UNIVERSITI TEKNOLOGI MARA

DIRECT ONE-STEP PCR REACTION FOR DETECTION OF *CYP2D6* POOR METABOLISER

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ABSTRACT

CYP2D6 is a highly polymorphic enzyme that mediates the metabolism of many currently prescribed drugs. Genetic variability within *CYP2D6* results in poor (PM), intermediate (IM), extensive (EM) and ultra-rapid metabolisers (UM) of *CYP2D6* substrates with PMs are at higher risk than other metabolisers of adverse reactions to these drugs. One of the most common mutant allelic variant of this gene in PMs is the **4* allele. In this study, we determined the **4* allele frequency in a random Malaysian subpopulation by using a direct one-step allele-specific PCR amplification approach in order to compare this allele frequency among Malaysian subpopulation with the previous findings on the occurrence of this allele in Malaysia. Frequencies for **4* mutated allele and genotypes have been evaluated in 123 unrelated Malaysians. Genotyping has been carried out on genomic DNA by the direct one-step allele-specific PCR amplification technique. *CYP2D6*4* occurred at a frequency of 16% and *CYP2D6*1*, 84%. The most frequent genotypes were *CYP2D6*1/*1* at 75.6%, 21 (17.1%) subjects had **1/*4* genotypes and 9 (7.3%) subjects had genotypes that predicted PM phenotype. This method however was unable to produce similar result as reported earlier where the **4* allele occurred at a very high frequency in this study, but further study should be carried out to increase the specificity of the PCR reaction. This method can also be commercialized as a tool for individualization of pharmacotherapy once the method is well validated as it is time saving and less prone to contamination.

CHAPTER 1

INTRODUCTION

Individual variation in response to drugs is a substantial clinical problem. Such variation ranges from failure in responding to a drug to adverse drug reactions and drug-drug interactions when several drugs are taken concomitantly. The clinical consequences range from patient discomfort through serious clinical illness to the occasional fatality [1]. Genetic polymorphisms are known to contribute to interindividual variations in the metabolism of numerous drugs in humans. Mutation in a gene coding for a drug metabolising enzyme can cause enzyme variants with high, low or no activity [2].

Cytochrome *P4502D6* (*CYP2D6*), also known as sparteine/debrisoquine hydroxylase, is among the most extensively studied enzymes in drug metabolism. It plays an important function in the metabolism of over 40 therapeutically used drugs [3]. The polymorphism of the enzyme results in poor (PMs), intermediate (IMs), efficient (EMs), or ultrarapid metabolisers (UMs) of *CYP2D6*-substrate drugs [4]. Clinical studies have shown that poor metabolisers of this gene are at higher risk than other metabolisers (IMs, EMs & UMs) of adverse reactions to these drugs. It is associated with the inefficient metabolism of over 25 drugs with a range of indications [5]. One of the most common mutant allelic variant of this gene in PMs is the **4* allele occurrence that is