UNIVERSITI TEKNOLOGI MARA

EVALUATION OF *VKORC1* HAPLOTYPES IN A COHORT OF PATIENTS TREATED WITH WARFARIN

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ABSTRACT

Introduction: Coumarin derivatives such as warfarin are the therapy of choice for the long-term treatment and prevention of thromboembolic events. Warfarin is administered orally as a racemic mixture of two enantiomers. It has very narrow therapeutic windows and large individual variability. The amount of dose needed to achieve therapeutic efficacy varies across the population due to differences in the genetic makeup of individual patients. Polymorphism of the *VKORC1* gene is one of the genetic factors that caused altered sensitivity to warfarin or warfarin resistance. Ten common SNPs have been identified, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041 of the *VKORC1* reference sequence (Gen Bank accession number AY587020). Five major haplotypes were derived and haplotypes H1 and H2 were assigned to group A which requires low warfarin dose while haplotypes H7, H8 and H9 were assigned to group B and require high-warfarin dose.

Objective: To develop genotyping method for detection of ten variants of *VKORC1* gene and establish the distribution of *VKORC1* haplotypes among patients prescribed with warfarin and study the relationship of haplotypes and warfarin dose, INR and bleeding.

Method: A total of 156 warfarin patients were recruited after they were screened for inclusion and exclusion criteria. Five ml of blood was drawn from each patient and DNA was extracted for genotyping of *VKORC1* variants. Two steps PCR method was developed to identify all the 10 SNPs. Two pairs of primers were designed specifically to detect each of the SNPs and the properties of the primers were checked using Oligo Analyzer software. The optimized method was validated by direct sequencing.

Result: Twelve haplotypes were inferred from 10 common SNPs. Four haplotypes H1, H6, H7 and H10 were found to be more frequent in among the patients. The haplotype with the highest frequency was H1 (72.9%) followed by H6 (4.2%), H7 (8.9%) and H10 (4.5%). The most common haplotype pairs was H1-H1 (55.1%) followed by H1-H7 (16%), H1-H6 (7.1%) and H1-H10 (4.5%). Two haplotype pairs, H1-H1 and H1-H10 were associated with a low warfarin dose requirement per day (2.93 mg, 95% CI: 2.70 – 3.16, MLR; $P < 0.05$ and 3.58 mg, 95% CI: 1.98 – 5.19, MLR; $P > 0.01$) while H1-H6 and H1-H7 were associated with high dose requirement (4.59 mg, 95% CI: 3.97 -5.22, MLR; $P < 0.01$ and 5.05 mg, 95% CI: 3.89 – 6.21, MLR; $P < 0.01$). The average INR was approximately 2.77 and did not differ significantly among the patients classified according to *VKORC1* haplotype groups (MLR; $P > 0.05$). The multiple regression analysis (Enter method) indicated that *VKORC1* haplotypes accounted for 28.1% of the variation in warfarin dose at the end of 6 months follow ups.

Conclusion: *VKORC1* haplotype provided a good correlation for different warfarin dose requirements. Genotyping of patients before warfarin is thus useful to predict more accurate dose required.

Keywords:
Single nucleotide polymorphisms (SNPs), polymerase chain reaction (PCR) machine, warfarin and haplotypes.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Many factors have been suggested to be responsible for a variety of drug responses and adverse reactions. Patient's individual genetic predisposition remains the major un-elucidated reason for inappropriate drug dosage (Spear *et al.,* 2001; Morley & Hall, 2004). This is generally caused by many genes which encode proteins that are associated with drug absorption, distribution, metabolism and elimination (Qing & Wolfgang, 2000; Spear *et al.,* 2001). Both the pharmacodynamics and pharmacokinetics variation have been shown to contribute to pharmacogenetics (Figure 1.1; Johnson, 2003).

Pharmacogenetics has begun since the 1950s when researchers found that some adverse drug reactions were caused by genetically determined variations in the enzyme activity (Laviero *et al.*, 2000; Meyer, 2000; Daly, 2010). Studies on pharmacogenetics have shown significant impact of inherited variability on the reaction to drugs. This knowledge has been used for improving individual drug treatment (Kirchheiner & Seeringer, 2007).

The goal of pharmacogenetic is to identify the genetic factors underlying the differences observed in individual response to drugs. The major problem in clinical practice is variability of drug response from person-to-person and single-nucleotide polymorphisms (SNPs) have been identified to play major roles in variations with heritable clinical phenotypes of drug response (Meyer, 2000).