Pharmacogenetics of cytochrome P4502D6: genetic background and clinical implication

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Abstract
Interindividual differences in the pharmacokinetics of a number of drugs are often due to hereditary polymorphisms of drug-metabolizing enzymes. Most important is cytochrome P4502D6 (CYP2D6), also known as debrisoquine/sparteine hydroxylase. It catalyzes hydroxylation or demethylation of more than 2% of drugs metabolized in the human liver, such as neuroleptics, antidepressants, some β-blockers and many others like codeine. About 7%–10% of Caucasians lack any CYP2D6 activity due to deletions and frame-shift or splice-site mutations of the gene. About 1%–3% of Middle-Europeans, but up to 29% of Ethiopians display gene duplications, leading to elevated so-called ultrarapid metabolism rates. Meanwhile there is now a much better understanding of the genetic background of poor, intermediate, extensive and ultrarapid metabolizers, enabling a more precise DNA genotyping-based prediction of plasma levels. Since there is evidence that deteriorated drug elimination partly accounts for drug side-effects, CYP2D6 genotyping could contribute to an individualized and therefore optimized drug therapy.

Introduction
Bioavailability of drugs depends to a significant degree on the extent of elimination by xenobiotic-metabolizing enzymes in the human liver. In a first step, mono-oxygenases like cytochrome P450 enzymes catalyze the transformation of the lipophilic compounds to more hydrophilic intermediates. Beside cytochrome P4503A, the polymorphic cytochrome P450 2D6 (CYP2D6) is one of the most studied cytochrome P450 enzymes, because it metabolizes approximately 25% of all medications in the human liver. As reviewed by Bertilsson et al. [1], nearly 40 years ago it was observed that the large interindividual variability of nortriptyline plasma concentration has a strong genetic background and is based on individually different metabolic rates. Later it was shown that the metabolisms of the anti-hypertensive drug debrisoquine [2] and the antiarrhythmic drug sparteine [3] are both polymorphic and metabolized by the same enzyme CYP2D6. Particularly antidepressants and neuroleptics, both having a number of adverse effects, are also metabolized by the polymorphic sparteine/debrisoquine hydroxylase [4]. Therefore, it became rapidly clear that this hereditary trait may have severe clinical consequences.

Now CYP2D6 is known to be one of the most important polymorphic genes involved in drug metabolism. Approximately 7%–10% of the European population are CYP2D6-poor metabolizers who only weakly metabolize drugs like antiarrhythmics, antidepressants, neuroleptics and some β-blockers or opiates (Table 1). Among this subgroup, clinically relevant drug side-effects are more likely compared with extensive metabolizers. The occasionally observed absence of the desired effect accounts in some cases for the phenomenon of gene duplications, occurring in 1%–3% of Middle-Europeans.

Apart from the important role in drug metabolism, it has been shown that CYP2D6 also metabolizes carcinogens like nitrosamines. Since such xenobiotics are constituents of cigarette smoke, it has been speculated that poor metabolizers might be more prone to lung cancer compared with extensive metabolizers. However, only a few studies have shown associations, whereas the majority could not confirm an overrepresentation of poor metabolizers among lung cancer patients [5].

This mini-review will summarize some major aspects of the genetic background of the CYP2D6 polymorphism and will give an overview on some important clinical applications.

Genetic background
The genetic background of the CYP2D6 polymorphism is well characterized and extensively described by Sachse et al.
The CYP2D6 gene is mapped to chromosome 22q13-1 [7,8]. It belongs to a gene cluster of the highly homologous inactive pseudogenes CYP2D7 and CYP2D8. Compared with CYP2D6, CYP2D8 was found to be a real pseudogene, while the CYP2D7 gene contained a single reading frame-disrupting insertion in its first exon [9]. The gene encompasses nine exons with an open reading frame of 1383 bp coding for 461 amino acids. Currently more than 73 different CYP2D6 haplotypes are recorded by the human cytochrome P450 (CYP) allele nomenclature committee (http://www.imm.ki.se/CYPalleles). The alleles may be classified on level of phenotypic activity to functional, nonfunctional and low active CYP2D6 enzymes. The most important genetic variations are splice-site variations, the introduction of premature stop codons, or large deletions, which result in a complete defect of the enzyme.

In order to predict the clinically relevant phenotype, however, it is mostly sufficient to investigate a limited number of variant alleles, enabling an accuracy of about 99%, as could be clearly demonstrated by a large genotype-phenotype association study in German Caucasians [6].

CYP2D6 4 was discovered as the primary gene defect at the cytochrome P450 CYP2D locus by Gough et al. [10]. Its frequency was estimated at 20-7% [6]. A G1846A transition generates a shift of the splice site at the boundary of intron 3 to exon 4, consequently leading to the generation of a nonfunctional protein. Further single nucleotide polymorphisms characterize additional subtypes of CYP2D6 4. In 4%-6% of Caucasian subjects, a complete deletion of the entire coding region of CYP2D6 occurs [11]. Such individuals are also completely lacking CYP2D6 activity. This allele, called *5, interestingly occurs with a similar frequency in different populations and is therefore believed to have an ancient origin. Further relevant variants are a 2549-A deletion in *3 (2-0%), and a 1707T deletion in *6 (0-9%), generating frame shifts. In contrast to these fatal polymorphisms, a triple-basepair deletion in allele *9 (1-8%) does not significantly alter enzyme activity [12], and a proline to serine exchange in codon 34 is associated with lower enzyme activity and particularly decreased stability of CYP2D6 10 [13]. This variant occurs in 1%-2% of Caucasians, but is the major cause of low CYP2D6 activity in Orientals [14].

Extremely high CYP2D6 activity in 1%-2% of Caucasians was identified as being due to gene duplications of allele *1 (1 × 2) and *2 (2 × 2). CYP2D6-2 has only weak diminished debrisoquine hydroxylase activity, however convincing evidence has recently come to light that a C/G polymorphism 1584 bp upstream of the start codon modulates CYP2D6 expression [15]. Homozygous carriers of the -1584C variant carriers of CYP2D6-2 exhibited only 50% of protein compared with carriers of the −1584G variant [16]. Therefore consideration of this frequent promoter polymorphism, now termed CYP2D6 41, improves the prediction of so-called intermediate metabolizers (Table 2).

Many other alleles have been reported, many extremely rare and existent only in specified populations [17]. Although the metabolic ratio of, for example, dextromethorphan may vary between individuals with the same genotype by more than one order of magnitude, genotyping enables a reliable prediction of the CYP2D6 poor, intermediate, extensive and ultrarapid metabolizer status.

**Interethnic variability**

The pattern of CYP2D6 polymorphisms differs dramatically between people with different ethnic backgrounds [14]. It is most pronounced with ultrarapid metabolizing. Whereas in Northern Europe a very low prevalence of the CYP2D6 gene duplication was detected [18], in some regions of Spain frequencies of more than 7% were observed [19], possibly due to the Arabic immigration in the eighth century. In Arabian countries [20] as well is in Ethiopia, a prevalence of ultrarapid metabolizers of up to 29% is reported [21]. In a few cases there were families with up to 13 gene copies identified. Apart from these striking findings, it is highly interesting that the phenotype may be influenced by environmental factors, because Ethiopian blacks have lower debrisoquine hydroxylase activity than Swedish Caucasians with the same CYP2D6 genotype. Moreover, Ethiopians living in Sweden have considerably higher CYP2D6 activity than Ethiopians in their home country, but still lower capacity than Swedes in Sweden. It was speculated that unknown genetic differences may explain these observations, or that dietary constituents such as local cereals or nondietary used shrubs like khat may influence CYP2D6 activity [22]. Central stimulating amphetamines, as contained in khat, are known to be substrates of CYP2D6 [23].

In contrast, however, in China CYP2D6 gene duplications are extremely rare. As mentioned above, Oriental populations lack poor as well as ultrarapid metabolizers,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Selected substrates of cytochrome P4502D6</th>
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<tbody>
<tr>
<td>β-blockers</td>
<td>Alprenolol, carvedilol, metoprolol, propranolol</td>
</tr>
<tr>
<td>Antiarrhythmics</td>
<td>Propafenone, encaïnide, flecaïnide, mexiletine, sparteine</td>
</tr>
<tr>
<td>Neuroleptics</td>
<td>Haloperidol, perhexiline, perphenazine, risperidon, clozapine, olanzapine, risperidone, thioridazine, zuclopenthixol</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>Amitriptyline, clomipramine, desipramine, fluoxetine, fluvoxamine, imipramine, maprotiline, nortriptyline, paroxetine</td>
</tr>
<tr>
<td>Antiemetics</td>
<td>Ondansetron, tropisetron</td>
</tr>
<tr>
<td>Others</td>
<td>Amphetamine, codeine, debrisoquine, dextromethorphan, methoxyamphetamine, phenacetine, tramadol</td>
</tr>
</tbody>
</table>
however the mean metabolic ratio of debrisoquine/4-hydroxydebrisoquine is increased compared with Caucasians [24]. This is due to the high prevalence of the low active (intermediate) CYP2D6 variant *10, e.g. in Hong Kong Chinese a CYP2D6*10 frequency of 42% was reported [25]. In blacks, however, large heterogeneity seems to exist [26,27] (Fig. 1).

### Interactions of CYP2D6

In contrast to CYP1A2 or CYP3A4, CYP2D6 does not undergo a substantial induction by xenobiotics or drugs like rifampine. An observed decrease of propafenone bioavailability after rifampine pretreatment can be clearly shown to be due to an induction of CYP3A4 leading to enhanced N-dealkylation as well as phase II pathways like glucuronidation or sulphation [28].

Inhibition, however, was observed for a number of drugs, which were able to compete with CYP2D6. Quinidine is the most potent inhibitor, virtually causing a poor metabolizer status [29], but many other different structures and specialties such as the tricyclic antidepressants chlorpheniramine, chlorpromazine and clomipramine, selective serotonin reuptake inhibitors, fluoxetine, paroxetine and sertraline, as well as moclubemide, and others like metoclopramide, methadone or mibefradil, are able to significantly inhibit the enzymatic activity of cytochrome P450 2D6 (Table 3).

In some cases, the inhibition of CYP2D6 by concomitant drugs may have beneficial effects: administration of high doses of antidepressants to patients having a ultrarapid metabolizer phenotype will possibly lead to high concentrations of potentially toxic metabolites and an increased risk for adverse reactions. In a clinical study investigating the interaction of paroxetine and nortriptyline among genetically defined ultrarapid metabolizers, a normalization of the metabolic status could be observed, offering a promising method to successfully treat ultrarapid metabolizers with CYP2D6-metabolized antidepressants [30].

### Clinical implications

Some early findings on the significance of CYP2D6 were based on investigations of the pharmacokinetics of tricyclic antidepressants. The clearance of such drugs like nortriptyline, desipramine and to some extent imipramine and amitriptyline [31–33] evidently depend on the CYP2D6 polymorphism. Additionally, the first case of a gene duplication was identified in a patient exhibiting extremely low plasma levels after oral treatment with nortriptyline [34,35]. Now the ultrarapid metabolizer phenotype of CYP2D6 has been well established as a relevant cause of nonresponse to antidepressant drug therapy. In contrast, specific serotonin reuptake inhibitors like fluoxetine, citalopram or paroxetine were shown to be inhibitors of CYP2D6 [36,37]. Interestingly, a study on side-effects like seizures and myoclonus after treatment with antidepressant drugs, could not identify any patient with a CYP2D6 poor metabolizer status, but half were concomitantly treated with drugs with potential inhibitory effects on CYP2D6 [38].

### Table 2 Major Caucasian CYP2D6 alleles, gene alteration, function and frequency [6,16]

<table>
<thead>
<tr>
<th>Allele</th>
<th>Major gene alteration</th>
<th>Function</th>
<th>Frequency (%)</th>
</tr>
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<tbody>
<tr>
<td>*1</td>
<td>Wild type</td>
<td>Normal activity</td>
<td></td>
</tr>
<tr>
<td>*2</td>
<td>2850C&gt;T, 4180G&gt;C</td>
<td>Normal activity</td>
<td></td>
</tr>
<tr>
<td>*1xN, *2xN</td>
<td>Gene duplication</td>
<td>Elevated activity</td>
<td>2%</td>
</tr>
<tr>
<td>*3</td>
<td>2549A&gt;del frameshift</td>
<td>Inactive enzyme</td>
<td>2%</td>
</tr>
<tr>
<td>*4</td>
<td>1846G&gt;A splice-site-mutation</td>
<td>Inactive enzyme</td>
<td>21%</td>
</tr>
<tr>
<td>*5</td>
<td>Gene deletion</td>
<td>No enzyme</td>
<td>2%</td>
</tr>
<tr>
<td>*6</td>
<td>1707T&gt;del frameshift</td>
<td>Inactive enzyme</td>
<td>1%</td>
</tr>
<tr>
<td>*9</td>
<td>2613–2615delAGA</td>
<td>Normal activity</td>
<td>2%</td>
</tr>
<tr>
<td>*10</td>
<td>100C&gt;T</td>
<td>Decreased activity</td>
<td>1.5%</td>
</tr>
<tr>
<td>*41</td>
<td>−1584G&gt;C</td>
<td>Decreased activity</td>
<td>10–20%</td>
</tr>
</tbody>
</table>

For detailed alleles populations and corresponding sequence alterations see [57].

### Table 3 Inhibitors of cytochrome P4502D6

- **Neuroleptics**: Clomipramine, levomepromazine, haloperidol (reduced), chlorpromazine
- **Antidepressants**: Fluoxetine, moclubemide, paroxetine, sertraline
- **Antiemetics**: Metoclopramide
- **Antihistamines**: Chlorpheniramine, cimetidine, clemastine, diphenhydramine, terbinafine, amiodarone, celecoxib, cocaine, doxorubicin, halofantrine, methadone, mibefradil, quinidine, ritonavir
The effects of the CYP2D6 polymorphism on antipsychotic therapy appear to be more pronounced in neuroleptics. Compounds like perphenazine, zuclopenthixol, thioridazine, haloperidol and risperidone are metabolized to a significant extent by CYP2D6. First studies investigating the relationship between the occurrence of extrapyramidal symptoms and CYP2D6 genotype revealed that indeed poor metabolizers appeared to possess an elevated risk of side-effects [1,39,40]. Moreover, the antipsychotic efficacy seems to be influenced by the number of active copies of CYP2D6 genes [40]. These findings indicate that genotyping should be performed before treatment with polymorphically metabolized antipsychotics [41].

The impact of polymorphic enzymes on the treatment of cardiovascular disorders is still open to discussion and more data are needed for verification, e.g. propafenone shows a stronger β-receptor blockage in poor metabolizers [42] and was believed to be causative for strong side-effects in a patient who was a poor metabolizer for CYP2D6 [43]. Flecainide, however, is partly metabolized by CYP2D6 [44], but apparently the electrophysiology of the heart was only marginally influenced by the genotype [45]. β-blockers like metoprolol, and to some extent carvedilol, are also CYP2D6 substrates [46,47]. Obviously, even after long-term treatment, in poor metabolizers, the median-adjusted metoprolol plasma concentrations were 6·2-fold higher compared with extensive metabolizers [48]. From a survey on adverse events after metoprolol treatment, the same group concluded that side-effects may be due to the CYP2D6 genotype, because the frequency of poor metabolizers was elevated in an albeit small group of patients, reportedly suffering from pronounced adverse symptoms [49]. However, currently there are no data available on whether the clinical outcome of β-blocker therapy is influenced by genetic polymorphisms of CYP2D6.

Absence of the desired effects may also be due to the poor metabolizer status. The analgetic property of codeine is caused by an O-demethylation reaction generating the potent opioid morphine. This demethylation is catalyzed by CYP2D6. Therefore, poor metabolizers are not able to profit from codeine given as an analgetic drug, as demonstrated in cold pressure tests, where poor metabolizers exhibited higher peak pain intensities [50,51]. The ability of quinidine to inhibit the formation of morphine through CYP2D6 gave rise to some studies to investigate the role of quinidine in the treatment of codeine dependence [52,53]. An example of the usefulness of CYP2D6 genotyping for drug therapy was published recently on the treatment of nausea and vomiting in cancer chemotherapy with the antiemetic 5-HT3 receptor antagonists tropisetron [54]. In a study including 42 cancer patients receiving chemotherapy, approximately 30% of all patients experienced nausea and vomiting. CYP2D6 ultrarapid metabolizers, however, had a significantly higher frequency of vomiting after treatment than all other patients. The authors concluded that antiemetic treatment with tropisetron or to a lesser extent with ondansetron could be improved by adjusting for the CYP2D6 genotype, and that approximately 50 subjects would have to be genotyped to protect one patient from severe emesis.

**Conclusion**

The early adaptation of a therapy regimen to genetic traits could help to avoid side-effects and improve the clinical outcome of pharmacotherapy. Genotyping instead of phenotyping with probe drugs should be preferred during an ongoing pharmacotherapy, because some drugs may inhibit CYP2D6 activity. For prediction of the phenotype by genotyping at least the major deficient alleles occurring in the particular ethnic group should be characterized. The benefit of genotype-related dose-adaption is best described by studies of psychotropic drugs [1], however there is a need for further prospective studies [55]. First dosage recommendations have been published recently for a set of antidepressants [56], which may be an important step in the attempt to improve individual drug therapy. More standardized clinical studies are required, testing the efficacy and side-effects of genotype-adapted and nonadapted dosage regimens.
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