# EVALUATION OF THE VKORC1 HAPLOTYPES IN INDIVIDUALISING WARFARIN THERAPY

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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 methods were 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. However, only 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,

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the study

Many factors have been suggested to be responsible for a variety of drug response and adverse reaction. 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 that 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 on inherited variability with respect to reaction to drugs. This knowledge has been used for improving individual drug treatment for more than 50 years (Kirchheiner & Seeringer 2007).

The goal of pharmacogenetic is to identify the genetic factors underlying the differences observed in individual response to drugs. Before it can be implemented in clinical practice, we need to develop pharmacogenetic tests. Pharmacogenetic tests when applied in medical practice helps to predict individual response, thus medicine can be given to maximize benefits and minimize risks of adverse drug reaction (ADR) for patients (Pirazzoli & Recchia, 2004).