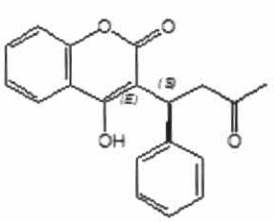
CHAPTER II

LITERATURE REVIEWS

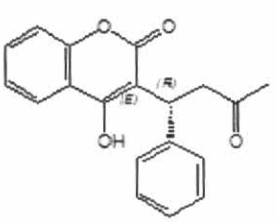
2.1 Warfarin

Warfarin, a coumarin-derivative anticoagulant, has been discovered since 1940 at the University of Wisconsin after there was reported that hemorrhagic death occurred in the cattle eat spoiled sweet clover. It was first synthesized since 1944 as rodenticide and used for treatment of thromboembolic diseases in human after 1953.

Warfarin ($C_{19}H_{16}O_4$, (RS) 4-hydroxy-3-(3-oxo-1-phenylbutyl) Coumarin) is white, odorless and crystalline powder. It is a racemic mixture of the 2 isomers (R- and S- warfarin). It is acidic, practically insoluble in water, readily soluble in acetone and dioxane and moderately soluble in alcohols. The sodium salts are very soluble in water, freely soluble in alcohol but insoluble in organic solvents. Warfarin sodium is discolored by light. [24]



S-Warfarin



R-Warfarin

Figure 2.1 The structure of Warfarin

2.1.1 Mechanism of action

As shown in figure 2.2, Warfarin exerts its effect by blocking the regeneration of reduced vitamin K, which is necessary for gamma carboxylation of glutamic acid residues in the precursor proteins of vitamin K-dependent clotting factors in the liver. Thus inhibiting synthesis of vitamin K-dependent clotting factors which include factors II (prothrombin), VII (proconvertin), IX (Christmas factor or plasma thromboplastin component) and X (Stuart-Prower factor), and the anticoagulant proteins C and S. [3-5]

Warfarin does not change catabolism of clotting factors. The rate of degradation of activated clotting factor depends on their individual rates of clearance.

Table 2.1 Half life of Vitamin K-dependent clotting factors

Clotting factors	Half life (Hours)
Factor II	60
Factor VII	6
Factor IX	24
Factor X	40
Protein C	8
Protein S	30

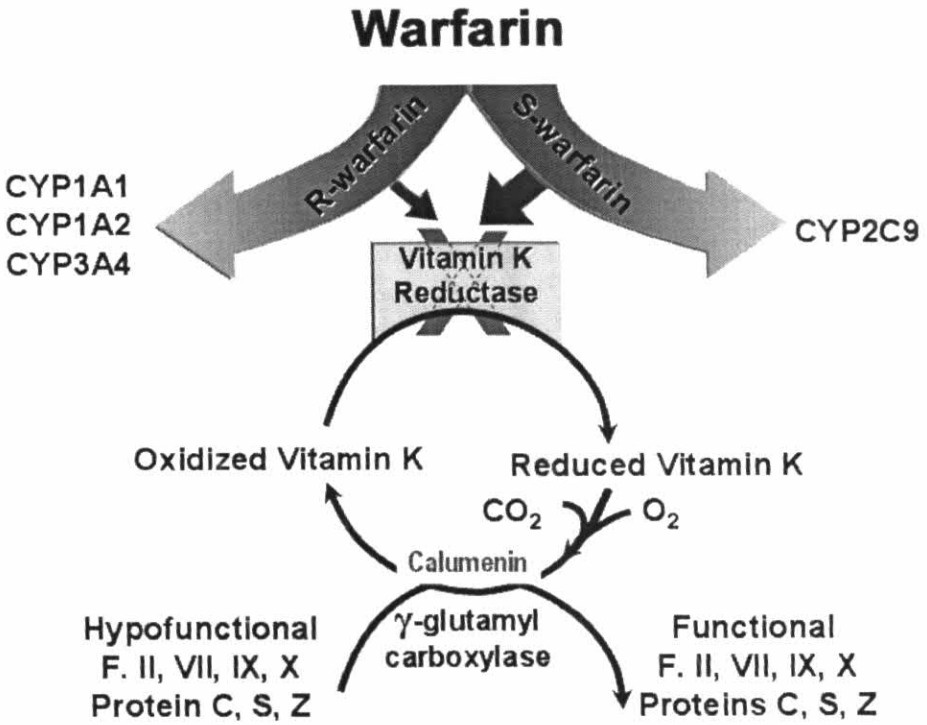


Figure 2.2 The mechanism of Warfarin [25]

2.1.2 Pharmacokinetics [5,24]

- Absorption

Warfarin sodium is rapidly absorbed from the GI tract with high bioavailability. Time to peak plasma concentrations are achieved 90 minutes after oral administration (within 4 hours).

- Distribution

Warfarin has high protein binding (approximately 99%). It is distributed to liver, lungs, spleen, kidneys and can crosses the placenta but not distributed into milk in human. Volume of distribution (Vd) of warfarin is approximately 0.14 L/kg (0.11-0.2 L/kg).

- Metabolism and Excretion

Warfarin is metabolized into inactive metabolites by cytochrome P450 enzyme system in the liver. S-isomer is oxidized by CYP2C9, CYP2C19 and CYP2C18 to 7-hydroxywarfarin which is excreted in bile. R-isomer is metabolized by CYP1A1, CYP1A2 and CYP3A4 to diastereoisomeric alcohols which are excreted in urine. The elimination half life of warfarin averages 40 hours (20-60 hours).

2.1.3 Indications and target INR

FDA approved warfarin for the prophylaxis and treatment of venous thrombosis, pulmonary embolism, thromboembolic complications associated with atrial fibrillation or cardiac valve replacement. Moreover, it is indicated to reduce the risk of death, recurrent myocardial infarction, and thromboembolic events such as stroke or systemic embolism after myocardial infarction.

Table 2.2 Indications and Target INR

Indications	Target INR (Range)
Prophylaxis for Venous thromboembolism	2.5 (2.0-3.0)
Treatment for Venous thromboembolism	2.5 (2.0-3.0)
Arterial thrombosis & stroke prevention	
Atrial fibrillation	2.5 (2.0-3.0)
Acute Myocardial Infarction	2.5 (2.0-3.0)
Valvular heart disease	2.5 (2.0-3.0)
Prosthetic tissue heart valve	2.5 (2.0-3.0)
Prosthetic mechanical heart valve	3.0 (2.5-3.5)

2.1.4 Drug Interactions

Drugs and foods that might interfere to warfarin treatment were shown in table 2.3.

Table 2.3 Drug and food interactions with warfarin by level of supporting evidence and direction of interaction. [5]

Level of Evidence	Potentiation	Inhibition	No Effect
I	Alcohol (if concomitant liver disease), amiodarone anabolic steroids, cimetidine,† clofibrate, cotrimoxazole erythromycin, fluconazole, isoniazid (600 mg daily), metronidazole, miconazole, omeprazole, *phenylbutazone*, piroxicam, propafenone, propranolol, † *sulfinpyrazone (biphasic with later inhibition)*	Barbiturates, carbamazepine, chlordiazepoxide, cholestyramine, *griseofulvin*, nafcillin, rifampin, sucralfate, high vitamin K content foods/enteral feeds, large amounts of avocado	Alcohol, antacids, atenolol, bumetadine, enoxacin, famotidine, fluoxetine, ketorolac, metoprolol, naproxen, nizatidine, psyllium, ranitidine‡
II	Acetaminophen, chloral hydrate, ciprofloxacin, dextropropoxyphene, disulfiram, itraconazole, quinidine, phenytoin (biphasic with later inhibition), tamoxifen, tetracycline, flu vaccine	Dicloxacillin	Ibuprofen, ketoconazole
III	Acetylsalicylic acid, disopyramide, fluorouracil, ifosphamide, ketoprofen, lovastatin, metozalone, moricizine, nalidixic acid, norfloxacin, ofloxacin, propoxyphene, sulindac, tolmetin, topical salicylates	Azathioprine, cyclosporine, etretinate, trazodone	
IV	Cefamandole, cefazolin, gemfibrozil, heparin, indomethacin, sulfisoxazole		Diltiazem tobacco vancomycin

* Italics indicate those drugs that have supporting level I evidence from both patients and volunteers.

† In a small number of volunteer subjects, an inhibitory drug interaction occurred.

‡ Level II evidence of potentiation in patients.

Drugs which can interfere S-warfarin metabolism (CYP2C9, CYP2C19 and CYP2C18) are more clinically important than those interfering R-warfarin metabolism (CYP1A1, CYP1A2 and CYP3A4). This is because S-warfarin is five times more potent than R-warfarin.[26] In addition, drugs may influence the pharmacodynamics of warfarin by inhibiting synthesis or increasing the clearance of vitamin K-dependent clotting factors. For example; second generation and third-generation cephalosporins, which inhibit the cyclic interconversion of vitamin K could increase the effect of warfarin.[5]

2.1.5 Clinical problems of warfarin

Warfarin is a very complicated drug due to narrow therapeutic index, serious side effects (major and fatal bleeding or thrombotic events). Palareti et al. [6] have found that rate of bleeding complications occurred about 7.6 per 100 patient-years (major and fatal bleeding were 1.1 and 0.25 per 100 patient-years, respectively). There were higher rate of bleeding in older patients, 10.5 and 6.0 per 100 patient-years among aged ≥ 70 and <70, respectively (relative risk 1.75, 95%Cl 1.29-2.39, $p < 0.001$).

Moreover, warfarin has several problematic drug interactions including drug-drug, drug-food and drug-herb interactions, and high intraindividual, interindividual and interethnic variability of warfarin doses. Warfarin maintenance doses are varied up to 20 folds. Therefore, it is difficult to adjust or predict the appropriate dose for individual patient. Moreover, several diseases could affect warfarin dose such as congestive heart failure which reduced blood flow to the liver and decreased warfarin metabolism, thus increase in warfarin concentration.

2.2 Factor associated with warfarin dose

There were many factors affecting warfarin dose such as age, weight, vitamin K intake, drug interactions and genetic variations including CYP2C9 polymorphisms, VKORC1 polymorphisms.

2.2.1 Age and weight

Advanced Age was associated with decreasing warfarin dose requirements. Routledge et al. [27] have studied 228 outpatients taking warfarin, age ranging from 19 to 90 years old (mean 55 years old). It was found that there was a reduction of warfarin dose requirement with increasing age. In addition, age could explain about 10% of the variance of warfarin dose, $p<0.001$ while weight explained only about 4%, $p<0.01$. Moreover, a retrospective longitudinal study of effect of age on warfarin dose was reported by Wynne et al. [28]. There was a negative correlation between dose and age at the start of therapy ($r = -0.30$, $p = 0.002$). Warfarin dose requirement was significantly declined over time, dose difference was significantly correlated with age difference ($r = 0.25$, $p<0.01$).

In Chinese population, Yu et al. [29] have shown that warfarin requirement was significantly negatively correlated with age ($r = -0.43$, $p<0.0001$) while there was weak correlation with body weight ($r = 0.20$, $p = 0.01$). Therefore, age was major factor affecting warfarin dose. Loebstein et al.[30] have reported that the warfarin doses were significantly different among different age groups ($p<0.0001$), dose was decreased while age was increased due to plasma warfarin clearance was decreased. Furthermore, they have concluded the effect of CYP2C9 genotypes and age on warfarin dose. Warfarin dose in patients with

CYP2C9*3 in age 66 years old and over group was about 4 folds lower than those in CYP2C9*1 in age 65 years old and younger group.

2.2.2 Cytochrome P450 2C9 (CYP2C9) Polymorphisms

Cytochrome P450, family 2, subfamily C, polypeptide 9 named CYP2C9 is a major component of the CYP2C subfamily. The CYP2C genes are located on chromosome 10q24 including CYP2C8, CYP2C9 and CYP2C19. *CYP2C9* gene has been mapped on chromosome 10q24.2, spanning approximately 55 kb in length including 9 exons and 490 amino acids.[31-33] All CYP2 gene family are consisted of 9 exons.[33] CYP2C9 is 92% homologous to CYP2C19 which is different only 43 of 490 amino

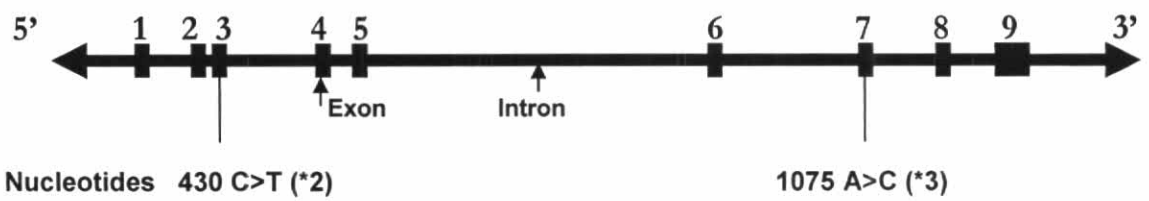


Figure 2.3 Structure of nine exons of CYP2C9 gene

Allele nomenclature is continuously updated by the Human Cytochrome P450 allele nomenclature committee in http://www.cypalleles.ki.se/cyp2c9.htm. To date, CYP2C9 polymorphisms have been found at least 30 alleles.[8] The most common in all population is CYP2C9*1 (wild type) with which normal enzymatic activity. Each variant represents single nucleotide polymorphism (SNPs) in which single amino acid is changed at a certain locus. Only 2 coding variants including CYP2C9*2 and CYP2C9*3 are common. CYP2C9*2, which cysteine (**T**GC) is substituted for arginine (**C**GC) at amino acid 144 [Arg144Cys] due to a 430C>T transition on exon 3, reduces enzymatic activity about 30%.[34,35] CYP2C9*3, which leucine (**C**TT) is substituted for isoleucine (**A**TT) at amino acid 359 [Ile359Leu] due to a 1075A>C

transition on exon 7, reduces enzymatic activity about 80%. Furthermore, the frequencies of CYP2C9 allelic variants were different among Caucasian, African and Asian populations.

As show in table 2.4, CYP2C9*2 is absent and CYP2C9*3 is rare in East Asian populations including Japanese, Chinese and Korean.[9]

Table 2.4 CYP2C9 allele frequencies in different ethnicity

	Ethnicity	Alleles	*Arg144*	*Cys144 (*2)*	Alleles	*Ile359*	*Leu359 (*3)*
Whites	American	200	92%	8%	200	94%	6%
	British	1,122	89.4%	10.6%	1,122	94.7%	5.3%
	German	988	88.7%	11.3%	734	92.2%	7.8%
	Swedish	860	89.3%	10.7%	860	92.6%	7.4%
Blacks	African-American	1,398	96.8%	3.2%	950	98.3%	1.3%
Asians	Chinese	1,016	100%	0%	896	96.7%	3.3%
	Japanese	1,512	100%	0%	1,402	97.8%	2.2%
	Korean	1,148	100%	0%	1,148	98.9%	1.1%

Several studies have revealed that CYP2C9 polymorphisms are significantly associated with warfarin sensitivity. Aithal et al.[10] have found that 29 (81%) of the 36 patients in the low-dose warfarin group ($\leq$ 1.5 mg per day) had one or more variant alleles compared with 40 (40%) of the 100 patients in normal population (OR = 6.21 [2.48-15.6]). Freeman, et al.[11] have reported that the presence of the variant alleles was associated with a significant reduction in weekly warfarin dose compared to a wild-type genotype (0.307$\pm$0.024 mg/kg/week vs 0.397$\pm$0.03 mg/kg/week, p=0.03). Taube et al. [12] have confirmed that warfarin dose was significantly related to genotype (Kruskall-Wallis, χ^2 = 17.985, p = 0.001). Scordo et al.[14] have shown that the frequencies of

CYP2C9 mutated alleles were 70.3%, 37.5% and 4.2% in the low-dose, medium-dose and high-dose group, respectively.

As Takahashi et al. [36] and Scordo et al. [14] have reported, the mean S-warfarin clearance was significantly different among the CYP2C9 genotype groups ($p<0.0001$). Moreover, Japanese patients with CYP2C9*1/*1 genotype had significantly greater median values than those in Caucasian patients ($p<0.01$).

Furthermore, prior studies by Aithal et al. [10] and Higashi et al. [13] have indicated that CYP2C9*2 and CYP2C9*3 polymorphisms are associated with an increased risk of over anticoagulation and bleeding complications during induction and maintenance phase. Peyvandi et al.[37] have concluded that 71.4% of the INR values in CYP2C9*3 homozygote were above 3.0 during the first 24 days of treatment.

Contribution of CYP2C9 polymorphisms and clinical factors could explain only 20-40% of the variation of warfarin dose requirement. Kamali, et al.[38] have revealed that age and CYP2C9 polymorphisms in the regression equation [dose = 8.05 – 0.06 x Age – 1.12 x (0 or 1; absent or presence CYP2C9*3 genotype)] account for 20.4 % of the variability in warfarin dose requirement. Gage et al. [39] have found that algorithm which consisted of factors [exp(0.385 - 0.0083 x age + 0.498 x BSA + 0.208 x CYP2C9*2 – 0.350 x CYP2C9*3 – 0.341 x amiodarone + 0.378 x target INR – 0.125 x simvastatin – 0.113 x race – 0.075 x female)] explained 39% of variance in the maintenance warfarin dose.

2.2.3 Vitamin K Epoxide Reductase Complex Subunit 1 (VKORC1) Polymorphisms

VKORC1 gene is located on chromosome 16p12-q21, spanning 5,126 base pairs including 3 exons and 163 amino acids.[17]

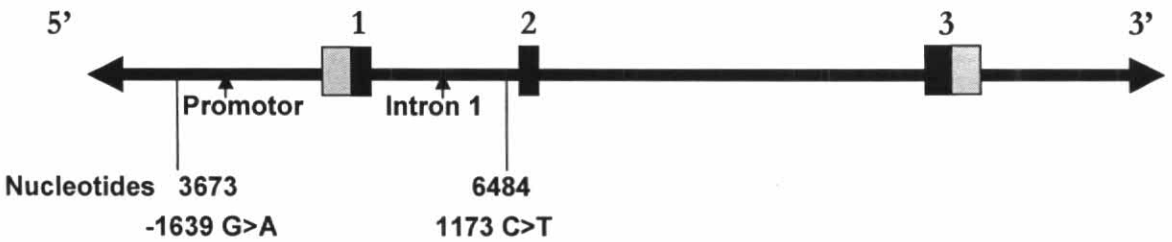


Figure 2.4 Structure of three exons of VKORC1 gene

In 2004, *VKORC1* gene has been identified.[16,17] *VKORC1* gene has been found to predict warfarin sensitivity. D'Andrea et al. [40] have studied VKORC1 1173C>T and VKORC1 3730 G>A and reported that *VKORC1* gene is associated with an interindividual variability of warfarin dose. For VKORC1 1173 in the intron 1, CC genotype had significantly higher in warfarin dose than those of TT or CT genotype. As Rieder et al. [18] have identified 2 haplotypes, VKORC1 haplotype group A consisted of H1 and H2 which is associated with a low warfarin dose requirement (approximately 3 mg/day) and is commonly found in the Asian-American population. Haplotype group B comprised of H7, H8 and H9 which is associated with a high dose of warfarin (approximately 5.5 mg/day) and is commonly found in the African-American (Table 2.5). The two main haplotypes (H1 and H7) cover about 99% of the genetic variability of the *VKORC1* gene in Asian populations. In addition, levels of VKORC1 mRNA expression were significantly correlated with haplotype group (P=0.002). The haplotype group A are associated with reduced mRNA expression and the haplotype group B are associated with increased mRNA expression.

Table 2.5 Distribution of VKORC1 Haplotypes in European-American, African-American, and Asian-American Populations.[18]

Haplotype distribution	Haplotype sequence*	European (N=119)	African (N=96)	Asian (N=120)
H1	CCGATCTCTG	0.12	0.07	0.89
H2	CCGAGCTCTG	0.26	0.06	0
H7	TCGGTCCGCA	0.21	0.42	0.10
H8	TAGGTCCGCA	0.14	0.01	0
H9	TACGTTCGCG	0.24	0.06	0
Others	-	0.04	0.38	0.01
Group distribution				
Group A (H1, H2)	-	0.37	0.14	0.89
Group B (H7, H8, H9)	-	0.58	0.49	0.10
Total group A and B	-	0.96	0.62	0.99

* For each haplotype sequence, the single-nucleotide polymorphisms are listed in sequential order along the *VKORC1* gene, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041.

Geisen et al. [41] have investigated 28 SNPs of VKORC1 polymorphisms and found that haplotype VKORC1*2 consists of four SNPs which were in complete linkage disequilibrium (position 3673 in the promotor, 6484 in intron 1, 6853 and 7566 in intron 2). VKORC1*2 corresponds to the low dose warfarin haplotype group A, VKORC1*3 and VKORC1*4 correspond to the high dose warfarin haplotype group B. VKORC1*1 has been found only in African-American. Asian population has VKORC1*2 and *3. In Asian, Yuan et al.[42] have studied VKORC1 promoter (G-1639A) and found that AA group had significant lower warfarin dose requirement than the GA and GG group, 2.6 and 3.8 mg/day, respectively ($p<0.0001$). Moreover, G allele in VKORC1 G-1639A had 44% higher activity than A allele. Furthermore, the frequencies of GG, GA and AA in Asian (2.7%, 17.6% and 79.7%) were significantly different from Caucasians (39%, 47% and 14%), $p< 0.0001$.

There are several studies that investigated the association of warfarin doses and pharmacokinetic and pharmacodynamic factors including *CYP2C9* and *VKORC1* genotypes in differences population. To date, contribution of clinical factors and genetic factors could explain warfarin dose about 33-63 %.[19-23,43-45] Sconce et al. [19] have found that Age, CYP2C9 and VKORC1 genotypes can explain 55 % of variation in warfarin dose. Wadelius et al. [20] have showed that there are highly significant difference among VKORC1 haplotype ($p<4.73x10^{-9}$) but not among GGCX (Gamma-Glutamyl Carboxylase) haplotype ($p=0.757$) and concluded that VKORC1 haplotype are good predictors of warfarin maintenance dose and explain about 30% of interindividual variability. In multiple regression model, CYP2C9, VKORC1, age, bodyweight, interacting drugs, and indication for treatment account for 56.0% of the total inter-individual variance in warfarin response. As Aquilante et al. [21] have reported, variables associated with lower warfarin dose requirements are VKORC1 3673 AA genotype, VKORC1 3673 GA genotype, variant CYP2C9 allele, increasing age, concomitant CYP2C9 inhibitors, and goal INR. Variables associated with higher warfarin dose requirements are weight, current smoker status, mean INR, concomitant CYP2C9 inducers, factor X insertion/deletion genotype, factor X insertion/insertion genotype, factor VII deletion/deletion genotype, and calculated vitamin K intake. The linear regression model explained 51.4% of the variability in warfarin dose requirements.

In Asian, Lee et al.[22] have studied three ethnicity populations in patients who lived in Singapore and found that dose requirement for Chinese and Malaysian were lower than for Indians. H1 are predominant in Chinese and Malaysian, but H7 is predominant in Indians. CYP2C9, VKORC1, age, gender and race could explain 48% of variance in warfarin dose. In Japanese populations, Takahashi et al. [23] have concluded that age, weight, CYP2C9 heterozygous variant, CYP2C9 homozygous variant,

VKORC1 1173 CT, VKORC1 1173 TT explain 57% of variance in warfarin dose. In addition, Kimura et al. [43] have found that age, sex, weight, CYP2C9, VKORC1 and GGCX accounted for 33.3% of variance in warfarin dose. In Chinese population, Miao et al. [44] have revealed that age, body weight, and genetic polymorphism of VKORC1 and CYP2C9 account for about 63% of the variability in warfarin daily dose requirements.

Recently, Kulkarni et al.[46] have investigated that INR:plasma total warfarin concentration are well correlated with weekly warfarin dose (R^2 = 0.65) to predict warfarin dose in long term treatment.

2.2.4 Coagulation Factors Polymorphisms

Recently, polymorphisms of the gene encoding vitamin K-dependent proteins (factor II, VII, IX, X) have been found to contribute to warfarin sensitivity. Shikata et al.[47] have found that the highest INR/Cp mean values and the lowest warfarin maintenance doses were observed in patients homozygous for the 165Met in the FII gene, -402G, $(37\text{-bp repeat})_6$, and -746T alleles in the FVII gene.

Moreover, FII and FVII polymorphisms are associated with alteration of those factor levels in the plasma. Poort et al.[48] have found that 87% of patients with the presence of the 20210A allele was in the high quartile of plasma prothrombin levels (>1.15 U/ml). Van't Hooft et al.[49] have found that the -401T allele in the factor VII was responsible for decreased transcriptional activity, whereas the -402A allele was associated with an increased rate of transcription thus leading to higher factor VII levels.

Furthermore, there was very little correlation between warfarin daily dose and FII ($r = 0.15$), FX ($r = -0.03$), and INR (-0.01), which nonlinear pharmacokinetic/pharmacodynamic model will also be necessary to explain the relationship between warfarin administration and FII and FX.

2.2.5 Estimation equations among Asians

Takahashi et al.[23] have studied among 115 Caucasians and 64 Japanese; included age, weight, CYP2C9*2, *3, *11, VKORC1 C1173T in the regression equation (R^2 = 57%).

Warfarin dose (mg/day) = 6.66 – 0.035 Age + 0.031 Weight – 1.706 (CYP2C9 heterozygous) – 2.815 (CYP2C9 homozygous variant) – 1.316 (VKORC1 1173 CT) – 2.941 (VKORC1 1173 TT)

In Chinese, Miao et al.[44] have reported the contribution of age, body weight, and CYP2C9 and VKORC1 G-1639A genotypes on warfarin dose (R^2 = 62.8%, $p<0.0001$).

Warfarin dose (mg/day) = 6.22 – 0.011 Age + 0.017 Weight – 0.775 (CYP2C9*3) – 3.397 (VKORC1-x1) – 4.803 (VKORC1- x2) (Input age in years, weight in kg, CYP2C9 genotype, input 1 for *1/*3, and input 0 for *1/*1;Input 0 in VKORC1-x1, input 1 in VKORC1-x2 for AA, input 1 in VKORC1-x1 and 0 in VKORC1-x2 for GA, input 0 in VKORC1-x1 and VKORC1-x2 for GG).

Moreover, Tham et al.[50] have studied Chinese, Malaysian and Indians population among patients who live in Singapore. Using the logarithmic derivation of warfarin dose in the multiple linear regression model including age, weight, VKORC1 381 CC, VKORC1 381 TC, and CYP2C9*3 (R^2 = 60.5%).

Warfarin dose (mg/day) = 10 [exp (0.838 – 0.05 Age + 0.003 Weight – 0.189 (CYP2C9*3) – 0.283 (VKORC1 381 CC) – 0.119 (VKORC1 381 TC)] (Input age in years, weight in kg, and CYP2C9*3, VKOR 381 CC, and VKOR 381 TC, input 1 if present and 0 if absent).

To date, many studies were performed to investigate the factors that effect on warfarin dose including genetics and non-genetic factors in different ethnic and try to establish the equation to predict warfarin maintenance dose requirement and reduce the bleeding risk in patients.

However, the estimation equation to predict warfarin dose requirement for Thai population has not been proposed.