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# Is CYP2D6 phenotype predictable from *CYP2D6* genotype?

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ABSTRACT

Genetic polymorphism of cytochrome P450s results in clinically significant modifications in patients' drug metabolizing capacities. CYP2D6 has a crucial role in the elimination of several clinically important drugs (antiarrhythmics, beta-adrenergic blockers, psychopharmacons and analgesics); however, the prediction of the phenotypic appearance of CYP2D6 is a challenge. Since single nucleotide polymorphisms and gene copy number variations (gene deletion and multiplication) frequently occur in *CYP2D6* gene, CYP2D6 activity particularly depends on the genetic factors.

Microsomal CYP2D6 activities (dextromethorphan *O*-demethylation) and CYP2D6 genotypes for the most frequent allelic variants (*CYP2D6*3*, **4*, **5*, **6*, **10*, **41* and duplication) were determined in 128 human liver samples derived from Hungarian organ donors. Substantial inter-individual variations were observed in CYP2D6 metabolic activities that were successfully predicted from the *CYP2D6* genotypes in 67% of the donors. The underestimation of CYP2D6 phenotypes in 12.5% of the donors was assumed to be originated from the overlapping ranges of CYP2D6 activity among similar diplotypes or from the presence of −*1584C>G* in the promoter region evoking increased transcription of the wild-type *CYP2D6* allele. In an appreciable number of donors (20.3%), the genotype-based CYP2D6 phenotype prediction was overestimated because of the rare *CYP2D6* allelic variants which were not included in our genotyping platform or some external factors that could alter CYP2D6 activity (medication with CYP2D6 substrate/inhibitor) and hepatic function (Augmentin therapy, chronic alcohol consumption). In conclusion, *CYP2D6* genotyping for the most frequent allelic variants was able to reliably predict CYP2D6 phenotypes in most donors; however, the external factors modifying the phenotypic appearance of CYP2D6 had to be taken into account. Personalized medication strategy should include monitoring of *CYP2D6* genotype in a more comprehensive manner and should take external factors into consideration for an appropriate prediction of CYP2D6 metabolizing capacity.



## 1. Introduction

Most drugs show inter-individual differences in their responses and efficacy due to the substantial variability of the capacity of drug metabolizing enzymes between individuals. The primary drug metabolizing enzymes belong to the cytochrome P450 (CYP) superfamily, which are responsible for the metabolism of >90% of the drugs on the market. In mammalian species, CYP enzymes are present in most tissues (e.g. gut wall, kidney, brain, lung); however, they are mainly expressed in the liver. Most of them are highly polymorphic resulting in clinically significant modifications in drug metabolizing capacities. The major genetic polymorphisms influencing drug metabolizing activity are related to the metabolism by CYP2D6. Although CYP2D6 presents only a small proportion of hepatic CYP enzymes (2%), it is one of the most important drug metabolizing enzymes, catalysing the metabolism of approximately 25% of the drugs on the market. CYP2D6 is involved in the biotransformation of numerous clinically used drugs, such as beta-blockers (metoprolol, bufuralol, propranolol), opioids (codeine, tramadol), antidepressants (amitriptyline, imipramine, fluoxetine, mianserin, venlafaxine), antipsychotics (aripiprazole, haloperidol, olanzapine, risperidone), antitussives (dextromethorphan) and anticancer drugs (tamoxifen) [1].

The *CYP2D6* gene is located on chromosome 22q13.1 nearby two homologous, non-functional pseudogenes, *CYP2D7P* and *CYP2D8P* [2]. *CYP2D6* gene is highly polymorphic; hitherto more than one hundred allelic variants were identified, a number of them containing mutations that have clinically relevant impact (http://www.cypalleles.ki.se/cyp2d6.htm) [34]. Both single nucleotide polymorphisms (SNP) and

*Abbreviations:* CNV, copy number variation; CYP, cytochrome P450; EM, extensive metabolizer; IM, intermediate metabolizer; PCR, polymerase chain reaction; PM, poor metabolizer; SNP, single nucleotide polymorphism; UM, ultra-rapid metabolizer.

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gene copy number variations (CNV) (such as gene deletion and duplication/multiplication) frequently occur. Among the allelic variants, there are fully functional alleles (e.g. *1, *2), alleles with reduced function (e.g. *9, *10, *41) and null (non-functional) alleles (e.g. *3, *4, *5, *6). Duplication of the functional allele leads to increased gene expression and enzyme activity [3,4]. Due to the wide range of CYP2D6 enzyme activity within the population, the patients can be divided into four metabolizer groups: poor (PM), intermediate (IM), extensive (EM) and ultra-rapid metabolizers (UM) [5]. Since *CYP2D6* gene expression is not inducible by xenobiotics, genetic factors are thought to play a substantial role in the phenotypic appearance of CYP2D6.

Typically, subjects considered to be PMs carry two non-functional alleles which do not encode a functional protein product; thus, they do not display detectable enzyme activity. Several studies established clinical risks in the case of PM patients treated with CYP2D6 substrate drugs [5–7]. The most frequent non-functional allelic variant is *CYP2D6*4* in Caucasians (European white population) with an allele frequency of 18–23% [8]. *CYP2D6*3* and *6 allelic variants, which occur at allele frequency of about 1% in Caucasians, contain one nucleotide deletion causing frame-shift mutation and the lack of functional CYP2D6 protein. *CYP2D6*5*, which occurs in 2–5% of Caucasians, means the deletion of the entire gene and the lack of CYP2D6 enzyme expression [9]. The alleles *CYP2D6*10* and *41 give rise to significantly decreased enzyme activity. However, the allele frequency of *CYP2D6*10* is 1–2% in Caucasians, this allele variant is typical in the Asian population with an allele frequency of 33–43% [10]. *CYP2D6*10* has a 100C>T SNP leading to the instability of the enzyme protein [11]. *CYP2D6*41* contains an intronic 2988G>A SNP causing increased levels of a non-functional splice variant and accordingly lower CYP2D6 metabolic activity [12]. *CYP2D6*41* frequently occurs in Caucasians (allele frequency: 7–10%) [13]. UM phenotype has also clinical relevance, since high enzyme activity can be responsible for adverse drug effects or for therapeutic failure depending on the used drug. Primarily, duplication or multiplication of the functional *CYP2D6* gene accounts for UM phenotype; however, copy number increase does not explain all cases of UMs [14].

Up to now, several in vivo and in vitro studies were implemented to investigate an applicable formula with which certain *CYP2D6* genotype could be translated to the corresponding phenotype. For example, Gaedigk et al. [15] introduced an activity score system, which assigns a number for each allelic variant depending on the functionality of the allele. Although more investigations were achieved at in vivo conditions to evaluate the effect of *CYP2D6* polymorphism directly on the enzyme activity, human derived systems, such as human liver microsomes, are recommended to be used. Incubation of the microsomes with CYP-specific substrates provides direct information about the specific CYP enzyme activity.

Due to the clinical significance of the medications metabolized by CYP2D6, it would be important to obtain accurate estimation of CYP2D6 activity based on a fast and simple genotyping method. The aim of the present work was to investigate the accuracy of CYP2D6 phenotype prediction from genotype and the reliability of *CYP2D6* genotype interpretation. Human liver microsomes were used to evaluate CYP2D6 metabolic activity, and the samples were genotyped for the most frequent allelic variants in the Caucasian population.

## 2. Materials and methods

### 2.1. Human liver microsomes

Human liver tissues (N = 128) were obtained from organ transplant donors at the Department of Transplantation and Surgery, Semmelweis University (Budapest, Hungary). Permission of the Hungarian Committee of Science and Research Ethics was obtained to use human tissues. Clinical histories of the donors are shown in Table 1. Human livers were perfused with Euro-Collin's solution (Fresenius AG, Bad Homburg vdH, Germany) and excised. The tissues were homogenized in 0.1 M Tris-HCl buffer (pH 7.4) containing 1 mM EDTA and 154 mM KCl. Microsomes were prepared by differential centrifugation as described by van der Hoeven and Coon [16]. All procedures of preparation were performed at 4 °C. Microsomal protein content was determined by the method of Lowry et al. [17] with bovine serum albumin as the standard.

**Table 1**
Demographic data of the human organ donors.

| Demographic data | | | |
|---|---|---|---|
| Donor number | | | 128 |
| Age (year) | Median (min; max) | | 45 (13; 74) |
| Gender | Male/female | | 67/61 |
| Cause of death | | | |
| | Cerebral hemorrhage/ hematoma | | 62 |
| | | Subarachnoid hemorrhage | 42 |
| | | Subdural hemorrhage | 5 |
| | | Epidural hematoma | 1 |
| | | Intraventricular hemorrhage | 5 |
| | | Ruptured cerebral aneurysm | 4 |
| | | Unknown | 5 |
| | Stroke | | 7 |
| | | Ischemic stroke | 6 |
| | | Hemorrhagic stroke | 1 |
| | Tumour | | 16 |
| | Accident | | 30 |
| | | Car/motor/bike accident | 12 |
| | | Seizure induced cerebral injury | 1 |
| | | Suicide | 4 |
| | | Asphyxia | 1 |
| | | Unknown cerebral injury | 12 |
| | Unknown | | 13 |
| Anamnesis | | | |
| | | Alcohol consumption | 11 |
| | | Medication with CYP2D6 substrate/inhibitor | 4 |
| | | Medication with agent which cause potential liver dysfunction | 2 |

### 2.2. CYP2D6 enzyme assay

Published method was followed to determine dextromethorphan *O*-demethylation selective for CYP2D6 enzyme [18]. The incubation mixture contained NADPH-generating system (1 mM NADPH, 10 mM glucose 6-phosphate, 5 mM $MgCl_2$ and 2 units/ml glucose 6-phosphate dehydrogenase), human liver microsomes (0.8 mg/ml) and the CYP2D6 selective substrate, dextromethorphan (1 mM). After a 20-min incubation, the reaction was terminated by ice-cold methanol and the incubation mixture was centrifuged for 10 min at 10.000 × *g*. The supernatant was analysed for the concentration of dextromethorphan and its metabolite dextrorphan by HPLC-UV using a LiChroSpher C18 column (125 × 4 mm, 5 μm) (Merck, Darmstadt, Germany). The mobile phase consisted of 1 mM Na-perchlorate (pH 2.6) and acetonitrile (71:29 v/v). The rates of enzyme activity were linearly dependent upon the amount of microsomal protein added for the incubation period. CYP2D6 enzyme assay for each donor was performed in triplicate and median (min, max) was calculated.

### 2.3. CYP2D6 genotyping

Hydrolysis single nucleotide polymorphism (SNP) analysis for *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*6*, *CYP2D6*10* and *CYP2D6*41* was performed by polymerase chain reaction (PCR) with TaqMan probes (Metabion, Planegg/Steinkirchen, Germany) using a CFX96 real-time detection system (Bio-Rad Laboratories). Primers and probes (Table 2) were designed based on the reference SNP sequences in the National

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