Occurrence of CYP2D6 Gene Duplication in Hong Kong Chinese, Mercè Garcia-Barceló, Lok Yee Chow, Kwok Lim Lam, Helen Fung Kam Chiu, Yun Kook Wing, and Mary Miu Yee Waye

The cytochrome P450 CYP2D6 enzyme debrisoquine 4-hydroxylase metabolizes many different types of drugs commonly used in psychopharmacotherapy, such as tricyclic antidepressants and neuroleptics. The CYP2D6 gene, which encodes for the CYP2D6 enzyme, is part of a cluster on chromosome 22 that includes two to three related pseudogenes. Starting at the 5’ end of the cluster, there are two nonfunctional pseudogenes termed CYP2D8P and CYP2D7AP (an additional pseudogene, CYP2D7BP, also can be found) followed by the active gene CYP2D6. Several mutated alleles of the CYP2D6 gene have been identified (3) and associated with alterations or polymorphisms in the metabolism of debrisoquine and related drugs metabolized by the CYP2D6 enzyme. As a result, CYP2D6 enzymatic activity ranges from ultrafast to a complete absence; the corresponding phenotypes are called ultrarapid (UM), extensive, and poor (PM) metabolizer. Unequal distribution of the alleles among different populations gives rise to interracial metabolic differences in addition to interindividual variation (4).

Individuals homozygous or heterozygous for deficient CYP2D6 alleles metabolize drugs at a low rate, increasing the risks of side effects and drug toxicity. On the other hand, individuals with duplication/multiplication of the active CYP2D6 gene metabolize drugs at ultrarapid rate, hence requiring more than average doses of drugs to reach therapeutic plasma concentrations (5). The mechanism behind the duplication involves mainly the active allelic variant CYP2D6*2 (6). The occurrence of gene duplication varies widely, from 29% in Ethiopians (7), to 20% in Saudi Arabians (8), to 1–7% in Caucasians (9, 10), to 1–2% in Orientals (11–13), although not many data are available for the latter. Establishing the prevalence of duplicated CYP2D6 active alleles may help prevent therapeutic failure in UM individuals treated with CYP2D6 enzyme-substrates at standard doses.

We investigated the prevalence of the duplication of the CYP2D6 gene in a Hong Kong Chinese population. This is the first time that a Hong Kong Chinese population has been screened for duplication of CYP2D6. We believe that our data will assist the identification and quantification of metabolic explanations for treatment failure, thus facilitating rational therapeutics in our population.

A total of 114 unrelated Hong Kong Chinese volunteers participated in the genotyping tests after giving their informed consent. This research project was approved by the local ethics committee.

Blood samples (12 mL) were collected in Vacutte® (Greiner) tubes containing EDTA as anticoagulant. DNA was extracted using QIAamp Blood kit (Qiagen). DNA samples were initially screened for the functional CYP2D6*2 allele and the PM-associated alleles *4D, *5, *8/*14, *10A, *10B, *15, *16 (3), and J9 (14) as we described previously (15).

Three different PCR tests were applied for the detection of duplicated CYP2D6 genes, two described by Lövlie et al. (16) and the third described by Lundqvist et al. (17). For test 1, primers cyp-17 and cyp-32 were used (16). This test yields a 5.2-kb and a 3.6-kb band in samples from subjects harboring duplicate alleles. The 5.2-kb band corresponds to the CYP2D7-CYP2D6 intergenic region, hence it is to be obtained from each sample and serves as internal control. The 3.6-kb band confirms the presence of the CYP2D6-CYP2D6 intergenic region formed during the duplication event (16). For test 2, amplification was carried out using the combination of primers cyp-207 and cyp-32, which yields a 3.2-kb product only from subjects carrying two copies of the CYP2D6 gene (16). For the third PCR assay, the primer combination used was 2D6dup-f and 2D6dup-r, which produce only a 3.5-kb amplification product from samples harboring the duplicated gene (17). For each test, 500 ng of genomic DNA was used as template and the Expand Long Template PCR System™ (Boehringer) was applied. Amplification reactions of 25–μL were performed on a PTC-200 DNA thermocycler (MJ Research). The reaction mixtures consisted of buffer 3 (supplied with the kit); 2.5 mmol/L MgCl2; 500 μmol/L; 2 U of the enzyme mixture (Taq and Pwo polymerases), and each of the primers at 0.3 μmol/L. For PCR assays 1 and 2, the cycle profile was as follows: predenaturation at 93 °C, 1 min; 37 cycles of 93 °C for 1 min; 63 °C for 30 s, and 68 °C for 6 min. The final elongation step was 10 min at 72 °C. For PCR assay 3, cycling conditions were as described previously (17). PCR products were detected in 1% agarose gels.

Thirteen of 114 samples were positive for tests 1, 2, and 3. Six samples were positive for tests 1 and 2 but negative for test 3, and two samples were positive for test 1 but negative for tests 2 and 3 (Table 1A). Interestingly, none of the samples positive for tests 1 and 2 harbored allele *2, whereas the samples that were positive only for test 3 both presented allele *2. Considering only the samples that scored positive for all three tests, the frequency of duplicated CYP2D6 alleles was 5.7% (11.4% of the Hong Kong Chinese population studied). However, only 7 of the 13 individuals had genotypes involving duplication of functional gene copies. The remaining samples positive for duplication presented a *10/*10 genotype. To our knowledge, this is the first time that duplication of allele *10 has been described. Allele *10, which is the most frequently mutated allele in Chinese populations, produces a CYP2D6 enzyme with diminished metabolic
activity (4). The consequences that the duplication of an allele of such characteristics (neither fully active nor inactive) may have on an individual phenotype are under study. Genotypes in which a functional gene was duplicated included *1/*2 or *1/*5, *2/*5 (two subjects), *1/*5 (one subject), and *2/*5 (one subject). These constellations were not further investigated.

To our knowledge, only two studies on duplicated CYP2D6 genes have been conducted on Chinese (Table 1B). Dahl et al. (12) reported a frequency of 2% for CYP2D6*2×2 in a Chinese population consisting of 21 subjects. In another study conducted on 113 Chinese subjects (11), the frequency reported for CYP2D6*2×2 was 0.9%.

This study has raised our concern about the methodology applied to screen for UM individuals in Chinese populations. In our experience, and as noted above, some PCR results are in disagreement. We wonder whether this could be attributable to differences in the structure of the CYP2D6 locus between races.

Duplication of the CYP2D6 gene has also been detected by the presence of a XbaI 42-kb fragment after restriction length polymorphism analysis (RFLP) (6). XbaI-RFLP analysis has identified several haplotypes of the gene cluster CYP2D6 (18, 19). The three most frequent XbaI fragments are 29 kb (active gene and two pseudogenes), 44 kb (in Caucasians, CYP2D6*4 and three pseudogenes), and 11.5 kb (deletion of the active gene) (17). The XbaI 42-kb fragment usually is indicative of a duplication of the functional CYP2D6*2 allele (6). However, the 42-kb haplotype may be heterogeneous and associated with both the PM and UM phenotypes. In a Zimbabwean population, the 42-kb XbaI fragment has been associated with the CYP2D6*4 mutation indicative of a duplication of a non-functional gene (20). Similarly, the 44-kb XbaI fragment, which is associated with 30% of mutant alleles in Caucasian PMs, is functional in Chinese (11, 21). The 44-kb XbaI fragments in Caucasians and Chinese differ in the composition of the genes of the CYP2D6 locus. Given the disagreement observed in PCR results and that XbaI-RFLP analysis could also be misleading in Chinese, we can only conclude that the composition of the 42-kb fragment in Chinese needs further study. Currently, our methodology does not allow us to proceed with further investigation. Pronounced differences between Caucasians and Orientals have been found in the CYP2D6 locus; consequently, primers used for PCR tests (designed from the CYP2D6 locus sequence in Caucasians) may not be specific enough.

PCR and RFLP tests detect the presence of duplicated genes but give no information about their activity or the metabolic capacity of an individual. Hence, an individual’s CYP2D6 complete genotype should be known, especially when interpreting the results from assays designed to detect duplicated CYP2D6 genes in “non-Caucasian” populations.
References


Quantification of the Aminosteroidal Non-Depolarizing Neuromuscular Blocking Agents Rocuronium and Vecuronium in Plasma with Liquid Chromatography-Tandem Mass Spectroscopy, Ursula Gutteck-Amsl er and Katharina M. Rentsch* (Institute of Clinical Chemistry, University Hospital Zürich, Rämistrasse 100, CH-8091 Zürich, Switzerland; *author for correspondence: fax 41-1-255-4590, e-mail rentsch@ikc.unizh.ch)

Rocuronium (RO) and vecuronium (VE) are widely used aminosteroidal non-depolarizing neuromuscular blocking agents. There are few methods published for the determination of VE, its metabolite 3-desacetyl-vecuronium (OHV) (1–4), and RO (5, 6) that use HPLC with ultraviolet, fluorescence, or electrochemical detection or gas chromatography with nitrogen-sensitive detection. To date, the methods published for the determination of VE and OHV use an exotic detection system (1), a time-consuming derivatization step (3), or a very laborious analytical technique (2, 4). The published methods for RO need a very sophisticated instrument that requires post-separation extraction of the drugs (6) or include a very time-consuming derivatization step (5). Despite the great analytical effort, the reproducibility (CV >10%) and detection limits (>10 μg/L) of these methods are not satisfying. To perform pharmacokinetic studies of RO, VE, and its metabolite OHV, we have established a robust, sensitive, and specific liquid chromatography electrospray ionization-tandem mass spectrometry method.

Immediately after blood collection into heparin-containing tubes and centrifugation at 4 °C, 1 mL of plasma was added to a tube containing 0.2 mL of a 1 mol/L sodium hydrogen phosphate solution to inhibit the degradation of the drugs. The samples were kept frozen at −70 °C until analysis. Before extraction of the plasma samples, 0.25 mL of a 1 mol/L sodium hydrogen phosphate solution and 50 μL of the internal standard pancuronium (PA; 10 mg/L) were added to 0.5 mL of plasma. After the addition of 1 mL of 6 mol/L potassium iodide, the drugs were extracted with 5 mL of toluene on a horizontal shaker (Infors HAT; Infors) for 20 min. After centrifugation for 5 min at 1000g, the organic layer was separated and dried by evaporation (Rotavapor; Büchi), and the residue was dissolved in 100 μL of mobile phase.

The HPLC system consisted of a RHEOS 2000 pump (Flux Instruments AG), an A200S autosampler (CTC) and a LCQ ion trap mass spectrometer (Thermoquest). The ionization mode was positive electrospray with a spray voltage of 3.8 kV and a capillary temperature of 230 °C. PA was detected by the most intense product ion of its divalent cation (m/z 286.4→236.7). RO was detected by the most intensive product ion of its monovalent cation (m/z 529.4→487.4). VE and OHV were detected by the most intensive product ions of their protonated molecules (dissociative cations; VE, m/z 279.2→249.4; OHV, m/z 258.5→100.2).

The different neuromuscular blocking agents were separated using a Nucleosil C18 HD column (12.5 cm × 4 mm; 5 μm particle size; Macherey-Nagel) protected with a