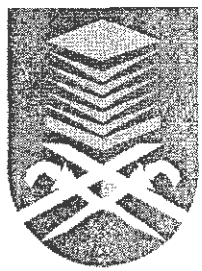

INSTITUT PENYELIDIKAN, PEMBANGUNAN DAN PENGKOMERSILAN
UNIVERSITI TEKNOLOGI MARA
40450 SHAH ALAM, SELANGOR
MALAYSIA

BY:

TEH LAY KEK, PhD
MOHD ZAKI SALLEH, PhD
RUSLI ISMAIL, PharmD
AMIRUDDIN AHMAD ISHAK, MPharm

Mac 2007

Tarikh : 9 Mac 2007
No. Fail Projek :

Penolong Naib Canselor (Penyelidikan)
Institut Penyelidikan, Pembangunan dan Pengkomersilan
Universiti Teknologi MARA
40450 Shah Alam

Y.Bhg. Prof.,

LAPORAN AKHIR PENYELIDIKAN

Merujuk kepada perkara di atas, bersama-sama ini disertakan 3 (tiga) naskah Laporan Akhir Penyelidikan bertajuk "GENETIC POLYMORPHISM OF CYP2C9 IN PATIENTS ON WARFARIN THERAPY AND THE CLINICAL RELEVANCE".

Penyelidik yang terlibat dalam projek ini adalah seperti yang berikut:

1	Dr. Teh Lay Kek	Fakulti Farmasi, UiTM
2	Prof. Dr. Mohd Zaki Salleh	Fakulti Farmasi, UiTM
3	Prof. Madya Dr. Rusli Ismail	Institut Penyelidikan Perubatan Molekul, USM
4	Prof. Madya Amiruddin Ahmad Ishak	Fakulti Farmasi, UiTM

Sekian, terima kasih.

Yang benar,

Teh Lay Kek, PhD
Ketua
Projek Penyelidikan

PROJECT TEAM MEMBERS

DR. TEH LAY KEK
(Faculty of Pharmacy, UiTM)
Project Leader

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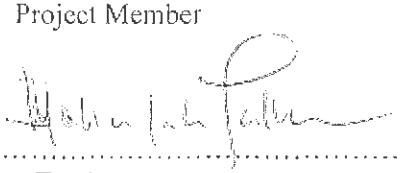
Tandatangan

PROF. DR. MOHD ZAKI SALLEH
(Faculty of Pharmacy, UiTM)
Project Member



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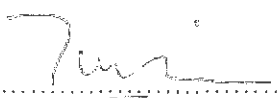
Tandatangan

ASSOC. PROF. DR. RUSLI ISMAIL
(Institute for Research in Molecular Medicine, USM)
Project Member



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Tandatangan

ASSOC. PROF. AMIRUDDIN AHMAD ISHAK
(Faculty of Pharmacy, UiTM)
Project Member

.....
Tandatangan

PENGHARGAAN

Setinggi-tinggi penghargaan dan ribuan terima kasih diucapkan kepada semua pihak yang terlibat secara langsung dan tidak langsung bagi membolehkan penyelidikan ini disiapkan dengan sempurna. Diantaranya :

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(UMBI/ HUKM)

Dr. Harris Ngow
(HUKM)

Riza Afzan, Lee Wee Leng, Mohd. Khairi
(Pembantu Projek/ Pelajar di Makmal Farmakogenetik)

Penghargaan juga ditujukan kepada pesakit-pesakit warfarin di Hospital Kebangsaan Malaysia kerana sudi menyertai projek ini.

GENETIC POLYMORPHISM OF CYP2C9 IN PATIENTS ON WARFARIN THERAPY AND THE CLINICAL RELEVANCE

ABSTRACT

Optimization of warfarin therapy has been difficult and pharmacogenomics has potential in offering clinically and economically useful interventions. We thus evaluated distribution of *CYP2C9* which metabolises warfarin among patients prescribed warfarin to bridge the importance of CYP2C9 genotyping in warfarin management. A total of 189 patients on warfarin therapy in a local hospital were recruited after written informed consent. Their medical records were reviewed. Five milliliters of blood was taken from each subject and DNA was isolated and used for identification of *CYP2C9* allele *2, *3 and *4 using nested-allele-specific-multiplex-PCR. Half of the patients were Malays and the remaining were Chinese. Two different genotypes were detected among the patients, 93.7% had *CYP2C9*1/*1* and 6.3% were *CYP2C9*1/*3*. With standard clinical practice, the warfarin doses prescribed ranged from 1 to 8 mg (mean = 3.31 mg) while the mean of INR achieved was 2.19 (S.D.0.86; range 0.86 to 5.69). The mean dose prescribed was higher in patients with genotype of *CYP2C9*1/*1* (3.38 mg; SD 1.35 vs. 2.37 mg; SD 1.05; p = 0.007). Twelve subjects had INR level less than 1 and doses ranged from 1.5 to 6 mg (mean= 3.83). All of them had genotype *CYP2C9*1/*1*. Forty-eight-percent of the patients with wild-type variant have INR value of 2 to 4 given mean dose of 3.38 (SD 1.34) while 2/3 of the patients with heterozygous **3* achieved desirable INR for a mean dose of 2.37. Seven patients with wild-type variant had INR value of more than 4 (mean 4.95; range 4.15 to 5.69) givens dose of 1 to 5 mg of warfarin. The discrepancies observed are due to other factors including patients' compliances, drug interaction or patients having alleles not determined in this study. Even the mean for doses and INR between the 2 genotypes groups were similar, the standard error means were 3 times larger for patients with *CYP2C9*1/*3* compared to wild-type. Current dosing protocol for warfarin lacked efficiency and screening for *CYP2C9* may allow clinicians to develop protocols with increased therapeutic effectiveness.

Keywords: CYP2C9 , Frequency, Genetic Polymorphism, Malaysian population, Warfarin

1.0 INTRODUCTION

1.1 CYTOCHROME P450

The cytochrome P450 (CYP) family is a heme containing monooxygenases; comprising the most important group of phase one enzymes. These enzymes are involved in the metabolism of a wide spectrum of endogenous as well as exogenous compounds (Wong, 1998). The cytochrome P450 gene family contains 60 to 100 different genes, of which the most important P450 isoenzyme is CYP3A4 (50% of the P450 metabolism) followed by CYP2D6 (20%), CYP2C9 and CYP2C19 (together 15%). The remaining is carried out by CYP2E1, CYP2A6 and CYP1A2 (Bertz and Granneman, 1997; Ingelman-Sundberg et al., 1999). The genes for CYP2D6, CYP2C9, CYP2C19 and CYP2A6 are functionally polymorphic. Therefore approximately 40% of human P450 dependent drug metabolism is carried out by polymorphic enzymes (Ingelman-Sundberg et al., 1999).

1.2 Clinical Relevance of the Polymorphic CYP2C9

CYP2C9 is one of the major drug-metabolizing CYP450 isoforms; one of several CYP2C genes clustered in a 500-kb region on proximal 10q24 (Gray et al., 1995).

Maekawa et al. (2006) sequenced the CYP2C9 gene in 263 Japanese individuals (134 diabetics and 129 healthy volunteers) and identified 62 variations, 32 of which were novel. Only 5 haplotypes accounted for more than 87% of the inferred haplotypes, and they were closely associated with the haplotypes of CYP2C19 in Japanese. The authors noted that although the haplotype structure of CYP2C9 was rather simple in Japanese, the haplotype distribution was quite different from those previously reported in Caucasians and Africans.

It metabolises a range of drugs used currently such as tolbutamide, diclofenac and warfarin (Table 1.1) This enzyme is polymorphic and resulted in a wide inter-racial differences in the responses to drugs (Kidds et. al., 2001; Goldstein, 2002). Two CYP2C9 alleles that produce a phenotype of poor metabolism occur in 11% and 8% of whites but only 3% and 0.8% of blacks (Xie et al., 2001). In a study by Aithal et al., (1999), the poor metabolisers have impaired metabolism of warfarin and thus increased plasma concentrations. Individuals with the genotype of impaired metabolism thus require lower doses of warfarin to achieve an anticoagulant effect similar to that in patients with the normal genotype and are more likely to have an excessive anticoagulant response (Aithal et al., 1999). In addition, bleeding episodes tend to be more common. Higashi et al. (2002) studied the association of these variants with over-anticoagulation and bleeding events during warfarin therapy in a retrospective cohort study. The results suggested that the 2 polymorphisms are associated with an increased risk of over-anticoagulation and of bleeding events among patients in a warfarin anticoagulation clinic. In a patient who was unusually sensitive to warfarin therapy, Steward et al. (1997) identified homozygosity for I359L, the so-called *CYP*2C9*3 allele. The patient, who was taking 0.5 mg of warfarin daily, had an S-to-R enantiomer ratio of 3.9:1, whereas control patients taking 4 to 8 mg of warfarin daily had S-to-R ratios of about 0.5:1. Steward et al. (1997) concluded that expression of CYP2C9*3 is associated with diminished clearance of the more potent S-warfarin, and that analysis of the plasma S-to-R warfarin ratio might serve as a useful alternative test to genotyping.

Kirchheiner et al. (2003) studied the effects of CYP2C9 on celecoxib, a nonsteroidal antiinflammatory drug (NSAID) that is used to treat rheumatoid arthritis and osteoarthritis. This drug exhibits antiinflammatory, analgesic, and antipyretic activity by selective inhibition of cyclooxygenase-2 (COX2; 600262). They found a more than

2-fold reduced oral clearance in homozygous carriers of *CYP2C9*3*; heterozygous carriers of 1 *CYP2C9*3* allele were in between, whereas *CYP2C9*2* had no significant influence on celecoxib pharmacokinetics. Kirchheiner et al. (2003) concluded that approximately 0.5% of Caucasians with a homozygous CYP2C9*3 genotype will have greatly increased exposure to celecoxib. It was not clear whether this is associated with greater efficacy or with an increased incidence and severity of adverse events.

Sullivan-Klose et al. (1996) demonstrated that the form of CYP2C9 in which ile359 is replaced by leucine is the basis of poor metabolizing of tolbutamide, the sulfonylurea hypoglycemic agent used in the treatment of diabetes mellitus. The frequency of the leu359 allele was found to be 6% in the Caucasian-American population and 0.5% in African-Americans. The frequency of the leu359 allele was 2.6% in Chinese-Taiwanese.

Kidd et al. (1999) described a 29-year-old male Caucasian who had participated in 6 bioequivalence studies over a period of several years. The patient displayed severe hypoglycemia after a single dose of glipizide, a second generation sulfonylurea structurally similar to tolbutamide and used as an oral hypoglycemic agent. His oral clearance of phenytoin was 21% of the mean of 11 other individuals, and his oral clearance of glipizide was only 18% of the mean of 10 other individuals. His oral clearance of nifedipine (a CYP3A4 substrate) and chlorpheniramine (a CYP2D6) substrate) did not differ from that of other individuals studied. Genotype testing demonstrated that the individual was homozygous for the leu359 allele and did not possess any of the known defective CYP2C19 alleles. These studies established that the leu359 allele is responsible for the phenytoin and glipizide/tolbutamide poor metabolizer phenotype. In a study of 281 epileptic patients treated with phenytoin, Tate et al. (2005) found a significant association between the maximum dose needed

and the CYP2C9*3 allele (I359L). Mean phenytoin doses for individuals with 0, 1, or 2 copies of the *3 allele were 354, 309, and 250 mg, respectively, indicating a trend of reduction in maximum dose needed to control symptoms.

1.3 WARFARIN MONITORING

In the past two decades, several measures have substantially improved the safety of oral anticoagulant therapy with coumarin derivatives in patients with venous and arterial thromboembolic disorders. Among these measures are:

i. the use of more sensitive prothrombin-time reagents and the introduction by WHO of the international normalised ratio (INR);

ii. the adoption of less intensive levels of anticoagulation (reflected by lower INR values), which had the same antithrombotic efficacy yet produced less haemorrhagic complications than did more intensive anticoagulation;

iii. the organisation of specialist anticoagulant clinics;

iv. the use of computerised systems for adjustment of drug doses.

On the whole, these measures have increased physicians' and patients' confidence, which has led to a world wide increase in the use of oral anticoagulant therapy. Yet, bleeding is still a dreaded complication with, and sometimes a deterrent to, the use of this therapy, particularly among the many elderly patients who need anticoagulants for the treatment of atrial fibrillation. A review of a large number of early studies gave average yearly rates as high as 0·8%, 4·9%, and 15% for fatal, major, and minor haemorrhages, respectively (The European Atrial Fibrillation Trial Study Group, 1995) More recently, lower rates were found in a prospective multicentre study of 2745 patients followed up from the start of anticoagulant therapy (0·25% fatal,1·1% major, and 6·2% minor) (Fihn et al., 1993). This perhaps reflects the progress in laboratory control. However, excessive bleeding was nearly twice as common among

patients aged 70 years or more, particularly in the first 3 months of treatment (Fihn et al., 1993). Early bleeding is likely to be a consequence of INR deviations towards severe anticoagulation that occur mainly in the early stages of treatment, when ideal doses are being established. Doses of warfarin needed to stabilise patients at INR values between 2·0 and 3·0 (the therapeutic range currently recommended for most clinical indications) vary from 1 mg per day to 20 mg or more.

Patients who carried one or more mutant alleles metabolised S-warfarin poorly and responded to small doses of the drug with greater lengthening of the prothrombin time and higher INR values than did carriers of the wild-type (Aithal et al., 1999). Most importantly, from a clinical point of view, genetically determined high-responders to warfarin had bleeding complications four times more commonly than did a control group stablised on larger doses of the drug (Aithal et al., 1999). Bleeding complications in carriers of hyper-responsiveness alleles are thought to be due to the more frequent and longer periods during which these patients have high INR values (Aithal et al., 1999).

Knowledge of carriage of the hyper-responsiveness alleles *CYP2C9*2* and *CYP2C9*3* might help the clinician to decide against the use of warfarin, particularly in high-risk elderly patients. It might also help in the choice of other coumarin derivatives, such as phenoprocoumon or acenocoumarol, the metabolism of which is little influenced by CYP2C9.

1.4 Research hypothesis

We hypothesized that CYP2C9 variants may be responsible for the inter-patients differences in warfarin doses in Malaysia. In this study, we will examine the frequencies of CYP2C9 variant alleles in among patients in Malaysia and correlate genotype association with sensitivity and outcome of warfarin therapy.

1.4.1 Objectives

1. To determine the types and frequencies of CYP2C9 variants in Malay and Chinese warfarin-treated patients;
2. To determine the association of CYP2C9 genotypes with anticoagulation related bleeding complications.

2.0 MATERIALS AND METHODS

2.1 Study Setting

Ethical approval was obtained from the local Research and Ethics Committee at UiTM and Hospital Universiti Kebangsaan Malaysia (HUKM). The study was conducted at the anticoagulation clinics and inpatient wards of HUKM. Patients who will be initiated warfarin therapy or who are already on warfarin maintenance therapy for venous thrombosis, atrial fibrillation and prosthetic valves implants were enrolled if they fulfilled the inclusion and exclusion criteria. The clinical management of the patients was according to the standard protocols of warfarin therapy initiation, dosing adjustments based on INR results, management of over-anticoagulation and under-anticoagulation and frequency of follow-up. Any dosing adjustment during the maintenance therapy was made according to the patient's INR result.

In addition, the genotype and allele frequencies of healthy blood donors were chosen as controls, i.e. patients who did not require warfarin treatment, were measured to test whether particular CYP2C9 genotypes were associated with an increased risk of requiring anticoagulant treatment.

2.2 Subjects

2.2.1 Inclusion criteria

The inclusion criteria included:

1. Adult patients older than 18 years old
2. Malay or Chinese descend with known origin for at least 3 generations
3. Undergoing anticoagulation therapy for atrial fibrillation, prostectic valve replacement and other thrombo-embolic events.
4. Will be on warfarin for at least 6 months

5. Willing and able to follow simple instruction by the researcher and
6. Willing to sign an informed consent form.

2.2.2 Exclusion criteria

The exclusion criteria were:

1. Of Indian descent because of difficulty in getting adequate Indian sample
2. Not willing or unable to agree to verbal or written consent
3. Not willing and unable to follow simple instructions
4. Product of mix marriages up to three generations
5. Not on warfarin therapy
6. Known or diagnosed to have chronic liver disease
7. Menstruating during the enrolment period because a drop in hemoglobin would cause misclassification as significant bleeding
8. Tendency for bleeding diathesis

Eligible patients were subsequently enrolled.

2.3 Clinical Data Collection

Inpatient and outpatient medical records for enrolled patients were reviewed and clinical data was extracted from anticoagulation and prescription records. The details of warfarin dosage regimen, peak INR and subsequent INR measurements, prescription drugs, co-morbid conditions and baseline investigation results (i.e. liver function test, renal function test, full blood count) were recorded. Subsequently, the patients were followed up for 6 months at intervals of 1, 3 and 6 months for any bleeding events or complications.

2.4 Blood Investigations

2.4.1 Serum Analysis

For both patients and control groups, blood sample (10 ml) was drawn at 12 to 14 hours after the last dose of warfarin for INR determination and baseline blood investigations (eg liver function test and renal function test). The exact time the patient took the medication would be recalled from the booklet given and recorded. An additional 5 ml of blood was taken at the first visit for CYP2C9 genotyping. The blood was collected into sodium citrate containing tubes. Whole blood sample was stored and kept at -20° Celsius until it was used for DNA extraction. Leukocytes DNA were extracted according to salting out method [Teh et al., 2002]. Blood samples (10 ml) would again be collected at intervals of 1 months, 3 months and 6 months after enrolment in the study for INR monitoring as per INR clinic protocol. Again the exact time the patient took the medication would be recalled from the booklet given and recorded.

2.4.2 Genotyping

A PCR genotyping method was developed by the Pharmacogenetic Research Group in the laboratory at UiTM to detect *CYP2C9*2*, **3* and **4*. Allele-spesific PCR was performed in parallel reactions to identify the single nucleotide differences for the variants. Allele specific primers at the 3' ends were designed to differentiate single nucleotide changes at the specific locus during PCR amplification. In order to avoid incompatibility of the primers sets, they were designed accordingly to have similar annealing temperature with appropriate length and GC contents. The primers were designed manually and the initial annealing temperature was determined using the formula"Tm = [2(A + T) + 4(C + G)]".

The PCR protocol (submitted for filing for patent) was performed in a 25 µl reaction mixture of 1 × PCR buffer (Biotools®, B & M Labs, S.A.), 2.0 mmol/L $MgCl_2$, 0.2 mmol/L dNTP (Promega Corporation, Madison U.S.A), primers (submitted for filing for patent), 200 ng (2 µl) genomic DNA as template and 1.0 U DNA Taq polymerase (Biotool® B & M Labs, S.A.). The PCR were performed with an initial hot start at 94 °C for 2 min, followed by 20 cycles of denaturation at 94 °C for 90 seconds and annealing extension at 64 °C for 90 seconds. The PCR was continued with another 18 cycles of denaturation at 94 °C for 90 seconds, annealing extension at 54 °C for 90 seconds using GeneAmp® PCR system 2700 Perkin Elmer (Applied Biosystems, Foster City).

The PCR products were subjected to electrophoresis on an ethidium bromide stained, 3% agarose gel (LE, analytical grade; Promega Corporation, Madison U.S.A) in 1X TBE (Tris, Borate, EDTA) buffer at 100 Volt for 45 minutes. (Appendix 3 and 4)

2..4.3 Direct PCR sequencing

The optimized nested PCR method was tested initially with 50 unknown DNA samples selected randomly. The results were reconfirmed by direct sequencing on ABI 3700 using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City). Prior to sequencing, the samples were purified using QIAquickR PCR Purification Kit (Qiagen, Hilden). The sequenced samples were used as positive controls in subsequent screening of the study samples.

Two independent gel reviewers were employed to analyze the gel documented result to ensure unequivocal result. Repeats were done for samples that did not have clear

bands or have equivocal results and scored as unsuccessful amplification if repeat failed.

2.5 Outcomes

In this study, the mean warfarin maintenance dose required to achieve the therapeutic INR was compared with CYP2C9 genotypes. Therapeutic INR was defined as the first INR measured within the optimal therapeutic range for a given indication. If the determined INR was between 2.0 and 3.0, then the INR was defined as to be within the "therapeutic range". "Above range" INRs were defined as measurements of greater than 4.0. Although any INR values between 3.0 and 4.0 were not defined as "above-range", this did not mean that clinicians necessarily considered these levels of anticoagulation "normal". Nevertheless, these levels were regarded as acceptable because INR values. The minimum cut off for above-range INR was considered to be 4.0 and any readings of INR between 2.0 to 4.0 were classified as "normal" (The European Atrial Fibrillation Trial Study Group, 1995).

The secondary outcomes were serious or life-threatening bleeding complications. Bleeding complications associated with raised INR above the therapeutic range of greater than 4·0 were classified as:

1. Minor if they required no additional testing, referral, or outpatient visits
2. Serious if th required medical evaluation, blood transfusion of two units or less)
3. Life threatening if they required surgical or angiographic intervention, transfusion of three or more units of blood, leading to irreversible sequelae)

2.6 Statistical Analysis

2.6.1 Genotype-outcome analysis

Data were presented as frequency ± 5% confidence interval. Data were compiled according to the genotype and allele frequencies with the 95% confidence intervals according to the following formula:

$(p \pm 1.96\sqrt{p(1-p)/n})$.

Expected genotype frequencies were calculated using the Hardy-Weinberg equation as follows:

$(p^2+2pq+q^2=1)$;

where p is the frequency alleles.

Chi-square test was used to compare the genotype frequencies in the populations. A p value of 0.05 or less was regarded as significant.

Patients' CYP2C9 genotypes were also investigated for correlation of genotypes and bleeding complications. The correlation was performed using appropriate regression analysis and tested by using 2-tailed t test. $P < 0.05$ was considered statistically significant. Comparisons of genotype frequency and bleeding complications of anticoagulation between the groups were made using Fisher's exact test.

All statistical tests were performed using the Statistical Package for the Social Sciences SPSS® version 12 (SPSS Inc, Ill)

3.0 RESULTS

3.1 Baseline demographic data on Warfarin-treated patient

The baseline demographic data for warfarin treated patients are shown in Table 3.1.The mean age of patients at the start of enrollment into the study was 60 years and half were female. Half were also Malays. The majority of patients (78%) were receiving warfarin for atrial fibrillation. There were no significant differences between patients with the wild-type genotype (**1/*1*) and patients with variant allele (*1/*3) with regards to demographic data.

Table 3.1: Comparison of Subjects Characteristics for patients with Variant versus patients with ' Wild-Type' CYP2C9 Genotypes.

Variables	*1/*1		*1/*3		All Patients	X^2 Test, P<0.05
	Malay	Chinese	Malay	Chinese		
Demographics						
Subjects, No.(%)	91(47.6%)	87(45.6%)	4(2.1%)	9(4.7%)	191	P=0.16
Men, No, (%)	44(46.3%)	46(48.4%)	0(0%)	5(5.3%)	95(49.7%)	P=0.40
Women, No, (%)	47(49.0%)	41(42.7%)	4(4.2%)	4(4.2%)	96(50.3%)	P=0.40
Age, Mean,(SD[a]),y	57.7(13.5)	62.9(10.7)	54.8(16.1)	61.6(10.3)	60.2(12.4)	P=0.93
BMI, Mean,(SD[a])	25.8(4.6)	24.4(3.9)	25.2(4.7)	23.6(2.8)	25.2(4.2)	P=0.29
Indication for Warfarin, No,(%)						
Atrial Fibrillation	70(36.6%)	70(36.6%)	3(1.6%)	6(3.1%)	149(78%)	P=0.43
[1]DCM	6(3.1%)	7(3.7%)	0(0%)	0(0%)	13(6.8%)	P=0.31
[2] DVT/PE	12(6.3%)	11(5.8%)	0(0%)	1(0.5%)	24(12.6%)	P=0.58
Valve Replacement	14(7.3%)	8(4.2%)	0(0%)	2(1.1%)	24(12.6%)	P=0.75
Thrombophilia	1(0.5%)	2(1.1%)	1(0.5%)	0(0%)	4(2.1%)	P=0.14
Co-morbid conditions, No, (%)						
Arrhythmias	70(36.6%)	70(36.6%)	3(1.6%)	6(3.1%)	149(78%)	P=0.42
[3]CCF	18(9.4%)	29(15.2%)	1(0.5%)	1(0.5%)	49(25.6%)	P=0.38

[4]DM	24(12.6%)	21(11.0%)	1(0.5%)	3(1.6%)	49(25.6%)	P=0.66
Hypertension	46(24.1%)	42(22.0%)	0(0%)	4(2.1%)	92(48.2%)	P=0.19
Malignancy	0(0%)	3(1.6%)	0(0%)	1(0.5%)	4(2.1%)	P=0.14
Hyperthyroidism	6(3.1%)	9(4.7%)	0(0%)	1(0.5%)	16(8.4%)	P=0.93
[5]IHD	20(10.5%)	20(10.5%)	1(0.5%)	3(1.6%)	44(23.0%)	P=0.49
Prescribed Medication, No, (%)						
Digoxin	28(14.7%)	29(15.2%)	1(0.5%)	3(1.6%)	61(31.9%)	P=0.93
Amiodarone	1(0.5%)	3(1.6%)	0(0%)	0(0%)	4(2.1%)	P=0.59
Losartan	8(4.2%)	8(4.2%)	0(0%)	3(1.6%)	19(10.0%)	P=0.10
Statin	46(24.1%)	41(21.5%)	1(0.5%)	3(1.6%)	91(47.6%)	P=0.21
Omeprazole	1(0.5%)	2(1.1%)	0(0%)	0(0%)	3(1.6%)	P=0.64
Thyroxine	6(3.1%)	2(1.1%)	0(0%)	0(0%)	8(4.2%)	P=0.44
Aspirin/Ticlid	25(13.1%)	29(15.2%)	2(1.1)	3(1.6%)	59(30.9%)	P=0.54
Traditional Drugs, No, (%)						
Yes	10(5.2%)	9(4.7%)	0(0%)	1(0.5%)	20(10.5%)	
No	15(7.9%)	23(12.0%)	0(0%)	1(0.5%)	39(20.4%)	P=0.43
NK	66(34.6%)	55(28.8%)	4(2.1%)	7(3.7%)	132(69.1%)	

Blood Parameter, Mean, (SD[a])						
Serum Creatinine	94.3(59.4)	87.3(37.1)	71.5(31.9)	94.6(29.6)	90.7(48.6)	P=0.21
Serum Albumin	40.5(3.8)	41.2(3.1)	39.5(3.8)	41.2(2.6)	40.8(3.5)	P=0.95
[6]Serum ALT	24.9(15.8)	22.6(13.1)	17.5(9.6)	21.6(10.3)	23.5(14.3)	P=0.13

[1]DCM = dilated cardiomyopathy, [2]DVT/PE= deep vein thrombosis/pulmonary embolism, [3]CCF= congestive cardiac failure, [4]DM= diabetes mellitus, [5]IHD= ischemic heart disease, [6]ALT= alanine transaminase, SD[a] = standard deviation, NK = not known

3.2 Allelic and Genotype frequencies

A total of 191 subjects were available for analysis.

Table 3.2.1 show the distribution of frequencies of genotypes among the study patients according to gender and ethnic groups. Table 3.2.2 shows the distribution of frequencies of alleles for all the study patients.

Among the warfarin treated patients, two different genotypes were detected, *CYP2C9 *1/*1* and *CYP2C9*1/*3*.The percentage frequencies were 93.2% and 6.8% respectively. *CYP2C9 *1/*2* was only found in the healthy Malays but not in the study patients' group.

The distribution of genotypes in the warfarin-treated patients according to ethnic groups and gender were also similar. (Chi Square Fisher's Exact Test; p=0.250 and p= 0.567)

Table 3.2.1: Warfarin-treated Patient according to ethnic groups and gender and genotype frequencies.

Ethnic groups / Genotype	Malay		Chinese		Total
Gender	Male	Female	Male	Female	
Total Number	44	51	51	45	191
*1/*1	44	47	46	41	178(93.2%)
*1/*3	0	4	5	4	13(6.8%)

Table 3.2.2: CYP2C9 allele frequencies among warfarin-treated patients.

	*CYP2C9*1*	*CYP2C9*2*	*CYP2C9*3*	*CYP2C9*4*
Malay	97.9%	nd	2.1%	nd
Chinese	95.3%	nd	4.7%	nd
Total	96.6%	nd	3.4%	nd

3.3 Warfarin Doses

The warfarin doses prescribed for the study patients ranged from 1 to 10 mg (Mean = 3.5 mg) and mean INR achieved was 2.59 (SD 0.46; range of 2.00 to 3.91).

Among patients with genotype of *CYP2C9 *1/*1*, the mean dose was 3.7 mg (SD=1.46) and the mean dose of warfarin prescribed for patients with genotype *CYP2C9 *1/*3* was 2.5 mg (SD= 1.03). here was a reduction by 32% of the warfarin dose to maintain INR between 2.0 to 4.0 for patients with *CYP2C9 *1/*3* genotype. The mean warfarin maintenance was highest among patients with *CYP2C9*1/*1* genotype compared to heterozygous *CYP2C9*1/*3*. The differences reached statistical significance. The doses used between the 2 genotypes groups were found to be statistically different (p = 0.004) (Figure 3.3.1) Mean maintenance warfarin dose was significantly related to the genotype.