UNIVERSITI TEKNOLOGI MARA

DETECTION OF CYP3A4*22 MUTATION IN HUMAN DNA USING ALLELE SPECIFIC POLYMERASE CHAIN REACTION

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ABSTRACT

CYP3A4*22 is one of the variant of CYP3A4, mainly expressed in human liver and small intestines. Many drugs clinically used today is metabolized by CYP3A4. It's broad substrate specificity makes it the most important metabolic enzyme for many drugs. Detecting this gene mutation can aid in deciding a more appropriate drug therapy for individual patient. The purpose of this study is to detect the CYP3A4*22 mutation in human DNA samples using allele-specific polymerase chain reaction, and evaluate the significance of presence or absence of the mutation among the DNA samples. Four DNA samples were amplified using allele-specific polymerase chain reaction (PCR) in a thermocycler. The amplicons of the first PCR were subjected through another round of PCR to detect the presence of wild type or mutant allele in the DNA samples. Results were obtained by using gel electrophoresis in 1.5% agarose stained with ethidium bromide. The first PCR produced amplicons of the DNA of interest while the second PCR showed bands for both wild type and mutant allele. All DNA samples tested for mutation were found to be heterozygous as both the wild type and mutant allele were detected. Although the mutant allele is known to significantly cause the reduction in metabolism of many drugs, it will not cause serious effect in the heterozygotes due to the presence of the wild type allele in the samples.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Cytochrome P450 is a superfamily of 57 genes that code for enzymes responsible for metabolism of drugs in human body (Nebert & Russell, 2016). The cytochrome P450 plays a major role in phase I drug metabolism and mutations of the CYP genes can cause alterations in drug metabolism that varies between individuals and subsequently give rise to drug interactions (Danielson, 2002).

One of the enzymes under the cytochrome P450 that is of importance is the CYP3A4. It was found that variations in CYP3A4 gene that alters enzyme activity is associated with genetic factors (Ozdemir et al., 2000). To explain the variations in CYP3A4 gene, common CYP3A4 coding variants cannot be used (Lamba, Panetta, Strom, & Schuetz, 2010).

To further understand the variations within CYP3A4 gene and its effects, extensive studies have been done to identify the genetic variations in the gene. Because of its importance in drug metabolism, the CYP3A4 is one of the most extensively studied gene under the superfamily of cytochrome P450 (Sevrioukova & Poulos, 2014).