Developing and Evaluating the HRM Technique for Identifying Cytochrome P450 2D6 Polymorphisms

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Background: Cytochrome P450 2D6 is one of the important enzymes involved in the metabolism of many widely used drugs. Genetic polymorphisms of *CYP2D6* can affect its activity. Therefore, an efficient method for identifying *CYP2D6* polymorphisms is clinically important. **Methods:** We developed a high-resolution melting (HRM) analysis to investigate *CYP2D6* polymorphisms. Genomic DNA was extracted from peripheral blood samples from 71 healthy individuals. All nine exons of the *CYP2D6* gene were sequenced before screening by HRM analysis. This method can detect the most genotypes (*1, *2, *4, *10, *14, *21, *39, and *41) of *CYP2D6* in Chinese. **Results:** All samples were successfully genotyped. The four most common mutant *CYP2D6* alleles (*1, *2, *10, and *41) can be genotyped. The single nucleotides polymorphism (SNP) frequencies of 100C > T (rs1065852), 1039C > T (rs1081003), 1661G > C (rs1058164), 2663G > A (rs28371722), 2850C > T

(rs16947), 2988G > A (rs28371725), 3181A > G, and 4180G > C (rs1135840) were 58%, 61%, 73%, 1%, 13%, 3%, 1%, 73%, respectively. We identified 100% of all heterozygotes without any errors. The two homozygous genotypes (1661G > C and 4180G > C) can be distinguished by mixing with a known genotype sample to generate an artificial heterozygote for HRM analysis. Therefore, all samples could be identified using our HRM method, and the results of HRM analysis are identical to those obtained by sequencing. Our method achieved 100% sensitivity, specificity, positive prediction value and negative prediction value. **Conclusion:** HRM analysis is a nongel resolution method that is faster and less expensive than direct sequencing. Our study shows that it is an efficient tool for typing *CYP2D6* polymorphisms. J. Clin. Lab. Anal. 29:220–225, 2014. © 2014 Wiley Periodicals, Inc.

Key words: high-resolution melting analysis; *CYP2D6*; direct sequencing

INTRODUCTION

Cytochrome P450 (CYP) is an enzyme that plays an important role in metabolism. The most important enzymes for drug metabolism are CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (1). CYP2D6 in particular can metabolize many widely used drugs, including antidepressants, antipsychotics, and antiarrhythmics (2). It contains nine exons and is located near two CYP pseudogenes, *CYP2D7* and *CYP2D8* (3). Together, the three genes have 92–97% similarity in nucleotides (4), making it difficult to distinguish mutations in these three genes. To date, 100 allelic variants have been reported for *CYP2D6* (<http://www.cypalleles.ki.se/cyp2d6.htm>). Different allele

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frequencies of *CYP2D6* have been noted within populations (5). The Taiwanese population, for example, can be divided into two groups according to their level of enzymatic activity: intermediate metabolizers (IM) and extensive metabolizers (EM). About 50% of the Taiwanese population are homozygous for *CYP2D6**10, resulting in the IM predicted phenotype (6).

Several methods can be used to detect gene mutations, including capillary electrophoresis (7), real-time PCR (8), the automated chip method (9), and single-base extension (10). These methods are laborious and require expensive instruments to perform the analysis. In contrast, high-resolution melting (HRM) is a novel, homogeneous, closed-tube, post-PCR method that offers considerable cost savings and is more time efficient (as it permits analyzing 96 or 384 wells at one time). It enables researchers to analyze genetic variations in PCR amplicons (11,12). This method used for *CYP2D6* genotyping was proposed by Pindurova et al. (13), however, they tested only hotspot-related exons in their population, an approach which may miss some new mutations in undetected exons and that may not be applicable to other populations. The aim of this project is to develop a more comprehensive HRM method and evaluate its performance as a method for identifying *CYP2D6* polymorphisms.

MATERIALS AND METHODS

DNA Samples

All the studied samples were collected from Kaohsiung Medical University Hospital. Genomic DNA samples isolated from ethylenediaminetetraacetic acid (EDTA) anticoagulated blood were obtained from 71 healthy individuals during health examinations in the hospital. The subjects consisted of 35 males and 36 females, and their ages were between 20 and 55 years old. The DNA was extracted using the NucleoSpin® Blood Kit (Macherey-Nagel, GmbH & Co. KG, Dueren, Germany) according to the manufacturer's instructions. We used agarose gel electrophoresis analysis to check the quality of the isolated genomic DNA, and the concentration was determined by Nano-Drop-1000 (Nano-Drop Technologies Inc, Wilmington, DE). The Institutional Review Board of Kaohsiung Medical University Hospital approved this study.

Primer Design

We designed the primer sets using Primer3 software with some modifications (Table 1). In order to avoid primer dimers and nonspecific amplification, we used free Primer-BLAST software to check each primer set.

Preamplification of the *CYP2D6* Gene

To isolate *CYP2D6* specifically, a 5.1-kb PCR product covering nine exons of the *CYP2D6* gene was amplified according to methods described previously (14). The long-PCR reaction mixture contained PCR buffer, 0.2 mM dNTPs, 0.5 μ M each primer, 1 U of Phusion DNA polymerase (New England Biolabs, Ipswich, MA) and 200 ng of genomic DNA in a volume of 50 μ l. PCR conditions were 98°C for 30 s, followed by 35 cycles of 98°C for 10 s and 72°C for 3 min, then 4°C. The PCR product was visualized on a 1% agarose gel. These PCR products were divided into two tubes. One was subjected for sequencing, the other was diluted with Tris-EDTA (TE) buffer and then subjected to further HRM analysis.

HRM Techniques

We used the LightCycler® 480 High Resolution Melting Master kit (Roche Diagnostics, Penzberg, Germany) according to the manufacturer's manual and analyzed the data using LightCycler® 480 Gene Scanning Software, version 1.5 (Roche Diagnostics). Polymerase chain reaction (PCR) reactions were performed in 10 μ l of final volume reaction with previously amplified 1:10 diluted PCR product. Thermal cycling consisted of an initial preincubation at 95°C for 10 min, after which the amplification was performed for 45 cycles as follows: 95°C for 15 s, 60°C for 15 s, and 72°C for 15 s. The melting curves were obtained under the following conditions: 95°C for 1 min, 40°C for 1 min, 60°C 1 s, and 90°C continuously, with a ramping rate of -0.02°C/s . Furthermore, an analysis of the melting curves with a default sensitivity of 0.3 was carried out using Gene Scanning Software; only exon 6 was analyzed with a high-sensitivity setting of 0.5.

Direct DNA Sequencing

We used direct sequencing to analyze the SNPs of the *CYP2D6* gene. The sequence reaction was performed in a final volume of 10 μ l, including 1 μ l of the purified PCR product, 2.5 μ M of one of the PCR primers, 2 μ l of the ABI PRISM terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA) and 2 μ l 5 $\times$ sequence buffer. The sequencing program started from 96°C for 1 min, and then was followed by a 25-cycle PCR program (denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and elongation at 60°C for 4 min). Direct DNA sequencing was performed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

TABLE 1. Primer Sets for the HRM Analysis of the *CYP2D6* Gene

	Sequence of primers	Amplicon (bp)	Main SNP change in Chinese	Related <i>CYP2D6</i> allele(s)
Exon 1	F: GTGTGTCCAGAGGAGCCCAT R: CTGGTCCAGCCTGTGGTTTC	310	100C > T (rs1065852)	*4, *10
Exon 2	F: AATAGGACTAGGACCTGTAGTCTGG R: GGTCCCACGGAATCTGTCT	280	1039C > T (rs1081003)	
Exon 3	F: TGGTGGATGGTGGGGCTAAT R: CTTCCCAGTTCCCGCTTT	253	1661G > C (rs1058164) 1758G > A (rs5030865)	*2, *4, *10, *21, *39, *41 *14
Exon 4	F: TGCCGCCTTCGCCAACCCT R: AGCTCGCCCTGCAGAGACT	312	1846G > A (rs3892097)	*4
Exon 5	F: GTGAACGCAGAGCACAGGA R: GCCGAGAGCATACTCGGGAC	407	2573_2574insC (rs72549352) 2663G > A (rs28371722)	*21
Exon 6	F: CCCGTTCTGTCCCGAGTATG R: GCTTACAGGATCCTGGTCAA	297	2850C > T (rs16947) 2988G > A (rs28371725)	*2, *14, *21, *41 *41
Exon 7	F: CAACATAGGAGGCAAGAAGG R: GGGCTATCACCAGGGGCTGG	320	3181A > G	
Exon 8	F: CCGTGTGTTTGGTGGCAGGG R: GGGGCTGTCCAGTGGGCACC	418	ND	
Exon 9	F: AGCACTTCAGCTTCTCGGTGC R: ACATTAGAGCCTCTGGCTAGG	159	4180G > C (rs1135840)	*2, *4, *10, *14, *21, *39, *41

ND, no polymorphism was detected.

CYP2D6 haplotype: *2, 1661C-2850T-4180C; *4, 100T-1661C-1846A-4180C; *10, 100T-1661C-4180C; *14, 1758A-2850T-4180C; *21, 1661C-2573_2574insC-2850T-4180C; *39, 1661C-4180C; *41, 1661C-2850T-2988A-4180C.

Analysis of the Whole Allele Duplication and Deletion

Two long PCR reactions were used to detect the whole allele duplication and deletion. To detect the entire *CYP2D6* duplication and deletion, we adopted methods reported by Lovlie et al. and Steen et al. (15, 16). To detect gene duplication, we used a set of three primers to amplify the duplication-specific 3.2-kb fragment and 3.8-kb control fragment: primers CYP-207-F (5'-CCCTCAGCCTCGTCACCTCAC-3'), CYP-32-R (5'-CACGTGCAGGGCACCTAGAT-3'), and CYP-13-F (5'-ACCGGGCACCTGTACTCCTCA-3'). To detect the gene deletion, we again used a set of three primers to amplify the deletion-specific 3.5-kb fragment and 3.0-kb control fragment: primers CYP-13-F, CYP-24-R (5'-GCATGAGCTAAGGCACCCAGAC-3'), and CYP-207-F. The PCR was conducted as follows: denaturation at 94°C for 2 min; 10 cycles of 94°C for 20 s and 68°C for 4 min; 25 cycles of 94°C for 20 s and 68°C for 4 min, plus 2 s per cycle; and a final extension at 68°C for 10 min.

Statistical Analysis

Sensitivity was expressed as the ratio between the true positive (TP) and the sum of the TP and false negative (FN): TP/(TP + FN). Specificity was expressed as the ratio between the true negative (TN) and the sum of the TN and false positive (FP): TN/(TN + FP). The positive prediction value was expressed as the ratio between the TP and the sum of the TP and FP: TP/(TP + FP). The

negative prediction value was expressed as the ratio between the TN and the sum of the TN and FN: TN/(TN + FN) (17).

RESULTS

All 71 samples analyzed for genotype by HRM were genotyped successfully, as shown by comparison with the sequencing results. The *10 allele (rs1065852) had the highest frequency (58%), followed by the wild-type *1 allele (26%) and the *2 allele (rs16947, rs1135840) (10%). The other two alleles, *41 (rs28371725) and *5 (deleted), were both relatively rare, with frequencies of 3%. The frequency of duplication was 1% ($n = 1$), and the duplicated allele was *10 $\times$ N.

HRM can detect the polymorphism at position 100 (100C > T) in exon 1 (Fig. 1A). This determination base of genotype *10 is predicted to produce phenotype IM. The 50% fluorescence value corresponded to $92.44 \pm 0.09^\circ\text{C}$ for the wild type, $91.48 \pm 0.07^\circ\text{C}$ for heterozygotes, and $91.88 \pm 0.15^\circ\text{C}$ for homozygotes. This polymorphism results in an amino acid change from proline to serine at codon 34 (P34S).

The polymorphism at position 1039 (1039C > T) in exon 2 can be detected by HRM (Fig. 1B). This SNP does not determine any genotype. The 50% value corresponded to $87.67 \pm 0.05^\circ\text{C}$ for wild type, $87.7 \pm 0.05^\circ\text{C}$ for heterozygotes, and $87.63 \pm 0.07^\circ\text{C}$ for homozygotes. This polymorphism has no amino acid change (F112F).

We found the heterozygote of polymorphism at position 1661 (1661G > C) in exon 3 can also be detected by HRM

Fig. 1. Detecting the polymorphisms of the *CYP2D6* gene by HRM analysis. Normalized and temperature-shifted difference plots for *CYP2D6* genes are shown. The polymorphisms were distinguished after grouping of different melting curves by shape. (A) For exon 1 mutations, the genotype was E-1 100C>T (P34S). (B) In exon 2 mutations, the genotype was E-2 1039C>T (F112F). (C) For exon 3 mutations, the genotype was E-3 1661G>C (V136V). (D) In exon 5 mutations, the genotype was E-5 2663G>A. (E) For exon 6 mutations, the genotype was E-6 2850C>T (R296C) and 2988G>A (splicing defect). (F) In exon 7 mutations, the genotype was E-7 3181A>G (D337G). (G) In exon 9 mutations, the genotype was E-9 4180C>G (S486T).

(Fig. 1C). This base is found in genotypes *2, *10, and *41 and has no definite correlation with enzyme activity. The 50% fluorescence value corresponded to $89.98 \pm 0.07^\circ\text{C}$ for heterozygotes and $90.11 \pm 0.07^\circ\text{C}$ for homozygotes. The homozygotes of 1661G and 1661C could not be distinguished by traditional HRM methods. However, these two homozygous genotypes can be clearly discriminated by generating artificial heterozygotes using a 1:1 mixture of the tested DNA and wild-type DNA. The 50% fluorescence value corresponded to $89.34 \pm 0.04^\circ\text{C}$ for homozygous 1661G, $89.42 \pm 0.05^\circ\text{C}$ for homozygous 1661C. This polymorphism has no amino acid substitution (V136V).

HRM can detect the polymorphism at position 2663 (2663G > A) in exon 5 (Fig. 1D). This SNP does not determine any genotype. The 50% fluorescence value corresponded to $90.24 \pm 0.02^\circ\text{C}$ for heterozygotes and $90.61 \pm 0.06^\circ\text{C}$ for the wild type.

HRM can also detect polymorphism at positions 2850 (2850C > T) and 2988 (2899G > A) in exon 6 (Fig. 1E). A single nucleotide C to T transition at position 2850 characterizes genotypes *2 and *41, and the predicted phenotypes are EM and IM, respectively. The transition of guanine to adenine at position 2988 characterizes genotype *41, and the phenotype predicted to the result is IM. The 50% fluorescence value corresponded to $89.91 \pm 0.15^\circ\text{C}$ for heterozygotes of polymorphism at positions 2850 and 2988, $90.09 \pm 0.07^\circ\text{C}$ for heterozygotes of polymorphism 2850 and wild types 2988, and $90.06 \pm 0.06^\circ\text{C}$ for wild types 2850 and 2988. The 2850 polymorphism results in R296C.

In addition, HRM can detect the polymorphism at position 3181 (3181A > G) in exon 7 (Fig. 1F). The 50% fluorescence value corresponded to 89.93°C for heterozygote, $89.78 \pm 0.08^\circ\text{C}$ for the wild type.

Furthermore, HRM can detect the heterozygote polymorphism at position 4180 (4180G > C) in exon 9 (Fig. 1G). This base is detected in genotypes *2, *10, and *41 and shows no correlation with enzyme activity. The 50% fluorescence value corresponded to $88.02 \pm 0.18^\circ\text{C}$ for heterozygotes, $88.21 \pm 0.08^\circ\text{C}$ for homozygotes. The homozygotes of 4180G and 4180C were undistinguishable by traditional HRM methods. Our strategy for differentiating these two homozygous genotypes was the same as that used for exon 3. The 50% fluorescence value corresponded to $87.91 \pm 0.05^\circ\text{C}$ for homozygous 4180G, $88.11 \pm 0.06^\circ\text{C}$ for homozygous 4180C. This polymorphism results in S486T.

No nucleotide change was shown in HRM analysis of exons 4 and 8 in our samples (data not shown).

In total, we found ten different allele combinations in our group: half of the genotypes had normal enzyme activity (EM) and the other half had decreased enzyme activity (IM). We found the following combinations of alleles for the EM: *1/*10, *2/*10, *1/*41, *1/*1 and *1/*2.

Combinations of alleles for IM were *10/*10, *10/*41, *5/*10, *1/*5, and *10 $\times$ N/10 (18).

DISCUSSION

The HRM method is rapidly becoming the most important mutation scanning methodology. It is a closed-tube method, which means that PCR amplification and subsequent analysis are performed sequentially in the well. There is no need to use expensive probe; two specific primers and saturating DNA-binding dye (commercial master mix) are sufficient. This makes it more convenient than other scanning methodologies. Although the HRM method is a powerful screening tool, it also has some limitations, one of which is that when unexpected polymorphisms are present, they may interfere with genotyping. The HRM method is not able to detect deletions of entire exons and genes (*CYP2D6**5 allele). Additionally, the possible effects of different template concentrations should be considered in using the HRM method (19). If a higher amount of DNA (2.5X) is used, deviation in results can be observed, which could give rise to an FP result (20).

Our study is the first to systematically use HRM to screen for variants of *CYP2D6* among the population of Taiwan, comparing these results with those from the Chinese populations, and we found eight genetic variants including one novel polymorphism, six alleles, and ten genotypes. The *CYP2D6**10, *10 $\times$ N and *41 alleles, all of which decrease the activity of *CYP2D6*, occur among the Chinese populations at frequencies of 48.4–52.53%, 1.43%, and 3.34–3.8%, respectively. *CYP2D6**10 is the most prevalent allele reported in Asian population. The *CYP2D6**1 and *2 alleles, which do not affect the activity of *CYP2D6*, occur among Chinese populations at frequencies of 24.65–28.1% and 11%, respectively. The *CYP2D6**5 allele results in the loss of *CYP2D6* function, which occurs among Chinese population at frequencies of between 4.66% and 6.1% (21,22). In the population of Taiwanese we tested, the allelic frequencies of *CYP2D6**1, *2, *5, *10, *10 $\times$ N, and *41 are similar to investigations of other Chinese population. Overall, we find 59% of the people in our study had genotypes associated with normal enzyme activity ($n = 42$) and the other 41% had genotypes associated with decreased enzyme activity ($n = 29$).

In conclusion, we demonstrated that the HRM method can efficiently and precisely detect the common *CYP2D6* genotypes in the Taiwanese population, and we suggest that this approach can also be applied to other population.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Bozina N, Bradamante V, Lovric M. Genetic polymorphism of metabolic enzymes P450 (CYP) as a susceptibility factor for drug response, toxicity, and cancer risk. *Arh Hig Rada Toksikol* 2009;60:217–242.
2. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): Clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005;5:6–13.
3. Kimura S, Umeno M, Skoda RC, Meyer UA, Gonzalez FJ. The human debrisoquine 4-hydroxylase (CYP2D) locus: Sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. *Am J Hum Genet* 1989;45:889–904.
4. Teh LK, Bertilsson L. Pharmacogenomics of CYP2D6: Molecular genetics, interethnic differences and clinical importance. *Drug Metab Pharmacokinet* 2012;27:55–67.
5. Ozawa S, Soyama A, Saeki M, et al. Ethnic differences in genetic polymorphisms of CYP2D6, CYP2C19, CYP3As and MDR1/ABCB1. *Drug Metab Pharmacokinet* 2004;19:83–95.
6. Liou YH, Lin CT, Wu YJ, Wu LS. The high prevalence of the poor and ultrarapid metabolite alleles of CYP2D6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 in Taiwanese population. *J Hum Genet* 2006;51:857–863.
7. Liao HW, Tsai IL, Chen GY, et al. Simultaneous detection of single nucleotide polymorphisms and copy number variations in the CYP2D6 gene by multiplex polymerase chain reaction combined with capillary electrophoresis. *Anal Chim Acta* 2013;763:67–75.
8. Eriksson S, Berg LM, Wadelius M, Alderborn A. Cytochrome p450 genotyping by multiplexed real-time dna sequencing with pyrosequencing technology. *Assay Drug Dev Technol* 2002;1:49–59.
9. Sukasem C, Sirachainan E, Chamnanphon M, et al. Impact of CYP2D6 polymorphisms on tamoxifen responses of women with breast cancer: A microarray-based study in Thailand. *Asian Pac J Cancer Prev* 2012;13:4549–4553.
10. Li J, Butler JM, Tan Y, et al. Single nucleotide polymorphism determination using primer extension and time-of-flight mass spectrometry. *Electrophoresis* 1999;20:1258–1265.
11. Safarikova M, Reiterova J, Safrankova H, et al. Mutational analysis of ACTN4, encoding alpha-actinin 4, in patients with focal segmental glomerulosclerosis using HRM method. *Folia Biol* 2013;59:110–115.
12. Cao CG, Sun HY, Zhou FF, Wang SM, Chen HY, Lu DR. Duplex genotyping of CYP2C19*2 and CYP2C19*3 by high-resolution melting curve analysis. *Yi Chuan* 2013;35:923–930.
13. Pindurova E, Zourkova A, Zrustova J, Jurica J, Pavelka A. Alternative reliable method for cytochrome P450 2D6 poor metabolizers genotyping. *Mol Biotechnol* 2013;53:29–40.
14. Sistonen J, Fuselli S, Levo A, Sajantila A. CYP2D6 genotyping by a multiplex primer extension reaction. *Clin Chem* 2005;51:1291–1295.
15. Lovlie R, Daly AK, Molven A, Idle JR, Steen VM. Ultrarapid metabolizers of debrisoquine: Characterization and PCR-based detection of alleles with duplication of the CYP2D6 gene. *FEBS Lett* 1996;392:30–34.
16. Steen VM, Andreassen OA, Daly AK, et al. Detection of the poor metabolizer-associated CYP2D6(D) gene deletion allele by long-PCR technology. *Pharmacogenetics* 1995;5:215–223.
17. Parikh R, Mathai A, Parikh S, Chandra Sekhar G, Thomas R. Understanding and using sensitivity, specificity and predictive values. *Indian J Ophthalmol* 2008;56:45–50.
18. Hoskins JM, Carey LA, McLeod HL. CYP2D6 and tamoxifen: DNA matters in breast cancer. *Nat Rev Cancer* 2009;9:576–586.
19. Er TK, Chang JG. High-resolution melting: Applications in genetic disorders. *Clin Chim Acta* 2012;414:197–201.
20. van der Stoep N, van Paridon CD, Janssens T, et al. Diagnostic guidelines for high-resolution melting curve (HRM) analysis: An interlaboratory validation of BRCA1 mutation scanning using the 96-well LightScanner. *Hum Mutat* 2009;30:899–909.
21. Man M, Farmen M, Dumaual C, et al. Genetic variation in metabolizing enzyme and transporter genes: Comprehensive assessment in 3 major East Asian subpopulations with comparison to Caucasians and Africans. *J Clin Pharmacol* 2010;50:929–940.
22. Qin S, Shen L, Zhang A, et al. Systematic polymorphism analysis of the CYP2D6 gene in four different geographical Han populations in mainland China. *Genomics* 2008;92:152–158.