CHAPTER V

DISCUSSION

In this study, we investigated the prevalence of CYP2C9 and VKORC1 genotypes and the impact of genetic factors on warfarin maintenance dose in Thai patients. The results showed that VKORC1 AA haplotypes and CYP2C9*3 are associated with low dose warfarin. Additionally, allele frequencies of CYP2C9*2 and CYP2C9*3 in Thais are 0% and 2%, respectively. This finding agrees with previous studies which indicated that CYP2C9*2 is absent and CYP2C9*3 is rare in Asian population (Chinese, Japanese, Korean).[9]

Moreover, VKORC1 1173C>T genotype was strongly correlated with VKORC1 -1639G>A genotypes, r = 1.0. This result is consistent with previous study of Geisen et al. [41] which indicated that VKORC1 position 3673 (G-1639A), 6484 (C1173T), 6853 and 7566 were in complete linkage disequilibrium. VKORC1 haplotypes of BB, AB and AA group in Thais are 7.1%, 36.2% and 56.7%, respectively. In agreement with previous studies by Reider et al.[18], Yuan et al.[42], Mushiroda et al.[56], Obayashi et al.[45], Miao et al.[44], this study found that the major genotype of VKORC1 in Thai patients is AA group which is similar to other Asian population. This confirms that the frequencies of VKORC1 in Asian are different from those of the Caucasian which indicated that BB genotype is the major genotype of VKORC1.[18,41,42] While earlier studies note that there are allele frequencies of VKORC1 group B in Asian about 10-14%, the present study found that the allele frequencies of VKORC1 group B in Thai population is 25% which is higher than those of the Japanese population (Chi-square = 17.02, $p<0.0001$). Mushiroda et al.[56] reported from 828 Japanese patients that BB, AB and AA group are 0.8%, 15.9% and 83.3%, respectively.

Genotyping using Realtime PCR, LightCycler® was the rapid and high sensitivity method to detect SNPs which the result of CYP2C9 and VKORC1 was completely in agreement with PCR-RFLP method. [59,60] It is likely to be practical in the clinical use. However, to date, those chemical were approved for Research Use Only (RUO), not for diagnostic since the chemical has not yet been approved for diagnostic by FDA.

Warfarin dose in CYP2C9*1/*3 and CYP2C9*3/*3 group are about 35% and 85% lower than those in the wild type group. This result is in agreement with previous research which demonstrated the association of CYP2C9*3 with low dose warfarin.[10-15] Takahashi et al.[23] studied the interethnic variability and has found that the mean warfarin maintenance dose in African-American, Caucasian and Japanese are 5.3 ± 2.6, 4.7 ± 2.4 and 3.5 ± 1.6 mg/day, respectively. In this study, mean warfarin dose in Thai patients is 3.7 ± 1.5 mg/day which is about 30% and 20% lowering than those required in African-American and Caucasian, respectively. This dosage is comparable to that required in Japanese. As prior studies have shown, our study confirms that mean warfarin dose in Asian population is lower than those in Caucasian and African because there are difference in the frequencies of VKORC1 haplotypes.[23,42] Haplotype group B related to high dose warfarin requirement is more common in Caucasian while haplotype group A associated with low dose requirement is more common in Asian.[18] Most of Thai populations are VKORC1 AA/CYP2C9*1/*1 genotypes which required about 50% lower dose from VKORC1 BB/CYP2C9*1/*1 genotypes.

The mean warfarin dose of Thais was about 0.46 ± 0.20 mg/kg/wk. There was a 24 years old woman who required warfarin up to 1.59 mg/kg/wk (extreme outlier in VKORC1 BB/CYP2C9 *1/*1 group). It is possible that she has the mutation in VKORC1 gene such as Val29Leu [17], 196G>A [61], 383 T>G transition in exon 2 [62] and Asp36Tyr [63], or other gene mutation which was the cause of warfarin resistance.

For plasma total warfarin concentration assay, blood was drawn in the morning before the next dose, thus, patients who took warfarin before drawing blood and who skipped the dose were excluded to prevent the error of analysis. Validation of the assay method for warfarin concentration indicated that the extraction recovery was about 57-66% which is comparable to earlier study.[52] In agreement with Herman et al.[64], this study found that CYP2C9*3 polymorphisms, but not VKORC1 had effect on pharmacokinetics which related to warfarin metabolism as demonstrated by the lowering in warfarin clearance Kulkarni et al.[46] found that there was a good correlation between INR : total warfarin concentration and the weekly warfarin dose. Also, this study show that the ratio of INR: total warfarin concentration was negatively correlated with warfarin dose ($r = -0.531$, $p<0.0001$). Furthermore, warfarin clearances in difference CYP2C9 genotypes were statistically significant differences between morning and bedtime groups. Since the plasma warfarin concentration was a one point estimate, the time that blood sample was drawn could be considered as trough concentration for those who took warfarin in the morning but for those patient who took warfarin at bedtime, the time when blood sample was drawn (in the morning) was approximately the middle of dosing interval, therefore, the drug concentration should not be considered as trough concentration, thus, should be higher (even if the same patient would take the same dosage of warfarin). However, there were no significant differences of clearance between different VKORC1 genotypes, $p=0.459$).

Time of taking warfarin had effect on short-half life clotting factor. FVII activity (half life about 6 hr) in morning group were significantly lower than those in bedtime group but there was no effect on FII activity. There were no significant differences between FII and FVII activities among different group of CYP2C9 genotypes. However, this study found that Factor II activities in VKORC1 BB & AB group were significantly higher than in VKORC1 AA group. This result is consistent with Yuan et al.[42] who studied the activity in SNPs of VKORC1 promotor (-1639) and had shown that the VKORC1 -1639G exhibited

about 44% increase of activity compared with the -1639 A. In addition, Rieder et al.[18] studied the associations between the haplotype group A and reduction in *VKORC1* mRNA expression while the haplotype group B associated with the increase in *VKORC1* mRNA expression. Additionally, Warfarin Sensitivity Index (WSI) among VKORC1 AA group was significantly greater than those in VKORC1 BB & AB groups, 2.99 ± 0.99 and 2.05 ± 0.98, respectively ($p<0.0001$) but CYP2C9*3 had no effect on Warfarin Sensitivity Index , $p=0.054$.

This study shows that CYP2C9*3 and VKORC1 AA group were associated with required lower dose of warfarin. CYP2C9*3 caused a decreased clearance of warfarin (pharmacokinetic effect), therefore required the lower dose of warfarin than wild type group. In addition, warfarin sensitivity index; INR:Cp (pharmacodynamic effect) in VKORC1 AA group was higher than those in VKORC1 BB and AB group, thus VKORC1 AA group required the lower dose. Furthermore, warfarin clearance were no significantly different among VKORC1 AA, AB and BB haplotypes, therefore, the cause of requiring low dose warfarin in AA group is not related with pharmacokinetics but it is likely to be the effect of pharmacodynamics.

The multiple linear regression model including the variables of age, weight, INR:Cp, CYP2C9*3 and VKORC1 produced the best model for estimating warfarin dose in Thais (R^2 = 59.5%). This finding indicates that genetic factors including VKORC1 and CYP2C9 genotypes have major contribution in the model used to predict the warfarin maintenance dose which is similar to the results of previous studies in Caucasian and Asians.[19-23,41,44,45,56,65] In addition, we have found that VKORC1 haplotypes are significantly associated with mean warfarin maintenance dose (r^2 = 32.4 %, $p<0.0001$). Factors associated with lower dose of warfarin requirement in Thai population are CYP2C9*3 polymorphisms, Warfarin Sensitivity Index and increasing age. Factors associated with higher warfarin dose are VKORC1 BB and AB haplotypes. VKORC1 is the major factor associated with warfarin maintenance dose. In Asians, there were

variations of VKORC1 haplotype happened more frequently than the variation of CYP2C9 polymorphisms. VKORC1 haplotypes frequencies in Thais were not comparable to other Asians such as Chinese, Japanese, Malaysian and Indian. [22,44,45,56] The highly variation of VKORC1 might be the cause of variations of warfarin dose within Asians.

For the clinical effect of genetic polymorphisms, patients with VKORC1 AA haplotype had risk of bruises and bleeding about 2.3 folds higher than the BB & AB haplotypes. However, major bleeding was not significant different among VKORC1 genotypes. Many factors combine and cause warfarin to have narrow therapeutic index and difficult to adjust an appropriated dose for the patient. To date, warfarin dose was given by trial and error based on age, INR monitoring and clinical symptoms.