Ultrarapid drug metabolism: PCR-based detection of CYP2D6 gene duplication

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The enzyme debrisoquine 4-hydroxylase (CYP2D6), which metabolizes many widely used drugs, is highly polymorphic. The activity of the enzyme ranges between subjects from ultrfast to a complete absence. Therefore, metabolic capacity varies, producing inter-subject differences in therapeutic efficacy and side effects at standard recommended doses. Up to 7% of Caucasians may demonstrate ultrarapid drug metabolism (UM) because of inherited alleles with multiplicative functional CYP2D6 genes, causing an increased amount of enzyme to be expressed. Identification of UM subjects is of potential clinical importance for adjustment of doses in drug therapy, as well as to avoid misidentification of noncompliance. In our study, we tested recently designed PCR assays for the detection of the UM genotype. We found a 3.5% prevalence of UM subjects from an inpatients psychiatric population consisting of 202 psychiatric patients.

The cytochrome P450 (CYP) enzyme debrisoquine 4-hydroxylase known as CYP2D6 is involved in the oxidative metabolism of many different classes of commonly used drugs, including neuroleptics, tricyclic antidepressants, selective serotonin reuptake inhibitors, β-adrenoceptor blockers, and antiarrhythmics (1, 2). The enzyme is highly genetically polymorphic. In addition to the wild-type gene (CYP2D6*1), at least 15 different alleles of CYP2D6, associated with deficient, reduced, normal, or increased enzyme activity, are known in Caucasians. As a result, metabolic capacity ranges between individuals from extremely slow to ultrfast (3). The various alleles are classified by the unified nomenclature developed by Daly et al. (4).

Approximately 5–10% of Caucasians are poor metabolizers (PMs), completely lacking CYP2D6 enzyme activity because of the inheritance of two mutant CYP2D6 null alleles (5). PM subjects have an impaired metabolism of CYP2D6 substrates. Treatment with standard recommended doses of the drugs listed above may produce higher steady-state plasma drug concentrations compared with extensive metabolizers, with an increased risk of concentration-dependent side effects and drug toxicity. On the other hand, when a drug, for example, codeine, requires metabolic activation by CYP2D6, the absence of the enzyme might produce a loss of therapeutic effect.

The majority of defective allelic variants of the CYP2D6 gene that, occurring homozygous or heterozygous together, give rise to the PM genotype have now been identified (3, 6–13). By screening for all these null alleles, i.e., CYP2D6*3, *4, *5, *6, *7, *8, *11, *12, *13, *14, and *16, CYP2D6 deficiency may be detectable with close to 100% accuracy (14). Identifying PM subjects before starting drug therapy may allow prediction of the adequate dose of CYP2D6 substrates in PMs, to assure therapeutic efficacy with a minimum risk of side effects. However, it is unnecessary and impractical to carry out routine screening for all known inactivating CYP2D6 mutations. In our psychiatric hospital, we perform PCR-based genotyping for the three most common defect gene variants, i.e., CYP2D6*3, CYP2D6*4, and CYP2D6*5, in all patients before drug therapy. Analysis of these mutant alleles is simple and allows identification of at least 95% of PMs in Caucasian populations (15). We only test for the remaining null alleles if poor metabolism appears present and is not caused by the three allelic variants mentioned above.

The other side of the range of metabolic capacity is ultrarapid drug metabolism, a result of excessively high CYP2D6 enzyme activity. Ultrarapid metabolizers (UMs), up to 7% of Caucasians, require more than average doses of drugs metabolized by CYP2D6 to reach therapeutic plasma concentrations (16). These subjects often have alleles with duplicate, or in some cases, amplified functional CYP2D6 genes, causing an excessive amount of CYP2D6 enzyme to be expressed. The active CYP2D6*2 variant is especially subject to multiplication (17–19). The
identification of gene duplication or amplification could help to avoid therapeutic failure in UMs when CYP2D6 substrates are administered at standard doses. Moreover, it could also be a tool to distinguish between genetically determined high metabolic capacity and low plasma drug concentrations caused by noncompliance. Noncompliance is a serious problem in the treatment of psychiatric patients. It is a common occurrence, especially in outpatients, and it is not easy to prove. Furthermore, a false accusation of noncompliance could be detrimental to a patient’s course of therapy.

Until recently, detection of multiple CYP2D6 genes required the use of restriction fragment length polymorphism (RFLP) analyses. However, in 1996 more simple and rapid PCR-based methods for efficient genotyping of UMs were developed (20, 21). In the present study, we have tested the CYP2D6 duplication assay described and evaluated by Lovlie et al. (21) for application in our laboratory. We also investigated the prevalence of ultra-rapid drug metabolism caused by CYP2D6 gene duplication in a group of 202 psychiatric inpatients of our hospital.

**Materials and Methods**

**SUBJECTS**
The blood samples used in this study were collected from a group of 202 anonymous psychiatric patients, all of North European Caucasian origin. The samples (86 men and 116 women; mean age, 46 years) were previously screened for the functional CYP2D6*2 allele and the PM-associated CYP2D6 mutations *3, *4, and *5 by PCR-based methods described elsewhere (7, 16, 22).

**CYP2D6 GENOTYPE DETERMINATION**

Genomic DNA was freshly isolated from leukocytes (EDTA-anticoagulated blood) by the GenomicPrep Blood DNA Isolation Kit (Pharmacia Biotech). To identify individuals carrying duplicate CYP2D6 genes, CYP2D6 duplication assays were performed according to the method of Lovlie et al. (21). Long-PCR was carried out on a Perkin–Elmer DNA Thermal Cycler, using the Gene Amp XL PCR kit (Perkin–Elmer), which contains rTth DNA polymerase, 3.3 × XL PCR reaction buffer, and 25 mmol/L Mg(OAc)₂ solution. PCR was performed in 50-μL reaction volumes containing 1 × XL PCR reaction buffer, 500 ng of genomic DNA, 200 μmol/L of each dNTP, 0.35 μmol/L of each primer, 1 mmol/L Mg(OAc)₂, and 1 U of rTth DNA polymerase. This differs somewhat from the original method: Lovlie et al. performed PCR in 100-μL reaction volumes containing no extra Mg(OAc)₂ and 2 U of rTth DNA polymerase. We modified these assay conditions to save reaction components, especially DNA polymerase.

With the primer combination cyp-17f (5′-TCCCC-CACTGACCCAACCTCT-3′) and cyp-32r (5′-CAGGTG-CAGGGCACCTAGAT-3′), a 3.6-kb PCR fragment amplified from the CYP2D6-CYP2D6 region is observed in subjects having duplicate alleles of the CYP2D6 gene. In addition, a 5.2-kb fragment from a CYP2D7-CYP2D6 intergenic region should be obtained from every sample as an internal control of the PCR reaction. Amplification of a 3.2-kb fragment, indicative of a CYP2D6-CYP2D6 intergenic sequence, by the CYP2D6-specific primer cyp-207f (5′-CCCTCAGCCTCCTACCT-CAC-3′) together with the cyp-32r primer is performed as a control reaction. This PCR assay yields product only in subjects carrying two CYP2D6 gene copies on the same allele, whereas no amplification is seen in individuals without duplicate genes. The conditions for amplification with the primer pairs cyp-17f/cyp-32r and cyp-207f/cyp-32r were as follows: an initial denaturing step of 93 °C for 1 min, followed by 37 cycles of 93 °C for 1 min, 67 °C for 30 s and 68 °C for 6 min, and a final elongation step of 72 °C for 10 min. The resulting long-PCR reactions for four patients are shown in Fig. 1.

**Results and Discussion**

With primer pair cyp-17f/cyp-32r, the expected 5.2-kb fragment was obtained from all 202 samples, indicating a reliable long-PCR-based DNA amplification in every patient. The 3.6-kb product, indicative of the presence of two CYP2D6 genes on the same allele, was seen in eight patients. Only in these subjects did the cyp-207f/cyp-32r PCR assay produce a 3.2-kb fragment, whereas no amplification was obtained in any other sample, as expected. The results of the long-PCR reactions for four patients are shown in Fig. 1.

Previous genotyping (unpublished data) showed five patients of the eight with CYP2D6 gene duplication (60%) to be homozygous for the functional CYP2D6*2 allele. In these subjects, gene duplication concerns CYP2D6*2, whereas the frequency of this allele in the entire population studied is only 30%. This fits with the observed preference of the CYP2D6*2 variant to be amplified compared with other CYP2D6 genes (3, 19). Two of the subjects with duplicate genes were genotyped *1/*1 and hence carry duplications of the wild-type gene. In one patient with the *4/*4 genotype, the defective CYP2D6*4
variant was duplicated. In contrast with the other seven cases, this last patient is not an UM, although a gene duplication is demonstrated. Because there are no functional CYP2D6 genes, this individual is classified as a PM. It is noteworthy that duplication of the *4 allele is rare and commonly associated with black American and African groups (21, 23). As far as we know, among Caucasians it has been described only once (3).

Screening for the most common inactivating CYP2D6 mutations is important to avoid misclassification of subjects with duplicate nonfunctional CYP2D6 genes, like the *4x2 carrier in our study, as UMs. This can be dangerous in a routine prediction of a patient’s metabolic capacity. To distinguish between different types of allele duplication, for example, in cases of questionable *2x2/*4 or *4x2/*2 constellation, additional PCR assays should be performed according to the method described by Sachse et al. (3).

The findings show that duplication of functional CYP2D6 genes in the patient population studied occurred in 3.5% of subjects. This is in agreement with the prevalences described earlier among subjects of Caucasian origin. Studies using RFLP analyses have found the frequency of UMs having two or more active genes per allele to be 1–2% in a Swedish population (19), 4% in a German group (3), and 7% among Spaniards (18). In an Ethiopian population, a prevalence of CYP2D6 gene multiplication as high as 29% was observed (24).

The set of PCR assays described easily identifies subjects having alleles with duplicate active CYP2D6 genes, which causes ultrafast drug metabolism. The method is reliable. False-negative results caused by technical failure of amplification or the use of DNA samples unfit for long-PCR will be noticed because of the absence of the internal control product in the cyp-17f/cyp-32r reactions. Positive results are confirmed by the cyp-207f/cyp-32r internal control product in the cyp-17f/cyp-32r reactions. Long-PCR will be noticed because of the absence of the long-PCR product in the cyp-17f/cyp-32r reactions. These results are confirmed by the cyp-207f/cyp-32r internal control product in the cyp-17f/cyp-32r reactions. Long-PCR will be noticed because of the absence of the long-PCR product in the cyp-17f/cyp-32r reactions. The method has been validated by Lovlie et al. (21) for the detection of the 42-kb XbaI allele of CYP2D6, which includes two copies of the CYP2D6 gene. Individuals of the 54-kb, 66-kb, or 175-kb haplotype, for example, which are indicative for alleles with 3, 4, or 13 CYP2D6 genes, were not included in their study. However, it is conceivable that all allelic variants having more than two CYP2D6 genes have been formed by multiple duplications (24). In addition, using another PCR-based assay for detection of CYP2D6 gene duplication, Johansson et al. (20) obtained a 10-kb fragment amplified from the region between two CYP2D6 gene copies not only from subjects carrying two genes per allele but also from individuals with triplicate or multiplicate genes. Hence, we suppose that the CYP2D6 gene duplication assays of Lovlie et al. will also yield positive results with these kinds of alleles, although research demonstrating the application of this method to alleles with more than two gene copies is not available. The number of extra CYP2D6 genes on one allele cannot be resolved by the PCR-based assays. This requires RFLP analysis as described elsewhere (17).

In conclusion, the method of long-PCR for detection of alleles having duplicate or probably multiduplicate CYP2D6 genes, together with the well-known PCR-based assays for nonfunctional CYP2D6 alleles, allow CYP2D6 genotyping with high predictability for both PMs and UMs. Identification of PMs and UMs is of potential clinical importance, e.g., as a tool for individualization and perfection of drug therapy. Knowledge of individual metabolic capacity can be helpful to avoid therapeutic failure in UMs or the development of adverse reactions in PMs, because differences in drug disposition could be compensated for by dose adjustment (25). Detection of gene duplication may also be of value in patients not responding properly to generally recommended doses of CYP2D6 substrates to distinguish between high metabolic capacity and bad compliance.

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