing that is adjusted according to their *CYP2D6* genotype.

Approximately 40–45% of all drug metabolism in phase I is carried out by CYP3A4. This enzyme is highly conserved across different individuals and essentially no functionally variant forms have been observed in Caucasians or Orientals [9,10]. However, there is a relatively high inter-individual variability in CYP3A4 activity, which has been suggested to be of genetic origin [11], although the reasons for this have not been explained.

Major causes that underlie inter-individual variability in drug metabolism of course also include poor compliance, unfavourable drug–drug interactions and pathophysiological conditions, factors that are always of great importance for the understanding of sub-optimal drug therapy.

The background to the major discoveries in the field of P450

Inter-individual differences in response to a xenobiotic was probably described first by Pythagoras in 510 BC when he noted that some, but not all, individuals develop
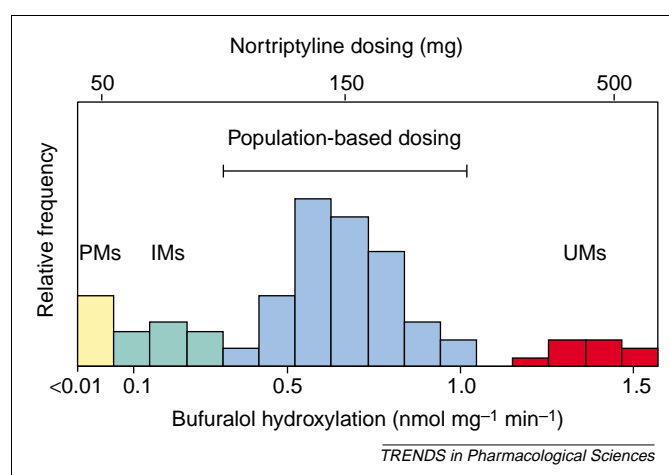
Figure 1. Variation in drug metabolism and nortriptyline dosing in the European population, based on cytochrome P450 CYP2D6 activity (hydroxylation of bufuralol). Within the population four phenotypes can be identified: poor metabolizers (PMs), who lack the functional enzyme; intermediary metabolizers (IMs), who are heterozygous for one functional allele or have two partially defective alleles encoding the enzyme; extensive metabolizers (EMs), who have two normal alleles; and ultrarapid metabolizers (UMs), who carry duplicated or multiduplicated functional *CYP2D6* genes. The relative frequency of these phenotypes refers to the European population as a whole. The doses of nortriptyline that are required to achieve therapeutic levels in all phenotypes are given. Despite this variation in metabolizing capability, population-based dosing is used today, and is based on the average plasma levels obtained in a given population for a given dose. Figure is adapted from Zanger et al. [84] and extrapolated to the whole European population.
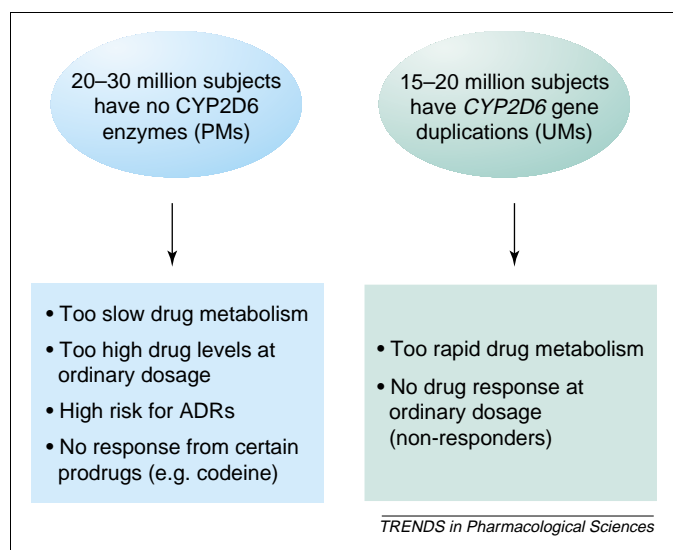
Figure 2. The consequences of outlier cytochrome P450 CYP2D6-dependent drug metabolism. 35–50 million Europeans are either poor metabolizers (PMs) (i.e. lack the functional enzyme) or ultrarapid metabolizers (UMs) (i.e. have multiple gene copies of *CYP2D6*, resulting in elevated enzyme levels), with respect to CYP2D6. As a result of the use of population-based dosing, drug treatment can result in many different effects in these subjects. Abbreviation: ADRs, adverse drug reactions.

haemolytic anaemia in response to fava bean ingestion. In a report by Gorrod and Oxon in 1902 [12], a genetic component was suggested to be involved in biochemical processes where the cause of inter-individual differences in ADRs was due to enzyme deficiencies. Thirty years later in 1932, Snyder [13] described the first population-based study to identify ethnic differences in a pharmacogenetic trait, namely the phenylthiocarbamate non-taster phenotype. In 1957, Motolosky [14] refined the ideas of Gorrod and Oxon, suggesting that inter-individual differences in drug efficacy and ADRs were in part due to genetic variation, and in 1959 Vogel [15] coined the term 'pharmacogenetics'. At the time of the discoveries of the polymorphic P450-dependent metabolism of several drugs, the polymorphic reactions of, for example, *N*-acetyltransferase, aldehyde dehydrogenase and paraoxone oxidase had already been described. Most of the major findings in the P450 field were based on ADRs observed in one individual or a small group of individuals. In general, it has taken a decade from the initial phenotypic description of the polymorphism to establish the genetic background.

The 'debrisoquine polymorphism'
The polymorphism of *CYP2D6*, termed the 'debrisoquine polymorphism' because CYP2D6 metabolizes debrisoquine, was discovered independently in three different laboratories. Early reports in 1967 by Folke Sjöqvist and collaborators regarding the metabolism of nortriptyline and desimipramine, later shown to be CYP2D6 substrates, revealed a tremendous inter-individual variation in plasma levels of the drugs following administration of the same dosage, and subsequently two metabolizer phenotypes were identified [16]. Alexandersson and Sjöqvist thereafter studied twins and concluded that the cause of this variation was genetic [17]. At St Marys hospital in London the metabolism of amphetamine was studied, and

Bob Smith, who carried out experiments on himself, was found to be metabolically 'odd' in this respect, but the studies were not followed up [18]. Instead, the researchers decided to study the metabolism of two antihypersensitive drugs, bethanidine and debrisoquine, that were in clinical use for the treatment of hypertension. In May 1975, when Smith took 40 mg of debrisoquine he became dizzy, faint and suffered from severe hypotension. This suggested that some individuals were non-metabolizers of the drug, and a subsequent study in which 94 subjects were given 10 mg of debrisoquine revealed this hypothesis to be true with the identification of two phenotypes [19].

In Bonn, the anti-arrhythmic effects of sparteine were studied and two subjects complained about unpleasant side-effects such as blurred vision, double vision (diploida), dizziness and headache. Analysis of the plasma levels of these subjects revealed that they possessed 4–5 times higher plasma levels of sparteine than the other subjects. This study was published both in abstract form and in Michel Eichelbaums Habilitationsschrift in 1975 [20] and prompted the authors to investigate the frequency of this effect. The two metabolizer phenotypes were published in 1978 [21] and the original publication came in 1979 [22].

The genetic basis of the debrisoquine polymorphism was elucidated 10–15 years following these initial studies. Urs Meyer, who was studying porphyria, became interested in P450 enzymes because it was known that 80% of haem synthesis is required to maintain levels of P450s and because P450s metabolize drugs that cause porphyria. In Meyer's liver bank, two PM livers for debrisoquine metabolism were identified. Meyer and colleagues were inspired by Luc Balant and Pierre Dayer in Geneva, who, in 1976, described a defect in the β-adrenoceptor blocking agent bufuralol in a family and later showed that it was a pharmacogenetic trait that was related to the debrisoquine polymorphism [23]. Bufuralol has fluorescent properties and could be used to monitor enzyme activity at high sensitivity, which was crucial for the purification of the enzyme from the rat liver and subsequently allowed the development of antibodies to CYP2D6 [24]. Fruitful discussions between Meyer and Frank Gonzalez led to a collaboration that resulted in the use of the polyclonal rat CYP2D antibodies, by Gonzalez, to clone the human CYP2D6 cDNA from a human liver λgt 11 cDNA library; the expressed cDNA had the expected bufuralol hydroxylase activity [25]. Using restriction fragment length polymorphism (RFLP), Meyers' laboratory, in 1990, confirmed altered CYP2D RFLP patterns in some PMs for debrisoquine, whereas the complete identification of the *CYP2D6*3* and *CYP2D6*4* alleles was published in 1990 [26]. The major genetic defect was also published simultaneously in the UK [27].

At this time, my colleagues and I were studying the genetic basis for slower debrisoquine metabolism in Chinese compared with Caucasians, as had been described by Leif Bertilsson and colleagues [28]. The studies were conducted in fruitful collaboration with Folke Sjöqvist and Bertilsson. We identified an RFLP analysis pattern in Chinese that was indicative of the presence of PMs in Chinese, but not in Caucasians [29], and later characterized the most common partially defective *CYP2D6* allele in

Orientals, *CYP2D6*10* [30]. A French group opposed our size identification of the Chinese *Xba* I haplotype [31]. Provoked by this, we re-screened several different DNA samples by *Xba* I RFLP using a lower density of the agarose gel and found some samples that by *Xba* I RFLP had much higher molecular weight DNA than was evident from the higher-density gels. We thought these were undigested. However, examination of their origin revealed that they came from individuals who possessed very rapid debrisoquine metabolism. A crucial experiment carried out by Inger Johansson was to digest the genomic DNA with *Eco* RI. This revealed 12 extra *CYP2D6* gene copies in the subjects who possessed very high metabolic activity. It turned out to be the first description of a stably amplified active gene in humans [32]. Retrospective analysis of genomic DNA from a non-responder to antidepressant therapy, described by Bertilsson in 1985, revealed that the non-responder possessed the UM phenotype and carried three copies of the *CYP2D6* gene [33]. Subsequent analysis in Ethiopians showed that this variant allele occurred in 29% of the subjects [34], and in a study of Spaniards, 10% were found to be UMs [35], revealing the high global significance of the UM phenotype. Indeed, 10% of the population in Italy and Turkey has now been found to carry this phenotype, whereas it is uncommon (1–2%) in Northern Europe and essentially absent in Asia [36]. We have proposed, based on results from phenotyping of Ethiopians in Ethiopia and Ethiopians in Sweden, that selection by diet has caused the frequent occurrence of this genotype in Northeast Africa in particular in Ethiopia, ~5000–12 000 years ago. Migration from Northeast Africa to the Mediterranean area is responsible for the frequent occurrence of UMs in the latter area [37]. Integrating the results obtained in different West European countries reveals that as much as 5.5% of the European population carry the UM genotype.

The polymorphisms of S-*mephenytoin and phenytoin metabolism*

Mephenytoin was developed as an anticonvulsant agent in the early 1940s and has been shown to be effective in the treatment of seizures. Adrian Küpfer identified a stereoselective metabolism of mephenytoin and reported that one individual complained of unacceptable sedation following a low dose, whereas other subjects did not react in such a manner to the same dose. Subsequent analysis of the urine of the subject who complained of the adverse side-effect revealed a marked impairment of the formation of 4-OH mephenytoin (a metabolite of mephenytoin) and absence of stereoselective elimination of the drug [38,39]. Küpfer examined the relatives of the affected individual and concluded that the trait was familial. An enzyme that was active in the metabolism of mephenytoin was purified in the laboratories of both Fred Guengerichs and Meyers. Antibodies against the purified enzymes were used to screen human λgt11 cDNA libraries and CYP2C8 and CYP2C9 were cloned [40]. The expressed enzymes were not found to hydroxylate mephenytoin but rather tolbutamide [41]. CYP2C9 was later shown to metabolize phenytoin and warfarin and to be of clinical relevance for the metabolism of these drugs.

The phenytoin and tolbutamide polymorphism was originally identified in 1964 and 1970, respectively. Joyce Goldstein became interested in this CYP2C9 polymorphism and, after some difficulties in recruiting interested clinical colleagues, collaborated with Don Birkett to study an individual who was a PM for phenytoin [42]. This subject, called the 'mythical poor metabolizer' was very difficult to find because he was a physician who was travelling around Australia. However, sequencing of his genomic DNA revealed the presence of an Ile359 mutation. Another individual who was a borderline PM for phenytoin possessed a Cys144 allele in addition to the Ile359 mutation. Goldstein successfully showed, by yeast expression, that the Ile359 allele (*CYP2C9*3*) was defective, a finding presented at the International Society for the Study of Xenobiotics (ISSX) meeting in October 1995 and published in 1996 [43]. Subsequent investigation by Crespi and collaborators showed the defective interactions of the *CYP2C9*2* allele with P450 reductase [44]. The correct S-mephenytoin hydroxylase was identified by Wrighton *et al.* [45]. After tedious work with the highly homologous *CYP2C8*, *CYP2C9* and *CYP2C19* genes, Goldstein and collaborators identified the major mutations responsible for the polymorphism as being *CYP2C19*2* and *CYP2C19*3* [46,47].

Polymorphism in nicotine oxidation

CYP2A6, which is the principal nicotine *C*-oxidase in humans, was cloned by Gonzalez and colleagues in 1989 [48]. Their subsequent work revealed two variant *CYP2A6* alleles: the inactive *CYP2A6*2* allele, which carries a L160H substitution; and the *CYP2A6*3* allele, which was reported as a hybrid between *CYP2A6* and the pseudogene *CYP2A7* (see [48] for references). The initial genotyping protocol did however cause unspecific amplification and an important step was the development of more-specific genotyping assays [49], thereby questioning the previous conclusions regarding the relationship between the *CYP2A6* genotype and nicotine dependence. Kamataki's group evaluated the structure of an allele in subjects with deficient SM-12502 (see Chemical names) metabolism, which suggested the presence of a gene deletion [50]. Parallel work in the my laboratory, in particular by Mikael Oscarson, led to a detailed characterization of the deletion involving the complete *CYP2A6* gene (*CYP2A6*4A*) [51]. This allele, which has been generated through an unequal crossover event with *CYP2A7*, explains the higher prevalence of reduced CYP2A6 activity in Asian populations. The reciprocal outcome of the crossover event, a duplication of the *CYP2A6* gene and thereby increased rate of nicotine metabolism, was subsequently presented by Tyndale and collaborators [52]. Now, a large number of additional single nucleotide polymorphisms (SNPs), some of importance for inter-individual variability in CYP2A6 activity, have been described (http://www.imm.ki.se/CYPalleles/cyp2a6.htm).

Chemical names

SM-12502: (+)-cis-3,5-dimethyl-2-(3-pyridyl) thiazolidin-4-one hydrochloride

Clinical relevance of cytochrome P450 polymorphism

Pharmacogenetics today, to a great extent, deals with genes encoding drug transporters, drug-metabolizing enzymes and drug targets. There is no doubt that the polymorphism of metabolizing enzymes, and in particular that of cytochromes P450s, has the greatest effect on inter-individual variability of drug response, as evidenced by many studies [2–7]. These polymorphisms affect the response of individuals to drugs used in the treatment of depression, psychosis, cancer, cardiovascular disorders, ulcer and gastrointestinal disorders, pain and epilepsy, among others (Table 2).

Depression

CYP2D6 is responsible for the metabolism of most psychoactive drugs, including antidepressants. Tricyclic antidepressants are almost entirely metabolized by CYP2D6 and the dosage required corresponds closely with the CYP2D6 phenotype. The kinetics of nortriptyline is dependent on the number of active *CYP2D6* genes [53] and the dosage required to reach the same plasma levels varies from 30–50 mg in PMs to 500 mg in UMs. Non-responders to nortriptyline have been found to possess the UM phenotype and a study at Huddinge University Hospital indicates that the UM phenotype is 10-fold more common in non-responders to antidepressant therapy than among responders to such therapy [54]. By contrast, the *CYP2D6* polymorphism has not been shown to be significant for treatment with selective serotonin (5-HT) reuptake inhibitors (SSRIs). However, treatment of CYP2C19 PMs with the SSRI drug sertraline, a CYP2C19 substrate, has been found to result in ADRs such as nausea and dizziness, effects that might be caused by toxic concentrations of the accumulated drug [55]. The pharmacokinetics of citalopram are also influenced by the *CYP2C19* polymorphism [56]. In addition, metabolism of valproate, commonly used to treat bipolar disorders, has been found to be influenced by the *CYP2C9* polymorphism, and the formation of all major metabolites is severely diminished by the presence of mutant alleles

[57]. However, the clinical implications remain to be investigated.

Psychosis

A study of antipsychotic drugs showed that the occurrence of ADRs in response to treatment of patients with CYP2D6 substrates increased from UMs to PMs [58]. Furthermore, it was necessary for the duration of treatment to be longer for PMs, and the costs for treatment of the patients were US$4000–6000 higher per year in patients of the UM and PM phenotypes, compared with those of the EM and IM phenotypes [58]. Parkinsonism-like side-effects have been shown to be present at higher frequency in PMs compared with EMs in several prospective and retrospective studies, and thus PMs were four times more likely to be given antiparkinsonian medication (see [59] for references). Oversedation has been observed in PMs in many studies following treatment of patients with perphenazine, thioridazine and other antipsychotics, whereas no significant relationship has been observed between *CYP2D6* polymorphism and tardive dyskinesia, acute dystonia, extrapyramidal symptoms or akathisia [60].

Ulcer and gastrointestinal disorders

Dosing with anti-ulcer agents to reach a specific plasma level is highly dependent on the CYP2C19 phenotype. In a study using a relatively low dose of omeprazole (20 mg) to treat ulcers, cure rates were very low in EMs (25%), higher in IMs (50%) and complete in PMs (100%) [61], illustrating the necessity of higher plasma levels for effective treatment. Similar results have been obtained in several other studies [62]. Examination of changes in gut pH in subjects of various *CYP2C19* genotypes receiving 20 mg omeprazole for 8 days revealed an increase in pH from 1.2 to 2.5 in EMs, from 1.2 to 5.5 in IMs and up to >6 in PMs, and long-term treatment has shown a higher extent of gastrin release in PMs compared with in EMs [63]. Dosing for long-term treatment is advantageously adjusted according to the CYP2C19 phenotype. Genotyping for *CYP2C19* is estimated to reduce the cost for anti-ulcer drug treatment

Table 2. Examples of the clinical impact of cytochrome P450 pharmacogenetics[a]

Disease	Enzyme	% of dose[b]		Examples
		UMs	PMs	
Depression	CYP2C9	–	–	Bipolar disorders and valproate
	CYP2C19	–	40	PMs and SSRIs
	CYP2D6	200	30	Non-responders (UMs) and side-effects of tricyclic antidepressants (PMs)
Psychosis	CYP2D6	160	30	Haloperidol and parkinsonian side-effects; oversedation and perphenazine, thioridazine
Ulcer	CYP2C19	–	20	Dosing of PPIs; pH and gastrin changes
Cancer	CYP2B6	–	–	Cyclophosphamide metabolism
	CYP2D6	250	60	Non-response to anti-emetic drugs (UMs)
Cardiovascular	CYP2C9	–	30	Warfarin dosing (acenocoumarol); irbesartan and blood pressure response
	CYP2D6	160	30	Perhexiline neuropathy and hepatotoxicity
Pain	CYP2D6	–	–	Codeine, no response (PMs)
Epilepsy	CYP2C9	–	–	Phenytoin pharmacokinetics and side-effects

[a]Abbreviations: CYP, cytochrome P450; PMs, poor metabolizers; PPIs, protein pump inhibitors; SSRIs, selective serotonin reuptake inhibitors; UMs, ultrarapid metabolizers.
[b]The doses shown for depression and psychosis are weighted as related to the size of samples in all studies published, as reviewed by Kirchheiner *et al.* [55]. The other doses are based on data presented in the main text. All doses are percentages of the normal dose.

by US$ 5000 per 100 patients in Orientals [64], but only on the basis that the 20 mg dose is the relevant dose.

Cancer
Tamoxifen is metabolized to its active metabolite endoxifen in a *N*-demethylation reaction and a 4-hydroxylation that is catalysed by CYP2D6 [65]. A smaller therapeutic effect has been observed in PMs for CYP2D6 and therefore predictive phenotyping and genotyping could be relevant before beginning the tamoxifen treatment. CYP2B6 metabolizes several anticancer drugs, including cyclophosphamide, and preliminary indications suggest that increased metabolism occurs in subjects who possess the *CYP2B6*6* allele [66]. The effects of anti-emetic drugs such as the 5-HT$_3$ receptor antagonists tropisetron and ondasetron were found to be highly related to the CYP2D6 phenotype. For example, lower plasma levels of these drugs and a higher frequency and intensity of vomiting were observed in subjects who possessed increased numbers of active gene copies of *CYP2D6* [67].

Cardiovascular disorders
Both warfarin and coumarols such as acenocoumarol, which are used as anticoagulants, are metabolized by CYP2C9. Several studies reveal an important influence of the *CYP2C9* genotype on warfarin dosing at equilibrium [68]. In general, subjects who are heterozygous for a *2 allele and *3 allele of *CYP2C9* require, on average, a 21% and 34% lower daily maintenance dose of warfarin, respectively, than homozygous wild-type patients, and subjects who are homozygous for the *2 allele or the *3 allele require a 60–75% lower dose of warfarin than homozygous wild-type patients (see [69] and references therein). A study by Higashi *et al.* [70] revealed that subjects who possessed variant alleles required dosing for 95 days longer than patients who possessed wild-type CYP2D6 to achieve stable warfarin dosing. In addition, the subjects who possessed the variant alleles had an increased risk (odds ratio = 2.3) for life-threatening bleeding events, findings that have also been observed in several other studies. Therefore, it appears of value to perform *CYP2C9* genotyping to achieve a safer and subject-specific warfarin treatment with fewer side-effects, particularly at the beginning of the treatment. Furthermore, the *CYP2C9* genotype has been shown to predict the blood pressure response to irbesartan [71] and CYP2D6 PMs had a 4–5-fold higher incidence of side-effects following treatment with metoprolol [72].

Monohydroxylation of the anti-anginal agent perhexiline is almost exclusively catalysed by CYP2D6, with activities being ∼ 100-fold lower in CYP2D6 PMs than in EMs [73]. Perhexiline causes concentration-related hepatotoxicity and peripheral neuropathy, and therefore determination of the *CYP2D6* genotype should predict dose requirements and reduce the risk of perhexiline concentration-related toxicity [74].

Pain
Codeine needs to be metabolized to morphine by CYP2D6 before pain-relieving effects are observed [75]. A similar phenomenon is also observed following tramadol

treatment [76]. ADRs have been observed in CYP2D6 UMs when treated with ethylmorphine [77], oxycodone and hydrocodone [78].

Epilepsy
CYP2C9 accounts for the majority of phenytoin metabolism and effective dosing of phenytoin is highly linked to the *CYP2C9* genotype. Several examples of ADRs, including CNS intoxication such as ataxia, diploidia and other neurological symptoms, have been described in patients with defective *CYP2C9* alleles following phenytoin treatment [79], although further studies are required before a general conclusion can be drawn.

Future aspects
It is to be assumed that the major allelic variants of P450 genes of clinical importance have now been identified. However, further genetic reasons that underlie variable P450 expression might remain to be identified in other genes encoding, for example, proteins with regulatory functions such as transcription factors. Furthermore, RNA-regulating proteins might to be relevant and add to the complexity of the field. In addition, P450 expression polymorphism might not only exist at the genomic level because alternative splicing [80] has already been shown to generate variable P450s.

With respect to depression and psychosis, Kirchheiner *et al.* [59] have carried out an impressive investigation and concluded that receptor polymorphism is of no value for predicting drug therapy; instead, they suggest that dosing of ∼ 50–60% of the drugs used in such therapy is dependent, to a large extent, on the *CYP2D6* and *CYP2C19* genotype.

At present, predictive genotyping for P450s in the clinic does not occur routinely for many reasons [81]. In my opinion, the lack of knowledge about genetics and pharmacogenetics among prescribers in addition to the lack of large, conclusive, prospective studies showing improvement of drug efficacy following genotyping are two major causes. P450 polymorphisms are emphasized in the Food and Drug Administration (FDA) draft guidelines [82,83] and clinical trials could be stratified according to P450 genotype with reduced costs as a consequence. All major drug companies take the pharmacogenetic aspect of P450s into account during drug development, and termination of the development of a candidate that has high affinity for a polymorphic P450 enzyme occurs if pharmacologically competitive candidates that are not substrates for the polymorphic enzymes are at hand. This might in the long term decrease the problem of P450 polymorphism in future drug therapy.

The costs of genotyping are decreasing rapidly and our knowledge about the benefits of predictive genotyping for a more effective therapy is increasing. Therefore, I believe that predictive genotyping for P450s will be routine for several specific drugs in the future. Based on the role of polymorphic P450s in drug metabolism, as evidenced, for example, by Kirchheiner *et al.* [59], I suggest that such action will improve the clinical efficacy of 10–20% of all drug therapy and reduce the incidence of ADRs by 10–15%. However, other aspects that underlie

inter-individual variability in drug metabolism, such as poor compliance, unfavourable drug–drug interactions and pathophysiological conditions, remain important and are often primary factors of concern in drug treatment.

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