ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7): 585-588
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical


*Original Research*

# Correlation between cytochrome P-450 CYP2D6 (CYP2D6) genotype and phenotype[1]

CHEN Shu-Qing[2] (*Department of Biochemistry, College of Medicine, Zhejiang University, Hangzhou 310006, China*); Peter J WEDLUND (*Department of Pharmacology and Experimental Therapeutics, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA*)

**KEY WORDS** cytochrome P-450 CYP2D6; genotype; phenotype; alleles; polymerase chain reaction

## ABSTRACT

**AIM**: To study the correlation between CYP2D6 genotype and its phenotype. **METHODS**: CYP2D6 genotyping was made by detecting CYP2D6 *3, *4, *6, and *7 alleles with an allele-specific polymerase chain reaction procedure. **RESULTS**: The CYP2D6 genotypes were well correlated with its phenotypes in all 125 extensive metabolizers and in 43 poor metabolizers. Extensive metabolizers had at least one wildtype CYP2D6 gene and the genotypes were *1/*1, *1/*3, and *1/*4. Poor metabolizers were found to be homozygous mutants of CYP2D6 gene and the genotypes were *3/*4, *4/*4, *3/*6, *4/*7, *4/*6, and *6/*6. **CONCLUSION**: Genotype could be used to screen variations of CYP2D6 expression.

## INTRODUCTION

The bimodalities in debrisoquine and sparteine metabolism were originally reported in 1977[1]. Since that time, the enzyme primarily responsible for this bimodal activity has been isolated, the protein and gene sequence defined, and the enzyme was designated as CYP2D6[2,3]. Today, over 50 drugs are included in the list of chemicals which are at least partially metabolized by the CYP2D6 enzyme. The clinical significance of this polymorphism was reviewed by Brosen and Gram[4] who defined the conditions under which screening patients for CYP2D6 activity might be performed. Case reports and indirect evidence over the years have implicated extremes in CYP2D6 activity with therapeutic problems for certain drugs. It suggested that screening for variations in CYP2D6 expression in clinical settings may improve patient therapy. CYP2D6 *3, *4, *6, and *7 were reported separately as enzyme deficient alleles[5-7]. The purpose of this research was to address correlation between phenotype and CYP2D6 genotype done by detecting those CYP2D6 *3, *4, *6, and *7 alleles with an allele-specific polymerase chain reaction (AS-PCR) procedure.

## MATERIALS AND METHODS

**Reagents** Taq-polymerase, rTth polymerase, dNTP, PCR buffers, PCR tubes and Ampliwax plugs were obtained from Perkin-Elmer/Applied Biomedical, USA. Primers were synthesized and high performance liquid chromatography (HPLC) purified by Operon Technologies Inc, USA (Tab 1). Perfect Match DNA polymerase enhancer was obtained from Stratagene, USA. Agarose Type Ⅴ, ethidium bromide were purchased from Sigma Chemical Co, USA and 1 kilobase ladder (molecular weight marker) from Gibco, USA.

**Subjects** Subjects 125 (71 male, 54 female, age 43 a ± 20 a) of extensive metabolizers (EM) and subjects 43 (24 male, 18 female, age 41 a ± 18 a) of poor metabolizers (PM) were picked out from a

[1] Project partially supported by Zhejiang Natural Science Foundation (Item No 396473) and most of the work was done in Dr Wedlund's Laboratory in *University of Kentucky, USA*.
[2] Correspondence to Associate Prof CHEN Shu-Qing.
Phn 86-571-721-7154. Fax 86-571-604-4817.
Received 1998-11-19 Accepted 1999-03-17

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

**Tab 1. Sequence and location of primers for the PCR detection of the CYP2D6 * 3, * 4, * 6, * 7, and wildtype (CYP2D6 * 1) alleles of the CYP2D6 gene. Numbering of bases from work by Kimura *et al*[3].**

| Oligonucleotide primers | Oligonucleotide sequence | Gene location |
|---|---|---|
| AB Fragment Primers | | |
| 1BF | 5′-GCATTTCCCAGCTGGAATCC-3′ | 1383 – 1402 |
| 2AR | 5′-CCGGCCCTGACACTCCTTCT-3′ | 3200 – 3181 |
| CYP2D6 * 3-Allele Primers | | |
| FA5-wildtype | 5′-CTGCTAACTGAGCACA-3′ | 2622 – 2637 |
| FA6-A allele | 5′-CTGCTAACTGAGCACG-3′ | 2622 – 2638 |
| CYP2D6 * 4-Allele Primers | | |
| RB7-wildtype | 5′-GGCGAAAGGGGCGTCC-3′ | 1949 – 1934 |
| RB8-B allele | 5′-GGCGAAAGGGGCGTCT-3′ | 1949 – 1934 |
| CYP2D6 * 6-Allele Primers | | |
| T1-wildtype | 5′-GCAAGAAGTCGCTGGAGCAGT-3′ | 1775 – 1795 |
| T2-T allele | 5′-GCAAGAAGTCGCTGGAGCAGG-3′ | 1775 – 1796 |
| T2R-common primer | 5′-AGCCCGACTCCTCCTTCAGTT-3′ | 2124 – 2102 |
| CYP2D6 * 7-Allele Primers | | |
| EF2-common primer | 5′-TGGCAAGGTCCTACGCTTCC-3′ | 2573 – 2592 |
| ER1-wildtype | 5′-CTCACGCTGCACATCCGGAT-3′ | 3041 – 3023 |
| ER2-E allele | 5′-CTCACGCTGCACATCCGGAG-3′ | 3041 – 3023 |

database of CYP2D6 phenotyping screening by using dextromethorphan as probe drug. More than 95 % of the subjects were Caucasians. A 20-mL blood sample was collected from each subject and DNA was extracted from blood for CYP2D6 genotyping according to Lahiri[8]. This study was approved by the Medical Institutional Review Board at the University of Kentucky and all subjects provided informed consent prior to their participation.

**Genotyping procedures** A 1818 base pair fragment (AB fragment; 1383 – 3200 bp region of the CYP2D6 gene) was generated by use of primer pairs 1BF and 2AR with rTth polymerase. This amplified fragment provided the template for testing the presence of the CYP2D6 * 3, * 4, * 6 and * 7 alleles. Briefly, 10 pmol · $L^{-1}$ of each oligonucleotide (1BF and 2AR) was added to 6.2 μL of sterile water, 6.0 μL of 3.3 XL buffer, 0.2 μmol of each dNTP, 2.2 μL of the $Mg(OAc)_2$ solution and a Ampliwax plug. After heating the wax plug to 80 ℃ for 5 min, tubes were cooled and 9.0 μL of 3.3 XL buffer, 0.5 μL of rTth polymerase, and 1.5 μL of DNA in 20 μL of sterile water were added on top of the wax layer and placed in the thermocycler at 94 ℃ for 1 min (first cycle), then 94 ℃ (0.5 min), followed by 60 ℃ (10 min) for 35 cycles. At the end of the program, a final extension was carried out at 72 ℃ for 10 min.

The AB fragment was diluted by 1/10 with sterile water and 1 μL of the diluted fragment probed by nested and hemi-nested PCR methods to detect the * 3, * 4, * 6, and * 7 alleles. The * 3 and * 4 alleles were detected under identical thermocycler conditions, 94 ℃ (1 min), 48 ℃ (1.5 min), for 16 cycles. Each tube contained 25 μL with 0.2 μmol of each primer (2 AR and FA5 [ * 3-wildtype]; or 2AR and FA6[ * 3-allele]; or 1BF and RB7 [ * 4-wildtype]; or 1BF and RB8 [ * 4-allele]); 0.2 mmol of each dNTP, 2.5 μL of 10 × buffer, 1.0 mmol $MgCl_2$, 18 μL of sterile water, 1 μL of AB diluted template, and 0.1 μL of Taq polymerase. The * 6 and * 7 alleles were detected under identical thermocycler conditions, 94 ℃ (1 min), 65 ℃ (1 min), 72 ℃ (1.5 min) for 16 cycles. Each tube contained 25 μL with 0.2 μmol of each primer (EF2 and ER1 [ * 7-wildtype], or EF2 and ER2 [ * 7-allele], or T1 and T2R [ * 6-wildtype], or T2 and T2R [ * 6-allele]), 0.2 mmol of each dNTP, 2.5 μL of 10 × buffer, 1.0 mmol $MgCl_2$, 0.1 μL of PCR enhancer, 18 μL of sterile water, 1 μL of AB diluted template, and 0.1 μL of Taq polymerase. All PCR solutions were overlayed with oil prior to placing in the thermocycler. PCR products were evaluated by gel electrophoresis on a 2 % agarose gel containing ethidium bromide 0.35 mg · $L^{-1}$.

**Quality control of genotyping** The positive controls were prepared from 25 μL aliquots of DNA solutions of known genotype and assigned a random

number for blinded analysis. Negative quality controls were generated by including all reagents except the DNA in a PCR tube for each allele evaluated.

## RESULTS

**PCR methods** Use of the long amplified AB fragment from the CYP2D6 gene provided the best quality and most efficient way to genotype patients. This method provided a single DNA fragment from which all alleles could be detected during the 2nd PCR step.

**Quality control** Negative quality controls did not detect any contamination problems with the PCR, and positive quality controls indicate that the methods in use are reproducible for the CYP2D6 * 3, * 4, * 6, and * 7 alleles.

**Agreement between genotype and its phenotype** One hundred and twenty five subjects identified as expressing the CYP2D6 enzyme based on the dextromethorphan metabolic ratio were characterized as expressing at least one functional CYP2D6 allele. Fourty three subjects who were phenotyped as deficient in CYP2D6 function, were found to have only the CYP2D6 * 3, * 4, * 6, and/or * 7 alleles. (Tab 2)

**Tab 2. Alleles distribution and metabolic ratio differences of CYP2D6 genotypes.**

| Genotypes | Subjects | Average (MR) | Range (MR) | Phenotype |
|---|---|---|---|---|
| * 1/ * 1 | 88 | 0.006 | 0.001 - 0.035 | EM |
| * 1/ * 3 | 3 | 0.011 | 0.001 - 0.028 | EM |
| * 1/ * 4 | 34 | 0.039 | 0.001 - 0.125 | EM |
| * 3/ * 4 | 6 | 2.484 | 1.416 - 4.86 | PM |
| * 4/ * 4 | 30 | 3.498 | 1.04 - 8.57 | PM |
| * 3/ * 6 | 1 | 0.69 | - | PM |
| * 4/ * 7 | 2 | 1.275 | 1.08 - 1.47 | PM |
| * 4/ * 6 | 3 | 2.04 | 1.52 - 2.53 | PM |
| * 6/ * 6 | 1 | 2.53 | - | PM |

MR: Metabolic ratio; EM: Extensive metabolizers; PM: Poor metabolizers.

**Alleles distribution** All EM were only found with * 1/ * 1 (wildtype homozygotes), * 1/ * 3 and * 1/ * 4 (heterozygotes) genotypes. EM had at least one functional allele. All PM were genotyped * 3/ * 4, * 4/ * 4, * 3/ * 6, * 4/ * 7, * 4/ * 6, and * 6/ * 6 (mutant homozygotes). PM did not have any functional allele. (Tab 2)

## DISCUSSION

Since the frequency of the CYP2D6 deficiency occurs in 5 - 7 of large Caucasian populations[9], all alleles responsible for this deficiency must occur in the population with a total frequency $P = 0.23 - 0.27$ ($P^2 = 0.05 - 0.07$). The frequency of the CYP2D6 * 3, * 4, * 6, and * 7 alleles occurred with a total frequency of 0.20 in this study, which is not significantly different from the expected frequency ($P = 0.23 - 0.27$) for Caucasian population. So the CYP2D6 deficiency may be detectable with an accuracy up to 100 %. This conclusion is bolstered by our ability to associate a deficient genotype in 43 unrelated subjects previously identified by phenotyping as deficient in CYP2D6 activity. This supports assertions[10] that nearly all alleles for the CYP2D6 deficiency have now been identified. The new ones which need to be identified must be very low chance alleles.

The amplification of a long region of the CYP2D6 gene followed by a nested PCR method to detect specific alleles in the CYP2D6 gene has been the most robust. The advantage of this approach lies in the ability to select oligonucleotides which are highly specific for the gene of interest. When amplifying short regions of DNA, the oligonucleotides may have substantial homology with genes within the same subfamily or with pseudo genes. This may explain the nonselective amplification observed with many of the PCR methods evaluated during this work. As the region of DNA to be amplified expands, the potential for identifying unique DNA sequences and more specific oligonucleotide primers improves. A long amplified gene segment eliminates the need for multiple separate amplification steps with various thermocycler conditions. The availability of rTth polymerase has substantially improved the ease of obtaining longer segments of DNA. Considerable difficulty was initially encountered when attempting to obtain a reproducible AB fragment with Taq polymerase or with the LA PCR polymerase from PanVera Corp, Madison, WI.

In theory, molecular genetics could provide clinicians with mechanistic information about patient therapy. In place of simple descriptive information

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Jul; 20 (7)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

provided by (or in many cases unavailable from) therapeutic drug monitoring, molecular genetics could produce information about why a patient may require a different dose, drug or treatment regimen prior to therapy. Whether such clinical applications will come to pass is unclear at this time. However, it is apparent the gap between our ability to measure and the practicality of defining genetic variability is narrowing. Eventually, molecular genetics may be applied to clinical practice to speed attainment of therapeutic drug levels, individualize dosing regimens, prevent toxicity, avoid inappropriate medications, and improve compliance by providing a mechanistic basis for drug treatment. As therapeutic agents continue to be characterized with regard to the specific enzyme(s) associated with their metabolism[11], clinical testing for genetic variability in those enzymes becomes a greater and more likely prospect for patient therapy.

## REFERENCES

1 Mahgoub A, Idle JR, Dring LG, Lancaster R, Smith RL. Polymorphic hydroxylation of debrisoquine in man. Lancet 1977; 2: 584-6.
2 Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, *et al*. Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. Nature 1988; 331: 442-6.
3 Kimura S, Umeno M, Skoda RC, Meyer UA, Gonzalez FJ. The human debrisoquine 4-hydroxylase (CYP2D6) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene and a pseudogene. Am J Hum Genet 1989; 45: 889-904.
4 Brosen K, Gram LF. Clinical significance of the sparteine/debrisoquine oxidation polymorphism. Eur J Clin Pharmacol 1989; 36: 537-47.
5 Kagimoto M, Heim M, Kagimoto K, Zeugin T, Meyer UA. Multiple mutations of the cytochrome P-450 Ⅱ D6 gene (CYP2D6) in poor metabolizers of debrisoquine. J Biol Chem 1990; 265: 17209-14.
6 Evert B, Griese EU, Eichelbaum M. A missense mutation in exon 6 of the CYP2D6 gene leading to a histidine 324 to proline exchange is associated with the poor metabolizer phenotype of sparteine. Naunyn-Schmiedebergs Arch Pharmacol 1994; 350: 434-9.
7 Evert B, Griese EU, Eichelbaum M. Cloning and sequencing of a new non-functional CYP2D6 allele: deletion of T1795 in exon 3 generates a premature stop codon. Pharmacogenetics 1994; 4: 271-4.
8 Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucl Acids Res 1991; 19: 5444.
9 Guttendorf RJ, Britto M, Blouin RA, Foster TS, John W, Pittman KA, *et al*. Rapid screening for polymorphisms in dextromethorphan and mephenytoin metabolism. Br J Clin Pharmacol 1990; 29: 373-80.
10 Heim M, Meyer UA. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. Lancet 1990; 336: 529-32.
11 Rodriques AD. Use of *in vitro* human metabolism studies in drug development. An industrial perspective. Biochem Pharmacol 1994; 48: 2147-56.

## 细胞色素 P-450 CYP2D6 基因分型与表型的吻合率[1]

陈枢青[2] (浙江大学医学院生物化学教研室, 杭州 310006, 中国)
Peter J WEDLUND[2] (*Department of Pharmacology and Experiment Therapeutics, College of Pharmacy, University of Kentucky, Lexington KY 40536, USA*)

**关键词** 细胞色素 P-450; CYP2D6; 基因分型; 表型分型; 等位基因

**目的**: 研究细胞色素 P-450 2D6 基因分型测定方法及其与表型的吻合率. **方法**: 利用等位基因特异扩增法基本原理, 对 CYP2D6 酶缺陷等位基因 CYP2D6 *3, *4, *6 和 *7 进行测定. **结果**: 通过 168 例基因分型, 并将结果与表型对照, 发现同时测定 CYP2D6 *3, *4, *6 和 *7 等位基因时, 125 例快代谢者和 43 例慢代谢者的基因分型结果与表型结果的吻合率为100 %. 快代谢者至少有一个野生型 CYP2D6 等位基因, 基因型为 *1/*1, *1/*3 和 *1/*4. 发现慢代谢者是 CYP2D6 突变型纯合子, 基因型为 *3/*4, *4/*4, *3/*6, *4/*7, *4/*6 和 *6/*6. **结论**: 对 CYP2D6 *3, *4, *6 和 *7 等位基因的测定能够准确预测其表型.

(责任编辑 周向华)