# CLINICAL APPLICATIONS OF PHARMACOGENOMICS OF WARFARIN

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**NATIONAL UNIVERSITY OF SINGAPORE**

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# CLINICAL APPLICATIONS OF PHARMACOGENOMICS OF WARFARIN

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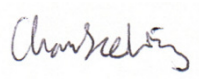
**DECLARATION**

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.



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Chan Sze Ling

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Date

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support. Completing a thesis aside, I now appreciate the transformation a PhD training brings, personally and also to my family, which recently welcomed a new little member Ming Ler.

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**SUMMARY**

Warfarin pharmacogenomic research has burgeoned considerably over the last one and a half decades. Hope (and hype) was initially high for warfarin pharmacogenomics to fulfill the promise of personalized medicine. Even though genetic association studies in various populations have firmly established the effects *CYP2C9* and *VKORC1* on warfarin dose variability, the medical community is still tentative on the adoption of warfarin pharmacogenetic testing (WPGT) in clinical practice due to its unclear clinical utility. While the results from ongoing clinical trials are being eagerly awaited, research in other aspects continues to pave the way. In this thesis, various aspects along the road from marker discovery to clinical implementation of warfarin pharmacogenomics, from scientific to economic, were explored in the Singaporean context. Firstly, selected genetic variants in *CYP4F2*, *GGCX* and *EPHX1* were investigated in the hope of finding markers that may further explain warfarin dose variability in our multiethnic Singaporean population. Of these, only *CYP4F2* rs2108622 (V433M) was significantly associated with warfarin maintenance dose (WMD), explaining an additional 2.8% of warfarin dose variability. Next, the value of genetic factors was evaluated from different angles to ascertain the potential of WPGT. The analysis showed that the currently known genetic factors, despite being highly correlated with ethnicity, provided additional predictive information towards WMD, demonstrating that ethnicity is not a sufficient surrogate for genetic information. Assessment of the population impact of WPGT using the population attributable fraction also found that Whites are likely to benefit from genotyping while Blacks, Japanese and Chinese may not. These findings highlight the need to study the benefits of WPGT in different races more carefully. Lastly, Singaporean Chinese were surveyed for their attitudes, preferences and willingness-

to-pay (WTP) for WPGT, which would be relevant in the implementation phase. The findings suggest that patient acceptance is not likely to be a major barrier, but possible social, ethical and legal issues should be addressed. With a WTP between S$160 and S$730, WPGT is also likely to be economically sustainable. Together, the findings herein help address some of the issues in the translation of warfarin pharmacogenomics, with particular relevance to the Singaporean population.

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**ABBREVIATIONS**

| | |
|---|---|
| *ABCB1* | ATP-binding Cassette Transporter B1 (gene) |
| ACC | Anticoagulation Clinic |
| ADHD | Attention Deficit Hypersensitivity Disorder |
| ADR | Adverse Drug Reaction |
| AIC | Akaike Information Criteria |
| ANOVA | Analysis of Variance |
| *APOE* | Apolipoprotein E (gene) |
| ApoE | Apolipoprotein E (protein) |
| *CALU* | Calumenin (gene) |
| CBA | Cost Benefit Analysis |
| CBC | Choice Based Conjoint |
| CDC | Centers for Disease Control and Prevention |
| CEA | Cost Effectiveness Analysis |
| CI | Confidence Interval |
| CUA | Cost Utility Analysis |
| CV | Contingent Valuation |
| CYP | Cytochrome P450 |
| CYP1A1 | Cytochrome P450 1A1 (protein) |
| CYP1A2 | Cytochrome P450 1A2 (protein) |
| *CYP2A6* | Cytochrome P450 2A6 (gene) |
| CYP2A6 | Cytochrome P450 2A6 (protein) |
| *CYP2C18* | Cytochrome P450 2C18 (gene) |
| CYP2C18 | Cytochrome P450 2C18 (protein) |
| *CYP2C19* | Cytochrome P450 2C19 (gene) |

| | |
|---|---|
| CYP2C19 | Cytochrome P450 2C19 (protein) |
| CYP2C8 | Cytochrome P450 2C8 (protein) |
| *CYP2C9* | Cytochrome P450 2C9 (gene) |
| CYP2C9 | Cytochrome P450 2C9 (protein) |
| CYP3A4 | Cytochrome P450 3A4 (protein) |
| *CYP3A5* | Cytochrome P450 3A5 (gene) |
| CYP3A5 | Cytochrome P450 3A5 (protein) |
| *CYP4F2* | Cytochrome P450 4F2 (gene) |
| CYP4F2 | Cytochrome P450 4F2 (protein) |
| dbSNP | Database SNP |
| DCE | Discrete Choice Experiment |
| DGT | Disease Genetic Testing |
| DNA | Deoxyribonucleic Acid |
| EGAPP | Evaluation of Genomic Applications in Practice and Prevention |
| *EPHX1* | Microsomal Epoxide Hydrolase (gene) |
| *F10* | Coagulation Factor X (gene) |
| *F2* | Coagulation Factor II (gene) |
| *F7* | Coagulation Factor VII (gene) |
| *F9* | Coagulation Factor IX (gene) |
| FDA | Food and Drug Administration |
| $F_{ST}$ | Wright's Fixation Index |
| GCE | General Certificate of Education |
| *GGCX* | Gamma-Glutamyl Carboxylase (gene) |
| GGCX | Gamma-Glutamyl Carboxylase (protein) |
| GST | Glutathione-S-Transferase |

| | |
|---|---|
| *GSTA1* | Glutathione S-Transferase Alpha 1 (gene) |
| GWAS | Genome Wide Association Studies |
| HB | Hierarchical Bayes |
| HBM | Health Belief Model |
| HDB | Housing Development Board |
| HR | Hazard Ratio |
| HWE | Hardy-Weinberg Equilibrium |
| INR | International Normalized Ratio |
| IWPC | International Warfarin Pharmacogenetics Consortium |
| $KH_2$ | Vitamin K Hydroquinone |
| KO | Vitamin K Epoxide |
| LD | Linkage Disequilibrium |
| MAE | Mean Absolute Error |
| MAF | Minor Allele Frequency |
| mEH | Microsomal Epoxide Hydrolase |
| MK-7 | Menaquinone-7 |
| mRNA | Messenger Ribonucleic Acid |
| mWTP | Marginal Willingness-to-Pay |
| NAD(P)H | Nicotine Adenine Dinucleotide Phosphate |
| NCBI | National Center for Biotechnology Information |
| NICE | National Institute for Health and Clinical Excellence |
| *NQO1* | NAD(P)H Dehydrogenase, Quinone 1 (gene) |
| *NR1I2* | Pregnane X Receptor (gene) |
| *NR1I3* | Constitutive Androstane Receptor (gene) |
| NUH | National University Hospital |

| | |
|---|---|
| NUS | National University of Singapore |
| OC | Osteocalcin |
| *ORM1* | Orosomucoid 1 (gene) |
| *ORM2* | Orosomucoid 2 (gene) |
| PAF | Population Attributable Fraction |
| $PAF_{100\%}$ | Population Attributable Fraction at 100% Prevalence of Exposure |
| PCR | Polymerase Chain Reaction |
| PGT | Pharmacogenetic Testing |
| PF | Prevented Fraction |
| *PROC* | Protein C (gene) |
| *PROS1* | Protein S (gene) |
| PSLE | Primary School Leaving Examination |
| QALY | Quality Adjusted Life Years |
| RP | Revealed Preference |
| SD | Standard Deviation |
| SE | Standard Error |
| SGVP | Singapore Genome Variation Project |
| SNP | Single Nucleotide Polymorphism |
| SP | Stated Preference |
| ucOC | Undercarboxylated Osteocalcin |
| VKOR | Vitamin K Epoxide Reductase |
| *VKORC1* | Vitamin K 2, 3-Epoxide Reductase Subunit 1 (gene) |
| WMD | Warfarin Maintenance Dose |
| WPGT | Warfarin Pharmacogenetic Testing |
| WTP | Willingness-to-Pay |

# CHAPTER 1: INTRODUCTION

## 1.1 Warfarin and Warfarin Pharmacogenetics

Warfarin has remained the mainstay of oral anticoagulant therapy for the treatment and prophylaxis of thromboembolism since the 1950s. An estimated 1 – 2% of the populations in developed countries are taking warfarin [1]. Though its efficacy has been well established, warfarin is challenging to use because of its narrow therapeutic index and wide variability in dose response, even within a population. Multiple factors including age, sex, weight, liver function, concomitant drugs, certain disease states, diet and genes, affect its dose response [2]. The goal of warfarin therapy is to prevent thrombosis while avoiding complications, especially bleeding. It is thus imperative that patients on warfarin be monitored regularly using the International Normalized Ratio (INR), a standardized measure of a patient's prothrombin time obtained by comparing with that of a healthy control. Currently, empirical starting doses of 5 to 10mg/day (2 to 5mg/day in Asians) are given and then adjusted to ensure that the patient's INR reaches and stays within the usual target range of 2 to 3 [3,4]. The initiation period is when the INR is most likely to be out of range and when risk of adverse events is the highest [2,3]. Even when managed under anticoagulation clinics (ACCs), patients are within their therapeutic INR range only about two-thirds of the time [3,5-7].

Clinical factors explain only about 20% of warfarin dose variability [8]. In addition, it has been observed that Asians required less warfarin to achieve the same level of anticoagulation compared to Caucasians, and the difference could not be fully explained by non-genetic factors [9,10]. This suggests that warfarin response may be partly genetic [9,11]. Facilitated by the completion of the Human Genome Project, warfarin pharmacogenetic research in the past decade has contributed much to our

understanding of the genetic determinants of warfarin response variability and how to better predict its response. There is now substantial evidence that genetic variations in cytochrome P450 2C9 (*CYP2C9)*(*2 and *3*), the gene coding for the main metabolizing enzyme of the more active S-isomer of warfarin, and vitamin K 2, 3-epoxide reductase subunit 1 (*VKORC1)*, the gene coding for the target enzyme for warfarin, affects warfarin dose requirements [12-20]. In view of the potential significance of these genetic findings, the Food and Drug Administration (FDA) updated the warfarin label in 2007 with pharmacogenetic information, and again in 2010 with dosage recommendations based on *CYP2C9* and *VKORC1* genotypes [21]. Despite these developments, the translation of warfarin pharmacogenomics into clinical practice has been slow.

### 1.2 Research Gaps

Dosing algorithms containing *CYP2C9*, *VKORC1* and non-genetic factors explain at most 50-60% of warfarin dose variability [16,22-27]. There are about 30 genes in the warfarin interactive pathway and it is possible that some of these, other than *CYP2C9* and *VKORC1*, may also affect warfarin dose requirements. They have been investigated accordingly, in particular gamma-glutamyl carboxylase (*GGCX*), microsomal epoxide hydrolase (*EPHX1*) and cytochrome P450 4F2 (*CYP4F2*), but results have generally been inconclusive. Replication of association findings across different populations is necessary to ascertain their authenticity. In addition, allele frequency differences between populations would also alter the relative contributions of genetic variants in different populations. For example, *CYP2C9*2* and *CYP2C9*3* are the 2 main *CYP2C9* variants contributing to warfarin dose variability in Caucasians, but only *CYP2C9*3* is of some importance in the Southeast Asian

population due to the rarity of *CYP2C9*2* [28]. In the local context, additional genetic markers may improve an existing dosing algorithm derived in the Singaporean multiethnic population [16].

The ultimate goal of discovering genetic markers of warfarin dose requirements is to improve clinical outcomes such as reducing bleeding risk and reducing thromboembolic events (as a result of underanticoagulation) via genetic testing for these markers. However, there are numerous steps and various issues to be addressed from marker discovery to clinical implementation. Recognizing that a systematic approach is needed to evaluate genetic tests, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative was established by the Office of Public Health Genomics at the Centers for Disease Control and Prevention (CDC) [29]. EGAPP largely adopts the ACCE framework, which covers **A**nalytical validity (how accurately and reliably the test measures the genotype of interest), **C**linical validity (how consistently and accurately the test detects or predicts the intermediate or final outcomes of interest), **C**linical utility (how likely the test is to significantly improve patient outcomes) and **E**thical, social and legal implications, to review the evidence and make recommendations for genetic tests (Figure 1) [30].

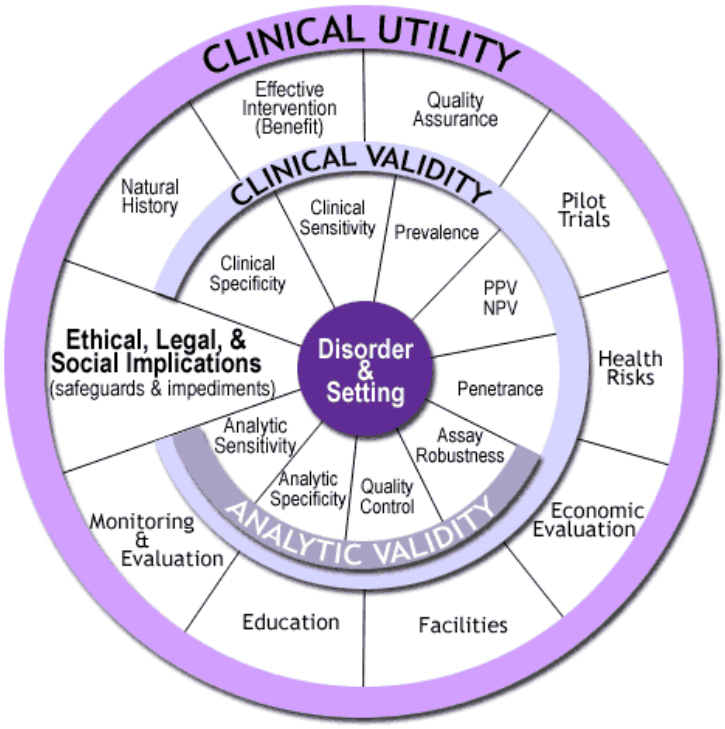


**Figure 1. Components of the ACCE Model Process for Evaluating Genetic Tests [Figure reproduced with credits to CDC]**

With much of this information lacking or inconclusive [31], there has been debate on whether warfarin pharmacogenetic testing (WPGT) is ready to be implemented in clinical practice [32,33]. From a regulatory standpoint, it has been argued that it may be inappropriate to demand evidence of clinical utility before advocating pharmacogenetic testing (PGT), due to the long lag time and uncertainty of obtaining such evidence [34]. It has even been argued that non-inferiority is sufficient for PGT [35]. Nevertheless, many clinicians remain uncomfortable with the uncertain clinical utility of WPGT. Several WPGT clinical trials are currently ongoing but clinical validity data, which can be obtained more readily, can contribute to the implementation debate in the meantime.

In addition, a policymaker would also want to know the population impact of WPGT to decide if it is justifiable. In public health, researchers are often interested in

estimating the impact of eliminating a known risk factor on the burden of disease by estimating the population attributable fraction (PAF) [36]. This measure can be adapted for WPGT to estimate its impact on the population level.

Part of the decision on advocating PGT would depend on its impact on healthcare delivery and costs [37]. Cost-effectiveness of WPGT is still inconclusive [38], but patients' willingness-to-pay (WTP) quantifies the health benefits in monetary terms and can facilitate a cost benefit analysis (CBA), an alternative method of economic analysis which may help answer questions on its economic sustainability. More studies on patients' preferences and WTP for PGT have also been proposed [37]. In addition, there may also be ethical, social and legal issues that need to be attended to [39,40]. Such data is certainly lacking in our Singapore population.

### 1.3 Research Objectives and Significance

To address the translational issues of WPGT especially in the local context, the research objectives in this thesis were:

i) To determine if the following genetic variants affect warfarin maintenance dose (WMD) in the Singapore multiethnic population

- *GGCX* rs699664 (R325Q)
- *GGCX* rs12714145 (intron 2)
- *GGCX* CAA microsatellite (rs10654848)
- *CYP4F2* rs2108622 (V433M)
- *EPHX1* single nucleotide polymorphisms (SNPs) (especially coding SNPs)

ii) To assess the utility of genetic markers in warfarin pharmacogenomics

iii) To estimate the population impact of WPGT using PAF and identify populations that may or may not benefit from WPGT

iv) To determine the attitudes, WTP and preferences for WPGT in Chinese warfarin patients and general public

### 1.4 Thesis Organization

The thesis will be organized as follows: chapter 2 will review the literature on warfarin, warfarin pharmacogenetics and the foundations of the 4 studies. Chapters 3 to 6 will detail the introductions, methods, results and discussions of each of the 4 studies addressing each of the research objectives stated above. Finally, an overall conclusion of the major findings, limitations and proposed future work will be presented in chapter 7.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 The Warfarin Interactive Pathway

The proteins and molecules that interact with warfarin in the body can be generally classified under 3 main groups: those involved in warfarin pharmacokinetics, the vitamin K cycle and those involved in the absorption and distribution of vitamin K.

### 2.1.1 Warfarin Pharmacokinetics

Warfarin is rapidly and completely absorbed from the gastrointestinal tract [41] and is >99% bound to plasma proteins, mainly albumin [42,43] and alpha-1-acid glycoproteins, encoded by the orosomucoid 1 (*ORM1*) and orosomucoid 2 (*ORM2*) genes [44,45]. There is limited evidence that P-glycoprotein, encoded by ATP-binding cassette transporter B1 (*ABCB1*), may be involved in the transport of warfarin across cell membranes [46].

Warfarin is extensively metabolized in the liver, mostly into hydroxylated metabolites before renal excretion [47]. The drug in clinical use is a racemic mixture of R and S enantiomers, and S-warfarin is about 3 to 5 times more potent than R-warfarin [48,49]. Phase I hydroxylation reactions are catalyzed by the Cytochrome P450 (CYP) enzymes. S-warfarin is primarily metabolized by CYP2C9 to 7-hydroxywarfarin, with cytochrome P450 2C8 (CYP2C8), cytochrome P450 2C18 (CYP2C18) and cytochrome P450 2C19 (CYP2C19) serving as minor pathways, while R-warfarin is mainly metabolized by cytochrome P450 1A2 (CYP1A2) (to 6- and 8-hydroxywarfarin) and cytochrome P450 3A4 (CYP3A4) (to 10-hydroxywarfarin), with cytochrome P450 1A1 (CYP1A1), CYP2C8, CYP2C9, CYP2C18, CYP2C19 and cytochrome P450 3A5 (CYP3A5) serving as minor

pathways [50-52]. There appears to be insignificant Phase II metabolism in humans [47]. Many of the CYP isoforms are inducible, and induction is mediated by the nuclear hormone receptors pregnane X receptor (encoded by *NR1I2*) and constitutive androstane receptor (encoded by *NR1I3*) [53-56].

### 2.1.2 The Vitamin K Cycle

The centre of the warfarin interactive pathway is the vitamin K cycle, comprising the vitamin K epoxide reductase (VKOR) which reduces vitamin K epoxide (KO), and GGCX which then uses the reduced vitamin K (vitamin K hydroquinone, $KH_2$) as a co-substrate to carboxylate the vitamin K dependent proteins, primarily factors II, VII, IX and X, protein C, protein S and protein Z. Warfarin, and other coumarin derivatives, inhibits VKOR, thereby interfering with the cyclic inter-conversion of $KH_2$ and KO. This in turn interferes with the post-translational gamma-carboxylation of glutamate residues by GGCX, which require $KH_2$ to function. [57].

The primary function of VKOR is to reduce KO to vitamin K, which then has to be further reduced to $KH_2$ [58]. There are 2 pathways in the vitamin K to $KH_2$ conversion: pathway I is catalysed by the warfarin-sensitive VKOR, which reduces both the epoxide and quinone form of vitamin K, and pathway II is thought to be catalysed by nicotine adenine dinucleotide phosphate (NAD(P)H) dehydrogenase (encoded by NAD(P)H dehydrogenase, quinone 1 (*NQO1*)), which reduces only the quinine form [59,60]. However, recent work suggested that an unknown warfarin-sensitive enzyme, instead of NAD(P)H, reduces vitamin K to $KH_2$ [58]. Pathway I is the most physiologically important one while pathway II only comes in when there is a high concentration of vitamin K, as would occur in the case of vitamin K

administration for coumarin overdose [60]. The vitamin K cycle is represented in Figure 2 below:

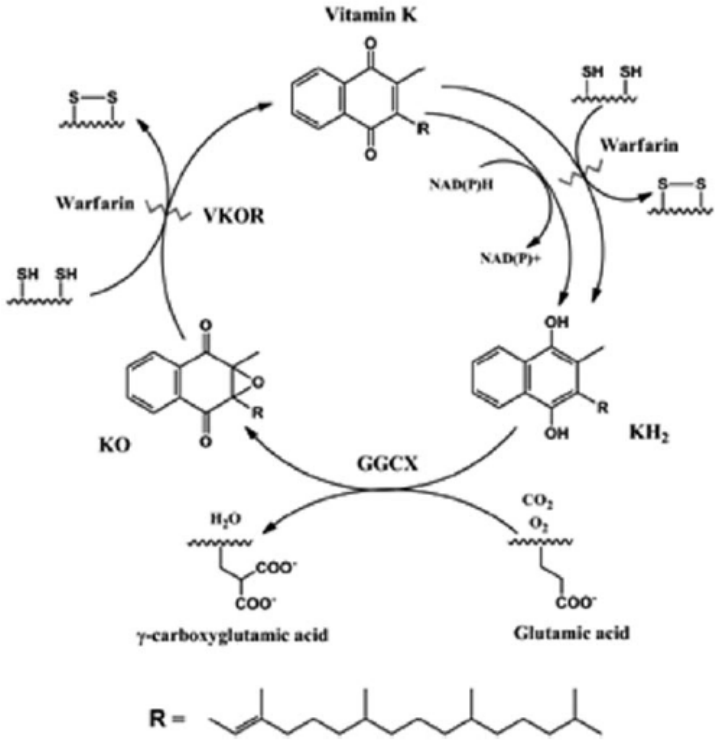


**Figure 2. Vitamin K Cycle**
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It was thought that VKOR is a multi-component system comsisting of microsomal epoxide hydrolase (mEH; encoded by *EPHX1*) and glutathione-S-transferase (GST; encoded by glutathione S-transferase alpha 1 (*GSTA1*)) [61,62], and that mEH may harbor a binding site for vitamin K 2,3 epoxide [60]. On the contrary, a mice mEH knockout study seemed to indicate that mEH did not play a critical physiologic role (the mice had no bleeding diathesis) [63]. Through linkage with combined deficiency of vitamin K dependent clotting factors type 2 and warfarin resistance, the gene for VKOR was later identified and named *VKORC1* (vitamin K

epoxide reductase complex subunit 1), in view of other possible unknown components [64]. Shortly after, VKOR was successfully purified as a single peptide, which seemed to be sufficient alone for VKOR activity [65]. On the other hand, the most recent attempt at characterizing VKOR again suggested that it may be a complex of VKORC1 and protein disulfide isomerase [66], which is a possible candidate for a thioredoxin-like domain that was found naturally fused with VKOR in a crystal structure of a bacterial homologue of VKOR [67]. Therefore, it is still unclear if VKOR is indeed a multi-component complex.

Another component of the vitamin K cycle is calumenin (encoded by *CALU*), an endoplasmic recticulum chaperone protein, which appears to inhibit the gamma-carboxylase system by associating with VKOR and GGCX in rat studies [68,69]. However, the effect of calumenin on warfarin response is uncertain as calumenin is expressed at low levels in humans [70].

### 2.1.3 Absorption and Distribution of Vitamin K

Vitamin K comprises of a group of compounds with similar biochemical properties, including phylloquinone (vitamin K1) and menaquinones (vitamin K2). Vitamin K1, a plant derived form, is the most important dietary vitamin K source in humans [71]. Vitamin K is a fat soluble vitamin, thus is absorbed from the intestines in the presence of fat [72]. In the blood it is transported by chylomicrons, which are subsequently broken down by lipases and the remnants cleared by the liver via an apolipoprotein E (apoE, encoded by *APOE*) receptor specific uptake [73-75]. This is also the probable mechanism by which it is transported to the liver for its participation in the vitamin K cycle, although low density lipoprotein and high density lipoprotein may also carry some of the vitamin K1 [76]. CYP4F2 has recently been characterized

as a vitamin K1 oxidase [77] after a SNP rs2108622 (V433M) in *CYP4F2* was found to be associated with warfarin dose response in a screen of metabolizing and transporter genes [78]. A diagram representing the genes involved in the warfarin interactive pathway is shown in Figure 3.

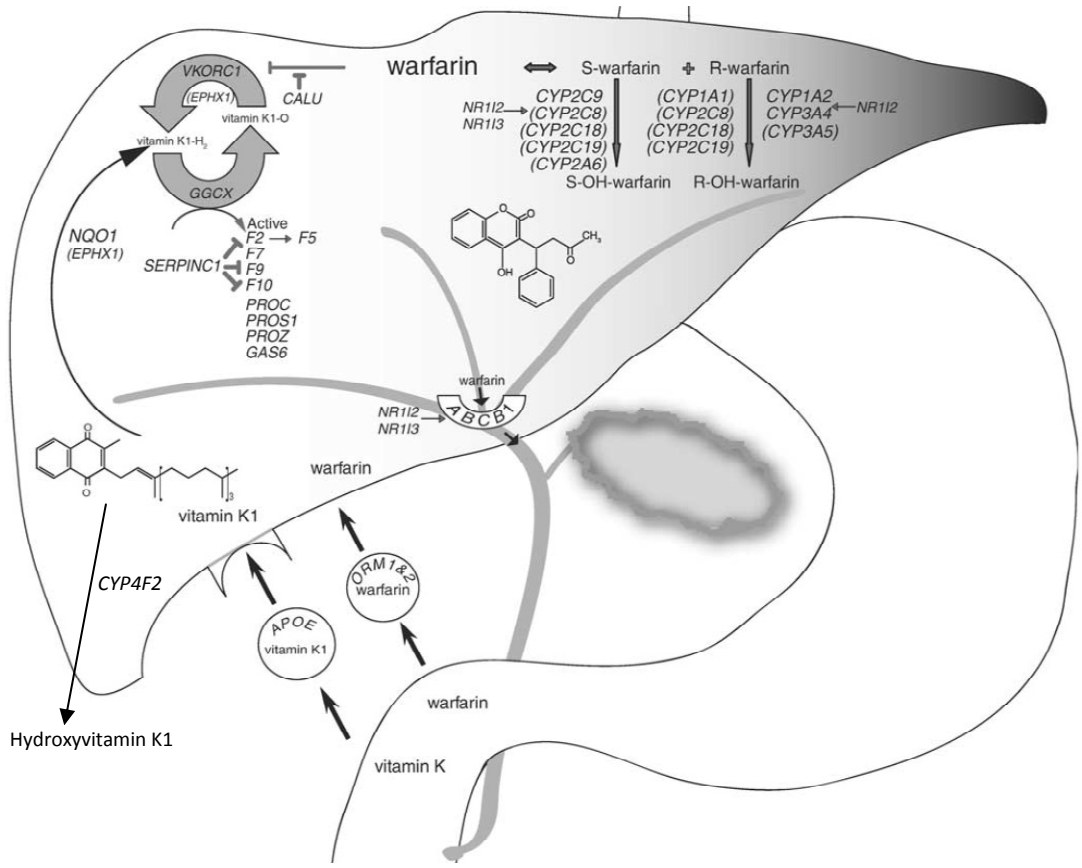


**Figure 3. Genes Involved in the Warfarin Interactive Pathway**
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## 2.2 Non-genetic Factors of Warfarin Response

Warfarin dose requirements are inversely correlated with age, weight and female gender [79-83]. The mechanism of age on lower warfarin dose requirements is unclear but may include factors such as hypoalbuminemia, decreased absorption and/or intake of vitamin K and polypharmacy. There may be some confounding

between gender and weight, since women are generally lighter than men, but intrinsic differences between men and women are also possible [83]. Weight is one measure of body size, which can affect warfarin dose requirements by affecting its volume of distribution or through its correlation with liver size, which is correlated with warfarin dose [82]. In some cases, body surface area or height have been found to be a better predictor of warfarin dose [8,84-88].

Chronic liver disease may increase sensitivity to warfarin due to impaired production of vitamin K dependent clotting factors [89], and patients in chronic renal impairment may need a lower warfarin dose due to alterations in protein binding, bioavailability and disposition [90-92].

Numerous drugs and herbal medicines have been documented to interact with warfarin [93]. They may affect warfarin pharmacokinetics by affecting its absorption or altering its metabolism. Some drugs may also influence its anticoagulation effect at the pharmacodynamic level by inhibiting vitamin K dependent clotting factors, interfering with other pathways of hemostasis or other unknown mechanisms [94]. Variation in diet composition resulting in large deviations from usual intake of vitamin K may also give rise to over- or under-coagulation [95]. Dietary vitamin K intake is also associated with warfarin sensitivity at initiation and warfarin dose requirements [96]. Interestingly, low vitamin K intake is associated with unstable warfarin response, which can be improved by vitamin K supplementation [97,98].

Long term alcohol consumption can induce hepatic enzymes and therefore increase warfarin clearance [94] but its clinical effect seem to be mixed [99,100]. Generally, consumption of a small amount of alcohol is unlikely to interact with warfarin [101]. A systematic overview of the drug and food interactions of warfarin has been undertaken by Holbrook *et al.* [93].

Smoking has been reported to increase warfarin metabolism and clearance [102,103]. Correspondingly, patients' INR have been found to increase upon smoking cessation, due to a resultant drop in warfarin clearance [104-106]. Higher warfarin doses were also required in smokers [107,108].

## 2.3 Genetic Factors of Warfarin Response

### 2.3.1 *CYP2C9*

CYP2C9 is the main metabolizing enzyme of the more active S isomer of warfarin. It is now well established that *CYP2C9*2* and **3* are associated with lower warfarin dose requirements, and a meta-analysis estimated that the dose reduction as compared to the wild type homozygote (**1/*1*) for genotypes **1/*2*, **1/*3*, **2/*2*, **2/*3* and **3/*3* were 19.6%, 33.7%, 36%, 56.7% and 78.1% respectively [109]. Furthermore, these *CYP2C9* variants have been found to be associated with an increased risk of major bleeding [110-119], and this risk persists even after dose stabilization [114]. *CYP2C9* variants are also a risk factor for unstable response [120], increased risk of above-range INRs and a longer time to achieve stable dosing [112,116,118,121-123].

*In vitro*, the products of *CYP2C9*2* and **3* were found to have only 12% and 5% metabolizing capacity of the wild type enzyme respectively [124,125]. *In vivo*, the unbound clearance of S-warfarin is decreased by 60% and 90% in heterozygous and homozygous *CYP2C9*3* carriers respectively [126-128]. Interestingly, further ethnic differences in S-warfarin clearance were found even after matching for *CYP2C9* genotypes, with Japanese and Chinese patients demonstrating higher clearance than Caucasians [129,130], suggesting a possible difference in activities of other enzymes involved in S-warfarin clearance.

The minor allele frequency (MAF) of *CYP2C9*2* and **3* vary in different ethnic groups. The MAF of *CYP2C9*2* is about 15%, 3% and 3% in Caucasians, Asians and African Americans respectively, while the MAF of *CYP2C9*3* is about 6%, 4% and 2% in Caucasians, Asians and African Americans respectively [131]. Among Asians, there also appear to be some interethnic differences. In our multiethnic Singaporean population, *CYP2C9*2* was present in 0%, 1% and 4% in Chinese, Malays and Indians respectively while *CYP2C9*3* was present in 7%, 9% and 18% in Chinese, Malays and Indians respectively [132].

Apart from *CYP2C9*2* and **3*, several rare *CYP2C9* variants such as **5*, **6*, **8*, **9*, **11*, **12* and **18* have also been associated with warfarin dose requirements [20,133-135], and these are likely to be more important in Black populations in which these variants are more common [131,136]. Other rare and novel *CYP2C9* variants have also been identified in Asian populations but their clinical significance is unclear [28,137,138].

### 2.3.2 *VKORC1*

Soon after *VKORC1* was identified, reports of common *VKORC1* variants being associated with warfarin dose requirements quickly followed. D'Andrea *et al.* first identified 2 common SNPs (1173C>T (rs9934438) and 3730A>G (rs7294)), and 1173 C>T was associated with lower warfarin doses [139]. Shortly after, a novel promoter SNP -1639G>A (rs9923231), which is in high linkage disequilibrium (LD) with 1173C>T, was reported to be associated with warfarin dose in Chinese and Caucasians in 2 separate studies [88,140]. Rieder *et al.* sequenced a 11kb region around *VKORC1* in Caucasians and inferred 9 haplotypes (H1 – H9) from 10 common (MAF > 5%) noncoding SNPs, including 1173C>T and -1639G>A. Of these, 5

common haplotypes were further grouped into the low-dose A (H1, H2) and high-dose B (H7, H8, H9) haplotypes, explaining about 25% of dose variability [141]. Patients homozygous for the low-dose AA *VKORC1* haplotype also had higher odds of over-anticoagulation (defined as an INR>5) [116] and required a longer time to therapeutic INR and time to INR>4 [123].

Haplotypes correlated with *VKORC1* messenger ribonucleic acid (mRNA) levels, suggesting that the effect on warfarin dose occurs at the transcriptional level [141]. There is some evidence that among the noncoding SNPs associated with warfarin dose requirements, -1639G>A is possibly the functional SNP. The SNP was found to alter promoter activity [140] and affect gene expression [142]. The G allele preferentially associates with active chromatin, which is consistent with increased mRNA expression, while the A allele generates a suppressor E-box binding site [142].

Though several *VKORC1* SNPs were found to be associated with warfarin dose requirements, -1639G>A, 1173C>T and haplotypes were equally and the most predictive of warfarin dose requirements across all races [143]. Therefore 1 SNP would be sufficient to capture the variation in *VKORC1*. A meta-analysis estimated that -1639GA and GG carriers required 52% and 102% more warfarin than AA carriers [144].

*VKORC1* -1639G>A also exhibits distinct interethnic differences in MAF. The A allele is present in 41%, 11% and 67% in Caucasians, African Americans and Asians respectively [131]. In our local population, the A allele was also most common in Chinese, followed by Malays and Indians [132]. Apparently, the high dose G allele is appropriately more common in populations that require higher doses and vice versa. Expectedly, these interethnic differences have been suggested to explain the interethnic differences in warfarin dose requirements [132] and dose variance

explained [143]. This implies that ethnicity may be a surrogate for *VKORC1* genotype, and this notion will be explored further in Study 2. Finally, several coding SNPs were associated with warfarin resistance [132,145-147], but their predictive value is uncertain.

### 2.3.3 Other Genes

After *CYP2C9* and *VKORC1*, other genes in the warfarin interactive pathway have also been investigated for their effects on warfarin response. Amidst the many studies which only investigated selected genes, a Swedish study comprehensively examined all 29 genes known to be in the warfarin interactive pathway at the time (therefore not including *CYP4F2*), suggesting that protein C (*PROC*), *APOE*, *EPHX1*, *GGCX* and *ORM1-2* may be potential additional genetic factors of warfarin response [148]. Subsequently, the first genome-wide association study (GWAS) in a Caucasian population did not find further genetic factors other than *CYP2C9* and *VKORC1* [19]. However, this study was underpowered (n = 181) to detect variants that explained <20% of warfarin dose variability. Another larger GWAS (n = 1053) in a Swedish population was able to detect *CYP4F2* in addition to *CYP2C9* and *VKORC1* [149]. With no further major genetic factors forthcoming, a recent study investigated copy number variations in *CYP2C9*, *VKORC1*, *CYP4F2*, *GGCX* and *CALU*, but found them to be rare in all the major races and thus have practically no role in explaining warfarin dose variation [150].

Genes with an important role in the warfarin interactive pathway and a growing body of data are discussed in more detail separately while other genes with scanty data are grouped and discussed together.

*GGCX*

The *GGCX* gene was re-sequenced in at least 3 groups of Japanese and European patients [18,151-153] and is one of the most intensively investigated. Three SNPs rs699664 (R325Q), rs12714145 (intron 2 variant) and rs11676382 (intron 14 variant) were found to have an effect on WMD in separate studies [152,154-157], but these findings were largely not replicated in others [18,153,156,158-162]. The contributions of these SNPs were also small, around 2 to 3% [152,154].

These studies were conducted in several races but the differences in results do not appear to be due to interethnic differences in MAFs. The MAF of rs699664 was about 0.27 – 0.33 for all populations except African Americans in which it was 0.682 [163], and the MAF of rs12714145 ranged from 0.2 to 0.4. However, rs11676382 is relatively common in Caucasians (MAF ~0.06 – 0.11) [152,156,163] but is almost absent in Asians and African Americans [18,156,163]. Even then, the association which was first detected in Caucasians [152] could not be replicated in another larger Caucasian population [156].

The rs699664 (R325Q) variant is a promising candidate SNP since it is non-synonymous and higher carboxylase activity has been demonstrated in the mutant enzyme (325Q) compared to the wild type (325R), by having a higher affinity for vitamin K [164]. This seems to coincide well with the finding that variant carriers require lower doses [155], since higher affinity for vitamin K would imply a lower need for vitamin K, which in turn implies that a lower warfarin dose would be needed to interfere with vitamin K levels needed for carboxylation. One explanation that the rs699664 association was not replicated in other populations may be that it is particularly relevant only in the Japanese population. A genotypic difference in the correlation between the ratio of undercarboxylated osteocalcin (ucOC) to intact

osteocalcin (OC) with serum menaquinone-7 (MK-7) has been observed, also suggesting that the vitamin K requirement for gamma-carboxylation may differ by rs699664 genotype [165]. ucOC is a sensitive index of bone vitamin K status and the ucOC/OC ratio has been correlated to dietary vitamin K intake. Interestingly, the ucOC/OCratio was not observed for vitamin K1 (phylloquinone) and menaquinone-4, which are found in leafy vegetables, and meat, liver, butter, egg yolk and cheese respectively, but only with MK-7 which is found almost exclusively in natto, fermented soybeans commonly eaten by the Japanese [165,166]. MK-7 has a much longer half life and is more potent than vitamin K1 in its effect on coagulation [167]. It was also proposed that there may be an interaction effect between R325Q genotype and vitamin K intake, since high vitamin K intake may cancel the genotypic effect of R325Q by overcoming the lower affinity of 325R [164]. The mixed results even among Japanese [17,155] may therefore be due to different dietary vitamin K intake in the subjects, which unfortunately was not captured.

Another variant of interest in *GGCX* is the CAA microsatellite repeat in intron 6. Japanese individuals who were heterozygous for 13 repeats (10/13 or 11/13) required higher doses [151]. A similar trend was found in Slovenian patients (only in *CYP2C9*1/*1* subgroup) [168], and with higher number of repeats in Swedish patients (13/13 or n/14-16 repeats) [169] and in African Americans (16 or 17 repeats) [160]. However, other studies in Caucasians, Japanese and Han Chinese did not find similar associations [18,22,148,152,153]. The reason for these conflicting results is not totally clear, but could be due to different repeat frequencies, different classification of the genotypes or even chance. In general, the CAA microsatellite appears to be associated with higher dose requirements at higher numbers of repeats, which are more common in Caucasians than Japanese (the frequency in Han Chinese was not reported),

although one study showed an opposite trend [152]. It is also possible that some of the significant findings were false positives, since the p-value was marginal in most cases. There is no data to date on the exact role of the CAA microsatellite repeat but it appears to be related to reduced sensitivity to warfarin, postulated to be due to increased GGCX activity [151].

*CYP4F2*

A non-synonymous SNP rs2108622 (V433M) in *CYP4F2* first emerged as an additional marker for warfarin dose requirements in a study using the Affymetrix drug metabolizing enzymes and transporters panel, explaining an additional 2% of dose variability on top of clinical factors, *CYP2C9* and *VKORC1* [78]. This finding was subsequently replicated in some Caucasian, Chinese and Japanese populations [149,161,170-175] but not in others [18,20,162,176-179]. Patients homozygous for the mutant alleles required about 1 – 2.5mg/day more warfarin than patients with the wild-type alleles [78,149,170]. The small but significant effect of *CYP4F2* has also been established in GWAS for both acenocoumarol and phenprocoumon [180,181]. The role of *CYP4F2* in the warfarin interactive pathway was initially unknown but functional studies subsequently found it to be a vitamin K1 oxidase (Figure 3) and the V433M polymorphism was associated with reduced capacity of the enzyme, explaining the higher doses required [77].

*EPHX1*

In light of the possible role of mEH in warfarin pharmacodynamics, common SNPs in *EPHX1* have been included in several pharmacogenetic studies. A common non-synonymous SNP rs1051740 (Y113H) showed possible association with WMD

in Israelis and Caucasians [158,163]. While this association was not replicated in subsequent studies [161,163], there were a few other signals from *EPHX1*. These include rs4653436 in the 5' flanking region in Caucasians and Han Chinese [148,159,182], rs2292566 (K119K) in Caucasians [161] and an intronic SNP rs1877724 in Han Chinese [18].

mEH is a biotransformation enzyme in the endoplasmic reticulum with an apparent dual role in detoxifying reactive epoxide intermediates of environmental toxins and drugs into less toxic dihydrodiols and bioactivation of carcinogenic polycyclic aromatic hydrocarbons [183], and has recently been associated with carbamazepine dose requirement [184,185] and risk of lung, colorectal and squamous cell esophageal cancers [186-189]. Functional studies indicate that the 2 common SNPs Y113H and H139R result in similar mEH activity but may alter enzymatic function by affecting mEH enzyme stability [190]. Furthermore, mEH protein content and hepatic enzyme activity exhibits large interindividual variation but much of this variability could not be accounted for by the 2 common SNPs. Instead most of it may be regulated by posttranscriptional controls [191]. *EPHX1* has 2 noncoding exon 1 sequences, E1 and E1-b, and their promoters drive tissue-specific expression of mEH [192]. The E1-b variant transcript, which is widely and preferentially expressed in most tissues, lies in a polymorphic region that is not in LD with Y133H or H139R [193]. Interestingly, rs4653436 and rs1877724 lie within this promoter region, suggesting that these SNPs may have a possible role in mEH expression, or tag other polymorphisms that do. Despite the conflicting biochemical evidence of the role of *EPHX1* in the warfarin interactive pathway, the multiple association signals might indicate a yet to be characterized role in the pathway.

*APOE*

ApoE is involved in vitamin K uptake and exists as 3 major isoforms, encoded by alleles ε2, ε3 and ε4. Although ε3 is the most common allele in all populations, the 3 alleles occur in different frequencies across populations [194]. There is conflicting evidence regarding the effect of *APOE* genotypes on warfarin dose requirement. Some studies found that ε4 homozygotes require a higher coumarin dose [148,195,196], while other studies found the opposite [197,198]. However, association of *APOE* genotype and WMD was largely not replicated, including in our Singaporean population [22,196,199-201].

Other Genes

The evidence with clotting factor genes is generally scanty, partly because different sets of variants were studied in different studies. A few significant associations have been detected in coagulation factor II (*F2*) rs5896 (T165M) [202,203], coagulation factor VII (*F7*) rs510335 [202], 10-basepair insertion at -323 [107]) and *PROC* rs5936 (S141S), rs1799808 [18], rs1799809, rs2069901, rs2069910, rs2069919 [148]). However, these results were not replicated in the few other studies that included them [22,151,159,161,178]. No significant associations were found with coagulation factor IX (*F9*), coagulation factor X (*F10*) or protein S (*PROS1*) variants [107,151].

*CYP2C18* and *CYP2C19* polymorphisms were associated with WMD, although the association was fully explained by LD with *CYP2C9*2* and/or **3* [148]. However, in other studies *CYP2C19* did not affect WMD [127,204]. Furthermore, a study also showed that although *CYP2C19* genotype affects R-warfarin pharmacokinetics, its effect is not translated into any significant pharmacodynamic

effect, especially when warfarin is given as a racemate [205]. *CYP3A5* also did not affect warfarin dosing, although it is one of the main enzymes responsible for R-warfarin metabolism [206]. Interestingly, one study found cytochrome P450 2A6 (*CYP2A6*) **2* (H160L) to be associated with lower warfarin dose [207] although CYP2A6 contributes negligibly to warfarin metabolism [208]. All other CYP enzymes did not affect WMD [148]. Other isolated findings include *ABCB1* (D haplotype) [206] and *CALU* (rs11653, rs1006023, rs2307040, rs339054 and rs339097 [148,179,209].

In summary, other than *CYP2C9* and *VKORC1*, there is limited and conflicting data on the effect of other candidate genes in the warfarin pathway. Their effect, if present, appears to be small as well. Other than *CYP2C9*, *VKORC1* and *APOE*, other candidate genes have also not been studied in the local population yet. Given the relative importance of *GGCX*, *EPHX1* and *CYP4F2* in the warfarin pathway, these 3 genes will be explored further in this thesis.

### 2.4 Dose Prediction from Genetic Factors

Dosing algorithms incorporating *CYP2C9*, *VKORC1* and non-genetic variables have been developed in various populations and are able to account for up to 50 to 60% of warfarin dose variability [16,22-26,88,155,210-213]. However, due to interethnic differences in MAF, the contribution of *CYP2C9* and *VKORC1* to warfarin dose variability differs between populations [13,214,215]. In African Americans, such dosing algorithms generally explain only up to 30% of dose variability [216].

Since *CYP2C9* and *VKORC1* have also been associated with early INR response, such as time to therapeutic range and risk of over-anticoagulation [116,123], it has been hypothesized that most of the genetic information may be captured in early INR values. However, several large recent studies showed that genes were still

relevant, albeit with lower contributions, even after including early INR values and thus are still useful for dose refinement [217-220].

The goal of a dosing algorithm is to improve dose accuracy and hopefully the associated clinical outcomes in patients needing warfarin, therefore that ability must be demonstrated to support its clinical use. Dosing algorithms have been compared with each other [221-224] and also compared against clinical and fixed dose regimens [134,225-228] in terms of their dose prediction accuracy in independent patient cohorts, therein validating the clinical utility of genetic factors. Generally, all pharmacogenetic algorithms have similar performances but performed best in Caucasians and Asians, and worst in African Americans. Surprisingly, the best performing algorithms in the different races were not necessarily the ones derived from the same races [222]. This may be due to differences between the sample populations used to derive these algorithms, such as certain unmeasured clinical factors or even target INR. Pharmacogenetic algorithms have consistently shown better dose prediction compared to clinical or fixed dose regimens [134,225-228]. For example, the International Warfarin Pharmacogenetics Consortium (IWPC) pharmacogenetic algorithm accurately predicted the warfarin dose in about 46% of patients, compared to 38% and 29% using a clinical algorithm and 5mg/day fixed dose respectively, with accurate dose being defined as within ±20% of their actual dose [225].

Although pharmacogenetic algorithms have generally been shown to improve warfarin dose prediction, the ultimate value of genetic factors lies in their ability to improve clinical outcomes. Several prospective clinical trials comparing pharmacogenetic dosing to clinical or standard dosing have been completed but the clinical validity of *CYP2C9* and *VKORC1* is still inconclusive due to limitations in

study design and sample size of the studies [229-235]. As such, large randomized trials such as the Clarification of Optimal Anticoagulation through Genetics (COAG) [236], Genetics InFormatics Trial (GIFT) [237], The EUropean Pharmacogenetics of AntiCoagulant Therapy (EU-PACT) [238] and Warfarin Adverse Events Reduction for Adults Receiving Genetic Testing at Therapy Initiation (WARFARIN) [239], are being undertaken to answer the clinical question more definitively. The results of another large trial (CoumaGen-II) have recently been published, indicating that pharmacogenetic dosing could improve anticoagulation control [240]. Locally a prospective randomized trial is also underway to evaluate the benefits of a pharmacogenetics-based dosing regimen compared to traditional dosing [241].

### 2.5 Population Impact of Genetic Factors

Despite being superior to clinical algorithms and fixed doses, pharmacogenetic algorithms are generally accurate in about 40% (ranging from about 30-50%) across various populations [222] and the margin of improvement appears modest. This leads to the question of what practical benefit can be expected from implementing WPGT in a population.

In terms of dose accuracy, there have been at least 2 attempts at quantifying the population impact of WPGT. Analysis of dose prediction accuracy in the multiethnic IWPC dataset revealed that patients requiring ≤3mg/day and ≥7mg/day (amounting to 46% of the cohort), benefit from WPGT. More patients in these dose groups had accurate dosing with WPGT than with the clinical algorithm (model with only clinical variables) or 5mg/day fixed dose, whereas the proportions achieving accurate dose for patients in the intermediate dose range (3 to 7mg/day) were similar with all 3 approaches [225].

Another way to estimate the impact of WPGT in a population might be to use the collective proportion of genotype combinations apart from the most common. For example, if the *CYP2C9* wildtype/*VKORC1* wildtype combination is the most combination, the sum of all other genotype combinations would represent those that may benefit from WPGT. This approach was adopted in 1 study which took the proportion of those carrying any variant *CYP2C9* and *VKORC*1 alleles, estimated to be 60% in Puerto Ricans [242].

However, these 2 groups of patients deemed to benefit may not coincide. That is, those requiring ≤3mg/day or ≥7mg/day may not necessarily be carriers of variant *CYP2C9* and *VKORC1* alleles. Though dose requirements of patients with different genotype combinations may differ, there is also likely to be substantial overlap between them. The proportions of patients requiring ≤3mg/day or ≥7mg/day thus depend on the effect sizes of the variant alleles and their frequencies, which differ by race [131,216,243,244]. The racial variation in allelic frequencies is most distinct with *VKORC1* (MAF of -1639G>A is 67% in Asians, 41% in Whites and 11% in Blacks), followed by *CYP2C9*2* (15% in Whites vs. 3% in Blacks and Asians) and *CYP2C9*3* (6%, 4% and 2% in Whites, Asians and Blacks respectively) [131]. Since different populations have different warfarin dose requirements [9,10], the simple proportion of variant carriers in a population, or proportion of patients needing low and high doses (defined as ≤3mg/day or ≥7mg/day) may not provide an accurate representation of the impact of WPGT.

The PAF is a metric commonly used in traditional epidemiology to quantify the contribution of risk factors to the burden of disease. The PAF represents the "proportional reduction in average disease risk over a specified time interval that would be achieved by eliminating the exposure(s) of interest from the population of

interest while distributions of other risk factors in the population remain unchanged" [36]. Extending the same idea to genetic variation, disease risk may be attributed to the presence of a variant allele at a particular SNP [245], and theoretically this may differ across populations as a function of the MAF and/or effect size of the SNP. This approach has been applied to several common disease such as diabetes [246-248], lung cancer [249], systematic lupus erythematosus [250], asthma and hay fever [251], Parkinson's disease [252] and cutaneous melanoma [253], among others.

In the case of warfarin, genetic variants (*CYP2C9* and *VKORC1*) have been mostly associated with WMD, which is a continuous outcome. To calculate an equivalent of the PAF, some manipulation would be necessary to obtain a dichotomous outcome. Furthermore, the value to the PAF of a genetic variant (in which the alternative is to carry the wild type allele) is questionable since it is not modifiable [36]. Applying the PAF to an outcome associated with WPGT, in this case dose prediction accuracy, by comparing to situations without WPGT, would give a practical representation of its population impact. In other words, this would estimate the population impact of *using* genetic information, rather than the genetic variation *per se*.

## 2.6 Social, Ethical and Economic Issues with WPGT

### 2.6.1 Patients' Attitudes towards PGT

In addition to clinical validity, patient acceptance is also essential for successful implementation of PGT in the clinic. A study of patients' and physicians' perspectives on PGT in Germany revealed that majority of patients are acceptive and optimistic about PGT but are concerned about adverse PGT results, privacy and possible detection of other diseases [254]. Indeed, privacy, confidentiality and cost

were the main concerns that emerged from other studies as well [255-257]. A more recent large survey of the US public also revealed strong enthusiasm for PGT, with interest being influenced by a combination of personal factors, awareness of genetics, and health and medication history. Notably, a risk of loss of confidentiality severely impacted interest in PGT [258]. A particular concern in PGT is the possibility of generating ancillary information unrelated to the purpose of the PGT, which optimistically did not negatively affect public interest in PGT in the same survey [259]. Currently, the *CYP2C9* and *VKORC1* variants related to warfarin dose requirements are not associated with any disease risk, but there is always a possibility that such information may emerge in the future.

It appears that patient demand is much stronger for PGT compared to genetic testing for late onset chronic diseases such as breast cancer (here termed as "disease genetic testing" (DGT)), possibly because patients perceive PGT similarly to a routine biochemical test [37]. Studies have been done to evaluate patients' attitudes towards DGT, including in our Asian population [260,261]. However, PGT differs from DGT in the nature of the information it conveys. Since PGT is intended to predict drug responses rather than disease risk, it is less laden with medical, social or personal significance [39]. PGT is therefore less risky than DGT due to lower potential of its results being misused or having unintended far-reaching consequences. Nevertheless, there are still ethical, social and legal issues associated with PGT which needs to be addressed [39,40]. Since Asians and Caucasians can have different knowledge and attitudes towards DGT as in the case of Parkinson's disease [261], it is possible that Asians have different perceptions of PGT as well. Attitudes predict intentions to undergo genetic testing and test uptake [262]. Therefore, knowledge of perceptions

and attitudes of Asians on WPGT would facilitate its clinical implementation by being able to address any misconceptions and concerns.

Actual uptake of a health intervention is influenced by more than just attitudes towards it. The Health Belief Model (HBM) is a widely used framework for predicting health behaviours and examples of its use include prediction of intention to undergo genetic testing for colorectal cancer [263], and its uptake [264,265]. The 4 core constructs of HBM are perceived susceptibility and perceived severity of diseases, perceived benefits and perceived barriers to preventive action. Two additional constructs that are often included are cues to action and health motivation [266]. As WPGT is not yet clinically available, not applicable to existing warfarin patients and a remote possibility for the general public, the intention here was to obtain a gauge of its acceptance, considering its potential benefits and possible ethical, social and legal risks, rather than to predict its actual uptake. As a result, not all the constructs in the HBM were systemically measured. Rather, similar studies mentioned above were used as the framework [254-259].

### 2.6.2 Economic Sustainability of WPGT

Due to the scarcity of resources, another necessary consideration for the clinical translation of WPGT is its economic sustainability. At around US$400, the cost of WPGT is one of the major barriers to routine clinical use [267]. The economic evaluation of a health intervention can take place under 2 major frameworks: cost effectiveness analysis (CEA) or cost utility analysis (CUA), where benefits are expressed as natural units or quality-adjusted life years (QALYs) respectively, and CBA, where both costs and benefits are expressed in monetary terms. Under the CBA framework, WTP is used to value the benefits, both health and non-health related, in

monetary terms [268,269]. It has been argued that WTP is superior to QALYs by enabling a more comprehensive valuation of benefits [270]. For example, in certain cases such as DGT, a non-health related benefit like diagnostic or prognostic information can have utility to patients [269] and this would not be captured in a CEA or CUA. Furthermore, the outcome in a CEA/CUA is one-dimensional and the incremental cost-effectiveness ratio is subject to variable interpretation [271]. In the UK, a threshold has been set by the National Institute for Health and Clinical Excellence (NICE) at £20-30000 per QALY, but the figure has been subject to constant debate [272]. In principle, a CBA can tell if an intervention is worthwhile and reflect what is more socially desirable [268]. However, most economic evaluations of healthcare interventions have been CEA/CUAs [269], possibly in part due to reservations on the accuracy and reliability of WTP methodologies [273].

So far, the cost-effectiveness of WPGT is inconclusive [38] and unsurprisingly no CBAs have been done. Verhoef *et al.*[38] undertook a systematic review of CEAs involving the use of PGT for the dosing of warfarin and other coumarin derivatives but could not conclude whether WPGT was cost-effective or not due to variability in study methodology, quality and choice of outcome measures. Moreover, effectiveness in those CEAs was either based on assumptions or scanty clinical data. However, the recently published results of the Coumagen-II trial, which demonstrated an absolute increase of 11% in time in therapeutic range and a relative risk of 0.48 in serious side effects with pharmacogenetic dosing compared to standard care, suggested that the input values were realistic and might even be slightly conservative [240].

Despite the potential advantages, there are several methodological issues and biases afflicting WTP estimation methods. These include hypothetical bias (where responses do not represent actual valuation), strategic bias (where respondents

deliberately gives a WTP amount different from their true WTP to attempt to influence the price of the good or service in question), under-sensitivity to the magnitude of benefit, over-inflation of the valuation of the intervention in question (i.e. budget constraint bias), dependence of WTP estimates on income and questionable external validity and reliability [274-278]. However, WTP estimation and CBAs in healthcare have increased in the last 2 decades, at least partly due to improvement in WTP estimation methods that reduce some of these problems [273].

WTP can be derived from revealed preference (RP) (observed market demand) or stated preference (SP) data (what people say they would do). Lacking RP data in most healthcare contexts, SP methods come in useful in generating information on the value of healthcare goods and services. SP methods can also value WTP and preferences for new products and services that are not yet in the market, such as WPGT in this case. SP methods can be further divided into 2 categories: contingent valuation (CV) and choice modeling, including discrete choice experiments (DCEs). DCEs has several advantages over CV, such as being able to value individual attributes (and thus are more information efficient), and are less prone to hypothetical bias and compliance (yea-saying) bias. However they tend to be more complex to design, complete and analyze, and take a longer time to complete [279]. Nevertheless, these disadvantages are minor relative to the significant advantages of DCEs.

The DCE is therefore an increasingly popular preference elicitation method used in healthcare, where respondents choose between hypothetical or real options in several choice sets, thereby implicitly indicating their relative preferences for various product attributes. When cost is one of the attributes, a DCE can be used to elicit WTP for individual attributes and/or the overall product [280]. In the DCE context,

preferences refer to the relative importance of attributes to respondents when making a choice among alternatives.

DCE has its underpinnings in consumer theory. The doctrine underlying DCEs is that the value of a good or service can be decomposed into its constituent parts (attributes), and consumers have preferences for and derive utility from these attributes rather than the good or service *per se* [280]. The technique elicits part-worth utilities for each attribute, which indicates their relative importance and how people trade between product features and price [281]. These utilities can then be used for policy analyses, such as assessing the relative impacts of attributes and predicting uptake rates [282]. A more detailed background on DCE is provided in Lancsar & Louviere (2008) and Louviere & Lancsar (2009) [280,282].

DCEs were initially used in transport and environmental economics but has been introduced and applied to a wide range of healthcare applications in the last 2 decades [280], such as eliciting WTP for PGT for the treatment of depression [283], DGT for colorectal cancer [284], meningococcal vaccine [285], a rapid malaria diagnostic test [286], health improvement in physical activity on prescription [287], and preferences for genetic counseling [288], colorectal cancer screening methods [289] and treatment modalities of attention deficit hypersensitivity disorder [290]. To date, it has not been applied to WPGT in any country.

# CHAPTER 3: ADDITIONAL GENETIC DETERMINANTS OF WARFARIN MAINTENANCE DOSE (STUDY 1)

## 3.1 Introduction

With the roles of *CYP2C9* and *VKORC1* well established, other genes in the warfarin interactive pathway have also been studied for association with warfarin response. In this study, 3 genes (*CYP4F2*, *GGCX* and *EPHX1*) that are closely involved in the vitamin K cycle, the site of action of warfarin (thus arguably relatively more important), and have demonstrated inconsistent results in other populations with respect to their effect on WMD, were chosen for investigation. Given the conflicting results regarding the effects of *CYP4F2* (rs2108622 (V433M)), *GGCX* (rs699664 (R325Q), rs12714145 and rs11676382) and *EPHX1* (rs1051740 (Y133H), rs4653436, rs2292566 and rs1877724), the objective of this study was to determine their effects on WMD in a multi-ethnic Asian population. All exonic regions of *EPHX1* were also studied to uncover other SNPs that may affect warfarin dose requirements, as the whole gene has not been studied with respect to warfarin response before. Three other *GGCX* SNPs (rs2592551 (R406R), rs10179904 (T414T), rs67988001 (intron 2 variant)) were also studied as a result of our genotyping methodology. *GGCX* rs11676382 (intron 14 variant) was not included because it was not expected to be present in an Asian population [291].

## 3.2 Materials and Methods

### 3.2.1 Study Population

The study subjects were patients receiving maintenance warfarin therapy with a stable therapeutic INR between 2 and 3 for at least 3 months, recruited from the ACCs at the National University Hospital (NUH) and Tan Tock Seng Hospital in

Singapore between June 2002 and June 2004 for a previous genotyping study [28,132]. Patients aged below 18 years or those with liver disease, malabsorption or chronic diarrheal diseases, or those taking drugs that may potentially interact with warfarin were excluded. Dietary advice to avoid foods that may interfere with warfarin pharmacokinetics was given to patients during warfarin therapy. Clinical information including age, weight, gender, ethnicity and mean daily WMD were recorded. A subject was classified as Chinese, Malay or Indian if he or she and his or her parents and paternal and maternal grandparents all belong to the same ethnic group. The mean daily WMD was the mean of 2 consecutive doses when INR readings were stable. The study was approved by the hospitals' ethics review committees and written informed consent was obtained from all patients. A copy of the ethics approval letter is appended in Appendix 1.

### 3.2.2 Genotyping

Sample Preparation and *CYP2C9* and *VKORC1* Genotyping

Deoxyribonucleic acid (DNA) extraction and genotyping of *CYP2C9* and *VKORC1* have been performed previously as reported, using direct sequencing and pyrosequencing respectively [28,132].

SNP Genotyping in *CYP4F2*, *GGCX* and *EPHX1*

Selected SNPs in *CYP4F2* and *GGCX*, and exons of *EPHX1* were genotyped by polymerase chain reaction (PCR), and direct sequencing of the PCR products was carried out using the BigDye Terminator Version 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc, Foster City, CA, USA) on the ABI 3130*xl* Automated Sequence Analyzer (Applied Biosystems) with the forward PCR primer,

reverse PCR primer or an internal primer, where appropriate. Primers were designed to flank exon 11 in *CYP4F2*, exons 8 and 9 in *GGCX* (covering SNPs rs699664 (R325Q), rs2592551 (R406R) and rs10179904 (T414T)) and a 793 base pair region in intron 2 of *GGCX* (covering rs12714145 and rs67988001). For *EPHX1*, primers were designed to flank all exons, intron-exon junctions and the SNP rs4653436 in the 5' flanking region. Both sense and antisense strands were sequenced to confirm variants. Briefly, PCR was carried out in 25µl reactions using MasterMix (Promega, Madison, USA), with an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 54°C to 61°C for 30 seconds and 72°C for 1 minute, followed by a final extension step at 72°C for 5 minutes. Generated sequences were compared with the reference sequences (NG_007971 for *CYP4F2*, EU135733.1 for *GGCX* and NG_009776 for *EPHX1*). The PCR and sequencing primers are provided in Appendix 2.

Microsatellite Genotyping in *GGCX*

PCR was performed to amplify the CAA microsatellite marker rs10654848 using a HEX-labeled forward primer. PCR conditions were similar to that for SNP genotyping except that the number of cycles was 25 instead of 35. The labeled PCR products and 500 ROX size standard (Applied Biosystems, Foster City, CA, USA) were separated on the ABI 3100 Automated Sequence Analyzer (Applied Biosystems) and analyzed using GeneScan software (Applied Biosystems). All experiments were carried out in at least duplicate. Each allele was then sequenced to confirm the number of CAA repeats.

### 3.2.3 Statistical Analysis

Analyses were performed using R version 2.11.1 [292] and the genetic analysis software PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) [293]. Deviation from Hardy-Weinberg Equilibrium (HWE) was assessed within each ethnic group using the exact test of Wigginton *et al.* [294]. A p-value <0.001 was used to indicate deviation from HWE and these SNPs were excluded from subsequent analyses. Differences in genotype distributions among the ethnic groups were tested using the chi-square test / Fisher's exact test.

The genotypes of the *GGCX* CAA microsatellite were clustered into 4 groups based on the number of repeats for analyses: group 1 (n/10 repeats), group 2 (n/11–12 repeats), group 3 (n/13 repeats) and group 4 (n/14–15 repeats), where 'n' refers to any smaller number of repeats. This clustering was similar to that used in most studies on this variant [151,152,169].

Association analysis of SNPs and *GGCX* CAA microsatellite with WMD was done using multiple linear regression and analysis of variance (ANOVA). SNPs with MAF <0.01 were first filtered away and a stepwise regression using Akaike Information Criteria (AIC) was done on the remaining variants from *CYP4F2*, *GGCX* and *EPHX1*. Variants that emerged as significant variables in the stepwise regression were then entered into a 'base' model consisting of previously known predictors (age, weight, presence or absence of *CYP2C9*3*, *VKORC1* 381 genotype) [16] and ethnicity, to determine their additional contributions. Finally, stratified analysis by ethnicity was performed to explore possible interethnic differences in genetic effects of the new variant(s). The analysis plan is represented in Figure 4 below.

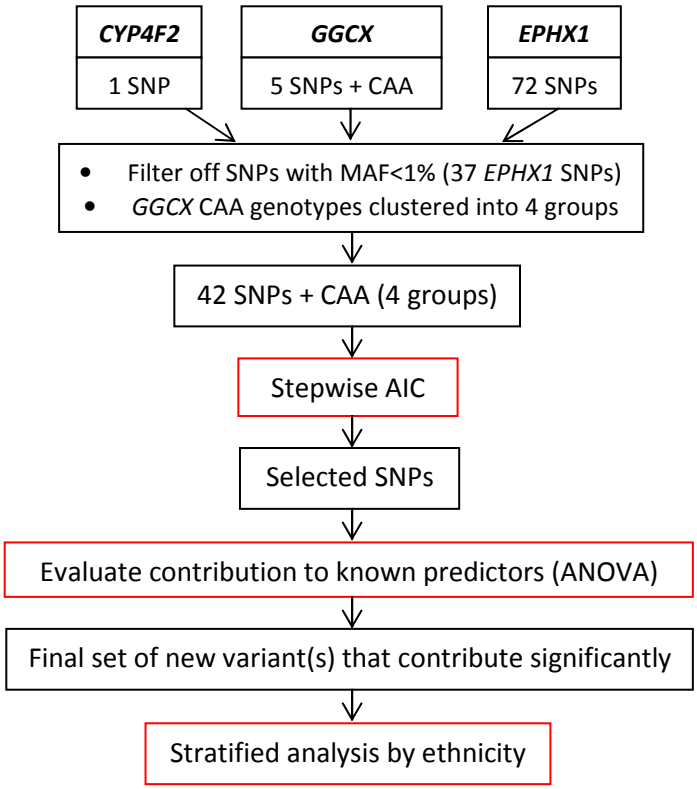



**Figure 4. Analysis Flowchart of SNPs and *GGCX* CAA Microsatellite**

The LD pattern between *EPHX1* SNPs was visualised using Haploview Version 4.2 [295]. Haplotype blocks were estimated using the confidence intervals method of Gabriel *et al.*[296], and tested for association correcting for the covariates mentioned above. Within each haplotype block, each haplotype was in turn tested for association with WMD, adjusting for the above mentioned predictors and ethnicity. Rare SNPs (MAF <0.01) were pooled and tested for association based on their presence or absence using multiple linear regression accounting for covariates. This was based on a method recently developed for analysis of rare variants discovered through resequencing within a functional unit such as a gene [297]. The log-transformed warfarin dose was used as the dependent variable in all univariate and

multivariate analyses. A p-value of <0.05 was considered statistically significant for association analyses.

### 3.3 Results

#### 3.3.1 Association of SNPs and *GGCX* CAA Microsatellite with WMD

Only the 248 patients belonging to 1 of the 3 major ethnic groups (Chinese, Malay or Indian) with complete covariate information were analyzed. Patient characteristics are shown in Appendix 3. The patient flowchart and breakdown of the 3 ethnic groups are shown in Figure 5 below.

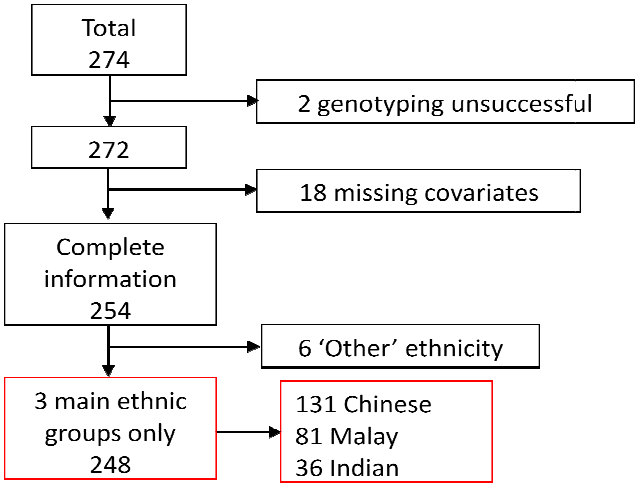



**Figure 5. Patient Flowchart for Study 1**

A total of 72 SNPs were detected in *EPHX1*, including 31 novel SNPs at the time of genotyping (database SNP (dbSNP) Build 130). Since then, 4 of the novel SNPs have been catalogued (dbSNP Build 135) but for the purposes of this thesis they shall still be considered novel. Of the 72 *EPHX1* SNPs, only 35 had a MAF ≥ 1%. The most common *GGCX* CAA microsatellite alleles were 10 and 11 repeats, accounting for 85% of all alleles, similar to that observed in Japanese and Han Chinese [18,151,153]. None of the SNPs deviated from HWE and the MAFs of the

variants are summarized in Appendix 4. Of the 35 common SNPs from *EPHX1* and 6 SNPs from *CYP4F2* and *GGCX*, there were significant differences in MAF among the ethnic groups for 17 SNPs (Appendix 4).

Five SNPs emerged from the stepwise regression but when adjusted for known predictors, only *CYP4F2* rs2108622 (V433M) was significantly associated with WMD, contributing an additional 2.8% to warfarin dose variability. GA or AA carriers required approximately 0.55mg/day more than GG carriers, when averaged across all levels of other covariates (adjusted means of log warfarin dose = 0.507, 0.578 and 0.569 for GG, GA and AA carriers respectively). The *EPHX1* intronic SNP rs1877724 which was borderline significant, did not contribute additionally after rs2108622 was accounted for (Table 1).

| Model | $r^2$ | $r^2$ change | ANOVA p-value* | Location of new SNP |
|---|---|---|---|---|
| Base** | 0.584 | - | - | - |
| 1) Base + rs2108622 | 0.612 | 0.028 | $2.4 \times 10^{-4}$ | *CYP4F2* V433M |
| 2) Base + rs1877724 | 0.594 | 0.010 | 0.05 | *EPHX1* intron 1 |
| 3) Base + rs3817268 | 0.584 | 0 | 0.90 | *EPHX1* intron 2 |
| 4) Base + rs67892231 | 0.585 | 0.001 | 0.31 | *EPHX1* K117R |
| 5) Base + rs2292568 | 0.589 | 0.005 | 0.22 | *EPHX1* P284P |
| 6) Base + rs2108622 + rs1877724 | 0.618 | 0.006*** | 0.16*** | |

* Compared with base model
** Base model = age + weight + *CYP2C9*3* + *VKORC1* 381 + ethnicity
*** Compared with model 1

**Table 1. Effects on WMD of 5 SNPs Emerging from Stepwise Regression after Adjusting for Known Predictors**

Upon stratified analysis by ethnicity, no big difference in the effect of *CYP4F2* rs2108622 (V433M) was observed among the 3 races, judging from its additional contribution of between 3.0 and 4.5%. However, there was substantial variation in $r^2$ among the 3 races (54.4% to 73.2%) and was lowest in Malays, possibly indicating more unknown factors in them (Table 2).

| Variable | Regression coefficient (p-value†) | | | |
|---|---|---|---|---|
| | All<br>n = 248 | Chinese<br>n=131 | Malay<br>n=81 | Indian<br>n=36 |
| Intercept | 0.886 (***) | 1.026 (***) | 0.825 (***) | 0.650 (**) |
| Age | -0.005 (***) | -0.005 (***) | -0.005 (***) | -0.006 (**) |
| Weight | 0.003 (***) | 0.002 (**) | 0.002 (.) | 0.006 (**) |
| *CYP2C9*3* | -0.152 (***) | -0.270 (***) | 0.016 | -0.140 (*) |
| *VKORC1* 381 TC | -0.130 (***) | -0.258 (*) | -0.100 (*) | -0.062 |
| *VKORC1* 381 CC | -0.310 (***) | -0.414 (***) | -0.276 (***) | -0.408 (***) |
| Indian ethnicity | -0.023 | - | - | - |
| Malay ethnicity | -0.059 (**) | - | - | - |
| *CYP4F2* rs2108622 GA | 0.071 (***) | 0.051 (*) | 0.079 (*) | 0.106 (.) |
| *CYP4F2* rs2108622 AA | 0.062 (.) | 0.122 (*) | 0.083 | 0.036 |
| $r^2$ | 0.612 | 0.595 | 0.544 | 0.732 |
| $r^2$ (without *CYP4F2*) | 0.584 | 0.563 | 0.499 | 0.702 |
| $r^2$ change due to addition of *CYP4F2* | 0.028 | 0.032 | 0.045 | 0.030 |

†p-value: *** 0.001 ** 0.01 * 0.05 . 0.1

**Table 2. Model Estimates of *CYP4F2* rs2108622 and Known Predictors in the 3 Ethnic Groups**

### 3.3.2 *EPHX1* Haplotype Association

The LD pattern across *EPHX1* appeared similar in the 3 ethnic groups (Appendix 5). Five haplotype blocks were estimated using the confidence interval method in Haploview. None of the haplotypes were associated with WMD in the haplotype test after adjusting for known predictors (Table 3).

| Block | SNPs | Haplotype | Frequency | p-value |
|---|---|---|---|---|
| 1 | rs4653436 rs12741681 rs12744609 | GAA | 0.754 | 0.614 |
| | | ACG | 0.185 | 0.861 |
| | | GCA | 0.057 | 0.106 |
| 2 | rs3738040 rs1877724 rs3738042 | GTGGGGG | 0.330 | 0.051 |
| | rs41266229 rs41266231 rs3738046 | GCGGGGG | 0.231 | 0.172 |
| | rs3738047 | ACGGGGA | 0.208 | 0.852 |
| | | GCAAAGG | 0.137 | 0.681 |
| | | GCGGGCG | 0.057 | 0.101 |
| | | GCGGGGA | 0.019 | 0.585 |
| 3 | rs3738048 rs55743622 | CA | 0.944 | 0.101 |
| | | TC | 0.057 | 0.101 |
| 4 | rs1051740 rs2292566 | CG | 0.466 | 0.957 |
| | | TG | 0.276 | 0.950 |
| | | TA | 0.258 | 1.000 |
| 5 | 33430A>G rs4149223 rs2234922 | AGAGCCCGCAGG | 0.336 | 0.560 |
| | rs2292567 rs4149226 rs2292568 | AGAGTCCGCAGG | 0.153 | 0.404 |
| | rs1051741 rs45467394 rs4149227 | ACGGTCTGCAGG | 0.119 | 0.226 |
| | rs4149228 rs4149229 rs4149230 | GCAGCTCTAGGC | 0.0766 | 0.287 |
| | | ACAGCCCGCAAG | 0.0726 | 0.761 |
| | | AGAATCCGCAGG | 0.0685 | 0.802 |
| | | ACAGCCCGCAGG | 0.0617 | 0.474 |
| | | ACAGCTCTAGGC | 0.0522 | 0.614 |
| | | ACAGTCCGCAGG | 0.0295 | 0.597 |

**Table 3. *EPHX1* Haplotype Blocks, Haplotype Frequencies and Multivariate Association Results**

### 3.3.3 Association of Rare SNPs with WMD

There were 37 rare SNPs in *EPHX1* (28 novel and 9 catalogued), and of the 248 patients, 45 had at least 1 rare SNP. The presence of rare SNPs was not associated with WMD ($p = 0.091$). Grouping and testing of the rare SNPs by features that may influence their function, such as whether they were coding or noncoding, synonymous or nonsynonymous, was also attempted but none of these groupings of rare SNPs were significantly associated with WMD. However, the presence of noncoding rare SNPs was marginally associated with WMD, with those carrying at least 1 rare noncoding SNP requiring about 0.34mg/day less than those without ($p = 0.088$).

### 3.4 Discussion

Exonic regions of *EPHX1* and selected variants in *CYP4F2* and *GGCX* were surveyed in this study and only the *CYP4F2* rs2108622 (V433M) variant was significantly associated with WMD after accounting for previously known predictors. This is in line with a recent study in another Singaporean multiethnic Asian population, which also found *CYP4F2* V433M to significantly influence warfarin dose requirements, albeit only in the subgroup of patients with low-dose *VKORC1* diplotypes [172]. Despite several studies failing to replicate this association [18,20,162,177,298], results from a recent meta-analysis found that heterogeneous and homogenous variant carriers require 10% and 21% more warfarin than wild-type carriers respectively [299] and the results herein are consistent with this finding. *CYP4F2* is now established as an additional genetic factor for response to warfarin as well as the other coumarin derivatives acenocoumarol and phenprocoumon [180,181,300,301]. However, its additional contribution is relatively small and is unlikely to dramatically improve dose prediction.

On the other hand, the associations of the previously described *GGCX* variants with WMD failed to replicate in our cohort, like in most other studies that included them [17,18,152,153,156,158,159,161,163,178]. The 2 *GGCX* SNPs (rs699664 and rs12714145) also failed to demonstrate an effect on acenocoumarol and phenprocoumon response [301,302]. The CAA microsatellite appears to have an effect on WMD only with higher number of repeats (≥14 repeats) [160,169], which is relatively rare in our population. Therefore its effect, if any, is unlikely to be relevant in our population.

This study also comprises the first exon sequencing of *EPHX1* to determine whether any coding or splice site variants of the gene affect WMD in our multiethnic

Asian population. Non-exonic variants reported to be associated with WMD (rs4653436 and rs1877724) were also deliberately captured. The intronic SNP rs1877724 was marginally associated with WMD after adjusting for known covariates and ethnicity, but failed to reach statistical significance after accounting for *CYP4F2* V433M. This is consistent with a Han Chinese study in which the association between rs1877724 and WMD was also marginal [18]. A Caucasian study investigating rs4653436 did not find an association with WMD as well [178]. Furthermore, in an earlier GWAS in Caucasians, no *EPHX1* SNPs reached genome-wide significance [19]. In those studies which reported a significant association between an *EPHX1* SNP and WMD the contribution of the SNP was small, in the range of 1–5% [18,148,159,161]. The effect of rs1877724 in our population, if any, is likely to be smaller than that of *CYP4F2* V433M.

One explanation for the failure to detect significant associations between the studied variants and WMD in our population is inadequate power. A post-hoc power calculation for MAF >1% at the 5% significance level using QUANTO Version 1.2.4 [303] revealed that the sample size in this study provided about 80% power to detect a genetic variant with marginal $r^2$ of at least 3%. MAF is one of the factors affecting statistical power in genetic association analyses, other than sample size and effect size [304]. In fact, the QUANTO software also takes in MAF as one of the parameters. Intriguingly, the output does not show any effect of MAF on power (Appendix 6). The fact that some of the *EPHX1* SNPs have low MAF, and that their numbers are relatively small (only 6 SNPs have MAF between 1 and 5%), should not pose a big problem of false negatives. Since numerous studies including much larger ones, have failed to replicate these associations, it is likely that the true effect size of the *GGCX* and *EPHX1* variants if any, are actually smaller. As such, the lack of association

between *GGCX* and *EPHX1* variants and WMD in this study is likely to be due to a lack of a meaningful effect in our population rather than a lack of power.

Defining the phenotype in genetic association studies is challenging, entailing completeness, reliability and validity [305]. It has also been demonstrated that phenotypic complexity, measurement bias and poor phenotypic resolution can affect the power to detect genetic variants in association studies, using the example of attention deficit hyperactivity disorder (ADHD) [306]. Warfarin response can be defined in many ways, such as WMD, time to stable dose, time to therapeutic range, % time in therapeutic range, INR achieved with a fixed starting dose, INR achieved on day X (usually ≤7), INR>4 in first week, variation in clotting factor activities, warfarin plasma concentrations, bleeding risk, and so on. In fact, several of these have been used as outcomes in genetic association studies, although WMD is the most commonly used one. Compared to phenotypes like ADHD, warfarin response is far less complex, albeit still fraught with variability and thus potential for poor phenotypic resolution. The usual target INR range is 2 to 3, implying that the WMD itself would contain some amount of variability. In other words, different warfarin doses may lead to an INR between 2 and 3, and qualify as WMD. An alternative target variable like WMD normalized to stable INR would theoretically remove much of this variability. However, a meta-analysis of *CYP2C9* and *VKORC1* effects on some of these outcomes (other than WMD) do not seem to indicate that they have better promise in detecting genetic associations [307]. Furthermore, there is no linear relationship between warfarin dose and INR [308]. Given the narrow therapeutic index of warfarin, the range of warfarin doses for INR to remain between 2 and 3 (i.e. the inherent variability of WMD) is likely to be very small. Finally, keeping in mind the ultimate goal of translating warfarin pharmacogenetic research into clinical practice,

the target variable should also be one that is convenient for clinical application. Considering all these factors, WMD is still a more suitable outcome variable than a INR-normalized WMD or INR achieved with a fixed starting dose, despite its shortcomings.

Despite the lack of a sizable effect, the present findings and others collectively still point to the possibility that mEH may have a yet to be defined role in the warfarin interactive pathway. Recent efforts in understanding the transcriptional regulation of *EPHX1* showed that the proximal promoter regions of the classic and alternative exons 1 (E1 and E1-b respectively) are key drivers of transcription in the liver and most body tissues, respectively [192,193]. Haplotype analysis of a region spanning both promoters and the whole gene also showed that Alu polymorphisms in the promoter regions, which affect transcriptional activity, reside in a haplotype block that extends to exon 3 [193]. rs1877724 and rs4653436 (associated with WMD in a Caucasian study [148]) are within this haplotype block, so it is possible that these SNPs tag other polymorphisms that affect transcription and thus protein activity.

Most of the novel SNPs discovered in the course of genotyping were rare. It was suggested that a large fraction of low frequency nonsynonymous SNPs have deleterious effects [309]. Interestingly, the presence of noncoding rare SNPs turned out to be marginally associated with WMD in this study. This is not totally surprising as some of the noncoding rare SNPs may exert their effects through transcriptional regulation since approximately 40% of them lie in the same haplotype block with the promoters, as defined by Yang *et al*. [193]. Nevertheless, nonsynonymous SNPs also seem to have different effects according to their predicted impact on the protein function. Among the 7 nonsynonymous rare SNPs, 4 were predicted to be benign by PolyPhen [310] and patients with these SNPs required approximately 4.3 mg/day

(after accounting for covariates) and most of them required a dose higher than predicted (by the pharmacogenetic model with previously known predictors and *CYP4F2*), while patients with the other 3 nonsynonymous SNPs predicted to be possibly damaging, required approximately 2.7 mg/day and slightly less than predicted. Although the number of SNPs and patients here were too small to draw any firm conclusions on the effect of rare SNPs by their possible effect on the gene product, these results suggest that rare SNPs may be worth studying on a larger scale. This is exemplified by a recent study which found that pooling rare variants in *CYP2C9* that tend to reduce the enzyme activity had an effect on the WMD [134].

Apart from *CYP2C9, VKORC1* and *CYP4F2,* other genetic factors of warfarin dose requirements have been elusive. Possibly, gene-gene and/or gene-environment interactions may explain part of the remaining variability. Considering the roles and interactions of the established and the herein studied genes (Figure 3), a possible gene-gene interaction may occur between *VKORC1* and *CYP4F2*, given that the amount of vitamin K1 available for GGCX would depend in part on the simultaneous activities of both gene products. However, the overall *VKORC1* and *CYP4F2* interaction was not statistically significantly (ANOVA between models with and without interaction terms; p-value = 0.375) and the $r^2$ was increased by only 0.68%. Gene-gene interactions may instead occur between *VKORC1* and *EPHX1* and/or *GGCX* but neither *EPHX1* nor *GGCX* have been shown to have a significant main effect, so indiscriminate testing for interactions is imprudent due to the rapid inflation in type 1 error. Moreover, statistical interaction may not necessarily reflect biological interaction, and vice versa [311]. The lack of a significant statistical interaction between *VKORC1* and *CYP4F2* simply means it does not explain warfarin dose variability any further, rather than demonstrate a lack of biological interaction.

*VKORC1* -1639G>A correlates with mRNA expression of the gene in the liver [141,142], and thus may result in different clotting propensity manifesting as different baseline INR values. Theoretically, if *VKORC1* or ethnicity (which is correlated with *VKORC1* genotype) is correlated with baseline INR, the baseline INR should be a better predictor of warfarin dose since it is closer to the phenotype along the causal pathway, and also incorporates variation from other factors such as vitamin K levels and clotting factor activities (Figure 6).

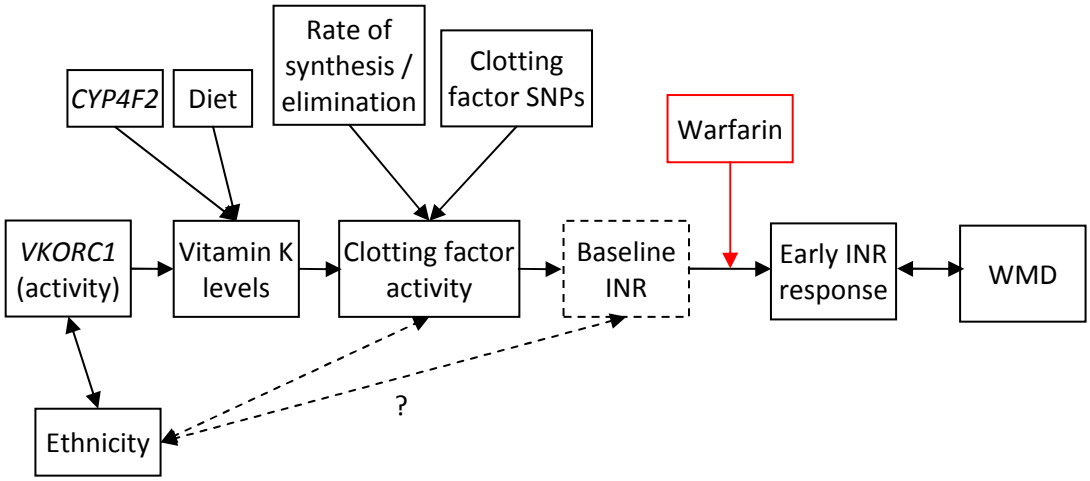



**Figure 6. Causal Pathway of Genetic and Non-genetic Factors on Warfarin Dose**

There is however no information on how baseline INR varies with *VKORC1* genotype or ethnicity. Interestingly, some studies have explored the incorporation of early INR readings to refine dose prediction [218,219,312] and a simulation study have even suggested that baseline clotting factors levels would be useful in predicting WMD [313]. Only 1 study can be found to consider the baseline INR as a predictor and it was disappointingly quite a poor one on its own [314]. This may be due to the canalization effect (which is "the relative robustness of key physiologic parameters, features and processes despite wide genetic and phenotypical variability of other factors"), that has been proposed to apply to the coagulation cascade as well

[313,315,316]. Despite the wide variability in clotting factor activities in normal subjects, INR exhibits low variability. This canalization effect is however expected to be reduced with anticoagulant therapy and the variation in clotting factor activities then results in variation of warfarin dose required [313]. If so, complex gene-gene and gene-environment interactions that stabilize the INR in normal individuals, may explain part of the remaining warfarin dose variability.

The presence of 3 distinct ethnic groups within our study population poses the risk of false positive associations due to population structure. The allele frequencies of *CYP4F2* rs2108622 differ significantly across the 3 ethnic groups. The minor allele A is more common in Indians than in Chinese and Malays (MAF = 0.472, 0.244 and 0.222, respectively) (Appendix 4). However, the association between the SNP with WMD does not appear to be an artifact since it is significant even after adjusting for ethnicity. In addition, it also has a significant effect within each ethnic group (Table 2). In light of significant associations in other populations as well, *CYP4F2* rs2108622 is likely to be a genuine finding.

To the best of our knowledge, this study has the largest cohort of Malays and Indians in addition to Chinese, enabling the exploration of genetic factors in explaining interethnic differences in warfarin dose requirements. Among these 3 ethnic groups, warfarin dose requirements in Malays are the least well understood. Current predictors explain only about 50% of their dose variability, compared to 57% in Chinese. More intriguingly, Malays have a *VKORC1* haplotype distribution which is intermediate between Chinese and Indians, yet require doses similar to the Chinese. Given the finding that the MAF of *CYP4F2* V433M in Chinese and Malays are similar, and that none of the studied *GGCX* and *EPHX1* variants were associated with warfarin dose requirements, none of the studied variants could account for the lower

than expected (from their *VKORC1* haplotype distribution) warfarin dose requirements in Malays, suggesting that other genetic loci, non-genetic factors, or joint effects of genetic variants with one another or with non-genetic factors may explain their warfarin dose requirements instead.

In conclusion, *CYP4F2* V433M has a small but significant effect on WMD in our multiethnic Asian population, while *GGCX* and *EPHX1* failed to demonstrate an effect.

# CHAPTER 4: TRANSLATIONAL ASPECTS OF GENETIC FACTORS IN PHARMACOGENOMICS (STUDY 2)

## 4.1 Introduction

It has recently been recognized that the relationship between association p-values and discriminative ability of genetic markers for complex diseases are not necessarily proportional [317,318]. Therefore, genetic markers should be evaluated by their ability to improve predictive accuracy, or clinical validity, apart from their association with disease [317,319]. Disappointingly, the genetic signals identified for complex diseases thus far are generally inadequate as disease classifiers despite being replicated across multiple populations and displaying statistically significant effect sizes [318,320,321]. As with complex diseases, pharmacogenetic traits are believed to be polygenic [322], although the observed effects are considerably larger than those detected in complex diseases, and are likely to involve fewer genes [323].

Prior to the proliferation of genetic analyses, warfarin dose requirements have been observed to vary between different ethnic and population groups, with Asians requiring lower doses than Caucasians [9,10]. In the multi-ethnic population of Singapore, Chinese and Malays have been observed to require lower doses compared to Asian Indians [28,132]. Interestingly, the frequency of the *VKORC1* haplotype associated with high warfarin dosage displayed considerable interethnic variability, and is appropriately more common in populations that require higher warfarin doses, and vice versa [132,243,324]. Furthermore, in dosing algorithms the contribution of race has been observed to drop drastically after the inclusion of these genetic factors, suggesting that the interethnic differences in dose requirements may be explained at least in part by genetic factors, and that race could be a surrogate for genetic factors [132,225]. If so, genetic factors would have much lower or even no additional utility

over ethnicity. However, there is also evidence that most genetic diversity occurs within members of the same race rather than between races [325,326]. This study thus aimed to explore the clinical relevance of *CYP2C9*, *VKORC1* and *CYP4F2* beyond the known and easily available clinical factors in predicting WMD. This was done using 2 approaches: exploring the contribution of genes over and above clinical variables in explaining variation in warfarin dose requirements, and comparing the predictive accuracy of WMD with and without genes.

## 4.2 Materials and Methods

### 4.2.1 Study Population

Data from the same 248 patients (131 Chinese, 81 Malays and 36 Asian Indians) in Study 1 were used in this study. Genotyping of *CYP2C9*3* [28], *VKORC1* (381; rs7196161) [132] and *CYP4F2* V433M (rs2108622) were previously reported or described in Study 1. It was previously established that *VKORC1* 381 alone is sufficient to distinguish between the 2 common haplotypes *H1* and *H7* in *VKORC1* [16]. Gene names mentioned henceforth in this study will refer to the described variants here. *CYP2C9*2* was excluded as this variant is considerably rare in our populations [16,28].

### 4.2.2 Statistical Analysis

Correlation between Genes and WMD

All analyses were performed in R Version 2.11.1 [292]. The contributions of *CYP2C9*3*, *VKORC1* and *CYP4F2* to warfarin dose variation have been established in this study population previously and in this thesis (Study 1) [28,132], but their

relationships with WMD was re-demonstrated in turn in this particular subset of 248 patients using linear regression with log warfarin dose as the response.

Correlation between Ethnicity and Genes

Given the concurrence between ethnic variation in warfarin dose requirement and inter-ethnic differences in the MAF of *VKORC1*, 2 hypotheses were investigated: (i) whether the 3 genes are predictive of self-reported ethnicity; and (ii) whether the genes are still useful in explaining warfarin dosage after adjusting for ethnicity. To explore the first hypothesis, the Wright's Fixation Index ($F_{ST}$), which is a measure of population differentiation, was used. For 2 populations with allele frequencies denoted as $p_1$ and $p_2$, the $F_{ST}$ is defined as:

$$F_{ST} = \frac{(p_1 - p_2)^2}{(p_1 + p_2)(2 - p_1 - p_2)}$$

**(Equation 1)**

For each $F_{ST}$, the empirical p-value was calculated as the proportion of 1,353,095 SNPs in the Singapore Genome Variation Project (SGVP) [243] with $F_{ST}$ values at least as large as the observed statistic using allele frequencies from the clinical sample. A multinomial regression was also performed, with the genes as predictors and ethnicity as the response to assess the relative contribution of each gene in explaining ethnicity. The statistical significance of each gene was evaluated using ANOVA. To investigate the second hypothesis, a linear model between log warfarin dose and the genes was constructed, after adjusting for previously established clinical factors like age and weight [16], and ethnicity. The additional contribution of the genes beyond age, weight and ethnicity in explaining WMD was also assessed with ANOVA.

Predictive Accuracy of WMD from Genetic and Clinical Factors

The comparison of accuracy of the 3 genes and clinical factors (age and weight) in predicting WMD was the second approach adopted in investigating the translational value of genetic factors and also a measure of clinical validity. A cross-validation exercise was performed where 80% of the 248 samples was used to train the regression model, which was subsequently used to predict the dose requirement for the remaining 20%. In each cross-validation, 2 statistics were measured: (i) the proportion of the variance explained by the model ($r^2$) with the training data; and (ii) the proportion of patients where the predicted dose was found within 20% of the actual dose. The second criterion is deemed to be within clinically acceptable limits and has commonly been used as a threshold for assessing dose prediction accuracy [221,222,225]. Some fixed dose regimens were also included for comparison. While 5mg/day is a common standard starting dose, it is not unusual in clinical practice to give lower doses to Chinese and Malay patients and so a race-specific fixed dose regimen was also included for comparison. 100 iterations of the cross-validation were performed for each model, and 8 models involving fixed dose regimens and various combinations of clinical and genetic factors were considered:

(A) Fixed dose of 5mg/day in all patients

(B) Fixed dose of 3mg/day for Chinese and Malays, and 5mg/day for Indians

(C) Ethnicity only

(D) Age, weight and ethnicity

(E) Two main genetic factors only (*CYP2C9*3* and *VKORC1*)

(F) Age, weight, ethnicity, *CYP2C9*3* and *VKORC1*

(G) Age, weight, *CYP2C9*3*, *VKORC1* and *CYP4F2*

(H) Age, weight, ethnicity, *CYP2C9*3*, *VKORC1* and *CYP4F2*

## 4.3 Results

### 4.3.1 Correlation between Genes and WMD

As expected, all 3 genes were significantly associated with WMD, explaining between 5 and 31% of the dose variance (Table 4). Of note, carriers of the T allele of *VKORC1* required higher doses, with almost 3mg/day difference expected between TT and CC carriers. This coincides with the interethnic difference in warfarin dose requirements and allele frequency of *VKORC1* (Appendix 3). Chinese had the lowest frequency of the T allele as well as the lowest dose requirement (MAF = 0.111, median dose = 3.00mg/day), followed by Malays (MAF = 0.346, median dose = 3.50mg/day) and Indians (MAF = 0.875, median dose = 5.11mg/day).

| Gene & variant | Genotype | Predicted dose, mg/day (SE)* | $r^2$ (%) | ANOVA p-value |
|---|---|---|---|---|
| *CYP2C9*3* | AA | 3.577 (1.031) | 5.5 | $1.97 \times 10^{-4}$ |
| | AC/CC | 2.457 (1.099) | | |
| *VKORC1* 381 | CC | 2.783 (1.033) | 31.3 | $2.20 \times 10^{-16}$ |
| | CT | 4.115 (1.047) | | |
| | TT | 5.455 (1.064) | | |
| *CYP4F2* rs2108622 | GG | 3.088 (1.102) | 8.8 | $1.25 \times 10^{-5}$ |
| | GA | 3.779 (1.048) | | |
| | AA | 4.802 (1.039) | | |

SE: standard error
* Predicted from linear model of each gene on log warfarin dose

**Table 4. Association Analysis between Each of the 3 Genes with WMD, Without Adjusting for Clinical Variables and Ethnicity**

### 4.3.2 Correlation between Ethnicity and Genes

Based on $F_{ST}$ calculations, *VKORC1* was highly differentiated among the 3 ethnic groups, with significant differences in all pair-wise comparisons. While the observed MAF differences between the Indians and the other 2 ethnic groups at *CYP2C9*3* and *CYP4F2* were similar to that at *VKORC1* (Appendix 3), there was no significant distinction between the Chinese and Malays at *CYP2C9*3* and *CYP4F2* based on $F_{ST}$ (Table 5).

| SNP | $F_{ST}$ (empirical p-value*) | | |
|---|---|---|---|
| | Chinese – Malays | Chinese – Indians | Malays - Indians |
| *CYP2C9*3* | 0.0005 (0.706) | 0.0220 (0.399) | 0.0163 (0.400) |
| *VKORC1* 381 | 0.0783 ($1.16 \times 10^{-3}$) | 0.5838 ($2.73 \times 10^{-5}$) | 0.2942 ($4.12 \times 10^{-4}$) |
| *CYP4F2* rs2108622 | 0.0007 (0.679) | 0.0565 (0.186) | 0.0690 (0.092) |

*Defined as the proportion of 1,353,095 SNPs in the SGVP with $F_{ST}$ values at least as large as the observed $F_{ST}$ in this analysis

**Table 5. SNP Level $F_{ST}$ Values between Ethnic Groups**

The multinomial regression between the ethnicity and the genotypes of the 3 genes identified *VKORC1* (p-value < $10^{-16}$) and *CYP4F2* (p-value = $4.66 \times 10^{-4}$) to be strongly associated with ethnic distribution, but not *CYP2C9*3* (p-value = 0.106).

Apart from individually having a significant effect on WMD, all 3 genes were also additionally important for explaining warfarin dose variation (p-value ranging from $6.2 \times 10^{-4}$ for *CYP4F2* to p < $10^{-16}$ for *VKORC1*, Table 6), even after adjusting for ethnicity and the 2 clinical factors (age and weight). In addition, *CYP4F2* significantly contributed in explaining dose variation (p = 2.42 x $10^{-4}$), even after taking into account ethnicity, clinical factors and the 2 main genetic factors *CYP2C9*3* and *VKORC1* (Table 6).

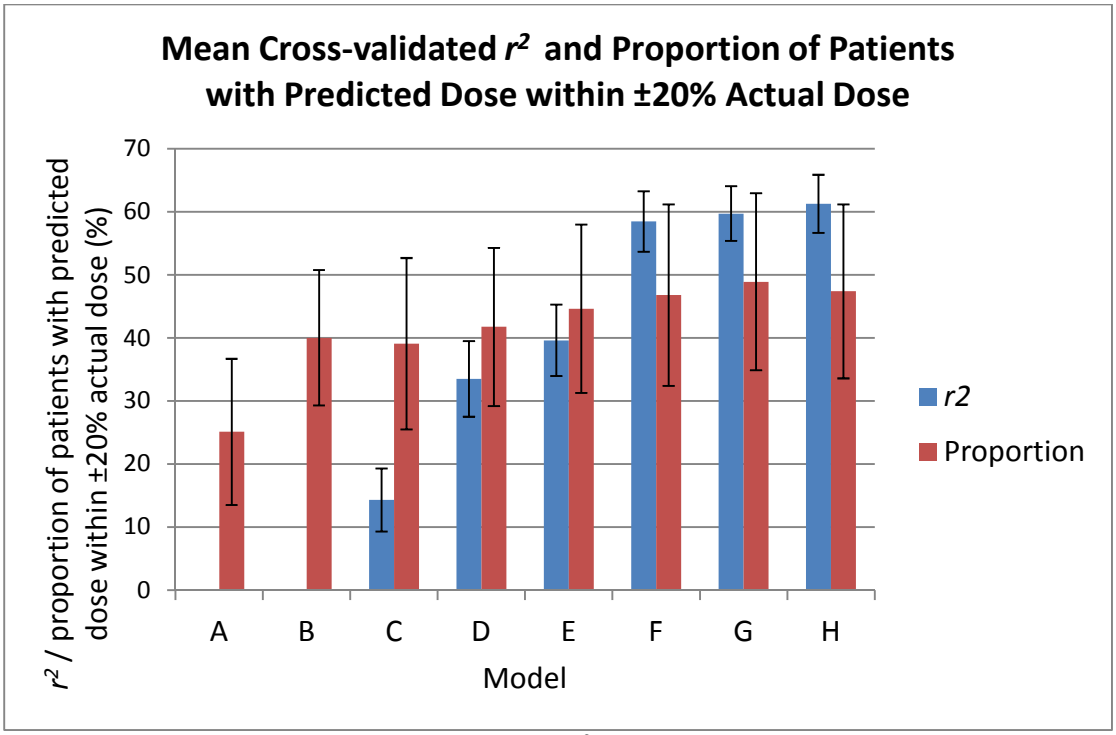
| Model variables | $r^2$ (%) | ANOVA p-value* |
|---|---|---|
| Age, weight, ethnicity | 33.4 | - |
| Age, weight, ethnicity + *CYP4F2* | 37.4 | 6.2 x $10^{-4}$ |
| Age, weight, ethnicity + *CYP2C9*3* | 38.8 | 6.1 x $10^{-6}$ |
| Age, weight, ethnicity + *VKORC1* | 53.2 | < $10^{-16}$ |
| Age, weight, ethnicity + *VKORC1* (+ *CYP2C9*3*) | 58.4 | 1.09 x $10^{-7}$ |
| Age, weight, ethnicity + *CYP2C9*3* + *VKORC1* (+ *CYP4F2*) | 61.2 | 2.42 x $10^{-4}$ |

* Base model consists of only age, weight and ethnicity. ANOVA p-value compares the stated model against the base model, except for the last 2 models where the ANOVA compares the stated model with the model immediately preceding it – thus evaluating the contribution of the locus in brackets.

**Table 6. Contribution of Genes other than Their Effect through Ethnicity**

### 4.3.3 Predictive Accuracy of WMD from Genetic and Clinical Factors

Through a cross-validation procedure, the accuracy of warfarin dose prediction in 8 settings involving fixed dose regimens and different combinations of clinical and genetic factors were compared (Figure 7). Due to the different dose requirements in the 3 ethnic groups, a standard dose of 5mg/day performed worst in terms of accuracy (model A), predicting the dosage for only 25% of the patients within acceptable error margin. As expected, a race-specific fixed dose, where 3mg/day was assigned to Chinese and Malays and 5mg/day assigned to Indians, performed better (model B), yielding very similar predictive accuracy to the use of ethnicity alone (model C). Incorporating clinical variables (age and weight) on top of ethnicity increased both the amount of dose variance explained ($r^2$) and also the proportion of patients that were accurately predicted (model D), but the improvement was marginal compared to when the genetic factors were incorporated (models E – H). The model incorporating only *CYP2C9*3* and *VKORC1* (model E) already outperformed models that utilize only clinical variables and ethnicity (models A - D), and the improvement was even more significant when these variables were incorporated in addition to the 2 genes (model F). The addition of *CYP4F2* without (model G) and with (model H) the inclusion of ethnicity marginally increased the amount of dose variance explained.




Bar chart showing the mean cross-validated $r^2$ (blue bars) and proportion of patients with predicted dose within ±20% actual dose (red bars) of 8 models. Error bars represent the 95% confidence interval (CI) of the respective measures. Model A: fixed dose of 5mg/day for all patients; model B: fixed dose of 3mg/day for Chinese and Malays, and 5mg/day for Indians; model C: ethnicity alone; model D: age, weight and ethnicity; model E: *CYP2C9* and *VKORC1*; model F: age, weight, ethnicity, *CYP2C9* and *VKORC1*; model G: age, weight, *CYP2C9*, *VKORC1* and *CYP4F2*; model H: age, weight, ethnicity, *CYP2C9*, *VKORC1* and *CYP4F2*

**Figure 7. Prediction Accuracy of Various Fixed Dose, Clinical and Genetic Models**

### 4.4 Discussion

While large-scale genetic studies in many common diseases and complex traits have unveiled numerous genomic loci that are associated with these phenotypes, the clinical relevance and utility of these findings have been relatively limited due to the marginal contribution by each locus. This then raises questions on the clinical relevance of pharmacogenomics, as drug response can also be considered a complex disease. In the case of warfarin, the observation of a strong correlation between the genotypes of *CYP4F2* and *VKORC1* with ethnicity poses the question of whether

there is any merit in performing genetic testing, since self-reported ethnicity may already function as an effective surrogate to represent the genetic information.

Using data from a multiethnic patient population consisting of 3 major ethnic groups in Singapore (consisting of Chinese, Malays and Asian Indians), this study established the additional contribution that genetic testing can yield beyond easily assayed clinical biomarkers like age, weight as well as self-reported ethnicity. The findings suggest that even within each ethnic group, genetic information from the 3 loci (*VKORC1*, *CYP2C9* and *CYP4F2*) was still important in assessing warfarin dose requirements. The broad classification of individuals by self-reported ethnicity thus can still result in under or over-dosing, with the severity and fraction of affected individuals differing depending on the ethnic group.

Based on statistical testing of various models (Table 6) and comparison of predictive accuracy (Figure 7), the small but significant contribution of *CYP4F2* was also confirmed. *CYP4F2* increased the $r^2$ by almost 3% even after including *VKORC1* and *CYP2C9*. This is consistent with study 1 and also in line with the only study to date which evaluated the additional role of *CYP4F2* in terms of prediction accuracy, where the difference in predictive accuracy in terms of $r^2$ with and without *CYP4F2* was 5% [134]. In addition, when evaluated in another study by another statistical criterion, the AIC, the inclusion of *CYP4F2* was also found to improve a basal dosing model of known clinical and genetic factors [327].

Out of the 3 loci surveyed, *VKORC1* was most predictive of ethnicity, while the associations between ethnicity and the genotypes of *CYP2C9* and *CYP4F2* were considerably weaker. This concurs with a previous genome-wide comparison of genetic variability across the Chinese, Malays and Indians in Singapore, where *VKORC1* was amongst the ten most differentiated regions across the genome between

these 3 ethnicities [243]. Despite the strong correlation between *VKORC1* and ethnicity, all the genes surveyed contributed additional predictive information for warfarin dose requirements on top of ethnicity. This is not particularly surprising since 85-90% of our genetic variation is due to differences *within* ethnic groups and only 10-15% due to differences *between* ethnic groups [325]. This suggests that even if self-reported ethnicity is statistically associated with warfarin dose variation, it is unlikely to be clinically useful on its own for predicting dose requirements, nor as a surrogate for the genetic factors that are involved in warfarin dose response. This finding concurs with those of a previous study which explored the contribution of race to genotypic variance across 3 pathways (irinotecan, fluorouracil and insulin) in the 4 Hapmap populations using hierarchical clustering and principal component analysis [326]. In addition, self-reported population or ethnic membership is likely to be even less useful with increasing rates of inter-ethnic marriages, particularly in multi-ethnic societies like Singapore, since self-reported ethnicity is likely to be misleading in the presence of genetic admixture.

The main objective of the predictive accuracy calculations here was not to evaluate specific dosing algorithms but to demonstrate the clinical validity of genetic factors to predict warfarin dosing in our population. The relative contribution of genetic information beyond conveniently attained clinical information such as age and weight will be important in deciding the importance of genetic testing. Studies assessing the prediction accuracy of warfarin dosing algorithms use measures such as mean absolute error (MAE), coefficient of determination ($r^2$), and proportion of patients with predicted dose within certain limits of the actual dose (±20% or ± 1mg/day) [134,221,225,226]. Similar measures were used in this study as these are easily interpretable and allow direct comparisons with published findings. However,

each measure represents only some aspect of dose prediction accuracy. This may explain why as genetic variables were added on to clinical variables, the $r^2$ increased considerably but the proportion of patients with predicted dose within ±20% actual dose only increased slightly. With the second measure, improvements in predictions that fall outside this range were undetected, yet these are likely where most of the improvements lie.

The prediction accuracy of clinical and genetic factors combined (model F) compared well with other different populations in terms of $r^2$ and proportion of patients with predicted dose within ±20% actual dose [134,221,225,226]. For example, the clinical relevance of genetic factors was similar to the observations by the IWPC, which similarly reported that genetic factors improved the prediction accuracy for an additional 6 - 8% of the samples beyond non-genetic biomarkers like age and weight [225].

In conclusion, genetic information adds value in addition to clinical biomarkers and self-reported population/ethnic membership for predicting warfarin dose requirements.

# CHAPTER 5: RELEVANCE OF WARFARIN GENOTYPING FROM A PUBLIC HEALTH PERSPECTIVE: THE POPULATION ATTRIBUTABLE FRACTION AS A MEASURE OF THE IMPACT OF WARFARIN PHARMACOGENETIC TESTING (STUDY 3)

## 5.1 Introduction

There is now substantial data to show that WPGT can improve dose prediction (clinical validity) [225-227,328] but whether routine genotyping is worthwhile also depend on its population impact, which can be expressed as the proportion of the population which would benefit, or the reduction in proportion of undesirable outcomes. Clinical utility data would be ultimately necessary and ideally the population impact is estimated based on relevant clinical outcomes but pending which, knowing the population impact of WPGT on intermediate outcomes such as dose accuracy can also be enlightening.

Two previous approaches in estimating the population impact of WPGT include taking the proportion of patients requiring low and high doses (≤3mg/day and ≥7mg/day ) and taking the collective proportion of genotype combinations apart from the most common [225,242]. However, these may not provide an accurate representation of the impact of WPGT.

Through a simple manipulation of the initial warfarin dose accuracy, where the outcome of interest (or 'disease' in classical PAF definition) was defined as 'inaccurate starting dose' (outside ±20% actual maintenance dose) and 'exposure' defined as 'no genotyping', the PAF was applied to assess the impact of WGPT in patients who need warfarin therapy. Although the PAF here was derived from a measure commonly used in assessing algorithm performance (i.e. proportion predicted within 20% actual dose), it offers a different perspective of the value of

WPGT, one that is more convenient for decision making on WPGT recommendations in a particular population. Indeed, several studies have evaluated the performance of published pharmacogenetic algorithms using the above-mentioned measure [134,225-228] and this was not the aim here.

The objective of this study was to estimate the potential population impact of WPGT and identify populations that may or may not benefit from WPGT. Using data from IWPC, the proportion of patients requiring ≤3mg/day or ≥7mg/day and their relationship with the different genotype combinations by race was first explored to determine the validity of the previous approaches, and then the population impact of WPGT estimated using the PAF.

## 5.2 Materials and Methods

### 5.2.1 Study Population

The expanded IWPC dataset containing clinical and genotype data for 6922 warfarin patients from 22 study sites was obtained from the PharmGKB database [329]. The dataset contained information on demographics, indication for warfarin therapy, stable therapeutic dose, treatment INR (INR achieved with a stable warfarin dose), target INR (desired INR), use of concomitant medications, and presence of genetic variants of *CYP2C9* and *VKORC1*. Some potentially important variables such as vitamin K intake, smoking status and adverse events were not included as they were not uniformly available across all the study sites [225]. *CYP4F2* genotype data was also not available. Those who did not reach stable doses, target INR not within 2 to 3 and those without dose information were excluded. Populations for which dose simulation could not be performed due to unavailability of genotype frequencies (Koreans) and undefined races (Asians and others/unknown) were also excluded. For

PAF calculation, those with missing clinical information (age, height, weight) needed for the IWPC dosing algorithms were further excluded.

### 5.2.2 Dose Simulation of Genotype Combinations

All analyses were performed in R Version 2.11.1 [292]. To predict the warfarin dose requirements for each genotype combination within each race due to variation in *CYP2C9* and *VKORC1*, effect sizes estimated from the study population and genotype frequencies from the Hapmap (Utah residents with northern and western European ancestry [CEU]; Yoruba in Ibadan, Nigeria [YRI]; Japanese in Tokyo, Japan [JPT]; Han Chinese in Beijing, China [CHB]) were used to simulate the dose distributions. *CYP2C9* genotype was expressed as the number of variant (**2* or **3*) alleles present, and all other variant alleles were considered as **1*. *VKORC1* -1639G>A (rs9923231) was used as the tagging SNP and missing genotypes were imputed using the same rules as reported by the IWPC [225].

Within each race, the square roots of warfarin doses were regressed on *CYP2C9* and *VKORC1* genotypes and the regression coefficients used as the effect size. Simulations were performed for 100,000 individuals in each race, where the genotype at each gene was sampled from a multinomial distribution with cell probabilities equal to the population-specific genotype frequencies from Hapmap. An additive nature was modeled to the effect of the 2 genes, and the root dose of each individual was simulated from a Gaussian distribution around the estimated effect size with the corresponding SEs as the standard deviation (SD), given the sampled genotype combination of the individual. The obtained distributions were back transformed to yield the actual dose distributions.

### 5.2.3 Calculation of PAF

In epidemiology, PAF is calculated for an exposure for a disease, which is a dichotomous state. Though the 'population' here would include only people requiring warfarin therapy, the PAF can be easily multiplied by the number of new warfarin patients in a given time interval to estimate the absolute number of patients who would benefit from WPGT. Here the predicted warfarin dose was dichotomized as 'accurate' (defined as within ±20% actual dose) and 'inaccurate' (outside ±20% actual dose). Ideally the first dose given is the dose the patient needs (i.e. maintenance dose) but an initial dose within ±20% of the actual dose was deemed to be within clinically acceptable limits, thus 'accurate', and this standard has been used in several studies assessing dose prediction accuracy [221,222,225]. The 'disease' was defined as 'inaccurate initial dosing', since this is what we wish to reduce. All patients receiving warfarin can receive their initial dose based on a fixed dose regimen (5mg/day), clinical or pharmacogenetic algorithm. The IWPC clinical and pharmacogenetic algorithms were used for initial dose prediction [225]. The 'exposure' was defined as 'no WPGT', and both scenarios (fixed dose and clinical algorithm) were compared against WPGT. The comparison between the clinical algorithm and WPGT would represent the true impact of WPGT (where the only difference is the addition of genetic factors) while the comparison between the fixed dose regimen and WPGT would represent the practical impact of WPGT for centers which routinely use a fixed dose regimen and do not formally incorporate clinical factors into initial dose choice.

In addition to a 5mg/day fixed dose regimen, a 3mg/day fixed dose regimen for Japanese and Chinese was also explored, since Asians are known to require lower doses [9,10]. A 6mg/day fixed dose was also tested in Blacks as it approximated the

mean simulated dose. The PAF was calculated using the formula below for a range of exposure prevalence (5 – 99.9%):

$$\boldsymbol{PAF = \frac{P_T - P_U}{P_T} \times 100\%}$$

**(Equation 2)**

where $P_T$ is the proportion with the outcome of interest (inaccurate dosing) in the whole (warfarin patient) population and $P_U$ is the proportion with the outcome in the unexposed (genotyped). The PAF at 99.9% prevalence of no WPGT was used to approximate that of 100% prevalence ($PAF_{100\%}$), the impact of implementing WPGT in all warfarin patients for a population that is currently not using WPGT.

## 5.3 Results

### 5.3.1 Study Population

After excluding those who did not reach stable dose, target INR not within 2 to 3 and those without dose information, 4237 patients remained. All Malays and almost all Indians did not have height information for PAF calculation so they were also excluded. After further excluding the Koreans, Asians and others/unknowns, 3672 remained for dose simulation (2543 Whites, 639 Blacks, 227 Japanese and 263 Chinese). Those with missing clinical information (age, height, weight) needed for the IWPC dosing algorithms were further excluded and 3252 patients remained for PAF calculation (2305 Whites, 627 Blacks, 195 Japanese and 125 Chinese).

### 5.3.2 Dose Simulation

Consistent with previous observations of interethnic variation in warfarin dose requirements [9,10], Blacks were found to require the highest doses, followed by Whites and then Chinese and Japanese (Figure 8, black lines). As a result, the

proportion falling into the ≤3mg/day and ≥7mg/day groups differed dramatically by race (Table 7). More Japanese and Chinese fell into these dose extremes (53 – 58%) compared to Whites and Blacks (36 – 43%).

Since only 2 genes were modeled here, the overall dose distribution is the sum of the distributions of 9 genotype combinations (3 genotypes per gene: wild type, heterozygous variant and homozygous variant for each SNP; $3^2 = 9$ combinations). The sizes of the distributions of each genotype combination are proportional to their expected frequency, so some colored lines may not be visible if their expected frequency is very low or zero (Figure 8, colored lines). A visual inspection of the distributions of the individual genotype combinations revealed that different combinations predominated in different populations (Figure 8, colored lines), and their contributions to the low and high dose groups also differed (Table 7). Whites displayed the largest genetic variation (with more colored lines being visible), while Japanese and Chinese displayed little variation, and Blacks exhibited the least. For Whites, several genotype combinations made up the ≤3mg/day group, while only 1 or 2 combinations dominated in Blacks (*CYP2C9*WT/*VKORC1*-1639GG), Japanese and Chinese (*CYP2C9*WT/*VKORC1*-1639AA and *CYP2C9*WT/*VKORC1*-1639AG) in that dose group. Similarly, 2 to 3 combinations dominated the ≥7mg/day group for Whites, and 1 combination (*CYP2C9*WT/*VKORC1*-1639GG) for Blacks. Very few Japanese and Chinese patients required ≥7mg/day.

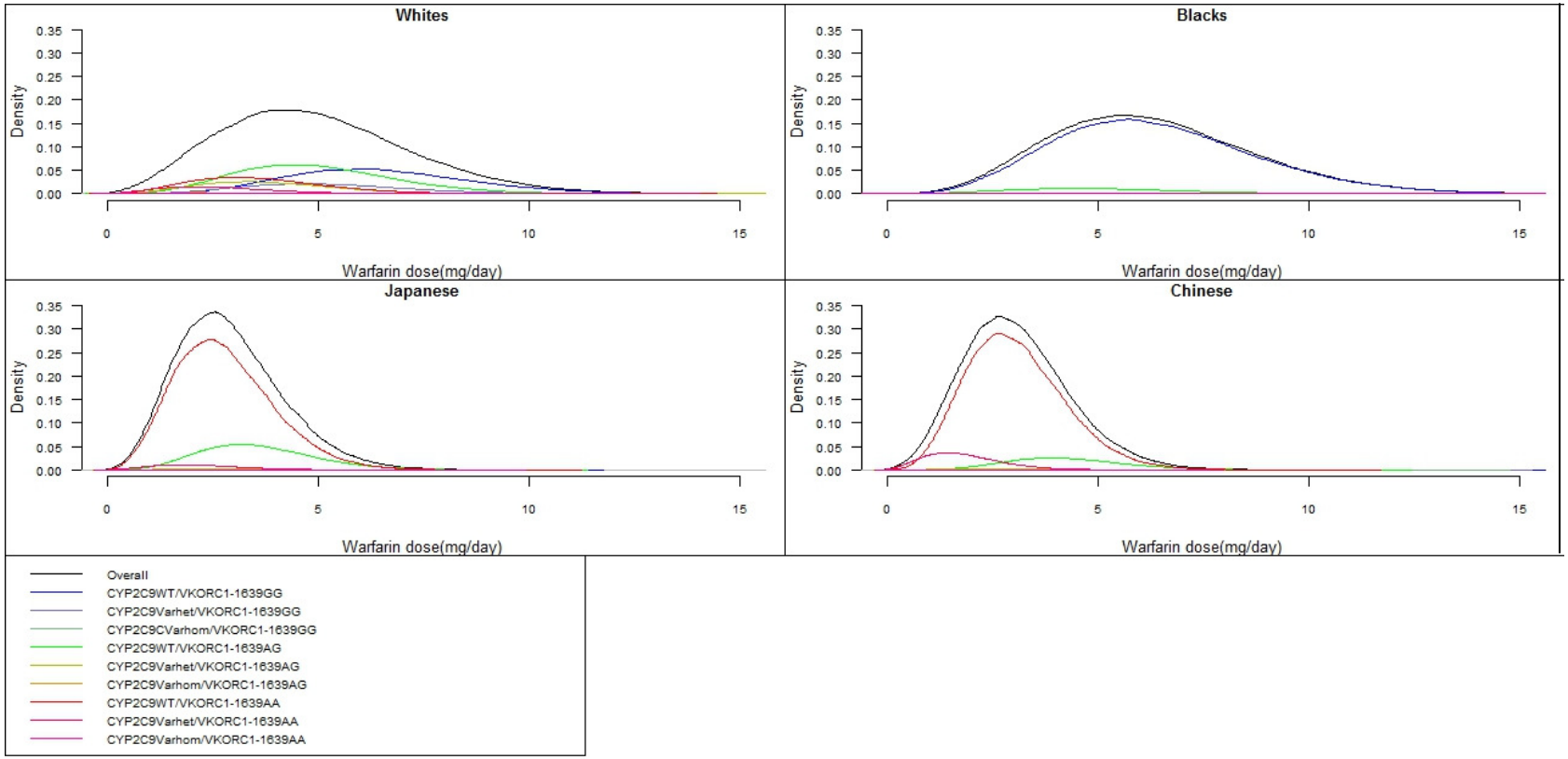
The predominant combination in each race (*CYP2C9*WT/*VKORC1*-1639AG and *CYP2C9*WT/*VKORC1*-1639GG for Whites, *CYP2C9*WT/*VKORC1*-1639GG for Blacks, and *CYP2C9*WT/*VKORC1*-1639AA for Japanese and Chinese) did not necessarily dominate the intermediate dose group (≤3mg/day and ≥7mg/day). Instead, the distributions spread over at least 2 dose groups. The dose group that was deemed

to benefit from WPGT (≤3mg/day and ≥7mg/day) thus did not coincide squarely with the presence of variant alleles.

| Genotype combination* | Proportion of population with dose requirement ≤3mg/day (%) | | | | Proportion of population with dose requirement ≥7mg/day (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Whites | Blacks | Japanese | Chinese | Whites | Blacks | Japanese | Chinese |
| WT/GG | 1.1 | 5.7 | 0.0 | 0.0 | 10.7 | 34.3 | 0.0 | 0.0 |
| Varhet/GG | 1.3 | 0.1 | 0.0 | 0.0 | 1.8 | 0.2 | 0.0 | 0.0 |
| Varhom/GG | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| WT/AG | 4.3 | 1.0 | 6.5 | 1.6 | 4.2 | 1.2 | 0.3 | 0.3 |
| Varhet/AG | 3.6 | 0.0 | 0.4 | 0.5 | 0.5 | 0.0 | 0.0 | 0.0 |
| Varhom/AG | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| WT/AA | 5.4 | 0.0 | 48.2 | 42.6 | 0.5 | 0.0 | 0.2 | 0.2 |
| Varhet/AA | 3.2 | 0.0 | 2.2 | 7.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Varhom/AA | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| All | 19.9 | 6.8 | 57.2 | 51.8 | 17.8 | 35.6 | 0.4 | 0.6 |

*Genotype combinations refer to *CYP2C9/VKORC1* -1639 genotype, where WT = no variant alleles (*CYP2C9 *1/*1*), Varhet = 1 variant allele (*CYP2C9 *1/*2* or **1/*3*), Varhom = 2 variant alleles (*CYP2C9 *2/*2*, **2/*3* or **3/*3*).

**Table 7. Proportion in Dose Extremes by Genotype Combination and Race**



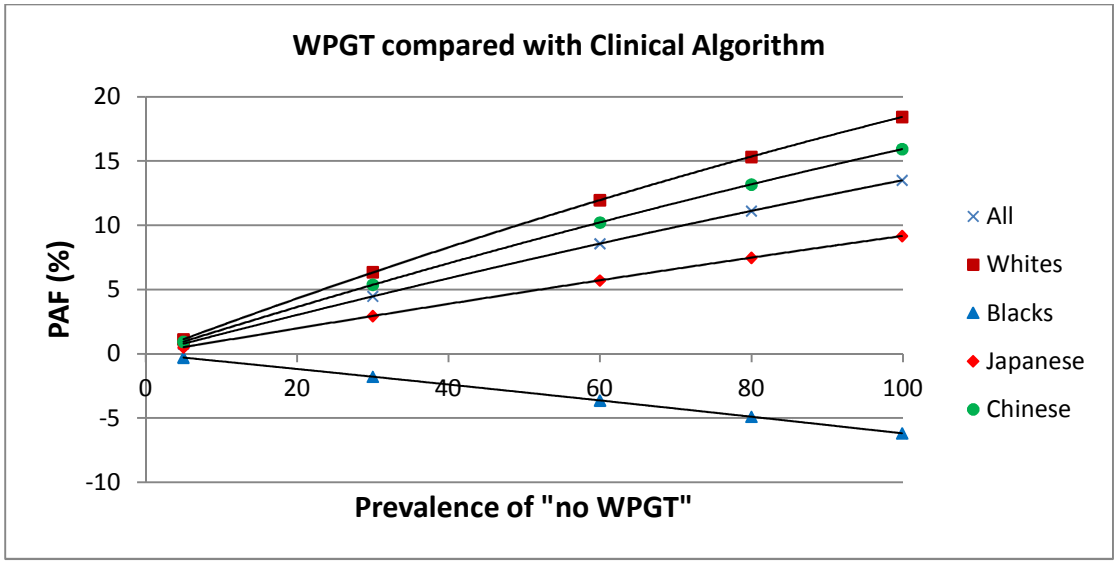

Simulated dose requirements using effect sizes from the IWPC dataset and genotype frequencies from Hapmap (CEU, YRI, JPT, CHS). The sizes of the density plots of individual genotype combinations (colored lines) and overall distribution (black lines) are proportional to their expected frequencies. *CYP2C9*WT = no variant alleles (**1/*1*), *CYP2C9*Varhet = 1 variant allele (**1/*2* or **1/*3*), *CYP2C9*Varhom = 2 variant alleles (**2/*2*, **2/*3* or **3/*3*).

**Figure 8. Warfarin Dose Requirements by Genotype Combinations and Race**

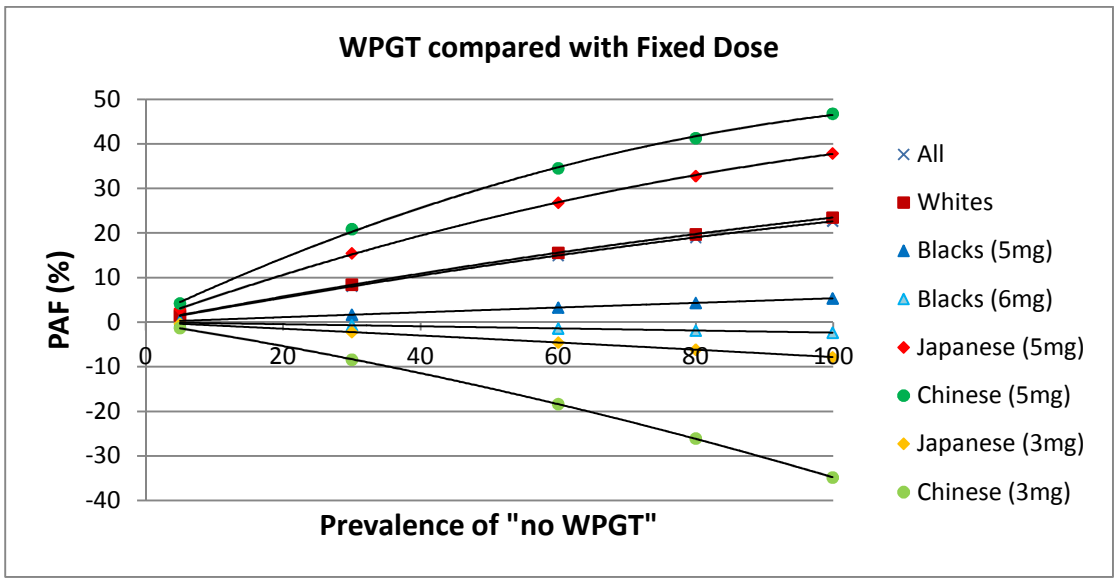
### 5.3.3 PAF

Overall, the addition of genetic information to clinical information could reduce inaccurate dosing by 13.7% (Figure 9a, WPGT vs. clinical algorithm). However, there was differential benefit across the populations. Whites stood to benefit the most, with 18.4% reduction in inaccurate dosing from complete implementation of WPGT, followed by Chinese and Japanese ($PAF_{100\%}$ = 15.9% and 9.2% respectively). Blacks actually did worse with WPGT compared to the clinical algorithm, as manifested by a negative PAF.

Overall, complete implementation of WPGT yielded greatest benefit when compared to the scenario where all patients received a 5mg/day fixed initial dose (Figure 9b, WPGT vs. fixed dose). In this case, overall inaccurate dosing could be reduced by 22.8% but again there were racial differences. Chinese and Japanese now stood to benefit most ($PAF_{100\%}$ = 46.8% and 37.9% respectively), followed by Whites ($PAF_{100\%}$ = 23.5%) then Blacks ($PAF_{100\%}$ = 5.3%) who would only benefit slightly. Since Asians are known to require lower warfarin doses and may often be given a lower fixed starting dose, WPGT was also compared to a 3mg/day fixed dose for Japanese and Chinese. In both populations, patients did worse with WPGT compared to the 3mg/day fixed dose. WPGT was also not better than a 6mg/day fixed dose in Blacks.




a) WPGT compared with Clinical Algorithm
In this scenario, those not receiving WPGT were assumed to be dosed initially using a clinical algorithm. For a population that is not implementing WPGT at all, the PAF corresponding to 100% prevalence is interpreted as the proportional reduction in inaccurate dosing if everyone were dosed using a pharmacogenetic instead of the clinical algorithm. Positive values indicate that WPGT is better than a clinical algorithm while negative values indicate the opposite. The negative values however do not have a meaningful interpretation.




b) WPGT compared with Fixed Dose
In this scenario, those not receiving WPGT were assumed to be dosed initially using a fixed dose of 5mg/day. For Japanese and Chinese, a 3mg/day initial fixed dose regimen, and for Blacks a 6mg/day fixed dose, were also added for comparison. Again, negative PAF values are non-interpretable except for indicating that WPGT is worse than the fixed dose.

**Figure 9. PAF by Prevalence of WPGT and Race**

It was previously demonstrated in the IWPC population that those requiring low (≤3mg/day) or high doses (≥7mg/day) benefit most from WPGT whereas those requiring intermediate doses (>3 to <7mg/day) do not [225]. To further explore how the PAF compared with this finding and the dose simulation findings herein, PAF was also calculated by dose groups. In the whole IWPC population, the impact in the low and high dose groups was the most and least in the intermediate dose group (Table 8). This was expected and consistent with the conclusions of the study by the IWPC [225]. However, when stratified by race, some distinct differences were revealed. In Whites, the overall pattern was still present though those in the intermediate dose range also benefited to some extent. Blacks in the intermediate dose range (which made up 57.5% of the Black population) however, did worse with WPGT. So even though the 35.9% in the high dose group benefited, there was little or no overall impact in this population. For Japanese, only those requiring low doses (57.3% of the Japanese population) benefited substantially while all Chinese benefited, when compared to a clinical algorithm or the 5mg/day fixed dose.

When the PAF values by dose groups (Table 8) were inspected against the simulated dose distributions (Figure 8), there appears to be no correlation between extent of genetic variation and PAF, whether across all doses or within each dose group. For example, in the low dose group, genetic variation increases in the order of Blacks, Japanese/Chinese and Whites but their corresponding PAFs did not increase in the same order. Furthermore, within Blacks alone PAF varies dramatically despite having almost the same amount of genetic variation across the 3 dose groups. This reiterates the unsuitability of using proportions of a population requiring low and high doses, or proportions carrying variant genotype combinations as a measure of the population impact of WPGT.

| Population | Total | Overall | Low dose group (≤3mg/day) | | | Intermediate dose group (>3 to <7mg/day) | | | High dose group (≥7mg/day) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | PAF (%) | n | PAF (%) | Proportion (%) | n | PAF (%) | Proportion (%) | n | PAF (%) | Proportion (%) |
| *WPGT vs. clinical algorithm* | | | | | | | | | | | |
| IWPC | 3252 | 13.7 | 768 | 22.4 | | 1914 | 2.6 | | 570 | 20.2 | |
| Whites | 2305 | 18.4 | 509 | 26.7 | 20.0 | 1414 | 8.9 | 62.0 | 382 | 23.8 | 18.0 |
| Blacks | 627 | -6.2* | 53 | 3.9 | 6.6 | 392 | -22.8* | 57.5 | 182 | 10.4 | 35.9 |
| Japanese | 195 | 9.2 | 130 | 14.2 | 57.3 | 63 | 0 | 42.3 | 2 | 0 | 0.4 |
| Chinese | 125 | 15.9 | 76 | 13.9 | 52.0 | 45 | 18.2 | 47.4 | 4 | 25.0 | 0.6 |
| *WPGT vs. 5mg/day fixed dose* | | | | | | | | | | | |
| IWPC | 3252 | 22.8 | 768 | 35.8 | | 1914 | 3.3 | | 570 | 33.0 | |
| Whites | 2305 | 23.5 | 509 | 33.2 | 20.0 | 1414 | 11.1 | 62.0 | 382 | 30.4 | 18.0 |
| Blacks | 627 | 5.3 | 53 | 5.7 | 6.6 | 392 | -38.6* | 57.5 | 182 | 39.0 | 35.9 |
| Japanese | 195 | 37.9 | 130 | 49.2 | 57.3 | 63 | 7.4 | 42.3 | 2 | 0 | 0.4 |
| Chinese | 125 | 46.8 | 76 | 51.3 | 52.0 | 45 | 37.9 | 47.4 | 4 | 25.0 | 0.6 |
| *WPGT vs. 3mg/day fixed dose* | | | | | | | | | | | |
| Japanese | 195 | -7.8* | 130 | -8.3* | 57.3 | 63 | -7.8* | 42.3 | 2 | 0 | 0.4 |
| Chinese | 125 | -34.8* | 76 | -76.3* | 52.0 | 45 | 0 | 47.4 | 4 | 25.0 | 0.6 |
| *WPGT vs. 6mg/day fixed dose* | | | | | | | | | | | |
| Blacks | 627 | -2.4* | 53 | 5.7 | 6.6 | 392 | -21.3* | 57.5 | 182 | 17.1 | 35.9 |

All PAF values listed are based on 100% no WPGT ($PAF_{100\%}$).

*Negative PAF values have no meaningful interpretation except for implying that WPGT is worse than the comparator.

**Table 8. PAF by Race and Dose Groups**

## 5.4 Discussion

Although the promise of pharmacogenomics is one of personalized medicine, the decision of whether to implement it ultimately relies on a population-based approach. Despite the recognition of the roles of *CYP2C9* and *VKORC1* on warfarin dose variation and the availability of several commercial genotyping platforms testing for these variants, whether or not to routinely genotype all new warfarin patients still remains a debate. The FDA has judiciously stopped short of recommending genetic testing prior to warfarin initiation due to insufficient evidence on its clinical utility and cost-effectiveness. Here, a different perspective to this problem is offered by estimating the potential impact of WPGT in several populations in terms of reduction in inaccurate initial dose.

In this study, the relationships between *CYP2C9* and *VKORC1* genotype combinations and dose requirements were first explored in different races among the IWPC population to examine the consistency and validity of previous methods of estimating WPGT population impact. The dose simulation results revealed much overlap between dose distributions of different genotype combinations and that some would have a substantial proportion falling within the intermediate dose range. The presence of variant *CYP2C9* and/or *VKORC1* alleles thus may not necessarily translate to benefit from WPGT according to the 3 and 7mg threshold. PAF calculations by dose groups also revealed that this dose threshold does not hold for all races. The overall pattern (where those requiring ≤3mg/day and ≥7mg/day benefit) was largely observed only in Whites, who make up the majority of the IWPC population. In most other races, patients in the intermediate dose range either did worse (Blacks) or benefited substantially (Chinese). The proportion of patients

deemed to benefit based on the 2 approaches used earlier thus do not apply across all races.

In trying to estimate the population impact of WPGT, it is not necessary to find race-specific dose thresholds for dividing the population into those who would benefit and those who would not. Moreover, there is little practical use in knowing these dose thresholds. Here the PAF was used as a measure of the population impact of WPGT and different races were found to benefit to different extents.

If WPGT is implemented in Whites, inaccurate dosing would be reduced by about 18% if everyone were currently dosed using a clinical algorithm, and by about 24% if everyone were currently given a 5mg/day fixed dose. For Blacks, WPGT was worse than the clinical algorithm and 6mg/day fixed dose, and only marginally superior to a 5mg/day fixed dose regimen. Moreover, the marginal benefit over the 5mg/day fixed dose was found to be at the expense of worse prediction in more than half of Blacks (Table 8). For Chinese and Japanese, switching from the clinical to the pharmacogenetic algorithm could reduce inaccurate dosing by about 16% and 9% respectively. However, the reduction would be much greater if all Chinese and Japanese were receiving 5mg/day initially ($PAF_{100\%}$ = 47% and 38% respectively). This is not surprising since Chinese and Japanese have lower dose requirements. However, a lower initial fixed dose of 3mg/day actually performed better than WPGT in Chinese and Japanese.

The IWPC has collated genotype and phenotype data from warfarin patients of different ethnicities from study sites in US, UK and Asia and developed a consensus dose prediction algorithm [225]. Differences in the ability of *CYP2C9* and *VKORC1* to explain warfarin dose variability in different races have been previously recognized. In general, pharmacogenetic algorithms perform better in Whites and Asians than in

Blacks [213,222]. When explored within the IWPC population, a similar trend was revealed with *VKORC1*, that it explains a greater proportion of warfarin dose variability in Whites than Blacks, even though the effect size was similar across all 3 racial groups. It was thus postulated that Whites, Blacks and Asians would have similar benefit despite differences in *VKORC1* allele frequencies, which accounted for differences in its contribution to warfarin dose variability in different populations [143]. However, interethnic differences and the lack of benefit from the pharmacogenetic algorithm compared to a fixed dose in Blacks, Japanese and Chinese was not revealed until these populations were analyzed separately here.

The poorer performance of WPGT compared to the clinical algorithm and 6mg/day fixed dose in Blacks, and 3mg/day fixed dose in Japanese and Chinese may be explained by a combination of a suboptimal pharmacogenetic algorithm and different dose distributions. Plots of predicted against actual doses provide clues on the explanation to this somewhat surprising finding (Appendix 7). In all 4 races, low doses tended to be over-predicted while high doses tended to be under-predicted, pointing to a sub-optimal dosing algorithm. Due to the right-shift in dose distribution in Blacks, and left-shift and 'compactness' of the dose distributions in Japanese and Chinese relative to that of Whites, the race-specific fixed doses were able to 'capture' enough of the respective populations to outperform the pharmacogenetic algorithm. The Blacks, Japanese and Chinese all have little genetic variation yet have different dose distributions (Figure 7). The observed dose distributions of the different races were therefore the result of their particular genetic makeup (of *CYP2C9*, *VKORC1*), though not necessarily the *amount* of genetic variation.

The IWPC pharmacogenetic algorithm was used as the representative WPGT here, since it was developed in a multiethnic population and is one of the better

performing algorithms across different races, outperforming even some ethnic specific algorithms [221,222]. Moreover, race-specific models have also been explored in the IWPC population and were found to be inferior to the general model. No details were provided on these race-specific models but it is likely that they were compared to the general model in terms accuracy measures used for model selection (i.e. MAE, % within 1mg/day and $r^2$ on the training set) [225]. If so, a better general model (compared to a race-specific model) does not necessarily translate to better performance compared to a race-specific fixed dose.

Studies that compared the IWPC pharmacogenetic algorithm with clinical and fixed dose regimens and found it to be superior were all performed in Caucasian or mixed (majority Caucasian) populations [134,225,226,228], except for 1 in Japanese [227]. The discrepancy between some of the results here (that races other than Whites do not benefit) and these studies may have been due to the influence of the Caucasians (who indeed do better with a pharmacogenetic algorithm), and methodological differences (for the Japanese study). Takeuchi *et al* [227] in the Japanese study have demonstrated a similar benefit pattern to the IWPC study [225] using different dose thresholds and an accurate dosing definition of within ±1mg/day of the actual dose, which would likely represent more than ±20% actual dose for a Japanese population who needs lower doses than Caucasians. The overall proportion with accurate dosing (according to their definition) with the pharmacogenetic and clinical algorithms were 71.5% and 64% respectively, not as dramatically different as it may appear to be when presented by dose groups. Such comparisons should be carried out in more non-Caucasian populations.

The validity of the PAF estimates depends on the absence of confounders. In the way the PAF was applied in this study, there was actually no confounding at all

since the 'disease' was measured under counterfactual conditions. The estimates are therefore valid as long as other factors affecting warfarin dose such as age, weight, diet, drugs and compliance do not change significantly in the near future. As mentioned in the methods, the PAFs calculated herein are applicable only to those who need warfarin. There is little data of the prevalence of warfarin use but it is estimated to be 1 – 2% in developed countries [1].

The PAF is typically used for exposures that increase disease risk and if the exposure decreases disease risk, PAF becomes negative, which occurred in several instances here. In this case, the negative values have no meaningful interpretation and the prevented fraction (PF) (i.e. fraction of potential total disease load prevented by the protective factor), obtained by reversing the exposure coding, should be used [330,331]. The interpretation of a negative PAF is difficult because it is no longer a true fraction as there would be some additional counterfactual 'cases' (patients with inaccurate dosing) which are not included in the denominator in the calculation of the PAF [332]. However, PAF is related to PF in the expression below [333], so a more negative PAF does correlate with a higher PF even though it is difficult to interpret its absolute value.

$$1 - PF = \frac{1}{1 - PAF}$$

**(Equation 3)**

Overall, the findings support WPGT in Whites but not in Blacks, Japanese and Chinese. However, there are several limitations to this analysis and more studies are needed in Blacks and Asians to confirm these results. Firstly, only the IWPC algorithm was used here and other algorithms may have different performances which will thus affect the PAF. This may explain why the results appear to contradict the results of Study 2, where dose prediction using a pharmacogenetic algorithm is

superior to an ethnic-specific fixed dosing. The IWPC pharmacogenetic algorithm was developed in a largely Caucasian population while the predictive accuracy obtained in Study 2 was based on cross-validation of models trained in the Singaporean Asian population and therefore would have predicted better. Secondly, the numbers of Chinese and Japanese were much smaller in comparison to Whites and Blacks and so the PAF estimates may not be as stable. Thirdly, only the impact of genotyping *CYP2C9* (**2* and **3*) and *VKORC1* was assessed here. Additional genetic factors may further improve dose prediction and thus PAF. For example, some variants associated with WMD that are almost exclusively found in African Americans, such as *CYP2C9 *5*, **6*, **8* and **11* [20,133,136], *CALU* rs339097 [209], *CYP2C9* rs7089580, *VKORC1* -8191 (rs61162043) [334], may improve dose prediction and thus make WPGT useful in this population. Fourthly, other potentially important clinical and genetic factors such as smoking status, alcohol consumption, vitamin K intake, *CYP4F2*, *APOE* and *GGCX*, were not available in the IWPC dataset [225], and thus the impact of WPGT may be over or under-estimated. Lastly, population impact was only measured in terms of initial dose accuracy. These results need to be considered together with evidence of clinical benefit (such as decrease in bleeding or thromboembolism), which are still forthcoming.

This analysis suggests that race is important, at least in informing policy decisions on the implementation of WPGT. Given the genetic diversity, potential benefit as expressed by the PAF and empirical evidence of clinical benefit in the reduction of hospitalization [235] and serious adverse drug reactions (ADR) [240], WPGT can be recommended in Whites. In 2008, the uptake of warfarin genotyping in the US is only 1.7% [335], so there is still much potential for benefit. On the other hand, there is currently insufficient evidence to recommend WPGT in Blacks and

Asians. More studies are needed to validate and compare the performance of different pharmacogenetic algorithms with a 3mg/day fixed dose in Asians to confirm whether genotyping is of benefit.

In conclusion, the PAF was used as a measure of the population impact of WPGT and Whites were found to benefit from genotyping while Blacks, Chinese and Japanese may not.

# CHAPTER 6: ATTITUDES, WILLINGNESS-TO-PAY AND PREFERENCES FOR WARFARIN PHARMACOGENETIC TESTING (STUDY 4)

## 6.1 Introduction

In addition to clinical validity, patient acceptance and economic sustainability are also necessary for successful implementation of PGT in the clinic. In this study, attitudes were measured as willingness to undergo WPGT, perceived benefits of WPGT, and concerns or barriers to taking WPGT. 'Perceived benefits' and 'concerns' are latent variables which were measured using 2 Likert scales, and were meant to measure how much respondents think WPGT can benefit them (after basic information on it has been provided), and their level of concern regarding ethical, social or legal issues pertaining to WPGT respectively.

In view of the inconclusiveness of cost-effectiveness studies on WPGT [38], this study will also use the DCE methodology to elicit the WTP and preferences for WPGT so as to have a grasp of its feasibility from the economic point of view as well as determine what potential patients would value in such a test. The WTP estimates may also potentially be used in a formal CBA. WPGT is still not a routine clinical procedure, so preferences here would pertain to the introduction of the test rather than delivery of the test. These present different scenarios, where different attributes would be relevant [283].

In the context of a CBA, all members of society benefit from a health intervention from a societal perspective. While users of the intervention directly benefit, others benefit from knowing that the intervention is available and from the fact that patients have access to the intervention [273]. Therefore, the general population would provide a societal perspective, allowing a more holistic value estimation of the intervention, and is therefore more relevant for policy making and

resource allocation. Since WPGT is most beneficial when administered to new warfarin patients before dose initiation, it may be argued that the general public is the more appropriate population to study. On the other hand, patients may be more suitable subjects as they are better able to value the benefits of the intervention [336]. Furthermore, FDA has recently issued a guidance on the use of patient-reported outcome instruments to support claims in approved medicinal product labeling, especially those pertaining to patients' symptoms, signs or an aspect of functioning [337], highlighting the importance of studying the patient population as well. In this study, both general public and warfarin patients were surveyed to allow estimation of ex-ante and ex-post WTP respectively. It also allows the exploration of attitudes on WPGT with and without the experience of the process of dose stabilization and counseling on warfarin side effects respectively. Though neither population can be considered ideal, both populations can offer valuable insight into the attitudes and WTP for WPGT of new warfarin patients, who are difficult to recruit. Both populations were also restricted to the Chinese, the predominant race in Singapore due to possible attitude and preference differences among races, and resource constraints.

The previous study (study 3) suggested that WPGT may not benefit the Chinese. However, this is still tentative and the PAF should be further investigated, such as using different pharmacogenetic algorithms and more importantly using clinical outcomes, to come to a more definitive verdict. WPGT thus may still be of benefit in the Chinese, especially if other genetic or non-genetic factors are found and added to the algorithm from future research. Furthermore, the findings of this study can also give some indication of the acceptance of PGT for other drugs. It should also

be mentioned again that given the hypothetical nature of WPGT to the particupants, a gauge of its acceptance was of a greater interest than actual intention or uptake.

The objective of this study was to determine the attitudes, WTP and preferences for WPGT among Singaporean Chinese. One of the specific aims was to estimate the price that new patients requiring warfarin would be willing to pay for WPGT. Uptake rates for 3 hypothetical warfarin pharmacogenetic tests at different prices (price sensitivity) were also estimated. In addition, the following relationships were also hypothesized:

i) Attitudes would be associated with socio-demographic and clinical variables related to warfarin intake in patients (length of warfarin therapy, number of INR tests needed till dose stabilization and history of ADR), or related to experience with drugs in the public (history of ADR and knowledge of friends/family with serious ADR),

ii) Higher perceived benefits would be associated with higher willingness to undergo WPGT,

iii) Higher concerns would be associated with lower willingness to undergo WPGT, and

iv) Preferences would be associated with socio-demographic and clinical variables (as defined above in i)).

## 6.2 Materials and Methods

### 6.2.1 Study Outline

The parts and flow of this study is represented in Figure 10 below, with brief details on the nature or purpose, final sample size and date of each part. Full details are given in the following method section. Pilot 1 formed the groundwork for the development of the main survey, while pilot 2 and pilot 3 served to pre-test and refine

the questionnaire that was developed after pilot 1, before the main surveys in the respective populations were conducted.

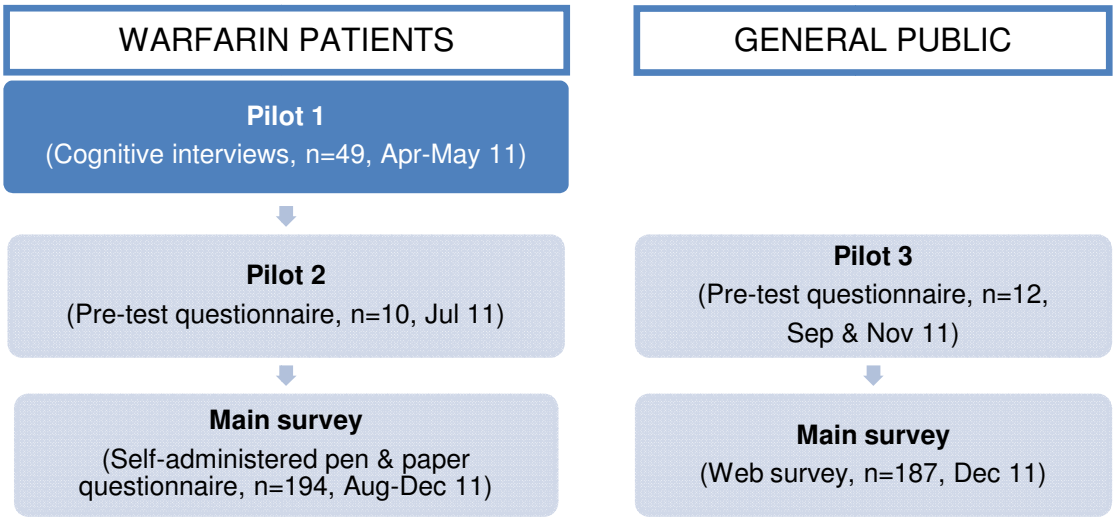



**Figure 10. Study Outline of Study 4**

### 6.2.2 Study Populations

Warfarin Patients

Patients were recruited from the ACCs in NUH between Apr to May 2011 for pilot 1, and between Jul to Dec 2011 for pilot 2 and main survey, using convenience sampling. Random sampling was not feasible due to logistical impracticalities (difficulties in screening and contacting potential subjects, and the need to collect personal particulars to do this) and a limited patient population size (i.e. practically all Chinese warfarin patients in NUH would have to be sampled, allowing for non-response). The inclusion criteria were age $\geq$ 21 years old, self-reported Chinese ethnicity, ability to give informed consent and currently on warfarin therapy. Patients with signs of cognitive function problems, as perceived by the interviewer, were excluded.

Previous studies [16] and personal communications with ACC pharmacists from various hospitals suggested that majority of warfarin patients in Singapore were elderly Chinese or Malay. Due to resource constraints and the likelihood that many Chinese patients cannot speak English (an observation which the ACC pharmacists also assent to), the initial target population (and thus pilot 1 participants) was restricted to only the Mandarin-speaking Chinese warfarin patients. However, a considerable number of English-speaking Chinese were encountered during the course of recruiting patients for pilot 1. Coupled with the finding in pilot 1 that higher education (and thus higher likelihood to be English speaking) was associated with better understanding of WPGT and DCE, English-speaking Chinese were also included in the subsequent phases of the study. Patients who have participated in pilot 1, but not pilot 2, were allowed to participate in the main survey again if they consent to.

Patients completed the main survey using a self-administered pen and paper questionnaire with DCE versions randomized by day (i.e. 1 version per day of recruitment). The study was approved by the hospital's ethics review committee and written informed consent was obtained from all patients. Pilot 1 patients were paid S$5 while pilot 2 and main survey patients were paid S$10 for their participation.

General Public

Pilot 3 participants were National University of Singapore (NUS) staffs (from libraries, administrative offices, bookshops and canteens) who were approached directly in Jul 2011 while main survey participants were recruited via referral from NUS students in Dec 2011. Eligibility criteria were age ≥ 40 years old, self-reported Chinese ethnicity and have never been on warfarin therapy. To avoid potential bias in

the main survey, a limit of 1 participant per household and up to 3 participants referred by each student, was imposed. Participants were required to supply their postal code, house number and email of the referring NUS student to verify this. The main survey was administered via a web survey generated using SSI Web 7.0.22 (Sawtooth Software Inc., Orem, USA), where DCE versions were automatically randomized. A lower age limit of 40 years old was set to avoid getting a disproportionately young sample considering the mode of administration of the main survey. This part of the study was approved by the NUS Institutional Review Board. All participants including those in pilot 3 were reimbursed S$10 for completing the survey.

Sample Size Calculation for Main Surveys

The sample size *n* required for each population was estimated based on requirements for the DCE using Orme's 'rule of thumb' formula

$$\frac{nta}{c} \geq 500$$

**(Equation 4)**

where $t$ = number of tasks/questions, $a$ = number of alternatives per task, $c$ = number of analysis cells (largest number of levels for any 1 attribute if considering main effects only) [281]. Values $t = 6$, $a = 2$ and $c = 4$ were used for calculation, as intended in the design. The DCE design (See DCE under Survey Design for details) was then iteratively run through the Choice Based Conjoint (CBC) Design Efficiency Test in SSI Web 7.0.22 (Sawtooth Software Inc., Orem, USA) using sample sizes in the region estimated by Orme's formula, assuming % choosing 'none' option is 15 – 20%. In the 'Advanced test' of the CBC Design Efficiency Test, the absolute precisions (SEs) of parameter estimates are estimated under conditional logit

estimation using simulated data. A suggested guideline for SEs of main effects is about 0.05 or less [338]. The estimated SEs by the CBC Design Efficiency Test using different sample sizes and % choosing 'none' option are shown in Appendix 8. No formula exists for DCE analysis using Hierarchical Bayes (HB) method (see Econometric Analysis under Statistical Analysis for details), but it does not appear to require greater sample sizes than traditional methods [339]. A sample size of 200 for each population was finalized as a compromise between precision and resource constraints.

### 6.2.3 Initial Pilot Study (Pilot 1)

Specific Aims

Pilot 1 is a semi-qualitative study for collecting relevant information for questionnaire development as well as assessing the feasibility of the study. The specific aims were to

i) determine the effectiveness of WPGT education,

ii) identify views and concerns about WPGT,

iii) identify the most relevant efficacy attribute(s) for the DCE, and

iv) determine the ability to complete the DCE.

Format and Content of Interviews

Individual face to face interviews with patients were conducted in Mandarin, using a semi-structured interview protocol (Appendix 9) with the aid of show cards (Appendix 10). All interviews were conducted by the same interviewer (SL Chan) and lasted approximately half an hour each. Voice recording was also done to aid data

recollection. The target sample size was 30 to 50, in line with recommendations for such pilot studies [340].

Patients were first asked some questions about their warfarin therapy, such as length of therapy, experience on the management process, worries about taking warfarin, length of time taken to stabilize dose, and adverse events. Some of these questions were intended to be possible warm-up questions in the DCE, depending on what attributes were eventually chosen, while others were important background information. Prior knowledge of pharmacogenetics was briefly ascertained and then WPGT explained with the aid of the first 4 show cards (Appendix 10). The first and second cards outlined why management of warfarin is difficult, the current management strategy and briefly described WPGT. The third and fourth show cards listed the potential benefits and risks of WPGT respectively. Post-education understanding of WPGT was then assessed based on the patient's ability to state at least 1 anticipated benefit (after show card 2) or explain it in their own words (after all 4 show cards). As it became apparent soon after the start of the interviews that a substantial proportion had difficulty understanding WPGT, both patient and interviewer ratings of the perceived level of post-education understanding were added to strengthen the evaluation of whether the patient could understand it. These ratings ranged from 1 to 5, with 1 representing no understanding and 5 representing good understanding. Patients rated 4 or 5 by the interviewer were considered able to understand WPGT. Patients were also asked if they would be concerned about any of the associated possible ethical or social risks.

Identification of relevant attributes and their levels is an important part in the design of a DCE. While the original plan was to simply identify the most important and relevant efficacy attribute(s), and verify the chosen WTP range for the DCE, a

decision was made to try out a mock DCE early on in the interviews to ascertain if patients can grasp the concept and complete a sufficient number of choice tasks. This decision was also in light that a sizable proportion might not be able to understand WPGT. Therefore, this data would be important in determining how feasible the main survey would be. In the last part of the interview, the DCE was briefly introduced and the patient asked to choose 1 or 2 of 5 shortlisted possible efficacy attributes. Those who chose 2 attributes were also asked if they thought them related and to rank them in order of importance. These 5 attributes were:

i) Chance of having accurate starting dose,

ii) Time to stable dose,

iii) Number of INR tests until stabilization,

iv) Risk of serious adverse events in the first 3 months, and

v) Risk of hospitalization due to serious adverse events in the first 6 months.

The DCE was then further explained and patients were then requested to complete a mock DCE with 3 attributes (number of INR tests until dose stabilization, risk of serious side effects in the first 3 months and total cost in first 6 months) and 2 alternatives (with or without WPGT). The first few mock DCEs contained 5 choice sets and subsequent ones had 7 in view of the need to include additional choice sets for validity. Details on the development of efficacy attributes and design of this mock DCE are found in Appendix 11.

Data Analysis

The ability to understand WPGT after education was assessed based on the patients' ability to explain it in their own words, state at least 1 potential benefit and interviewer rating of perceived level of understanding by the patient of at least 4. The

2 most important efficacy attributes were chosen based on frequency of the most important attribute chosen by the patients. The second choice by the respondents was not used in the final decision as a substantial proportion only chose 1 attribute. Finally, the ability to understand and complete the DCE was assessed based on the post-DCE evaluation and the ability to complete all the choice sets offered. In the post-DCE evaluation, those who said they had no problems and/or were able to verbalize their though process while doing the exercise, or made comments that indicate their understanding (such as stating other factors that may affect their choice or state which attribute was most important to them while choosing), were deemed to be able to understand the DCE.

Descriptive and bivariate statistical analyses were carried out using Stata/SE 10.0 for Windows (StataCorp LP, College Station, TX).

### 6.2.4 Pilot 2 and 3

A structured questionnaire was constructed based on input from pilot 1 and 10 participants were asked to complete it on paper before being debriefed to find out if they found any part of it problematic or difficult to understand. Questions were also asked to elicit their thought processes while answering some of the questions to determine if they understood them as intended (see debrief questions in Appendix 12). Before the main survey in the general public, pilot 3 was conducted using an almost identical questionnaire (except for background questions on warfarin intake) in 12 members of public in the same manner. The last 4 participants in pilot 3 completed the questionnaire via web survey on a laptop to further identify any technical or formatting problems.

### 6.2.5 Survey Design

Questionnaire Structure and Development

The structured questionnaire was refined based on pilot 2 and 3 findings, which are summarized in Appendix 13. To ensure meaningful responses, basic information on warfarin and WPGT (which was also used in the pilot studies) were provided at appropriate points in the survey and true/false questions were included to ascertain the level of understanding of the information provided. The rationale and structure of the DCE and the definitions of the attributes were then provided, and 2 questions designed to test their understanding of the efficacy attributes were also included before the DCE tasks. After pilot 3, the 4 true/false questions which were meant to test warfarin knowledge were dropped to shorten the survey and also considering that most patients scored well, thus providing little information.

Apart from the attitude questions and DCE, background questions on warfarin intake, demographics, socio-economic status, disease risk perception and self-evaluation survey cognitive burden were also collected. Housing type was used as an approximate proxy for socio-economic status (see Footnote[1]). For the general public, questions on ADR history, knowledge of friends' or relatives' ADR history and perception of value of a new test that could potentially decrease the number of INR tests needed (should they need warfarin) were asked instead of questions on warfarin intake.

The disease risk perception question asked respondents to rate their risk of developing 6 diseases (stroke, heart attack, diabetes, cancer, H1N1 infection and hepatitis B) in their lifetime on a 6 point scale. These 6 conditions are a selection of

[1] Housing typaes in Singapore can be categorized into public (also known as HDB (Housing Development Board)) or private (condominiums or landed), and HDB flats range from 1-room to 5-room or executive types. HDB is the government board providing public housing in Singapore. Housing type is closely correlated with monthly household income, Source: Census 2010 (Available from: http://www.singstat.gov.sg/pubn/popn/c2010sr2/t31-38.pdf)

common chronic diseases as well as commonly known infectious diseases, and were intended to give an indication of general self-perception of disease. Presence of these diseases was also captured under the 'already have it' option. A sizable proportion of patients used the 'don't know' option (which was added because 2 patients in pilot 2 found it taboo to rate one's risk or found it hard to put down a risk), which provided no information on disease risk perception. This question was thus revised ('don't know' option removed, number of response categories reduced to 4 and H1N1 infection and hepatitis B removed) after pilot 3 to further shorten the survey and improve the quality of responses for the public main survey.

For self-evaluation of survey cognitive burden, respondents were asked to rate the ease of understanding the instructions, level of concentration needed and how offensive they found the survey contents on an 11-point scale. However, it was noticed that some patients (during patient main survey) have misread the scale labels and/or gave responses that were inconsistent with the interviewer's perception of their understanding of the questionnaire (based on the need for explanation). These scales were subsequently dropped for the public survey (after pilot 3) and replaced with 1 question to ask if any difficulty was encountered with the DCE instead.

The questionnaire consisted of 5 sections: information on warfarin, DCE, information on WPGT, attitudes towards WPGT and demographics, background information and quality of survey. Questionnaires for both populations were also available in Mandarin and translation was checked by a second person. Representative questionnaires for warfarin patients and the general public are given in Appendix 14 and Appendix 15 respectively.

Attitudes

Respondents' willingness to undergo WPGT was captured on a 5-point scale. A supplementary question was also included to capture possible reasons for being 'very unwilling' to undergo WPGT.

Perceived benefits and concerns of WPGT were 2 separate concepts and were measured using several items each to capture their relevant dimensions. The items chosen to represent each concept were adapted from previous studies [254,260] or self-constructed, and were refined through pilot 2. Items for the 2 scales were interspersed and participants were asked to express their agreement to the statements on a 5-point Likert scale (Section 4 Question 2, Appendix 14 & Appendix 15).

DCE

Based on pilot 1 results, 2 efficacy attributes most relevant to patients were selected. The levels of these 2 efficacy attributes cover the range of values reported in prospective clinical trials comparing WPGT and standard management [230,231,233] (see Appendix 11 for development of these attributes). Instead of an alternative-specific design as presented in the mock DCE in pilot 1 (show cards 8 – 16, Appendix 10), the nature of test was incorporated as an attribute to capture possible preferences for the genetic nature of WPGT and to overcome design efficiency issues with an alternative specific design. WPGT is not yet available in clinical practice in Singapore but reported costs of genotyping *CYP2C9* and *VKORC1* in the US range from US$200-600 (S$260 – 780, based on Dec 2010 exchange rates) [267,341-343]. With decreasing costs of genotyping, the price of WPGT when it eventually becomes available in Singapore is expected to fall within the range of S$100 – S$600. The 2

efficacy attributes, together with nature of test and cost, describe WPGT. The 4 attributes and their levels are shown in Table 9 below.

| Attribute | Levels |
|---|---|
| Nature of test | Genetic, Non-genetic |
| Number of INR tests needed till dose stabilization | 5, 13, 21 |
| Risk of serious side effects (major bleeding or clotting) | 1%, 5%, 9% per year |
| Cost of test | $100, $225, $375, $600 |

**Table 9. DCE Attributes and Levels**

The DCE design was generated using SSI Web 7.0.22 (Sawtooth Software Inc., Orem, USA). Each choice set contained 2 alternatives (hypothetical tests) where respondents were asked to choose the one they preferred. Following the completion of each choice set, respondents would be asked if they would actually take the test they had chosen. This is a technique known as 'dual-response none', which guards against information loss (thus preserving efficiency and power) while retaining market realism [344]. As a balance between the cognitive burden on respondents (as assessed in the initial pilot study), statistical efficiency and practicalities of administering a pen and paper survey (among patients), the final design contained 4 versions with 6 random choice sets each. Up to 15 versions of 6 random choice sets were first generated and then 4 with the least number of dominant choice sets were chosen and tested for efficiency using the CBC Design Efficiency Test in SSI Web 7.0.22. The final design and design efficiency test results are given in Appendix 8. Two fixed choice sets (not used for model estimation) were also added to all 4 versions as holdout tasks for assessment of predictive ability of the estimated model. The holdout tasks were placed at the fifth and seventh positions in all versions for both populations. All respondents had to complete a total of 8 choice sets.

### 6.2.6 Statistical Analysis

Transformation of Questionnaire Variables

Knowledge scores were calculated for DCE attributes pertaining to WPGT efficacy (Section 2 questions 5 & 6, Appendix 14 & Appendix 15) and WPGT (Section 2 question 1, Appendix 14 & Appendix 15) by taking the number of questions correctly answered respectively. These scores were used as an indicator of survey quality since the self evaluation of survey cognitive burden was dropped in the patient population and removed for the public. Due to a sizable proportion of patients having missing disease risk perception information and a different scale being used in the public, this was also dropped from the analyses. Presence of the 4 chronic diseases (as indicated under 'already have it') was used instead.

The following demographic and clinical variables were collapsed for analysis due to the small numbers in some cells.

i) Marital status:

- Divorced/separated + widowed

ii) Educational status:

- No qualification/lower primary + primary
- Secondary + upper secondary
- Diploma + degree

iii) Housing type:

- 1-2 room HDB + 3-room HDB
- 4-room HDB + 5-room HDB or executive
- Private condominium + landed

iv) Length of warfarin therapy (Section 2 question 1, Appendix 14):

- ≤1 week + >1 week to <3 months

- 3 to 6 months + 7 to 12 months
- >1 to 3 years + >3 years

v) Number of INR tests needed till stabilization (Section 2 question 3, Appendix 14):

- <5 + 5 to 9
- 10 to 14 + 15 to 20 + ≥21

All analyses were performed using Sawtooth Software or Stata.

Attitudes

Univariate associations between willingness to undergo WPGT and respondent characteristics were explored using the Fisher's exact test or one-way ANOVA, where appropriate. Perceived benefits and concerns about WPGT were measured on 2 Likert scales, so Cronbach's alpha was first used to assess their internal consistencies. Item analysis was also performed to identify and eliminate any problematic items. Perceived benefits and concerns about WPGT were expressed as summary scores from the Likert items designed to measure each concept, calculated as the average score on the 5 point scales. Higher scores indicated higher perceived benefits or higher concerns respectively. The scores were assumed to be on a continuous scale and univariate associations between perceived benefits and concerns with respondent characteristics were analyzed using the student's t-test, one-way ANOVA or Pearson's correlation, as appropriate. A p-value of $<0.05$ was considered statistically significant.

Econometric Analysis

Individual utility coefficients were estimated from DCE data using HB in CBC/HB Version 5.2.8 (Sawtooth Software Inc., Orem, USA). HB estimates

individual utilities from an optimal mix of an upper level model, or prior (which assumes a multivariate normal distribution), and a lower level model, or posterior (which assumes a multinomial logit model for individual utilities) iteratively using a Gibbs sampler, a Monte Carlo Markov Chain algorithm. More details are provided in the Sawtooth Software manual [344]. All attributes except nature of test were specified as linear. Validity of the HB estimates was verified using predictive accuracy on the 2 holdout tasks.

Using the individual HB utilities, 3 welfare measures were calculated: i) marginal WTPs (mWTPs), ii) relative attribute importances and iii) WTP for 3 hypothetical WPGTs. mWTPs were calculated using the following formula [345]:

$$mWTP_i = \frac{\beta_i}{\beta_p}$$

**(Equation 5)**

where $\beta_i$ is the estimated coefficient on attribute *i* and $\beta_p$ is the coefficient for price. Relative importance scores for each attribute were calculated by expressing the range of each attribute's utility as a proportion of the sum of the utility ranges of all attributes [281]:

$$IS_n = \frac{r(X_n)}{\sum_{i=1}^{N} r(X_i)}$$

**(Equation 6)**

where $IS_n$ is the importance score of the nth attribute ($X_n$) among N attributes, and $r(X_n)$ is the utility range. As the actual clinical benefit of WPGT over no WPGT is still uncertain, 3 hypothetical scenarios representing the possible benefits of WPGT were constructed (presented as different combinations of attributes) and the WTP-values for them calculated using the following formula [345,346]:

$$WTP = \sum_i \frac{\beta_i}{-\beta_p}(\Delta X_i)$$

**(Equation 7)**

mWTPs are for single attributes while WTPs are for entire goods or services, in this case the hypothetical WPGTs, which are composed of 4 attributes.

Uptake rates at different prices (price sensitivity) for these 3 hypothetical WPGTs were also simulated in SMRT 4.20.2 (Sawtooth Software Inc., Orem, USA), using the 'shares of preference' simulation method [347].

Summary statistics of mWTPs, attribute importances and WTPs were presented as medians with their 95% confidence intervals (CI). The 95% CIs were obtained by bootstrapping using 1000 replications using the bias correction method to account for the skewed distributions, as described in Haukoos and Lewis (2005) [348].

To explore the association between preferences and socio-demographic and clinical characteristics, hierarchical multiple linear regression was performed with attribute importances (calculated using (Equation 6) as the dependent variable. The first level of independent variables in the model consists of socio-demographic variables, followed by clinical variables in the second level. A p-value of ≤0.05 is considered statistically significant.

## 6.3 Results

### 6.3.1 Pilot 1

Patient Characteristics

Of 174 patients approached, 43 were ineligible (28 cannot speak Mandarin and 15 were deemed to have poor cognitive function), 81 refused to participate and 50 agreed to participate, giving a response rate of 38.2%. Of those who refused participation, 47 (58%) were males. One patient had to be dropped from the analysis

as she was thought to have poor cognitive function and was not giving meaningful answers during the interview, resulting in a final sample size of 49. The patient characteristics are summarized in Table 10.

| Patient characteristics | n (%) |
|---|---|
| Age, median (range) | 58 (22 – 82) |
| Gender | |
| Female | 14 (28.6) |
| Male | 35 (71.4) |
| Highest educational level attained | |
| No formal education or lower Primary | 7 (14.3) |
| Completed Primary | 13 (26.5) |
| Completed Secondary | 16 (32.7) |
| Completed 'A' levels | 4 (8.2) |
| Diploma | 5 (10.2) |
| Degree or higher | 4 (8.2) |
| Housing type | |
| 3-room HDB | 6 (12.5) |
| 4-room HDB | 16 (33.3) |
| 5-room HDB | 15 (31.3) |
| Private condominium or landed | 9 (18.8) |
| Others | 2 (4.2) |
| Length of warfarin therapy | |
| <3 months | 8 (16.3) |
| 3 to 6 months | 7 (14.3) |
| 7 to 12 months | 1 (2.0) |
| >1 to 3 years | 9 (18.4) |
| >3 years | 24 (50.0) |
| History of serious adverse events | |
| Bleeding* | 8 (16.3) |
| Clotting | 0 |
| Time taken to stabilize dose† | |
| <4 weeks | 6 (12.5) |
| 1 to 2 months | 8 (16.7) |
| >2 months | 11 (22.9) |
| Don't know | 16 (33.3) |
| Not stable yet | 7 (14.6) |
| Participating in WPGT clinical trial | 3 (6.1) |
| Taken any genetic test in the past | 1 (2.0) |

* Based on patient's self report. If bleeding occurred in a critical organ or required medical intervention it was considered serious. This mirrors closely the definition of major bleeding in McMillin *et al* [233].

† Stable dose was defined as the time when 2 consecutive INR readings at least 2 weeks apart were within therapeutic range and when there were no dose changes. Based on patient's recall or verification against their anticoagulation record card, where available.

**Table 10. Pilot 1 Patient Characteristics**

Experience and Feelings on Warfarin Management

Most patients have been taking warfarin for a while and have since adapted to the demands of being on warfarin therapy. Many still found it troublesome to have to do INR tests and see the pharmacist regularly and the degree of this feeling varied quite widely, depending on their frequency of review and work commitments. Other hassles included having to watch their diet and other drugs they take, and having to take the necessary precautions before dental procedures. Despite these inconveniences, most patients recognized the importance of these monitoring and restrictions. Some expressed that they would trade inconvenience for the assurance of good INR control. On the other spectrum, a few patients were rather non-compliant to ACC reviews and diet restrictions as they haven't felt that doing so have adversely affected them or simply found it too troublesome.

Most patients also did not remember or have any extraordinary experience during the initiation period, except 1 who had purple toe syndrome and another who remarked that daily INR tests and injections were a torture. Frequent INR monitoring was not mentioned until prompted by the interviewer, except by 4 patients. Some mentioned having to watch diet and remember to take their medicines initially, suggesting that the lifestyle adjustments in the initial period affected patients more than the inconveniences of frequent INR monitoring.

Eighteen patients (37%) said they had no worries taking warfarin. Of the remaining 31 patients, most were worried about hurting themselves and bleeding. Those who had no worries tended to be older than those who did, and surprisingly half of those who had a bleeding episode before still did not worry.

Understanding and Concerns about WPGT

Patients' prior knowledge of pharmacogenetics was briefly ascertained by asking if they have heard of it and to briefly explain what they understood by it if they had. Only 3 patients had heard of pharmacogenetics and 2 of them gave a correct explanation. 28 patients were able to either state at least 1 anticipated benefit and/or explain WPGT. Overall, 65% were deemed to be able to understand WPGT. Those who were able to understand WPGT were younger (mean age: 52.1 vs. 64.8 years, t-test $p = 0.0029$) and more educated ($\chi^2$ $p = 0.007$). Those who did not know their time to stable dose also tended not to understand WPGT, compared to those who were able to state a duration ($\chi^2$ $p = 0.002$). Asking patients to state anticipated benefits after show card 2 (Appendix 10) was also intended as a way to assess if patients were hopeful that WPGT would benefit new patients should it become available. Interestingly, 1 patient thought it would not be of significant benefit as other factors like diet would still affect the dose.

Three patients (6.3%) had some concerns about WPGT prior to being shown the possible risks in show card 4. One patient was concerned about what was actually tested (i.e. whether other unintended tests would be done using the DNA) and another stated anxiety as a concern. The third patient could not verbalize her exact concerns but was opposed to genetic testing. After being shown the possible risks of WPGT, 7 patients (14.6%) said they would be concerned about at least 1 of the risks. The 3 patients who expressed prior concerns were among these 7. The most commonly cited concerns were the risk of other disease risks being revealed from WPGT results in the future, and being labeled, which can affect self-perception and cause anxiety.

Most Important Efficacy Attributes

Forty-three patients were asked to choose 1 or 2 from 5 shortlisted possible efficacy attributes which they felt was more important or relevant. Five patients were asked only if the 2 mock DCE efficacy attributes were important and to rank them. One patient terminated the interview before reaching this part. Thirty-one patients (72.1%) chose 2 attributes but most of them (77.4%) felt that the 2 attributes they chose were related. While the number of INR tests till stabilization was the most commonly chosen attribute (Table 11), all 5 attributes were important as they were all chosen with somewhat comparable frequency. This is not particularly surprising as all 5 are inherently related and many patients indeed had a hard time choosing 1 or 2. INR tests and ACC reviews were a bane to many patients, thus attribute 3 was most relevant and tangible. While many patients were also concerned about ADR, as revealed in earlier parts of the interview and in their choice of attributes 4 or 5, some appeared to have difficulty grasping the concept of risk. The time frame (3 months and 6 months for attributes 4 and 5 respectively) used to define the cumulative risk caused confusion in some patients. Some other patients did not chose attributes 4 or 5 saying that "the risk will still be there… can't do anything about it". Attribute 1 was relatively more difficult to explicate. Several patients could not understand it and more explanation was required for others to understand it.

| Most important attribute* | Second most important attribute*, n(%) | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Nil | |
| 1 | 0 | 1 (2.3) | 0 | 0 | 0 | 2 (4.7) | 3 (7.0) |
| 2 | 1 (2.3) | 0 | 1 (2.3) | 0 | 1 (2.3) | 1 (2.3) | 4 (9.3) |
| 3 | 2 (4.7) | 3 (7.0) | 0 | 1 (2.3) | 4 (9.3) | 6 (14.0) | 16 (37.2) |
| 4 | 4 (9.3) | 4 (9.3) | 1 (2.3) | 0 | 1 (2.3) | 1 (2.3) | 11 (25.6) |
| 5 | 0 | 1 (2.3) | 1 (2.3) | 5 (11.6) | 0 | 2 (4.7) | 9 (20.9) |
| Total | 7 (16.3) | 9 (20.9) | 3 (7.0) | 6 (14.0) | 6 (14.0) | 12 (27.9) | 43 (100) |

*Attribute 1: Chance of having accurate starting dose, 2: Time to stable dose, 3: Number of INR tests till stabilization, 4: Risk of serious ADR in first 3 months, 5: Risk of hospitalization due to serious ADR in first 6 months.

**Table 11. Combinations of Efficacy Attributes Chosen by Pilot 1 Patients**

Understanding of DCE

The mock DCE was conducted on 44 patients. Five of them attempted 5 choice sets and 39 attempted 7 choice sets. Overall 30 patients (68.2%) were able to understand it, and most could handle all the choice sets given to them. Unsurprisingly, a large majority (93.3%) of those who could understand WPGT also understood the DCE. Interestingly, 9 patients also commented that a different payment mode, particularly the ability to use Medisave (see Footnote[2]), would affect their choice.

### 6.3.2 Main Survey

Study Populations

Out of 580 warfarin patients approached, 413 met the eligibility criteria but only 222 of them consented to do the survey, giving a response rate of 53.8%. Nineteen subsequently withdrew after finding the questionnaire too difficult or tedious and 3 responses were found to be completed by the same individuals. The second of these 3 duplicate responses were therefore deleted. After removing another 6 cases with missing data, 194 warfarin patients remained for analysis. There were more males among the valid respondents (73.7%) compared to those who refused (56.5%), so the sample may not be representative of the general warfarin patient population. Forty-one warfarin patients (21.1%) needed substantial assistance in completing the questionnaire.

For the general public, 224 logged into the web survey but 17 did not meet eligibility criteria and 20 did not complete the survey. A total of 187 respondents

[2] Medisave is a national medical savings scheme in Singapore which helps individuals put aside part of their income into their Medisave Accounts to meet their future personal or immediate family's hospitalization, day surgery and certain outpatient expenses. Under the scheme, every employee contributes 6.5-9% (depending on age group) of his monthly salary to a personal Medisave account. (source: http://www.moh.gov.sg/mohcorp/hcfinancing.aspx?id=304)

completed the survey and no information could be captured on non-respondents. Characteristics of both patient populations are summarized in Table 12.

| | Patients (n = 194) | Public (n = 187) |
|---|---|---|
| Questionnaire completed in English, n(%) | 117 (60.3%) | 159 (85.0%) |
| Age, mean (SD) | 57.3 (13.8) | 52.5 (5.2) |
| Male gender, n(%) | 143 (73.7%) | 51 (27.3%) |
| Religion, n(%) | | |
| Christianity | 55 (28.4%) | 53 (28.3%) |
| Buddhism | 80 (41.2%) | 76 (40.6%) |
| Taoism | 15 (7.7%) | 14 (7.5%) |
| Free thinker | 43 (22.2%) | 44 (23.5%) |
| Others | 1 (0.5%) | 0 |
| Marital Status, n(%) | | |
| Single | 24 (12.4%) | 11 (5.9%) |
| Married | 150 (77.3%) | 159 (85.0%) |
| Divorced / Separated / Widowed | 20 (10.3%) | 17 (9.1%) |
| Highest educational Status, n(%) | | |
| PSLE | 45 (23.2%) | 22 (11.8%) |
| GCE 'O' or 'A' levels | 94 (48.5%) | 87 (46.5%) |
| Diploma / Degree | 55 (28.4%) | 78 (41.7%) |
| Housing type, n(%) | | |
| 1 – 3 room HDB | 49 (25.3%) | 19 (10.2%) |
| 4 – 5 room HDB | 118 (60.8%) | 116 (62.0%) |
| Condominium/landed | 27 (13.9%) | 52 (27.8%) |
| Participated in WPGT clinical trial, n(%) | 13 (6.7%) | NA |
| Taken genetic test in the past, n(%) | 5 (2.6%) | 3 (1.6%) |
| Number of chronic diseases, n(%) | | |
| 0 | 127 (65.5%) | 172 (92.0%) |
| 1 | 44 (22.7%) | 14 (7.5%) |
| 2 | 17 (8.8%) | 0 |
| 3 | 6 (3.1%) | 0 |
| 4 | 0 | 1 (0.5%) |
| Length of warfarin treatment, n(%) | | |
| Up to 3 months | 29 (15.0%) | NA |
| 3 to 12 months | 32 (16.5%) | |
| >1 year | 124 (63.9%) | |
| Don't know | 9 (4.6%) | |
| Number of INR tests needed till stabilization, n(%) | | |
| ≤9 | 96 (49.5%) | NA |
| ≥10 | 38 (19.6%) | |
| Don't know | 60 (30.9%) | |
| History of ADR, n(%) | | |
| Yes | 52 (26.8%) | 25 (13.4%) |
| No | 124 (63.9%) | 135 (72.2%) |
| Don't know | 18 (9.3%) | 27 (14.4%) |
| Know of friends/relatives with history of ADR, n(%) | NA | 56 (30.0%) |
| Have friends/relatives taking warfarin, n(%) | NA | 25 (13.4%) |
| DCE attribute knowledge score, mean (SD)* | 1.40 (0.71) | 1.58 (0.67) |
| WPGT knowledge score, mean (SD)** | 3.70 (0.62) | 3.65 (0.56) |

PSLE: Primary School Leaving Examination (the qualification of Primary education in Singapore), GCE 'O' or 'A' levels: General Certificate of Education 'Ordinary' or 'Advanced' levels (academic qualifications in the Commonwealth countries including Singapore), HDB: Housing Development Board, NA: not applicable

* DCE attribute knowledge score ranges from 0 to 2.

** WPGT knowledge score ranges from 0 to 4.

**Table 12. Characteristics of Main Survey Populations**

Willingness to Undergo WPGT

About 38% of patients and 60% of the public indicated that they were 'somewhat willing' or 'very willing' to undergo WPGT (Table 13). A greater proportion of patients were neutral (46.4%) compared to the public (30.0%). Of the 10 patients and 3 public respondents who indicated 'very unwilling', the top reason indicated was cost. Four also indicated being uncomfortable with a genetic test and 3 did not think it would benefit them. Overall, relatively few respondents (10% of public and 16% of patients) were unwilling to undergo WPGT.

Willingness to undergo WPGT was significantly associated with gender, educational status, length of warfarin treatment and number of chronic diseases present in warfarin patients (Table 13). Willingness was higher in males, the better educated and those with more chronic diseases. The trend with length of treatment appeared U-shaped; those who had been on warfarin for less than 3 months or more than a year were more willing to undergo WPGT, compared to those who were on warfarin for between 3 months and 1 year. In the public, willingness was associated with history of ADR and number of chronic diseases present. Those with no ADR history were more willing to undergo WPGT while those who were not sure tended to be neutral.

Perceived Benefits and Concerns about WPGT

The perceived benefits and concern scales in both populations had acceptable internal consistencies, with Cronbach's alpha >0.7 for all of them (Table 14). The 4 items on the perceived benefits scale were administered to warfarin patients but item analysis revealed item (ii) to be problematic. Cronbach's alpha for the perceived benefits scale before removing that item was 0.60, and 0.76 when the item was

removed. The direction of item (ii) was consequently reversed, and item (iii) dropped (due to similarity with item (i)) in the public survey. Both warfarin patients and the public have relatively high perceived benefits (mean scores 3.77 and 3.97 respectively) and moderately high concerns (mean scores 3.30 and 3.33 respectively) about WPGT (Table 13). Since the perceived benefits and concern scores are actually ordinal scale data, their cumulative frequencies are also shown in Figure 11 and Figure 12 respectively to show their medians and distributions as well.

Perceived benefits of benefits from WPGT was not significantly associated with any socio-demographic or clinical variables in patients but was associated with housing type and the value placed in a new test that may potentially decrease the number of INR tests needed in the public population. Those with higher socioeconomic status (as measured by housing type) and those who placed more value in the new test had higher perceived benefits. Concern score again was not significantly associated with background variables in patients, but was associated with housing type and educational status in the public. Higher socioeconomic status and educational status were associated with decreased level of concern about WPGT in the public.

| Characteristics | Willingness to undergo WPGT, n(%*) | | | | | | Perceived benefits[†] | | Concern[†] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | VU | SU | N | SW | VW | p-value | Score, mean (SD) | p-value | Score, mean (SD) | p-value |
| ***WARFARIN PATIENTS*** | 10(5.2) | 21(10.8) | 90(46.4) | 50(25.8) | 23(11.9) | | 3.77 (0.63) | | 3.30 (0.69) | |
| Age, mean (SD) | 62.7(10.8) | 57.9(12.9) | 56.9(14.0) | 56.2(15.8) | 58.5(10.4) | 0.715 | -0.101[††] | 0.163 | 0.066[††] | 0.364 |
| Gender | | | | | | | | | | |
| Male | 6(4.2) | 10(7.0) | 69(48.3) | 38(26.6) | 20(14.0) | **0.032** | 3.80(0.62) | 0.210 | 3.29(0.68) | 0.823 |
| Female | 4(7.8) | 11(21.6) | 21(41.2) | 12(23.5) | 3(5.9) | | 3.67(0.65) | | 3.32(0.72) | |
| Religion | | | | | | | | | | |
| Christianity | 1(1.8) | 6(10.9) | 24(43.6) | 17(30.9) | 7(12.7) | 0.243 | 3.85(0.79) | 0.730 | 3.16(0.88) | 0.349 |
| Buddhism | 4(5.0) | 10(12.5) | 42(52.5) | 13(16.3) | 11(13.8) | | 3.75(0.49) | | 3.34(0.63) | |
| Taoism | 1(6.7) | 3(20.0) | 5(33.3) | 4(26.7) | 2(13.3) | | 3.73(0.40) | | 3.40(0.61) | |
| Free thinker | 4(9.3) | 2(4.7) | 19(44.2) | 16(37.2) | 2(4.7) | | 3.71(0.70) | | 3.36(0.53) | |
| Others[‡] | 0 | 0 | 0 | 0 | 1(100) | | 4.33(0) | | 3.00(0) | |
| Marital status | | | | | | | | | | |
| Single | 1(4.2) | 3(12.5) | 10(41.7) | 8(33.3) | 2(8.3) | 0.844 | 3.83(0.54) | 0.361 | 3.33(0.59) | 0.720 |
| Married | 8(5.3) | 15(10.0) | 73(48.7) | 35(23.3) | 19(12.7) | | 3.74(0.65) | | 3.28(0.70) | |
| Divorced/Widowed | 1(5.0) | 3(15.0) | 7(35.0) | 7(35.0) | 2(10.0) | | 3.93(0.53) | | 3.41(0.70) | |
| Highest educational status | | | | | | | | | | |
| PSLE | 3(6.7) | 13(28.9) | 14(31.1) | 7(15.6) | 8(17.8) | **0.001** | 3.61(0.59) | 0.110 | 3.30(0.58) | 0.885 |
| GCE ‘O’ or ‘A’ levels | 5(5.3) | 6(6.4) | 49(52.1) | 22(23.4) | 12(12.8) | | 3.78(0.72) | | 3.28(0.76) | |
| Diploma / Degree | 2(3.6) | 2(3.6) | 27(49.1) | 21(38.2) | 3(5.5) | | 3.88(0.45) | | 3.33(0.65) | |
| Housing | | | | | | | | | | |
| 1 – 3 room HDB | 2(4.1) | 6(12.2) | 25(51.0) | 10(20.4) | 6(12.2) | 0.709 | 3.63(0.65) | 0.187 | 3.33(0.62) | 0.916 |
| 4 – 5 room HDB | 6(5.1) | 14(11.9) | 51(43.2) | 31(26.3) | 16(13.6) | | 3.82(0.64) | | 3.29(0.71) | |
| Condominium/landed | 2(7.4) | 1(3.7) | 14(51.9) | 9(33.3) | 1(3.7) | | 3.81(0.53) | | 3.28(0.72) | |
| Participated in WPGT clinical trial | | | | | | | | | | |
| Yes | 0 | 0 | 8(61.5) | 2(15.4) | 3(23.1) | 0.359 | 3.46(1.14) | 0.068 | 3.00(0.95) | 0.103 |
| No | 10(5.5) | 21(11.6) | 82(45.3) | 48(26.5) | 20(11.1) | | 3.79(0.57) | | 3.32(0.66) | |
| Taken genetic test in the past | | | | | | | | | | |
| Yes | 0 | 0 | 4(80.0) | 0 | 1(20.0) | 0.576 | 3.93(0.72) | 0.552 | 3.00(0.91) | 0.329 |
| No | 10(5.3) | 21(11.1) | 86(45.5) | 50(26.5) | 22(11.6) | | 3.76(0.63) | | 3.30(0.68) | |

*Table 13 continued*

| Characteristics | Willingness to undergo WPGT, n(%*) | | | | | | Perceived benefits† | | Concern† | |
|---|---|---|---|---|---|---|---|---|---|---|
| | VU | SU | N | SW | VW | p-value | Score, mean (SD) | p-value | Score, mean (SD) | p-value |
| Length of treatment | | | | | | | | | | |
| Up to 3 months | 0 | 2(6.9) | 12(41.4) | 10(34.5) | 5(17.2) | **0.033** | 3.82(0.73) | 0.740 | 3.24(0.79) | 0.717 |
| 3 to 12 months | 1(3.1) | 1(3.1) | 22(68.8) | 7(21.9) | 1(3.1) | | 3.69(0.76) | | 3.28(0.65) | |
| >1 year | 8(6.5) | 17(13.7) | 54(43.6) | 32(25.8) | 13(10.5) | | 3.77(0.56) | | 3.33(0.67) | |
| Don't know | 1(11.1) | 1(11.1) | 2(22.2) | 1(11.1) | 4(44.4) | | 3.93(0.72) | | 3.08(0.77) | |
| Number of INR tests needed till stabilization | | | | | | | | | | |
| ≤9 | 4(4.2) | 11(11.5) | 41(42.7) | 33(34.4) | 7(7.3) | 0.114 | 3.82(0.54) | 0.312 | 3.38(0.68) | 0.187 |
| ≥10 | 2(5.3) | 2(5.3) | 21(55.3) | 8(21.1) | 5(13.2) | | 3.79(0.58) | | 3.15(0.58) | |
| Don't know | 4(6.7) | 8(13.3) | 28(46.7) | 9(15.0) | 11(18.3) | | 3.67(0.76) | | 3.26(0.75) | |
| History of ADR | | | | | | | | | | |
| Yes | 0 | 7(13.5) | 25(48.1) | 14(26.9) | 6(11.5) | 0.530 | 3.87(0.51) | 0.316 | 3.37(0.66) | 0.268 |
| No | 9(7.3) | 12(9.7) | 54(43.6) | 33(26.6) | 16(12.9) | | 3.72(0.70) | | 3.24(0.69) | |
| Don't know | 1(5.6) | 2(11.1) | 11(61.1) | 3(16.7) | 1(5.6) | | 3.81(0.37) | | 3.48(0.73) | |
| Number of chronic diseases present | | | | | | | | | | |
| 0 | 7(5.5) | 15(11.8) | 64(50.4) | 34(26.8) | 7(5.5) | **0.017** | 3.77(0.59) | 0.295 | 3.33(0.65) | 0.179 |
| 1 | 2(4.6) | 4(9.1) | 17(38.6) | 13(29.6) | 8(18.2) | | 3.73(0.65) | | 3.30(0.73) | |
| 2 | 0 | 2(11.8) | 8(47.1) | 2(11.8) | 5(29.4) | | 3.98(0.67) | | 3.25(0.88) | |
| 3 | 1(16.7) | 0 | 1(16.7) | 1(16.7) | 3(50.0) | | 3.44(1.03) | | 2.70(0.33) | |

*Table 13 continued*

| Characteristics | Willingness to undergo WPGT, n(%*) | | | | | | Perceived benefits† | | Concern† | |
|---|---|---|---|---|---|---|---|---|---|---|
| | VU | SU | N | SW | VW | p-value | Score, mean (SD) | p-value | Score, mean (SD) | p-value |
| ***GENERAL PUBLIC*** | 3(1.6) | 16(8.6) | 56(30.0) | 73(39.0) | 39(20.9) | | 3.97(0.55) | | 3.33(0.68) | |
| Age, mean (SD) | 54.0(6.6) | 51.2(3.4) | 52.9(6.1) | 51.7(4.5) | 53.8(5.5) | 0.202 | 0.019†† | 0.796 | -0.054†† | 0.466 |
| Gender | | | | | | | | | | |
| Male | 0 | 3(5.9) | 16(31.4) | 19(37.3) | 13(25.5) | 0.747 | 4.05(0.57) | 0.245 | 3.23(0.73) | 0.203 |
| Female | 3(2.2) | 13(9.6) | 40(29.4) | 54(39.7) | 26(19.1) | | 3.94(0.54) | | 3.37(0.66) | |
| Religion | | | | | | | | | | |
| Christianity | 2(3.8) | 5(9.4) | 13(24.5) | 19(35.9) | 14(26.4) | 0.737 | 4.08(0.53) | 0.332 | 3.28(0.75) | 0.200 |
| Buddhism | 1(1.3) | 5(6.6) | 27(35.5) | 31(40.8) | 12(15.8) | | 3.94(0.55) | | 3.43(0.69) | |
| Taoism | 0 | 1(7.1) | 6(42.9) | 5(35.7) | 2(14.3) | | 4.00(0.41) | | 3.49(0.50) | |
| Free thinker | 0 | 5(11.4) | 10(22.7) | 18(40.9) | 11(25.0) | | 3.88(0.60) | | 3.18(0.62) | |
| Marital status | | | | | | | | | | |
| Single | 1(9.1) | 0 | 2(18.2) | 4(36.4) | 4(36.4) | 0.201 | 4.00(0.71) | 0.154 | 3.47(0.98) | 0.778 |
| Married | 1(0.6) | 15(9.4) | 47(29.6) | 63(39.6) | 33(20.8) | | 3.99(0.52) | | 3.32(0.66) | |
| Divorced/Widowed | 1(5.9) | 1(5.9) | 7(41.2) | 6(35.3) | 2(11.8) | | 3.73(0.66) | | 3.33(0.72) | |
| Highest educational status | | | | | | | | | | |
| PSLE | 1(4.6) | 1(4.6) | 6(27.3) | 11(50.0) | 3(13.6) | 0.287 | 3.97(0.44) | 0.089 | 3.59(0.51) | **0.039** |
| GCE 'O' or 'A' levels | 1(1.2) | 9(10.3) | 32(36.8) | 31(35.6) | 14(16.1) | | 3.88(0.56) | | 3.38(0.66) | |
| Diploma / Degree | 1(1.3) | 6(7.7) | 18(23.1) | 31(39.7) | 22(28.2) | | 4.07(0.55) | | 3.20(0.73) | |
| Housing | | | | | | | | | | |
| 1 – 3 room HDB | 2(10.5) | 1(5.3) | 4(21.1) | 10(52.6) | 2(10.5) | 0.168 | 3.61(0.65) | **0.007** | 3.58(0.84) | **0.010** |
| 4 – 5 room HDB | 1(0.9) | 12(10.3) | 37(31.9) | 44(37.9) | 22(19.0) | | 3.98(0.49) | | 3.39(0.62) | |
| Condominium/landed | 0 | 3(5.8) | 15(28.9) | 19(36.5) | 15(28.9) | | 4.07(0.58) | | 3.11(0.70) | |
| Have friends/relatives taking warfarin | | | | | | | | | | |
| Yes | 1(4.0) | 0 | 4(16.0) | 14(56.0) | 6(24.0) | 0.073 | 4.08(0.39) | 0.279 | 3.16(0.65) | 0.177 |
| No | 2(1.2) | 16(9.9) | 52(32.1) | 59(36.4) | 33(20.4) | | 3.95(0.57) | | 3.36(0.68) | |
| Taken genetic test in the past | | | | | | | | | | |
| Yes | 0 | 0 | 1(33.3) | 0 | 2(66.7) | 0.207 | 4.33(0.33) | 0.246 | 3.13(1.01) | 0.613 |
| No | 3(1.6) | 16(8.7) | 55(29.9) | 73(39.7) | 37(20.1) | | 3.96(0.55) | | 3.33(0.68) | |

*Table 13 continued*

| Characteristics | Willingness to undergo WPGT, n(%*) | | | | | | Perceived benefits† | | Concern† | |
|---|---|---|---|---|---|---|---|---|---|---|
| | VU | SU | N | SW | VW | p-value | Score, mean (SD) | p-value | Score, mean (SD) | p-value |
| History of ADR | | | | | | | | | | |
| Yes | 0 | 4(16.0) | 4(16.0) | 14(56.0) | 3(12.0) | **0.001** | 4.08(0.49) | 0.199 | 3.40(0.57) | 0.559 |
| No | 2(1.5) | 9(6.7) | 36(26.7) | 53(39.3) | 35(25.9) | | 3.98(0.55) | | 3.30(0.72) | |
| Don't know/not sure | 1(3.7) | 3(11.1) | 16(59.3) | 6(22.2) | 1(3.7) | | 3.81(0.57) | | 3.43(0.58) | |
| Know of friends/relatives with history of ADR | | | | | | | | | | |
| Yes | 0 | 5(8.9) | 14(25.0) | 24(42.9) | 13(23.2) | 0.754 | 4.08(0.49) | 0.078 | 3.28(0.65) | 0.471 |
| No | 3(2.3) | 11(8.4) | 42(32.1) | 49(37.4) | 26(19.9) | | 3.92(0.56) | | 3.35(0.69) | |
| "If you need to take warfarin, how much would you value a new test that can potentially decrease the number of INR tests needed?" | | | | | | | | | | |
| Not at all | 0 | 1(33.3) | 1(33.3) | 1(33.3) | 0 | **<** | 2.78(0.77) | **<** | 2.53(0.50) | 0.158 |
| A little | 1(5.3) | 6(31.6) | 7(36.9) | 5(26.3) | 0 | **0.001** | 3.96(0.41) | **0.001** | 3.57(0.70) | |
| Somewhat more | 0 | 3(6.0) | 20(40.0) | 21(42.0) | 6(12.0) | | 3.75(0.55) | | 3.31(0.68) | |
| Quite a lot | 2(2.5) | 4(5.1) | 19(24.1) | 39(49.4) | 15(19.0) | | 4.08(0.51) | | 3.34(0.64) | |
| Very much | 0 | 2(5.6) | 9(25.0) | 7(19.4) | 18(50.0) | | 4.14(0.47) | | 3.29(0.73) | |
| Number of chronic diseases present | | | | | | | | | | |
| 0 | 2(1.2) | 15(8.7) | 53(30.8) | 68(39.5) | 34(19.8) | 0.089 | 3.97(0.56) | 0.850 | 3.31(0.67) | 0.114 |
| 1 | 1(7.1) | 0 | 3(21.4) | 5(35.7) | 5(35.7) | | 3.95(0.43) | | 3.49(0.76) | |
| 4 | 0 | 1(100) | 0 | 0 | 0 | | 3.67(0) | | 4.60(0) | |

p-values <0.05 are bolded. VU: Very unwilling, SU: somewhat unwilling, N: neutral, SW: somewhat willing, VW: very willing

*% are within row.

†Perceived benefits and concern scores range from 1 to 5, with higher scores representing higher perceived benefits or concerns respectively. Item (ii) from the perceived benefits scale (Table 14) was not included in the calculation.

††Pearson's correlation coefficient

‡The patient with 'others' religion was combined with 'free thinkers' for analysis.



**Table 13. Attitudes on WPGT and their Relationships with Socio-demographic and Clinical Variables**

With regards to WPGT,

| | | Cronbach's alpha | |
|---|---|---|---|
| Perceived benefits scale | | Patients | Public |
| i) | I am hopeful that it can detect which dose works best. | | |
| ii) | I don't think it will lower my risk of warfarin side effects. | | |
| iii) | I think it can predict a more suitable starting dose for me. | 0.76* | 0.71† |
| iv) | I am hopeful that there may be less trial and error in finding my warfarin dose. | | |
| Concern scale | | | |
| i) | If it reveals that I need a very low or very high dose, I would feel anxious. | | |
| ii) | I am worried that it may subsequently reveal that I possess additional risk factors for another disease that I was unaware of. | | |
| iii) | I am worried that the results may be passed onto unauthorized persons. | | |
| iv) | Apart from the fact that I'm taking warfarin or have a pre-existing condition, if it reveals that I need a very low or very high dose, I may be additionally disadvantaged when buying health insurance. ‡ | 0.72 | 0.71 |
| v) | Apart from the fact that I'm taking warfarin or have a pre-existing condition, if it reveals that I need a very low or very high dose, I may be additionally treated unfairly at work or job-seeking. ‡ | | |

Agreement to all items was answered on a 5-point Likert scale.
*Calculated with item (ii) removed as it worsened the overall Cronbach's alpha when included.
†Item (ii) was reversed (i.e. 'I think it will lower my risk of warfarin side effects.') and item (iii) was removed for the public.
‡A "NA" option was added to these 2 items as some warfarin patients do not buy insurance or work.

**Table 14. Perceived Benefits and Concern Scales and their Internal Consistencies**

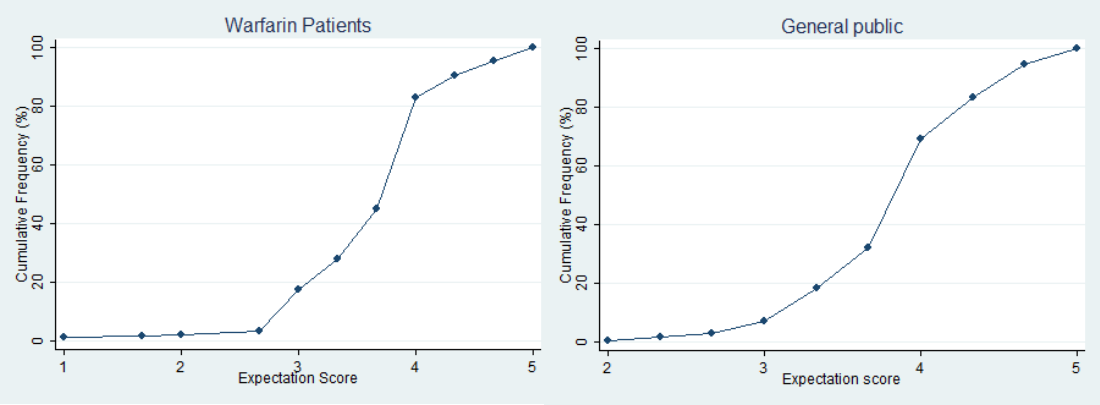



**Figure 11. Cumulative Frequencies of Perceived Benefits Scores**

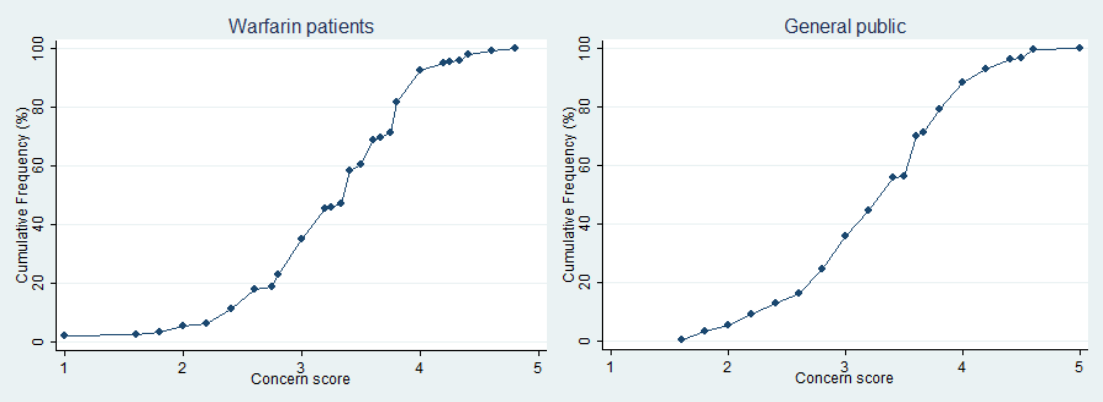



**Figure 12. Cumulative Frequencies of Concern Scores**

Relationship between Perceived Benefits and Concerns with Willingness to Undergo WPGT

As hypothesized, higher perceived benefits of WPGT was associated with higher willingness to undergo it ($p < 0.001$ in both populations). Higher concern was significantly associated with lower willingness to undergo WPGT in public ($p = 0.004$), but not in patients although a similar trend was present ($p = 0.072$).

WTP and Attribute Importances

The mWTP and attribute importances for warfarin patients and the general public are summarized in Table 15. Among warfarin patients, the median mWTP was S$89 for a genetic vs. non-genetic test, S$17 for every decrease in number of INR tests needed and S$63 for every % decrease in annual risk in serious side effects. The respective values in the general public were S$20, S$25 and S$109. The public was less willing to pay for a genetic test as compared to a non-genetic test but was more willing to pay for reduction in number of INR tests needed and serious ADR risk than warfarin patients. In both populations the attribute importances were generally

consistent with the respective mWTPs and both populations placed a similar emphasis on cost of test.

Only educational status was statistically associated with side effect importance in patients. Importance placed on side effect risk increased with increasing educational status ($p = 0.0405$). No other demographic or clinical variables were associated with any of the attribute importances in patients or the public. The prediction accuracies for the 2 holdout tasks using the HB model parameters are given in Table 16.

| | Warfarin patients (n = 194) | General Public (n = 187) |
|---|---|---|
| mWTP, median (95% CI) | | |
| Genetic vs. non-genetic test | $88.61 ($55.11 to $123.33) | $19.80 ($12.15 to $42.84) |
| Every decrease in number of INR tests | $16.61 ($10.50 to $18.42 | $24.94 ($20.04 to $31.36) |
| Every decrease in % risk of serious side effects | $63.37 ($49.10 to $85.04) | $109.36 ($86.59 to $133.84) |
| Attribute importances (%*), median (95% CI) | | |
| Nature of test | 7.90 (7.28 to 8.87) | 3.57 (3.25 to 3.89) |
| Number of INR tests needed before warfarin dose stabilizes | 18.88 (16.27 to 22.74) | 24.65 (21.28 to 27.96) |
| Risk of serious side effects (Major bleeding or clotting) | 34.26 (30.59 to 40.61) | 44.11 (41.50 to 46.72) |
| Cost | 26.14 (23.39 to 27.91) | 24.69 (23.08 to 26.44) |

* Importances are relative proportions across all 4 attributes and add up to 100% within every individual. Estimates here are the medians across all individuals for each attribute and thus may not add up to 100%.

**Table 15. mWTP and Attribute Importances**

| | Warfarin patients | General public |
|---|---|---|
| Holdout task 1 | 93.3% | 93.0% |
| Holdout task 2 | 80.9% | 88.2% |

**Table 16. Prediction Accuracy of Holdout Tasks**

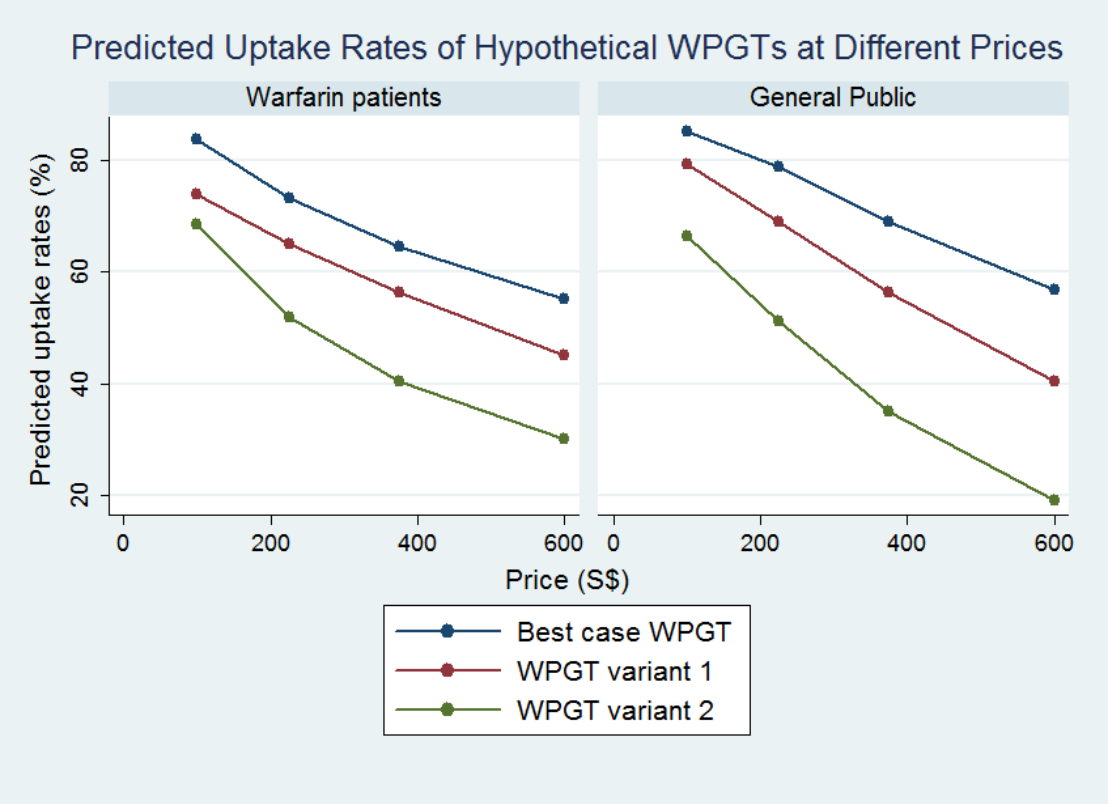
WTP and Price Sensitivity of Uptake Rates of Hypothetical WPGTs

The WTP for the 3 hypothetical WPGTs are shown in Table 17. The public had higher median WTPs for all 3 hypothetical WPGTs than patients. The best case WPGT naturally commanded the highest WTP and both groups were more willing to pay for a WPGT that decreased their risk of serious side effects than one that decreased the number of INR tests needed (WPGT variant 1 vs. WPGT variant 2). This is consistent with the observation that the side effects attribute had higher importance score than the INR test attribute. The predicted uptake rates of the 3 hypothetical WPGT as price varied from $100 to $600 are shown in Figure 13. The rates represent the proportion that are predicted to choose each hypothetical WPGT over no WPGT at different prices, and therefore should be interpreted as relative indications of preference rather than actual uptake rate. In line with mWTP and WTP estimates, a higher uptake rate was expected in the public compared to patients at almost every price point. In both populations, 'best case WPGT' was the most preferred, followed by 'WPGT variant 1' then 'WPGT variant 2' at every price point. As expected, predicted uptake rates fell with increasing price.

| Attribute | No WPGT (baseline) | Best case WPGT* | WPGT variant 1* | WPGT variant 2* |
|---|---|---|---|---|
| Nature of test | Non-genetic | Genetic | Genetic | Genetic |
| Number of INR tests needed before warfarin dose stabilizes | 13 | 5 | 13 | 5 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 1% per year | 1% per year | 5% per year |
| WTP (warfarin patients), median (95% CI) | | $571.34 ($419.87 to $702.26) | $397.33 ($282.91 to $523.11) | $164.30 ($146.88 to $224.68) |
| WTP (general public), median (95% CI) | | $730.45 ($605.06 to $801.04) | $454.01 ($340.76 to $553.98) | $227.65 ($175.98 to $292.58) |

* Each scenario was compared with the baseline for calculation of WTP and price sensitivity simulation. For price sensitivity simulation, price of no WPGT was specified as $0. A total of 3 variants representing different combinations of the attributes were presented.

**Table 17. Hypothetical WPGTs and WTP**




Lines represent proportion of patients and public predicted to choose each hypothetical WPGT over no WPGT at prices ranging from $100 to $600. The specifications of the 3 hypothetical WPGTs are given in Table 16.

**Figure 13. Price Sensitivity of Uptake Rates of the Hypothetical WPGTs**

### 6.4 Discussion

Patients' acceptance is an important consideration in the clinical implementation of PGT and their views on pharmacogenomics and PGT has duly been studied qualitatively and quantitatively [254-257]. Since attitudes may be influenced by cultural factors, results from these (Caucasian) studies may not be applicable to our population. To the best of our knowledge, this is the first study on attitudes, WTP and preferences for WPGT. Two populations (warfarin patients and general public) were surveyed to estimate the likely attitudes, WTP and preferences for WPGT in new warfarin patients. Current warfarin patients, having experienced the process of repeated INR tests during the initial dose stabilization, as well as

counseling on warfarin side effects, are likely to be better able to appreciate the value of improving these aspects. On the other hand, normal members of public usually do not actively consider the value of such clinical interventions and may place a lower value on their benefits until the need befalls them, in this case needing to take warfarin. New warfarin patients, who are at the transition between these 2 populations, are assumed to be intermediate in their attitudes, WTP and preferences for WPGT.

The results indicate that Singaporean Chinese (both patients and public) were generally willing to undergo WPGT or were neutral about it. Only a small proportion indicated unwillingness. They also had relatively high perceived benefits and concerns about WPGT. These results are consistent with those of Rogausch *et al.* [254], although no direct comparison could be made on the figures due to different question structure and analysis methods. As expected, higher perceived benefits of benefit from WPGT was associated with higher willingness to undergo it while the opposite was true for level of concern. Willingness to undergo WPGT tended to be higher in the public. One possible explanation of this is that the public's lack of experience with being on warfarin therapy had led them to under or overestimate the benefits of WPGT. In this case, it appeared that they perceived the benefits of WPGT more positively, as reflected by the slightly higher perceived benefits score.

Patients who were males, better educated and had more chronic diseases were more willing to undergo WPGT. The relationship between chronic disease burden and willingness may be explained by a higher desire to reduce further health-related problems or inconveniences, while the relationships with gender and educational status may possibly be related to the ability to understand the information provided and appreciate the potential benefits of WPGT. To investigate this, further analyses

was done to explore if willingness differed by WPGT knowledge scores and whether patients needed substantial assistance with completing the questionnaire. Though WPGT knowledge score was not associated with willingness to undergo WPGT, those who needed substantial assistance with completing the questionnaire were generally less willing to undergo WPGT. Females and the less educated tended to need substantial assistance, so it appears that the ability to comprehend the questionnaire is a likely explanation for the observation. Several cognitive biases, including difficulty in understanding certain medical information, can affect patients' ability to make good decisions [349], so this highlights the importance of making the information on WPGT more easily understandable and taking time to explain it to patients. The reason for the U-shaped pattern with length of treatment and willingness to undergo WPGT is also not immediately obvious. One speculation is that new patients (<3 months) were still overwhelmed by the inconvenience of frequent INR tests and fear of ADR, while long term patients (>1 year) desired relief from the burdens of long term warfarin therapy, since the need for regular INR monitoring and the risk of side effects, among other factors, have an impact on quality of life [350]. If so, it is possible that the duration that patients need to be taking warfarin may be the underlying factor. To further understand the decision making process at the time when WPGT would be needed, it would be useful to study the effect of anticipated length of therapy on willingness to undergo WPGT.

Respondents from the public who valued more a hypothetical new test that may reduce the number of INR tests needed, were logically more willing to undergo WPGT. While most of the public were willing to undergo WPGT, those without an ADR history tended to be more willing, and those who were not sure of their ADR history tended to be neutral. Patients who had experienced an ADR may have

negative emotions and less trust towards their healthcare providers [351,352], so a possible explanation for this observation is that some of those who experienced an ADR are now skeptical of medical interventions.

Assuming new warfarin patients just about to start their first dose are intermediate between warfarin patients and normal individuals in terms of their WTP, the results suggest that if the number of INR tests needed till stabilization with WPGT can be reduced to 5 and risk of serious side effects reduced to 1% per year, at least half of new warfarin patients would be willing to pay between S$570 and S$730 for this test. This is in comparison with no WPGT, where the number of INR tests needed till stabilization was specified at 13 and risk of serious side effects at 5% per year. If the risk of serious side effects can be reduced to 1% per year but patients still require the same number of INR tests to reach stabilization with WPGT (WPGT variant 1), the median WTP would fall to between S$400 and $450. If patients require fewer INR tests but have the same risk of side effects (WPGT variant 2), the median WTP would be between S$160 and S$230. The former (WPGT variant 1) appears to be more likely as the recently published results of the CoumaGen-II clinical trial comparing 2 pharmacogenetic algorithms and standard care suggested that WPGT is associated with lower risk of serious ADR but not reduced number of INR tests needed. In the trial, the 90 day incidence of serious ADR was 4.5% and 9.4% in the combined pharmacogenetic arm and controls respectively, $p = 0.001$), while number of INRs measured were similar (mean 7.70 and 7.47 respectively, $p = 0.35$) [240]. It should be noted again that these WTP estimates were based on assumed benefits of WPGT and data from more clinical trials would be needed to establish its actual clinical benefits, which would affect patients' WTP. Placing the WTP estimates in context, for a diagnostic test, Singaporean Chinese are willing to pay a sum (best case WPGT)

similar to a subsidized colonoscopy in Singapore [353], which may need to be repeated at least every 10 years. With falling genotyping costs, WPGT is likely to cost much less than the estimated WTPs and thus be economically sustainable. In fact, it has even been argued that CBAs are not necessary for PGT as great increase in medical spending is unlikely [35].

From the WTP as well as mWTP estimates, both warfarin patients and the general public were more willing to pay for safety rather than convenience. This is also reflected in their preferences for the various attributes. This is reasonably expected since an episode of a serious side effect is likely more detrimental than the inconvenience and pain of a few additional blood tests. Unsurprisingly, both patients and public placed a similar emphasis on cost, but the nature of test appeared to affect the choice of patients more than that of the public. This may be due to genuine concerns about the genetic nature of WPGT or an artifact from inadequate understanding of the information provided and/or the DCE methodology. In WTP studies of a rapid diagnostic test for malaria, ex-post WTP tended to be higher than ex-ante WTP [286,354]. In contrast, ex-ante WTP was higher than ex-post WTP in this study. Again, the relationships between attribute importances with attribute knowledge and WPGT knowledge scores were explored to determine if lack of understanding of the information provided might have affected the results.

Higher attribute knowledge score was associated with lower 'nature of test' importance ($p = 0.0003$), higher side effect importance ($p = 0.0014$) and lower cost importance ($p = 0.0186$) in patients but not the public. Notably, the patient population was older and less educated than the public and many had problems grasping even the concept of genes. Though general knowledge of genes was not measured here, another study in Singaporean Chinese, which assessed some basic concepts of

genetics and the genetic basis of Parkinson's disease using 6 items, revealed poor knowledge of genetics [261]. In addition, patients who needed substantial assistance with completing the questionnaire also had lower side effect importance ($p = 0.0004$) and higher cost importance ($p = 0.0405$), suggesting that lack of understanding may have affected their computed WTP. Patients with lower attribute knowledge score or had difficulty with the questionnaire probably also had difficulty understanding the concept of risk, and thus may put less importance on the side effect attribute than those who had better knowledge scores or had no problem with the questionnaire. The public generally scored better on attribute knowledge and thus no association between attribute knowledge score and importances were detected. Another sign that a higher emphasis on 'nature of test' was due to problems with the DCE was that the 4 respondents from the general public who indicated having 'a lot' of problem with the DCE (as opposed to 'a little' or 'not at all') had significantly higher mWTP for a genetic test (median mWTP = \$130.44, \$5.88 and \$33.99 respectively, kruskal-wallis rank sum test p-value = 0.0174).

Of all demographic and clinical variables, only educational status was significantly associated with side effect importance in patients. No associations between demographic or clinical variables and attribute importances were detected in the public. This is not surprising as demographic variables are generally not very useful for delineating attribute preferences [355,356] and preferences may be better separated by some unmeasured attitudinal or behavioral factors. The association between educational status and side effect importance in patients is also in line with the above mentioned observations that a higher ability to understand the concepts in the questionnaire was associated with higher side effect importance.

There are several limitations to this study. Firstly, sampling was non-random so generalizability of the results may be limited. Random sampling was not feasible due to logistical impracticalities (difficulties in screening and contacting potential subjects, and the need to collect personal particulars to do this) and a limited patient population size. For warfarin patients, the coverage was probably quite high, since recruitment took place at almost every ACC session in a 4 – 5 month period. In other words, most patients would have been encountered at least once. Secondly, there may be some non-response bias. Males were somewhat overrepresented in the patient sample while females were overrepresented in the public sample. Given that male patients tended to have higher willingness to take WPGT, the actual willingness among patients may be lower. The samples were also more highly educated than the general population (% with diploma or degree = 28.4%, 41.7% and 22.1%, in patients, public sample and general population (Census 2010 ≥40 year old [357]), respectively), and with educational status being a significant predictor of willingness (in patients) and concern score (in public), the actual willingness to undergo WPGT may be lower, and concern level may be higher. Nevertheless, the public sample is quite comparable in terms of educational status with the general population of age 30 to 60 years old (% with diploma or degree = 41.2% [357]), a group who might become future warfarin patients. With an increasingly educated population, the results may actually have future applicability. Thirdly, there was evidence of inadequate understanding and difficulty with the concepts put forth and DCE in some respondents, which may have given rise to inaccurate results. However, this may reflect the actual situation in the clinic, where some patients would have difficulty understanding information on the WPGT even if it is explained by a doctor, and therefore make a similar choice as they would in an artificial DCE setting if the choice was completely voluntary. Usually

though, this is not the case for many medical decisions especially in Asian countries. Even in a western society, not all patients want to participate in decision making [358]. This brings on the fourth limitation, which is that the effect of agency on choice was not studied. Many patients, especially the older and less educated, tend to leave medical decisions to their doctor and may be persuaded to take a test if it is recommended by the doctor. Majority of doctors would also persuade the patient and/or the family members to accept a treatment he/she thinks is best for the patient [359]. Since in such cases some patients who wouldn't choose the test voluntarily end up doing the test under their doctor's recommendation, not considering the agency effect would produce a more conservative estimate of the WTP and uptake rate for WPGT. Fifthly, only the Chinese were studied and thus the results could not be extrapolated to other ethnic groups. Sixthly, the perceived benefits and concern scores were analyzed as interval scale data although they are strictly speaking ordinal scale. The decision was made to analyze them as such since non-parametric methods are not as robust and the results are more difficult to interpret. Accordingly, the scores were simply used as gauge of the level of perceived benefits and concern, rather than having any further meaning in themselves. And lastly, only the WTP of out-of-pocket payment was evaluated but not other payment schemes such as the use of Medisave (a national healthcare savings scheme for working Singaporeans), or government subsidies. Though this was suggested by at least 1 patient in pilot 1, it was not included due to potential complexity in the design and analysis of the DCE. Besides, WPGT is unlikely to be of priority to be considered for Medisave usage or government subsidies.

As discussed, there are several selection and measurement biases that may affect the validity of the results. From information available about the possible

sources of these biases, the actual willingness to undergo WPGT may be lower. Similarly, actual perceived benefits may be lower and concerns higher. However, there is no reason to believe that the sentiment would be overly negative. It should also be emphasized that actual uptake of WPGT, when it does become available, would likely differ from the indicated levels of willingness, as other factors that affect the actual behavior are not measured. This would be the case even if willingness was measured without bias here. The estimated WTP for WPGT was also affected by differential understanding of the DCE but it is difficult to predict whether actual WTP (a function of actual price and uptake) would be higher or lower than estimated. Apart from the effect of this bias, other factors affecting uptake were not measured. The directions of the part worth estimates in the expected directions provide face validity, and relatively good prediction accuracies of the holdout tasks provide internal validity. External validity of WTP estimates from DCEs has been largely untested due to lack of RP data and is an ongoing issue in the field [360].

In conclusion, patient acceptance is not likely to be a major barrier to clinical implementation of WPGT. However, patient education is necessary and the ethical, social and legal issues should be addressed in the process. The median WTP for WPGT in Singaporean Chinese ranged from about S$160 to S$730, depending on the actual clinical benefits WPGT can bring. Both patients and public generally placed most emphasis on side effects, followed by cost, number of INR tests and nature of test. With falling costs of genotyping, WPGT is likely to be economically sustainable.

# CHAPTER 7: CONCLUSIONS

## 7.1 Major Findings

This thesis comprises of 4 studies focusing on different aspects of warfarin pharmacogenomics, from marker discovery to specific issues pertaining to clinical application. In study 1, genetic variants in *CYP4F2*, *GGCX* and *EPHX1* were investigated for association with WMD in the hope of finding markers that may further explain warfarin dose variability in our multiethnic Singaporean population. *CYP4F2* V433M (rs2108622) was found to be significantly associated with WMD after accounting for known factors such as age, weight, *CYP2C9*, *VKORC1* and even race. *CYP4F2* V433M contributed an additional 2.8% to dose variability, which is relatively small compared to the effects of *CYP2C9* and *VKORC1*. On the other hand, *GGCX* and *EPHX1* did not contribute additionally to warfarin dose variability in our population, or at least have too small an effect to reach statistical significance.

Study 2 further dissected the role of genetic factors in the context of race and the analysis revealed that while race was closely correlated with *VKORC1* genotype, genetic information (including *VKORC1* genotype) still provided additional input in explaining warfarin dose variation, as manifested in the ANOVA analysis and improvement in predictive accuracy of WMD. Furthermore, the addition of *CYP4F2* led to a statistically better prediction model despite its small contribution, a slight improvement in prediction accuracy as measured by the proportion of patients with predicted dose within ±20% actual dose. Race is thus not a sufficient surrogate for the known genetic factors, and dosing algorithms that include *CYP4F2* should be further evaluated.

Having established the value of the genetic factors, study 3 went on to explore the population impact of WPGT by adopting the PAF as a measure. Using the

multiethnic IWPC dataset, the analysis suggested that WPGT (testing for the well established variants in *CYP2C9* and *VKORC1* only) would likely benefit Caucasians but not Blacks, Chinese and Japanese, in whom 6mg/day (for Blacks) or 3mg/day (for Chinese and Japanese) fixed doses seemed to achieve the right dose just as well as WPGT if not better.

Study 4 was an attitudinal and econometric study surveying warfarin patients and the general public to infer the acceptability and WTP for WPGT in potential warfarin patients. Both populations had relatively high perceived benefits of WPGT and were generally willing to undergo it, but also had some concerns. When choosing between WPGTs (or between undergoing and not undergoing WPGT in practice), the ability of WPGT to reduce side effect risk was most important, followed by cost, number of INR tests needed till stabilization and lastly nature of test. The estimated median WTP for WPGT ranged from S$160 to S$730 depending on its actual clinical performance, and so is likely to be economically sustainable with falling genotyping costs. Due to the higher educational status of the study populations, the actual willingness and perceived benefits may be lower, while level of concern may be higher. In view of the methodological biases in this study, the results should therefore be interpreted cautiously.

### 7.2 Clinical Significance

The findings in this thesis have potential practical implications on the improvement of warfarin management through application of pharmacogenomics in Singapore and beyond. In the local context, addition of *CYP4F2* V433M may improve the previously developed dosing algorithm [16], albeit by a small degree. With falling genotyping costs, the cost of including an additional variant on a genotyping platform

is likely to be marginal and thus worthwhile. Unsurprisingly, a recent analysis proposed that multiplexed preemptive genotyping may be more efficient that single 'reactive' testing, given that two-thirds of the population would use at least 1 drug with an established pharmacogenetic association within a 5 year period [361]. Furthermore, with the cost of whole genome sequencing approaching US$1000 in the next few years [362], established and potential genetic markers lay waiting in one single test if it gains a foothold in routine medical practice someday.

Currently, there appears to be a general acceptance that although race is a key factor determining WMD, it becomes irrelevant after genotype is taken into account [143,225] and that the major races studied thus far (Caucasians, Blacks, Asians) may benefit from WPGT [134,225,226]. The role of race was brought back into focus in studies 2 and 3, in which the results concurred with the first inference but not the second. It appears contradictory that while study 2 confirmed that race alone is not a sufficient surrogate for genotype (in particular *VKORC1*) as previously suggested [132], but study 3 suggests that WPGT may be no better than a race-specific fixed dose. The contradiction may partly be explained by methodological differences and discrepancy in the definition of superiority from statistical and clinical viewpoints. Nevertheless it cautions practitioners against assuming that statistical association between genetic factors and WMD in different races or several races combined necessarily leads to clinical benefit and more importantly, highlights the pitfall of extrapolating findings in a mixed population to its individual races. Individual populations may also have genetic variants specific or more relevant to them, or additional undiscovered variants that might make WPGT useful. Apart from racial differences associated with genetics, differences in cultural aspects such as diet, lifestyle and behavior may also affect warfarin control. Therefore populations

differing sufficiently in these aspects may be worth studying individually, especially with regards to clinical outcomes. In the local context, it would be particularly useful to compare WPGT against a 3mg/day fixed dose in Chinese and Malays.

Evidence of clinical benefit from ongoing clinical trials is still necessary before most practitioners would be willing to adopt WPGT. However, for it to reach the end of the translational road to have its intended effect, social, ethical, legal, logistical and economic issues have to be addressed. Some of these issues were explored in study 4 and there was indication of reasonable acceptance and economic sustainability in the local Chinese population, giving assurance that these are not likely to be major barriers. Study 4 also highlighted the need to educate potential patients on WPGT and counsel them to allay any concerns, should WPGT be eventually implemented in Singapore. Looking forward, outreach programs to educate the public on pharmacogenomics and personalized medicine could already be started given the possibility of more PGTs being incorporated into clinical practice. Social media is one channel, a powerful and efficient one, to reach out to and to garner views and concerns about PGT from the public. Through constant engagement with the public on these matters, hopefully they would be more prepared and receptive when the need for a PGT comes.

### 7.3 Limitations

Although the patient cohort used in studies 1 and 2 for SNP discovery and validation was one of the largest multiethnic population in the region (the next largest being a Malaysian cohort [363]), the number of Malays and Indians were still too small for meaningful analysis on their own and thus genetic variants that may be relevant to certain races (especially Malays) could not be detected. The range of

genetic variants studied were limited and the overall sample size was also not large enough to detect variants with smaller effects. In addition, potentially important clinical variables such as dietary vitamin K intake, alcohol intake, smoking status and level of compliance were not available in the dataset for Studies 1 and 2.

It appears that Study 1 in this thesis, and other studies, which failed to replicate the associations for the variants in *GGCX* and *EPHX1,* were victims of the winner's curse, a phenomenon where the true genetic effect size of a newly identified association tends to be biased upwards [364]. Therefore, future studies attempting to replicate these or other isolated associations should consider correcting for this bias, perhaps by using the one-parameter maximum likelihood method recently proposed for quantitative traits, so that the appropriate sample size required can be calculated [365].

The final IWPC dataset used for PAF calculations in study 3 also contained a relatively small number of Japanese and Chinese. Coupled with the fact that only the IWPC algorithms were assessed, the conclusion that Blacks, Japanese and Chinese do not do better with WPGT compared to a race-specific fixed dose needs to be confirmed and further examined. Furthermore, assessment of the utility of genetic factors in studies 2 and 3 was made only with respect to warfarin dose accuracy, an intermediate outcome. Applying the same analysis on clinical outcomes such as reduction in INR tests needed or bleeding events, when available, would yield a more complete picture of the value of genetic factors. Another limitation of this analysis was that information for genes other than *CYP2C9* (**2* and **3*) and *VKORC1* were not available and therefore it could not be determined if these additional factors may make WPGT superior to clinical and fixed dose regimens, especially in Blacks, Japanese and Chinese.

In the last study, the main limitation was the possible bias in the samples from non-random sampling and non-response. Inadequate understanding of the survey by some participants (more so in the patient population) may also have affected the results. Patients who needed substantial assistance with the survey, an indicator of difficulty in understanding the survey, responded slightly differently in terms of their willingness to undergo WPGT and preferences. Also, the survey asked for the participants' autonomous choices on WPGT, which may not accurately reflect clinical practice in Singapore, where the doctor usually has a strong influence on the healthcare decisions of patients. In view of these limitations, the elicited willingness to undergo WPGT and WTP may not reflect the true state of affairs when WPGT is eventually ready for the clinic. In the DCE, 2 fixed tasks were included as an internal validity check but it was not possible to evaluate the external validity of the WTP estimates. However this is a common challenge for WTP studies in healthcare and is an area of active research [277].

### 7.4 Future Directions

Alongside warfarin pharmacogenomic research, new oral anticoagulants has been developed, garnering hope of doing away with the problems of using warfarin altogether. Two agents, dabigatran (a direct thrombin inhibitor) and rivaroxaban (a factor Xa inhibitor), are now FDA-approved and a third agent apixaban (another factor Xa inhibitor) is in phase III clinical trials [366]. These are attractive alternatives to warfarin, given that routine monitoring is not needed. If these agents become the main anticoagulant of choice, warfarin pharmacogenomics would be redundant except for being a fine case study. However, it appears likely that there will be a considerable number of years before this happens, if it does. Weighing the risks and benefits

between the new agents and warfarin, currently only a few select subgroups (those with poor control despite good compliance or due to unavoidable drug interactions, and warfarin naïve patients needing anticoagulation for atrial fibrillation) may be suitable for the new agents, if cost is not a concern [367]. Clinicians are a lot more familiar with warfarin and how to handle its problems compared to the new agents, so the lack of a monitoring test may actually unnerve some, not to mention the lack of an antidote, lack of experience on how to handle drug interactions (though fewer) and lack of data in patients with relevant co-morbidities. The (in)practical aspects (needing to take twice daily) and high costs are also not in the favor of new agents at the moment [367,368]. Continued research on these new agents is needed to fill these clinical gaps.

Since warfarin will still be the dominant oral anticoagulant for some time, there are aspects of warfarin pharmacogenomics worth pursuing, especially in the local context. With the advancement of genotyping technologies and next generation sequencing being the new workhorse of genetic studies, GWAS in less well studied Asian populations like Malays and Indians would be valuable in uncovering possible genetic variants that are relevant to these populations. Stratified analysis in study 1 showed that warfarin dose variability in Malays was the least well understood among the 3 Asian ethnic groups, so discovery of additional genetic factors might be beneficial to this ethnic group, which is not only the second largest ethnic group in Singapore but also the majority in Malaysia and Indonesia.

Existing and potential warfarin patients have been studied here with regard to their attitudes and WTP for WPGT. However, healthcare professionals taking care of warfarin patients are instrumental in the delivery of WPGT and so their knowledge and attitudes on WPGT should also be studied. One such study in the US suggests that

much needs to be done in terms of educating and convincing healthcare professionals about WPGT [369], but it has several methodological limitations. Besides, the situation may be different now (after more time for pharmacogenomic news to assimilate in the medical community) and in our local setting. Identification of potential barriers and needs of healthcare professionals can then help direct the necessary efforts to prepare them effectively for WPGT implementation. Patients' decision to undergo WPGT can be considerably affected by their doctor's recommendation, so understanding how the attributes of WPGT affect doctors' decisions to recommend WPGT (by way of a DCE) would also be valuable. The effect of different payment vehicles has also been suggested to affect patients' WTP. However, instead of another DCE to elicit WTP using a different payment vehicle, it may suffice to determine the desire for Medisave usage and subsidies, conditioning on the actual cost of warfarin genotyping. Ultimately, warfarin genotyping would be recommended based largely on its clinical utility; Medisave and subsidies are ways to make it is affordable for most patients.

Cutting edge technology aside, more aggressive and systematic effort in managing diet and compliance may be what some patients need. These factors have long been known to play a role in warfarin response but are difficult to measure and are often conveniently omitted from pharmacogenetic algorithms. In fact, there is recently renewed interest in the investigation of the effect of vitamin K on warfarin dose variability [370,371]. In a recent study, partial least squares regression was used to dissect and detect the correlation between warfarin dose and vitamin K intake, which is confounded by other non-genetic variables such as body weight and physical activity [371]. To facilitate such a study locally, research is also needed to first develop an instrument to quantify vitamin K intake, such as a local version of the K-

card [372]. Low vitamin K intake has been associated with unstable anticoagulation control [97] and supplementation with low dose vitamin K has been shown to improve stability [98,373]. Therefore, a validated instrument to measure vitamin K intake would also be useful in identifying such patients for early intervention and appropriate dietary counseling. Having to watch one's diet was one of the main challenges to new warfarin patients (as revealed in pilot 1 of study 4), so a better ability to quantify and control its effect may not only help doctors and pharmacists achieve better anticoagulation control but may be reassuring and empowering for patients.

Lastly, apart from vitamin K intake per se, dietary fat intake may be an important but overlooked factor affecting warfarin response. Vitamin K is a fat-soluble vitamin and it has been found that changes in its plasma concentrations were mirrored by changes in triglyceride levels in healthy individuals [374]. Although 1 small study did not find a significant correlation between dietary fat intake and WMD [375], there are case reports of such a possibility, such as this case of orlistat enhancing warfarin effect, possibly by reducing vitamin K absorption [376]. Larger studies should be designed to investigate both the effects of vitamin K and fat intake, as well as their possible interaction.

In essence,

*"In all affairs it's a healthy thing now and then to hang a question mark on the things you have long taken for granted." - Bertrand Russell*

*"Make everything as simple as possible, but not simpler." - Albert Einstein*

## LIST OF PUBLICATIONS

Published

**Chan SL**, Thalamuthu A, Goh BC, Chia KS, Chuah B, Wong A, Lee SC. Exon sequencing and association analysis of *EPHX1* genetic variants with maintenance warfarin dose in a multi-ethnic Asian population. Pharmacogenet and Genomics 2011 Jan; 21(1): 35-41.

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**Chan SL**, Suo C, Lee SC, Goh BC, Chia KS, Teo YY. Translational aspects of genetic factors in the prediction of drug response variability: a case study of warfarin pharmacogenomics in a multi-ethnic cohort from Asia. Pharmacogenomics J. 2012;12(4):312-8. Epub 2011 Mar 8.

**Chan SL**, Suo C, Chia KS, Teo YY. The Population Attributable Fraction as a Measure of the Impact of Warfarin Pharmacogenetic Testing. Pharmacogenomics. 2012 Aug;13(11):1247-56.

**Chan SL**, Low J, Lim YW, Finkelstein EA, Farooqui MA, Chia KS, Wee HL. Willingness-to-pay and Preferences for Warfarin Pharmacogenetic Testing in Chinese Warfarin Patients and General Public. Per Med 2013; 10(2): 127-37

Manuscripts Under Review

**Chan SL**, Chia KS, Wee HL. Informing the Design of a Discrete Choice Experiment for Evaluating Warfarin Pharmacogenetic Testing among Mandarin-speaking Chinese Warfarin Patients in Singapore.

**Chan SL**, Low J, Chia KS, Wee HL. Attitudes on Warfarin Pharmacogenetic Testing in Chinese Warfarin Patients and General Public.

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# APPENDIXES

## Appendix 1. Ethics Approval for Warfarin Genotyping Study (Study 1)


*Adding years of healthy life*

DSRB Ref: **C/09/669**

6 Commonwealth Lane
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Singapore 149547
Tel: 6496 6500 Fax: 6496 6501
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RCB No. 200002150H

08 January 2010

Dr Lee Soo Chin
Department of Haematology Oncology
National University Hospital

Dear Dr Lee

**NHG DOMAIN-SPECIFIC REVIEW BOARD (DSRB) APPROVAL**

**Project Title: Study of genetic factors other than CYP2C9 and VKORC1 that influence warfarin dose requirements in a South-east Asian population**

We are pleased to inform you that the NHG Domain Specific Review Board has approved the above research project to be conducted in National University Hospital.

The documents reviewed are:

a) IRB / DSRB Application Form: Study of genetic factors other than CYP2C9 and VKORC1 that influence warfarin dose requirements in a South-east Asian population, **Version 01**

The DSRB has approved your request for waiver of informed consent

The approval period is from **08 January 2010 to 07 January 2011.** The reference number for this study is **DSRB-C/09/669**. Please use this reference number for all future correspondence.

Continued approval is conditional upon your compliance with the following requirements:

2. No deviation from, or changes of the protocol should be implemented without documented approval from the NHG DSRB, except where necessary to eliminate apparent immediate hazard(s) to the study subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g. change of monitor or telephone number).

3. Any deviation from, or a change of, the protocol to eliminate an immediate hazard should be promptly reported to the NHG DSRB within seven calendar days.

4. Please submit the following to the NHG DSRB:

   a. All unanticipated problems involving risk to subjects or others should be reported. In order to assist the DSRB, all reports should be accompanied by the NHG DSRB Unanticipated Problems Involving Risk to Subjects or Others Reporting Form. Please find all forms and guidelines on reporting on the internet at www.b2bresearch.nhg.com.sg.

1 of 2

National Healthcare Group

DSRB Ref: **DSRB-C/09/669**

b. Report(s) on any new information that may adversely affect the safety of the subject or the conduct of the study.

c. NHG DSRB Project Status Report Form – this is to be submitted 4 to 6 weeks prior to expiry of the approval period. The study cannot continue beyond **07 January 2011** until approval is renewed by the NHG DSRB.

d. Study completion – this is to be submitted using the NHG DSRB Project Status Report Form within 4 to 6 weeks of study completion or termination.

6 The NHG Research QA Program was launched in May 2006. The program aims to promote responsible conduct of research in a research culture with high ethical standards, and to identify potential systemic weaknesses and make recommendations for continual improvement. This research project may be randomly selected for completion of self assessment worksheet or for a study review by the QA team. For more information please visit www.b2bresearch.nhg.com.sg.

Yours sincerely,

A/Prof Tan Huay Cheem
Deputy Chairman
NHG Domain Specific Review Board C

Cc: Director of Research, NUH (via fax only)
c/o Office of Biomedical Research, NUH

Chief, Department of Haematology-Oncology , NUH
Research Administration, Haematology Oncology, NUH (via fax only, 68723137)

**Appendix 2. PCR and Sequencing Primer Sequences**

| Gene | Fragment | PCR Primers | | PCR product size (basepairs) | Sequencing primers (forward/reverse) |
|---|---|---|---|---|---|
| | | Forward (5' → 3') | Reverse (5' → 3') | | |
| *CYP4F2* | Exon 11 | TGCCACAGTGCCACACTATT | GAGCTGGAACTTGGACCATC | 819 | PCR primers |
| *GGCX* | Intron 2 | CTTGGTGGCCAGAAATGC | ATCAGGAAGCCAGCTTGAGA | 793 | GTAGAGACGGGGTTTCACCA/ AGCCAGCTTGAGATAAAGCAG |
| | Intron 6 | TGCCCTTGCTTATTACATAGGA | GTGGCTGGGTAGATGCCTAA | 441 | PCR primer* |
| | Exon 8 | ACTTTCCTTTTCAGTGGAACAC | AAAGGCAAAGCAGACTCAAA | 778 | PCR primers |
| *EPHX1* | 5' promoter | TATCCAAGATTGCCTCCCAC | GGTTAATCATCCATCCAGCG | 319 | PCR primers |
| | Exon 1 (alternative) | GCCCTTTAAGTAGCCCGTTT | AGTGTTCCGACTTGGGTGAC | 609 | PCR primer/ GGGCGGACCAACTACAAGT |
| | Exon 1 (classic) | CCTTCTTTTAGATGGGACTCG | TCTTTCATTTCCAGAAAGACG | 850 | GATGGGACTCGAGCACTGAT/ PCR primer |
| | Exon 2 | TTTTCCCAGGATGATGAACAG | GGGCATAGAGGAGGTGATGA | 791 | TAAAATCAGGGACAGGGTTG/ PCR primer |
| | Exon 3 | GAGGCATGACTGGCTTGAAC | GGACTGGATGGTGCATTTCT | 758 | AAGAAATGCGAAGTCTACAGTGA/ PCR primer |
| | Exon 4 | GAAACCGGGAGGCAATAATC | GGCCCTCTGGTGTTCTATGA | 755 | PCR primers |
| | Exon 5 | GTGTTCCCACCAGGCTCTC | GGAGTTGGAGAAGGGAGGTT | 746 | PCR primer/nil† |
| | Exon 6 | CGTTGACTTGGATCCTCCTG | GGGGTGAAAGCCACTATGAA | 789 | PCR primers |
| | Exon 7 | GATGCCGAGGCAGAGTTAAG | GGGAGTCAGGCATGTAGAGG | 754 | PCR primers |
| | Exon 8 | CTGCCTGTGACACGAGGATA | CACCCACAAGGAGCCATAAA | 701 | PCR primers |
| | Exon 9 | ATTTAGAGGCTGTCCCATGC | GGTGCCATTGGTCTGGTG | 795 | PCR primers |

* Only forward was sequenced to confirm number of CAA repeats

†No primer could be designed for sequencing in the reverse direction due to a string of TG repeats located just after the exon. Attempts to sequence using the reverse primer during the optimization stage resulted in multiple peaks after that repeat region, a possible symptom of enzyme slippage.

## Appendix 3. Study 1 Patient Characteristics

| | **All (n=248)** | **Chinese (n=131)** | **Malays (n=81)** | **Indians (n=36)** |
|---|---|---|---|---|
| Age(yr), mean (SD) | 56.4 (13.2) | 57.3 (13.3) | 55.2 (13.0) | 55.9 (13.5) |
| Weight (kg), mean (SD) | 63.5 (14.0) | 62.5 (14.2) | 63.9 (13.3) | 66.0 (14.8) |
| Daily maintenance warfarin dose (mg), median (range) | 3.50 (0.79 – 14.50) | 3.00 (1.00 – 8.00) | 3.50 (0.79 – 7.00) | 5.11 (1.25 – 14.50) |
| Indication for warfarin use (%) | | | | |
| Thromboembolism | 23.4 | 16.0 | 40.7 | 11.1 |
| Atrial fibrillation | 35.1 | 32.8 | 32.1 | 50.0 |
| Heart valve replacement | 31.5 | 38.2 | 19.8 | 33.3 |
| Others | 10.1 | 13.0 | 7.4 | 5.6 |
| *CYP2C9*2* (%) | | | | |
| Present | 0.8 | 0 | 1.2 | 2.8 |
| *CYP2C9*3* (%) | | | | |
| Present | 9.3 | 6.9 | 8.6 | 19.4 |
| *VKORC1* 381 genotype (%) | | | | |
| CC | 56.0 | 78.6 | 42.0 | 5.6 |
| CT | 28.2 | 20.6 | 46.9 | 13.9 |
| TT | 15.7 | 0.8 | 11.1 | 80.6 |

## Appendix 4. MAF of Genetic Variants Genotyped in *CYP4F2*, *GGCX* and *EPHX1* (Study 1)

| *GGCX* CAA microsatellite grouping | Genotypes | All (n = 248) | | Chinese (n = 131) | | Malays (n = 81) | | Indians (n = 36) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Frequency (%) | n | Frequency (%) | n | Frequency (%) | n | Frequency (%) | n |
| Group 1 | 8/10 | 0.8 | 88 | 0 | 48 | 1.2 | 24 | 2.8 | 16 |
| | 10/10 | 34.7 | | 36.6 | | 28.4 | | 41.7 | |
| Group 2 | 10/11 | 30.6 | 100 | 38.2 | 68 | 21.0 | 20 | 25.0 | 12 |
| | 11/11 | 8.1 | | 12.2 | | 3.7 | | 2.8 | |
| | 10/12 | 0.8 | | 0 | | 0 | | 5.6 | |
| | 11/12 | 0.8 | | 1.5 | | 0 | | 0 | |
| Group 3 | 8/13 | 0.4 | 25 | 0 | 11 | 1.2 | 13 | 0 | 1 |
| | 10/13 | 6.9 | | 6.1 | | 9.9 | | 2.8 | |
| | 11/13 | 0.8 | | 2.3 | | 4.9 | | 0 | |
| Group 4 | 8/14 | 0.4 | 35 | 0 | 4 | 1.2 | 24 | 0 | 7 |
| | 10/14 | 7.7 | | 0.8 | | 14.8 | | 16.7 | |
| | 11/14 | 2.8 | | 0.8 | | 6.2 | | 2.8 | |
| | 13/14 | 2.0 | | 0.8 | | 4.9 | | 0 | |
| | 14/14 | 0.8 | | 0 | | 2.5 | | 0 | |
| | 11/15 | 0.4 | | 0.8 | | 0 | | 0 | |

| Position[a] | SNP | Location | Amino acid change | Allele | | MAF | | | | $\chi^2$ *P*-value[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Major | Minor | All (n=248) | Chinese (n=131) | Malay (n=81) | Indian (n=36) | |
| ***CYP4F2*** | | | | | | | | | | |
| | rs2108622 | exon 11 | V433M | G | A | 0.270 | 0.244 | 0.222 | 0.472 | <0.001 |
| ***GGCX*** | | | | | | | | | | |
| | rs12714145 | Intron 2 | | G | A | 0.399 | 0.401 | 0.457 | 0.264 | 0.056 |
| | rs67988001 | Intron 2 | | G | A | 0.294 | 0.351 | 0.278 | 0.125 | 0.003 |
| | rs699664[c] | Exon 8 | R325Q | G | A | 0.292 | 0.351 | 0.278 | 0.111 | 0.001 |
| | rs2592551[c] | Exon 9 | R406R | C | T | | | | | |
| | rs10179904 | Exon 9 | T414T | C | T | 0.105 | 0.050 | 0.173 | 0.153 | <0.001 |
| ***EPHX1*** | | | | | | | | | | |
| 224061834 | rs4653436 | 5' flanking | | G | A | 0.188 | 0.176 | 0.179 | 0.250 | 0.189 |
| 224061994 | 2575C>T[d, e] | 5' flanking | | C | T | 0.036 | 0.050 | 0.031 | 0 | 0.337 |
| 224064364 | rs12741681 | 5' flanking | | A | C | 0.246 | 0.244 | 0.241 | 0.264 | 0.896 |
| 224064596 | rs12744609 | Alternative intron 1 | | A | G | 0.188 | 0.172 | 0.179 | 0.264 | 0.275 |
| 224064670 | 5251G>T[d] | Alternative intron 1 | | G | T | 0.002 | 0 | 0.006 | 0 | |
| 224064793 | 5374C>T[d] | Alternative intron 1 | | C | T | 0.002 | 0.004 | 0 | 0 | |
| 224064836 | 5417A>G[d] | Alternative intron 1 | | A | G | 0.004 | 0 | 0.012 | 0 | |
| 224079660 | rs3738039 | Exon 1 | | G | T | 0.010 | 0.019 | 0 | 0 | 0.595 |
| 224079664 | rs3738040 | Exon 1 | | G | A | 0.216 | 0.244 | 0.210 | 0.125 | 0.332 |
| 224079729 | rs55948105 | Exon 1 | | A | G | 0.006 | 0.011 | 0 | 0 | |
| 224079807 | 20388A>G[d] | Exon 1 | | A | G | 0.002 | 0.004 | 0 | 0 | |
| 224079882 | 20463T>C[d] | Intron 1 | | T | C | 0.002 | 0 | 0 | 0.014 | |
| 224079978 | rs1877724 | Intron 1 | | C | T | 0.337 | 0.271 | 0.401 | 0.431 | 0.008 |
| 224080011 | rs3738042 | Intron 1 | | G | A | 0.149 | 0.137 | 0.136 | 0.222 | 0.108 |
| 224083004 | rs41266229 | Intron 1 | | G | A | 0.149 | 0.137 | 0.136 | 0.222 | 0.071 |
| 224083020 | rs41266231 | Intron 1 | | G | A | 0.149 | 0.137 | 0.136 | 0.222 | 0.071 |
| 224083083 | 23664G>A[d] | Exon 2 | V10V | G | A | 0.002 | 0.004 | 0 | 0 | |
| 224083181 | rs3738046 | Exon 2 | R43T | G | C | 0.056 | 0.073 | 0.056 | 0 | 0.028 |
| 224083256 | rs3738047 | Intron 2 | | G | A | 0.230 | 0.267 | 0.204 | 0.153 | 0.408 |
| 224083318 | rs3738048 | Intron 2 | | C | T | 0.056 | 0.073 | 0.056 | 0 | 0.028 |
| 224083518 | rs55743622 | Intron 2 | | A | C | 0.056 | 0.073 | 0.056 | 0 | 0.028 |
| 224085956 | 26537A>C[d] | Intron 2 | | A | C | 0.002 | 0 | 0.006 | 0 | |
| 224085969 | 26550G>A[d, f] | Intron 2 | | G | A | 0.002 | 0 | 0.006 | 0 | |
| 224085994 | rs3817268 | Intron 2 | | G | T | 0.163 | 0.176 | 0.136 | 0.181 | 0.768 |
| 224086055 | rs56237740 | Intron 2 | | T | G | 0.002 | 0.004 | 0 | 0 | |
| 224086150 | rs55798709 | Exon 3 | E77E | G | A | 0.022 | 0.042 | 0 | 0 | 0.005 |
| 224086256 | rs1051740 | Exon 3 | Y113H | T | C | 0.466 | 0.470 | 0.537 | 0.292 | 0.013 |
| 224086269 | rs67892231 | Exon 3 | K117R | A | G | 0.010 | 0 | 0.006 | 0.056 | <0.001 |
| 224086276 | rs2292566 | Exon 3 | K119K | G | A | 0.258 | 0.290 | 0.216 | 0.236 | 0.027 |
| 224086397 | rs2260863 | Intron 3 | | C | G | 0.067 | 0.034 | 0.080 | 0.153 | 0.002 |
| 224086492 | 27073A>G[d] | Intron 3 | | A | G | 0.002 | 0.004 | 0 | 0 | |
| 224092849 | 33430A>G[d, g] | Intron 3 | | A | G | 0.077 | 0.088 | 0.086 | 0.014 | 0.060 |
| 224092864 | rs4149223 | Intron 3 | | G | C | 0.436 | 0.408 | 0.432 | 0.542 | 0.371 |
| 224093028 | rs55784606 | Exon 4 | H139Y | C | T | 0.004 | 0.008 | 0 | 0 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 224093029 | rs2234922 | Exon 4 | H139R | A | G | 0.131 | 0.099 | 0.148 | 0.208 | 0.056 |
| 224093239 | rs2292567 | Intron 4 | | G | A | 0.071 | 0.069 | 0.056 | 0.111 | 0.390 |
| 224093268 | rs4149224 | Intron 4 | | C | T | 0.004 | 0.008 | 0 | 0 | |
| 224093284 | rs56178222 | Intron 4 | | C | T | 0.002 | 0.004 | 0 | 0 | |
| 224093343 | 33924G>A[d] | Intron 4 | | G | A | 0.002 | 0 | 0 | 0.014 | |
| 224093354 | 33935C>T[d, h] | Intron 4 | | C | T | 0.004 | 0.004 | 0.006 | 0 | |
| 224093364 | 33945C>T[d] | Intron 4 | | C | T | 0.002 | 0.004 | 0 | 0 | |
| 224093946 | rs4149226 | Intron 5 | | C | T | 0.387 | 0.420 | 0.303 | 0.458 | 0.009 |
| 224093948 | 34529G>A[d] | Intron 5 | | G | A | 0.002 | 0.004 | 0 | 0 | |
| 224094091 | 34672C>G[d] | Intron 5 | | C | G | 0.002 | 0 | 0.006 | 0 | |
| 224094171 | rs34143170 | Exon 6 | H247H | C | T | 0.008 | 0 | 0.012 | 0.028 | |
| 224094198 | rs56300109 | Exon 6 | N256K | C | A | 0.002 | 0 | 0.006 | 0 | |
| 224094253 | rs35073925 | Exon 6 | T275A | A | G | 0.020 | 0.019 | 0.031 | 0 | 0.396 |
| 224094276 | 34857G>C[d] | Exon 6 | L282L | G | C | 0.002 | 0.004 | 0 | 0 | |
| 224094282 | rs2292568 | Exon 6 | P284P | C | T | 0.133 | 0.149 | 0.111 | 0.125 | 0.833 |
| 224094312 | 34893G>T[d] | Exon 6 | M294I | G | T | 0.002 | 0.004 | 0 | 0 | |
| 224094383 | 34964C>T[d] | Intron 6 | | C | T | 0.004 | 0 | 0.006 | 0.014 | |
| 224096779 | 37360G>A[d] | Exon 7 | E341K | G | A | 0.002 | 0.004 | 0 | 0 | |
| 224097191 | 37772G>C[d] | Intron 7 | | G | C | 0.018 | 0.011 | 0.037 | 0 | 0.102 |
| 224098852 | rs1051741 | Exon 8 | N357N | C | T | 0.135 | 0.111 | 0.136 | 0.222 | 0.040 |
| 224098944 | 39525A>T[d] | Exon 8 | E388V | A | T | 0.002 | 0.004 | 0 | 0 | |
| 224099099 | 39680G>A[d] | Intron 8 | | G | A | 0.002 | 0.004 | 0 | 0 | |
| 224099152 | rs45467394 | Intron 8 | | G | T | 0.133 | 0.145 | 0.117 | 0.125 | 0.923 |
| 224099153 | rs4149227 | Intron 8 | | C | A | 0.133 | 0.145 | 0.117 | 0.125 | 0.923 |
| 224099300 | rs4149228 | Intron 8 | | A | G | 0.133 | 0.145 | 0.117 | 0.125 | 0.923 |
| 224099321 | 39902C>T[d] | Intron 8 | | C | T | 0.002 | 0 | 0 | 0.014 | |
| 224099374 | 39955G>A[d] | Intron 8 | | G | A | 0.002 | 0.004 | 0 | 0 | |
| 224099481 | 40062A>G[d] | Exon 9 | Y393C | A | G | 0.002 | 0.004 | 0 | 0 | |
| 224099542 | rs45540739 | Exon 9 | V413V | G | A | 0.002 | 0.004 | 0 | 0 | |
| 224099550 | 40131A>G[d] | Exon 9 | K416R | A | G | 0.002 | 0 | 0.006 | 0 | |
| 224099551 | rs4149229 | Exon 9 | K416K | G | A | 0.073 | 0.099 | 0.056 | 0.014 | 0.021 |
| 224099602 | rs45550332 | Exon 9 | A433A | G | A | 0.006 | 0 | 0.019 | 0 | |
| 224099653 | rs4149230 | Exon 9 | S450S | G | C | 0.133 | 0.145 | 0.117 | 0.125 | 0.923 |
| 224099686 | 40267C>G[d] | 3' UTR | | C | G | 0.002 | 0.004 | 0 | 0 | |
| 224099706 | rs4653695 | 3' UTR | | A | C | 0.063 | 0.084 | 0.031 | 0.056 | 0.375 |
| 224099766 | 40347T>A[d] | 3' UTR | | T | A | 0.002 | 0 | 0 | 0.014 | |
| 224099854 | 40435C>T[d] | 3' UTR | | C | T | 0.002 | 0.004 | 0 | 0 | |
| 224099912 | 40493C>T[d] | 3' flanking | | C | T | 0.004 | 0.008 | 0 | 0 | |

[a]Chromosome position based on National Center for Biotechnology Information (NCBI) Genome Build 36.3

[b]Genotypic frequencies compared among Chinese, Malays and Indians using Pearson's $\chi^2$ test or Fisher's exact test for those MAF>0.01

[c]SNPs were in perfect LD ($r^2 = 1$)

[d]Variants not catalogued in NCBI dbSNP Build 130 are considered novel, and are numbered based on positions on Genbank genomic sequence NG_009776 for *EPHX1*

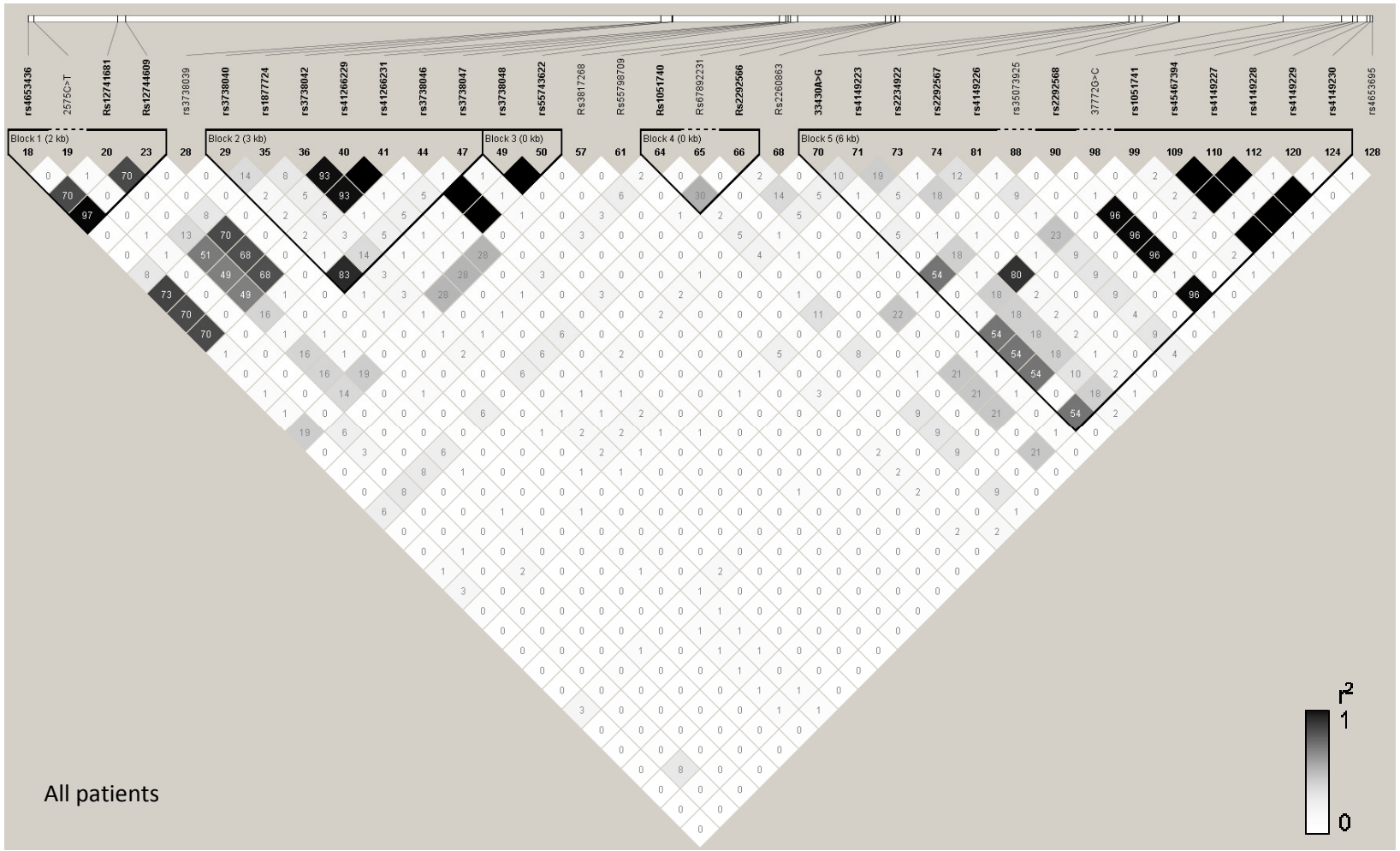
[e]2575C>T is rs117582469 in dbSNP Build 135

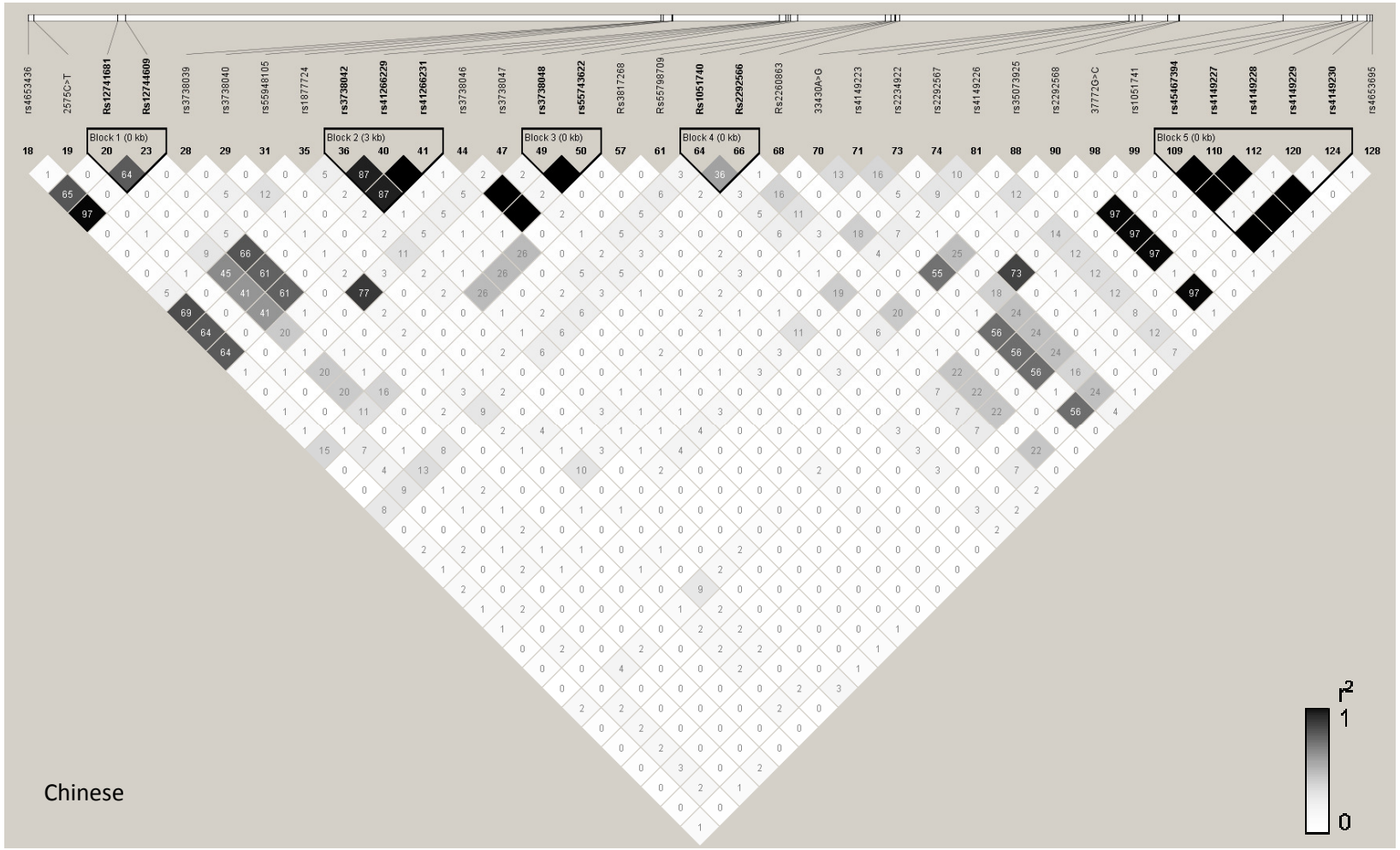
[f]26550G>A is rs146555972 in dbSNP Build 135

[g]33430A>G is rs79860830 in dbSNP Build 135

[h]33935C>T is rs189380029 in dbSNP Build 135

**Appendix 5. LD Maps of *EPHX1* in All Patients and Each of the 3 Ethnic Groups (Study 1)**

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rs41266231
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rs3738047
rs3738048
rs55743622
Rs38172668
Rs55798709
Rs1051740
Rs2292566
Rs2260863
33430A>G
rs4149223
rs2234922
rs2292567
rs4149226
rs35073925
rs2292568
37772G>C
rs1051741
rs45467394
rs4149227
rs4149228
rs4149229
rs4149230
rs4653695
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Block 2 (3 kb)
Block 3 (0 kb)
Block 4 (0 kb)
Block 5 (0 kb)
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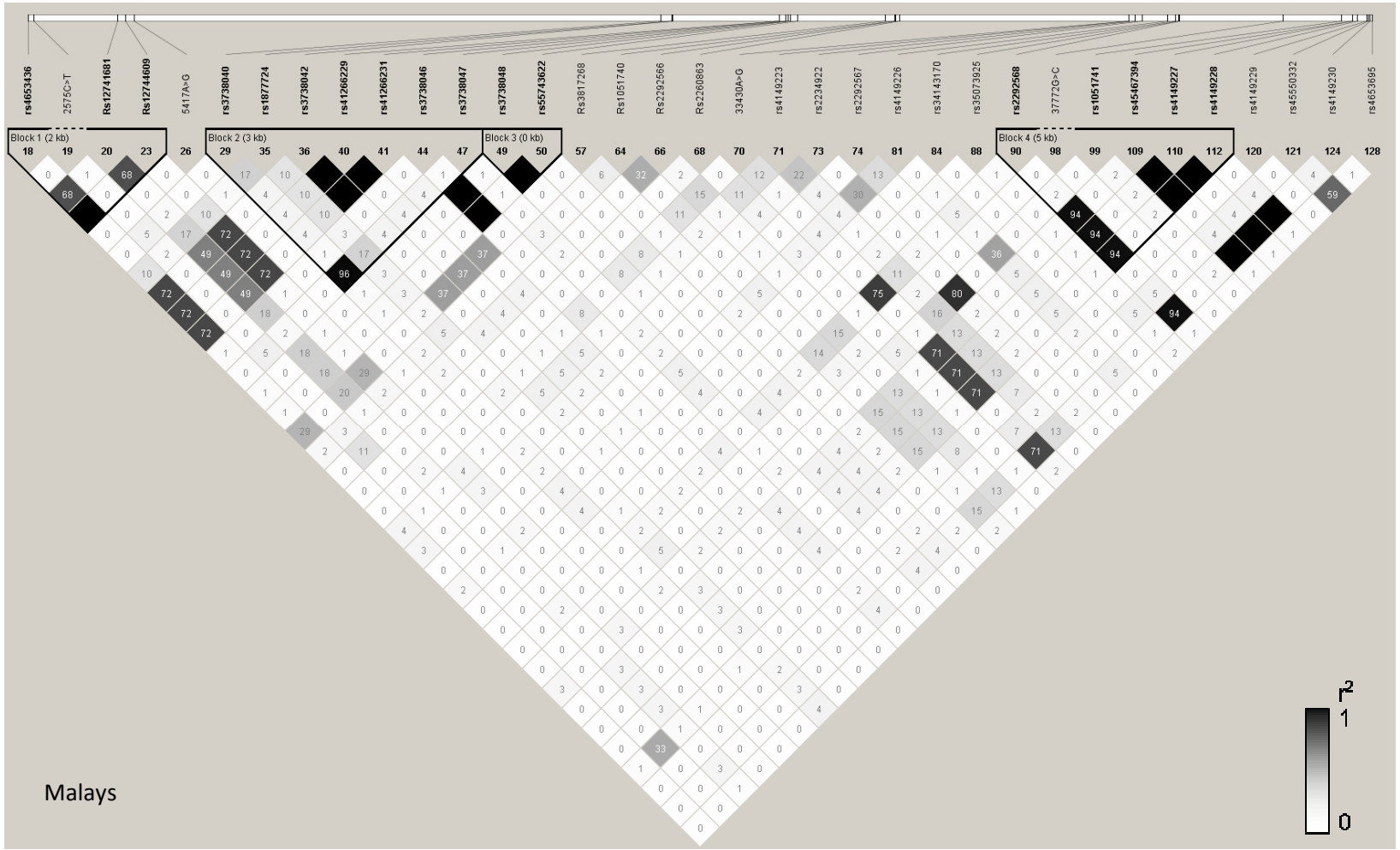
r2
1
0
Chinese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2575C>T
Rs12741681
Rs12744609
5417A>G
rs3738040
rs1877724
rs3738042
rs41266229
rs41266231
rs3738046
rs3738047
rs3738048
rs55743622
Rs3817268
Rs1051740
Rs2292566
Rs2260863
33430A>G
rs4149223
rs2234922
rs2292567
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rs34143170
rs35073925
rs2292568
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rs45467394
rs4149227
rs4149228
rs4149229
rs45550332
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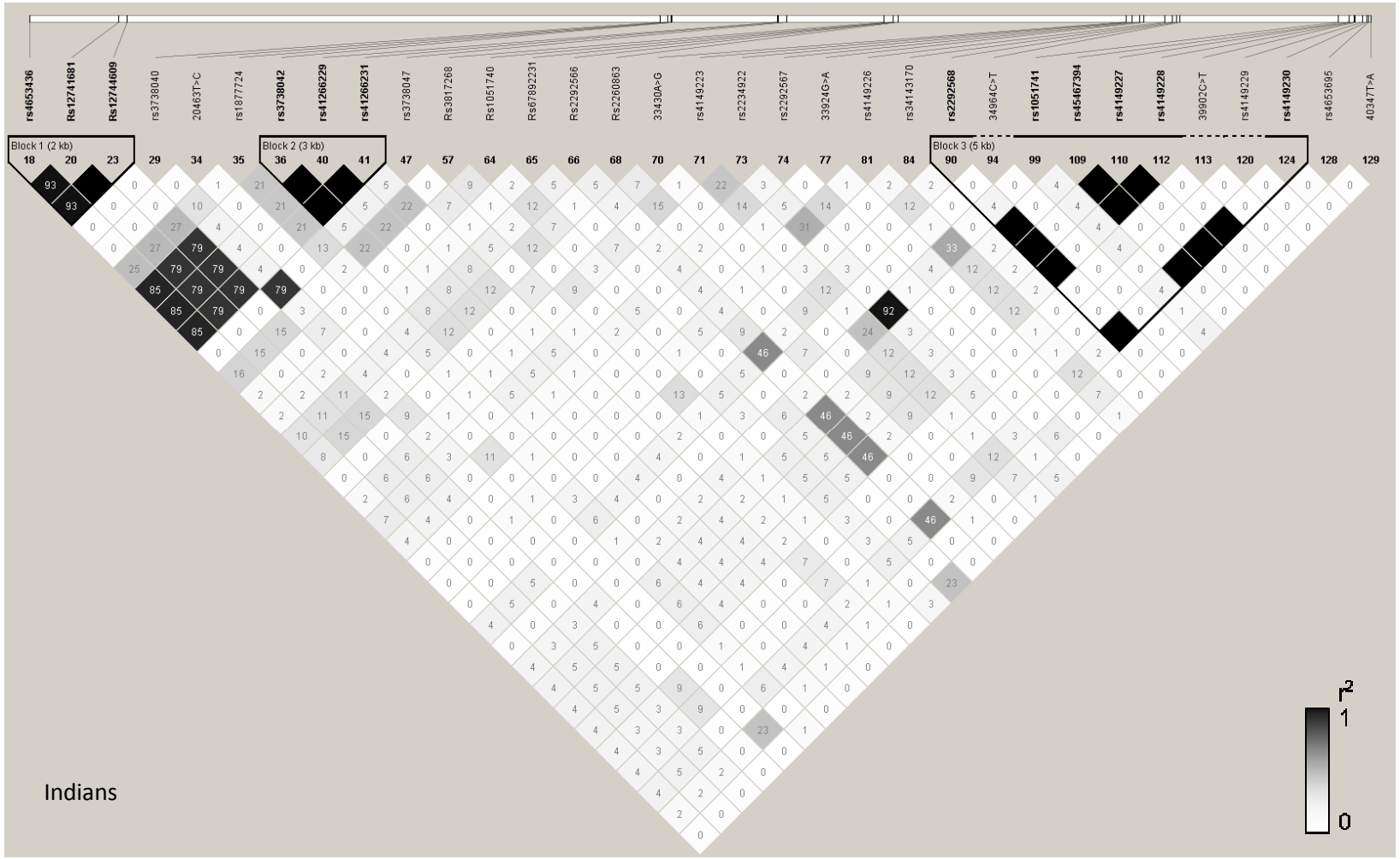
Block 3 (0 kb)
Block 4 (5 kb)
Malays
r²
1
0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Rs12741681
Rs12744609
rs3738040
20463T>C
rs1877724
rs3738042
rs41266229
rs41266231
rs3738047
Rs3817268
Rs1051740
Rs67892231
Rs292566
Rs2260863
33430A>G
rs4149223
rs2234922
rs2292567
33924G>A
rs4149226
rs34143170
rs2292568
34964C>T
rs1051741
rs45467394
rs4149227
rs4149228
39902C>T
rs4149229
rs4149230
rs4653695
40347T>A
Block 1 (2 kb)
Block 2 (3 kb)
Block 3 (5 kb)
Indians
r²
1
0

**Appendix 6. Power Calculation for Study 1 (QUANTO output)**

Model # 1
Outcome: Continuous
Design: Independent individuals
Hypothesis: Gene only
Sample size: 248 individuals
Significance: 0.050000, 2-sided
Gene
Mode of inheritance: Additive
Allele frequency: 0.0100 to 0.1000 by 0.0200
Continuous trait settings
Main 0.5400
Std. dev. 0.2000

Marginal R2 Main effect
R2G 0.0100 *bG 0.1421
(*indicates calculated value)

| Parameter | Null | Full | Reduced |
|---|---|---|---|
| Gene | bG=0 | bG | ---- |

| | | Power | |
|---|---|---|---|
| Frequency | R2G | Gene | bG |
| 0.010000 | 0.0100 | 0.3517 | 0.1421 |
| | 0.0200 | 0.6097 | 0.2010 |
| | 0.0300 | 0.7848 | 0.2462 |
| | 0.0400 | 0.8891 | 0.2843 |
| | 0.0500 | 0.9459 | 0.3178 |
| 0.030000 | 0.0100 | 0.3517 | 0.0829 |
| | 0.0200 | 0.6097 | 0.1172 |
| | 0.0300 | 0.7848 | 0.1436 |
| | 0.0400 | 0.8891 | 0.1658 |
| | 0.0500 | 0.9459 | 0.1854 |
| 0.050000 | 0.0100 | 0.3517 | 0.0649 |
| | 0.0200 | 0.6097 | 0.0918 |
| | 0.0300 | 0.7848 | 0.1124 |
| | 0.0400 | 0.8891 | 0.1298 |
| | 0.0500 | 0.9459 | 0.1451 |
| 0.070000 | 0.0100 | 0.3517 | 0.0554 |
| | 0.0200 | 0.6097 | 0.0784 |
| | 0.0300 | 0.7848 | 0.0960 |
| | 0.0400 | 0.8891 | 0.1109 |
| | 0.0500 | 0.9459 | 0.1239 |
| 0.090000 | 0.0100 | 0.3517 | 0.0494 |
| | 0.0200 | 0.6097 | 0.0699 |
| | 0.0300 | 0.7848 | 0.0856 |
| | 0.0400 | 0.8891 | 0.0988 |
| | 0.0500 | 0.9459 | 0.1105 |

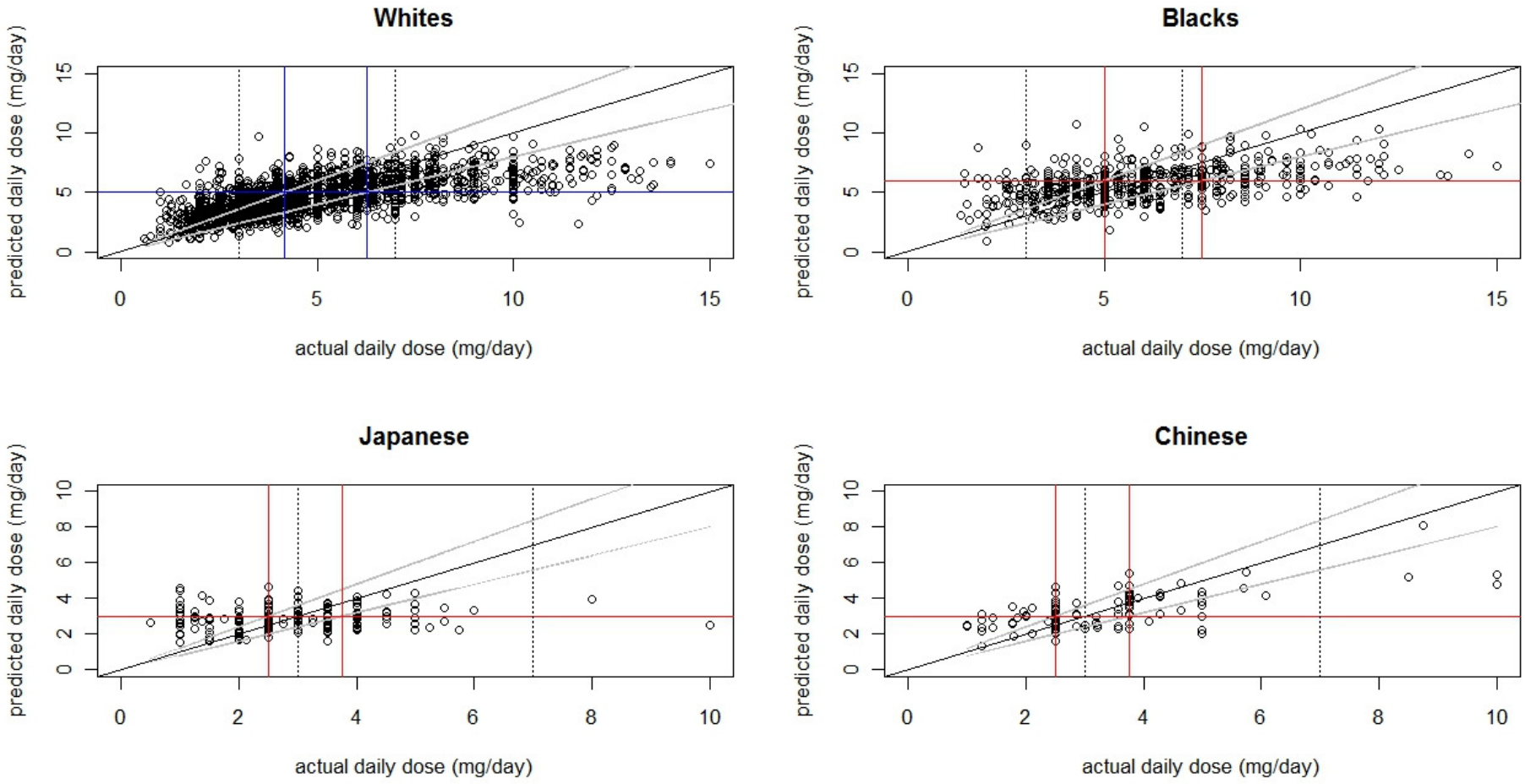
Model # 2
Outcome: Continuous
Design: Independent individuals
Hypothesis: Gene only
Sample size: 248 individuals
Significance: 0.050000, 2-sided
Gene
  Mode of inheritance: Additive
  Allele frequency: 0.1000 to 0.4000 by 0.1000
Continuous trait settings
  Main 0.5400
  Std. dev. 0.2000

  Marginal R2 Main effect
  R2G 0.0100 *bG 0.0471
  (*indicates calculated value)

| Parameter | Null | Full | Reduced |
|---|---|---|---|
| Gene | bG=0 | bG | ---- |

| | | Power | |
|---|---|---|---|
| Frequency | R2G | Gene | bG |
| 0.100000 | 0.0100 | 0.3517 | 0.0471 |
| | 0.0200 | 0.6097 | 0.0667 |
| | 0.0300 | 0.7848 | 0.0816 |
| | 0.0400 | 0.8891 | 0.0943 |
| | 0.0500 | 0.9459 | 0.1054 |
| 0.200000 | 0.0100 | 0.3517 | 0.0354 |
| | 0.0200 | 0.6097 | 0.0500 |
| | 0.0300 | 0.7848 | 0.0612 |
| | 0.0400 | 0.8891 | 0.0707 |
| | 0.0500 | 0.9459 | 0.0791 |
| 0.300000 | 0.0100 | 0.3517 | 0.0309 |
| | 0.0200 | 0.6097 | 0.0436 |
| | 0.0300 | 0.7848 | 0.0535 |
| | 0.0400 | 0.8891 | 0.0617 |
| | 0.0500 | 0.9459 | 0.0690 |
| 0.400000 | 0.0100 | 0.3517 | 0.0289 |
| | 0.0200 | 0.6097 | 0.0408 |
| | 0.0300 | 0.7848 | 0.0500 |
| | 0.0400 | 0.8891 | 0.0577 |
| | 0.0500 | 0.9459 | 0.0645 |

**Appendix 7. Predicted versus Actual Warfarin Doses in IWPC Populations (Study 3)**



Predicted doses using the IWPC pharmacogenetic algorithm were plotted against actual daily doses by race. Within each plot, the black diagonal line is the y=x line, indicating perfect prediction, and the 2 grey lines demarcate the boundaries within which predicted doses were considered accurate (i.e. within ±20% actual dose). The 2 vertical dotted lines demarcate low dose (≤3mg/day), intermediate and high dose (≥7mg/day) groups. In Whites, the 5mg/day fixed dose (horizontal blue line) and the segment of the population which would be thus accurately dosed are demarcated within the 2 vertical blue lines (i.e. all patients between the 2 vertical blue lines were accurately dosed using the 5mg/day fixed dose). Similar demarcations are made for Blacks (6mg/day), Japanese (3mg/day) and Chinese (3mg/day) using their respective race-specific doses in parentheses. In these 3 races, the region between the vertical red lines captures a substantial proportion of the population such that the proportion between the grey lines is outnumbered.

## Appendix 8. Final DCE Design (Study 4)

| Version* | Task | Concept | Nature of test | Number of INR tests till stabilization | Risk of serious ADR (% per year) | Cost of test |
|---|---|---|---|---|---|---|
| 0 | 1 | 1 | Genetic | 5 | 1 | $225 |
| 0 | 1 | 2 | Non-genetic | 13 | 5 | $375 |
| 0 | 2 | 1 | Genetic | 5 | 5 | $375 |
| 0 | 2 | 2 | Genetic | 13 | 1 | $100 |
| 1 | 1 | 1 | Genetic | 5 | 5 | $375 |
| 1 | 1 | 2 | Non-genetic | 21 | 1 | $225 |
| 1 | 2 | 1 | Genetic | 5 | 1 | $600 |
| 1 | 2 | 2 | Non-genetic | 13 | 9 | $100 |
| 1 | 3 | 1 | Genetic | 21 | 5 | $600 |
| 1 | 3 | 2 | Non-genetic | 13 | 5 | $225 |
| 1 | 4 | 1 | Genetic | 5 | 9 | $100 |
| 1 | 4 | 2 | Non-genetic | 21 | 1 | $100 |
| 1 | 5 | 1 | Non-genetic | 13 | 1 | $600 |
| 1 | 5 | 2 | Genetic | 21 | 9 | $375 |
| 1 | 6 | 1 | Non-genetic | 5 | 5 | $375 |
| 1 | 6 | 2 | Genetic | 13 | 1 | $225 |
| 2 | 1 | 1 | Genetic | 13 | 9 | $100 |
| 2 | 1 | 2 | Non-genetic | 21 | 5 | $225 |
| 2 | 2 | 1 | Genetic | 5 | 5 | $600 |
| 2 | 2 | 2 | Non-genetic | 13 | 1 | $375 |
| 2 | 3 | 1 | Non-genetic | 13 | 1 | $225 |
| 2 | 3 | 2 | Genetic | 5 | 9 | $100 |
| 2 | 4 | 1 | Genetic | 5 | 1 | $375 |
| 2 | 4 | 2 | Non-genetic | 21 | 5 | $600 |
| 2 | 5 | 1 | Genetic | 21 | 5 | $225 |
| 2 | 5 | 2 | Non-genetic | 5 | 9 | $100 |
| 2 | 6 | 1 | Genetic | 13 | 9 | $600 |
| 2 | 6 | 2 | Non-genetic | 21 | 1 | $600 |
| 3 | 1 | 1 | Non-genetic | 5 | 9 | $100 |
| 3 | 1 | 2 | Genetic | 13 | 1 | $375 |
| 3 | 2 | 1 | Non-genetic | 5 | 5 | $375 |
| 3 | 2 | 2 | Genetic | 21 | 9 | $225 |
| 3 | 3 | 1 | Genetic | 21 | 1 | $600 |
| 3 | 3 | 2 | Non-genetic | 13 | 5 | $375 |
| 3 | 4 | 1 | Genetic | 13 | 9 | $600 |
| 3 | 4 | 2 | Non-genetic | 21 | 1 | $225 |
| 3 | 5 | 1 | Non-genetic | 5 | 5 | $100 |
| 3 | 5 | 2 | Genetic | 5 | 1 | $375 |
| 3 | 6 | 1 | Non-genetic | 13 | 9 | $100 |
| 3 | 6 | 2 | Genetic | 13 | 1 | $225 |
| 4 | 1 | 1 | Genetic | 13 | 1 | $100 |
| 4 | 1 | 2 | Non-genetic | 5 | 9 | $225 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | 2 | 1 | Genetic | 21 | 1 | $375 |
| 4 | 2 | 2 | Genetic | 13 | 5 | $375 |
| 4 | 3 | 1 | Non-genetic | 5 | 5 | $600 |
| 4 | 3 | 2 | Non-genetic | 21 | 9 | $100 |
| 4 | 4 | 1 | Genetic | 5 | 1 | $225 |
| 4 | 4 | 2 | Non-genetic | 13 | 5 | $600 |
| 4 | 5 | 1 | Genetic | 21 | 9 | $225 |
| 4 | 5 | 2 | Non-genetic | 13 | 9 | $375 |
| 4 | 6 | 1 | Genetic | 21 | 5 | $100 |
| 4 | 6 | 2 | Non-genetic | 13 | 5 | $600 |

* Version 0 = fixed tasks

Summary of Design Efficiency Tests

| Simulation conditions** | Estimated SEs of parameters* | | |
|---|---|---|---|
| | n = 150, 15% none | n = 200, 15% none | n = 200, 20% none |
| Nature of test | | | |
| Genetic | 0.03688 | 0.03199 | 0.03182 |
| Non-genetic | 0.03688 | 0.03199 | 0.03182 |
| Number of INR tests | | | |
| 5 | 0.06256 | 0.05433 | 0.05334 |
| 13 | 0.05472 | 0.04752 | 0.04707 |
| 21 | 0.06216 | 0.05413 | 0.05326 |
| ADR risk per year | | | |
| 1% | 0.05781 | 0.05017 | 0.04961 |
| 5% | 0.06867 | 0.05942 | 0.05782 |
| 9% | 0.07111 | 0.06173 | 0.06045 |
| Cost | | | |
| $100 | 0.08820 | 0.07641 | 0.07485 |
| $225 | 0.07603 | 0.06605 | 0.06502 |
| $375 | 0.08010 | 0.06954 | 0.06774 |
| $600 | 0.07870 | 0.06803 | 0.06640 |
| None | 0.09233 | 0.08228 | 0.06803 |

SE: standard error

* From 'Advanced test' of the CBC design efficiency test in SSI Web 7.0.22. Random responses of sample size n were simulated and parameters estimated under the aggregate logit framework in this test. The recommended guideline for SEs of main effects is ≤0.05.

** Sample sizes and % of respondents choosing the 'none' option.

**Appendix 9. Study 4 Pilot 1 Interview Protocol**

**Interview protocol for pilot 1 (30 – 50 patients)**

**Introductory Script**

| **Notes:** |
| --- |
| To be used to approach potential subjects (Chinese patients heading for ACC) for both cognitive interviews and main survey. Change details as necessary.<br>Note demographic details (age & gender) of all patients approached to capture details on non-responders.<br>Assess suitability of patient while engaging patient<br>Ability to speak Mandarin<br>Ability to give consent / signs of cognitive function problems |

Good morning/afternoon, my name is Sze Ling, a graduate student from NUS and I'd like to invite you to participate in a survey on warfarin pharmacogenetic testing (PGT). Do you have a few minutes to let me explain more about the study?
早上好/下午好，我的名字叫思玲，是新加坡国立大学的研究生。我想邀请你参加一项关于华法林（Warfarin）药物基因测试的研究调查。能给我几分钟的时间向你解释一下吗？

[Continue if patient hasn't flatly refused]

May I know if you have heard about genetic testing? Research in recent years has now enabled us to predict your warfarin dose by testing your genes. This PGT may benefit new patients who need warfarin but its use in clinical practice depends on whether patients are willing to undergo the test and whether they are willing to pay for the test. Hence, the purpose of this study is to find out what you think of warfarin PGT and whether you will be willing to pay for it. Please note that NUHS currently does not have any official plans to implement warfarin PGT.
请问您有听过基因测试吗？近年来的研究现在已经能让我们测试基因就能够预测你需要的华法林剂量。这个基因测试有可能让需要华法林的新病人受益，不过它在临床的成功使用取决于病人愿不愿意接受和支付。所以，这项研究的目的是要知道你对华法林基因测试的看法以及愿意支付的价格。我要先说明国大医院目前还没有正式实施华法林基因测试的计划。

So I'd like to do an in-depth individual interview with you in Mandarin. This is the first phase of this study and the purpose here is to find out more about your experience with warfarin therapy and what is important to you, especially if choosing between having and not having a PGT. This interview will take about 30 minutes and will be voice recorded. The voice recording is solely intended to help us recall the contents and will be kept strictly confidential. With the digital recordings, we do not need to return to you at a later date for any clarifications. Hence, this will be more convenient for you as well.

我想和你以华语做个单独深入访谈。这是本研究的第一阶段，而这里的目的是要了解你服华法林的经验和什么效益指标对你最重要，尤其在选择于做不做华法林基因测试之间。访谈将持续大约 30 分钟，而会被录音。这录音完全是为了帮助我们回忆访谈内容，而将会严格保密。有了录音我也不需要日后再跟你澄清，所以这样对你也比较方便。

*[If patient seem uncomfortable]*: If you are not comfortable with the digital voice recording, we shall not proceed further. 如果你对录音不自在，我们恐怕不能继续。

In appreciation of your taking time to take part in this research study, we will reimburse you $5 for your time and inconvenience.
为了感谢您抽空参与这项研究，我们将会支付你 5 元来报销您的时间和不便。

[Proceed to consent taking if patient is suitable & agreeable]

**Consent Taking**

| Notes: |
| --- |
| To be done at interview venue |
| Re-emphasize some points in patient information sheet |

- You are invited to take part in this research study because you are a warfarin patient
- Phases of the study and no. of patients involved
- This interview will be recorded but participation is voluntary and you may stop at any time Your medical care will not be affected in any way
- All records, including the voice recording, will be kept confidential

Do you have any other questions about the study?
你对这项研究还有别的问题吗？

[Give $ and complete consent document (2 sets)]

**Semi-structured Interview Protocol**

**PART 1: INTRODUCTORY QUESTIONS & BACKGROUND INFORMATION**

| |
|---|
| Aim: To get patients thinking about different warfarin therapy monitoring measures that are possible efficacy attributes in the discrete choice experiment.<br>Note: Point here is not to get exact answers but to get them thinking |

There are no right or wrong answers. Your responses will also not be judged.
这里没有正确或错误的答案。您的回答也不会被评定。

1. How long have you been on warfarin therapy?
   你服华法林（Warfarin）多久了？

- ☐ <3 months
- ☐ 3 – 6 months
- ☐ 7 – 12 months
- ☐ >1 – 3 years
- ☐ > 3 years
- ☐ Don't know/Not sure

2. What do you think of your warfarin management so far?
   到现在为止，你觉得你的华法林（Warfarin）管理怎么样？

3. What was your experience like in the first few weeks when you first started warfarin?
   在你开始服华法林（Warfarin）的头几个礼拜的经验是怎么样的？

4. What is your biggest worry with regards to the use of warfarin?
   你服华法林（Warfarin）最大的担心是什么？

*If on warfarin for <3 months:*

5. Is your dose stable now?
   你的剂量现在稳定了吗?

(i.e. 2 consecutive INR values at least 2 weeks apart that was within the target therapeutic range and during which no changes to warfarin dose was made)
(i.e. 连续 2 次分开至少 2 个星期的 INR 结果在治疗目标范内，也没有更改剂量)

- ☐ Yes (go to 6)
- ☐ No
- ☐ Don't know/ Not sure

6. (When you first started warfarin), how long did it take to stabilize your dose?
   （当你开始服华法林时），花了多少时间才稳定你的剂量?

- ☐ < 2 weeks
- ☐ 2 – 4 weeks
- ☐ 1 – 2 months
- ☐ >2 months
- ☐ Don't know/Not sure (Go to 6a)

6a) Do you remember how many clinic visits it took to stabilize your dose?
你记得需要复诊几次才稳定你的剂量吗?

☐ Yes: _____ visits
☐ No

7. How many INR tests have you had since beginning warfarin therapy/until your dose was stable?
从你服华法林开始/从开始到你剂量稳定为止，你做了几次 INR 测试？

☐ <5
☐ 5 – 9
☐ 10 – 14
☐ 15 – 20
☐ ≥ 21
☐ Don't know/Not sure

8. Have your doctor or pharmacist told you what the risk of bleeding or clotting side effects is?
你的医生或药剂师曾经告诉你出血或凝血副作用的风险是多少吗？

☐ Yes
☐ No

9. What do you think is the risk of bleeding or clotting side effects in the first 3 months?
你觉得在头 3 个月出血或凝血副作用的风险是多少？

☐ <1%
☐ 1 – 5%
☐ 6 – 10%
☐ ≥11%
☐ Don't know/Not sure

10. Have you experienced any side effects due to over- or under-anticoagulation (for eg., clotting, bleeding or use of vitamin K)?
你有没有经历过因为抗凝过度或不足而引起的任何副作用 ？（例如: 凝血,流血或需要服用维他命 K）

☐ Yes, clotting
☐ Yes, bleeding
☐ Yes, used Vitamin K
☐ Yes, but not sure of the details
☐ No
☐ Don't know/Not sure

11. Are you enrolled in the warfarin PGT clinical trial?
你有参加华法林基因测试的临床试验吗？

☐ Yes
☐ No

12. Have you ever taken any genetic test in the past?
你在过去有接受过任何基因测试吗？

☐ Yes
☐ No

If yes, what was the genetic test?
如果有，是什么基因测试？_____

13. May I know your age?
请问你的年龄是什么？_____

14. Gender [observe]: _____

14a. Highest educational level attained:

14b. Housing type:

14c. Did anyone accompany you here today?

14d. If yes, is that person the decision maker for your healthcare matters?

**PART 2: KNOWLEDGE ABOUT WARFARIN PHARMACOGENETIC TESTING & OBTAINING RELEVANT INFORMATION FOR MAIN SURVEY**

**Aim:**

- To assess baseline knowledge about warfarin pharmacogenetic testing (PGT) and post-education understanding
- To find out what anticipated benefits and concerns patients have about warfarin PGT.
- To determine the most relevant efficacy attribute for the discrete choice experiment (DCE)
- To estimate the WTP range for warfarin PGT (for DCE)

**Aid**: Show cards to provide basic information on warfarin PGT

**Note:** Encourage patients to be as specific as possible. Eg. "Can you be more specific?", "Can you explain that in detail?" "Can you give some examples?"

Knowledge, anticipated benefits & concerns

15) Have you heard of pharmacogenetics?
你有听过药物遗传学吗？

*Yes → go to 16*
*No → [Show cards on warfarin PGT (slide 1 & 2 only)] & go to 17*

16) What do you think the term pharmacogenetics mean?
你觉得"药物遗传学"这名称是什么意思？

Go through *show cards (slide 1& 2 only) after patient answers*

17) Can you explain what warfarin PGT is to me in your own words?
你可以向我解释什么是华法林基因测试吗?

18) What benefits do you think a warfarin PGT can bring?/ How do you think warfarin PGT can help a new warfarin patient?
你觉得华法林基因测试可以带来什么好处？/你觉得华法林基因测试可以怎么帮助一个新的华法林病人？

19) What concerns would you have, considering it involves testing your DNA?
因为这涉及到测试你的 DNA，你有什么担心吗？

Go through *show cards (slide 3&4) after patient answers*

20) Do you think the show cards are helpful in explaining what warfarin PGT is?
你觉得这些显示卡对于解释什么是华法林基因测试有帮助吗？

21) Do you have any comments on these show cards? / Is there any part which is not clear in the show cards?
你对这些显示卡有什么意见吗？/ 这些显示卡有哪里不清楚吗？

21a) On a scale of 1 to 5, can you rate how well you think you understood what warfarin PGT is? 1 represents cannot understand at all and 5 represent understand very well.

按 1 到 5 的尺度,你觉得你有多了解什么是华法林基因测试？1 代表很不了解，5 代表很了解。

21b) Rate respondent on understand on same scale.

22) Do you think any of these would be of concern to you? [show slide 4]
你觉得这些对你是顾虑吗？

**Mock DCE to test understanding (To replace questions on attributes & cost)**

In the next part of the study I intend to do a choice experiment where patients will choose between having or not having the warfarin PGT. So I'll now like to try a short version of it to see where it needs to be improved. To decide whether or not to take the warfarin PGT you would want to compare some things, and that will be mainly the benefit and cost.

23) Which 1 or 2 of these 5 attributes would you choose to represent benefit? In other words, which of these are most important or relevant to you? [Show list of 5 attributes]

24) Why?

25) Can you explain why you didn't pick this? [choose 1 or 2 other attributes]
Here I'm using no. of INR tests and risk of ADR to represent benefit. [Show blank DCE format]

The purpose of the choice experiment is to see how you make trade-offs between benefit and cost, and from there deduce how much patients are willing to pay for the test. Therefore I'll show you 5 choice sets where the numbers here will vary. [show 2 examples] Pretend you are a new warfarin patient, the warfarin PGT is available and that taking it is optional. For each choice set, choose the option you would go for as though it is the real situation.

Let me explain the attributes:

1) The number of INR tests is a reflection of how accurately the warfarin PGT predicts your starting dose. This is the average number you can expect to have from beginning until your dose stabilizes.

2) In the initial period your INR will take some time to stabilize so the risk of ADRs such as bleeding or clotting are the highest during this period. We are referring to risk of serious ADRs during the first 3 months, such as major bleeding, thromboembolism or vit K use. [show and explain risk using box grid]

3) Cost is expressed as the total cost incurred in the first 6 months. This includes the cost of INR tests, ACC visits, drugs and the warfarin PGT for this case (PGT).

Do you have any questions?

在这研究的下一步我打算做个选择实验，要求病人选择要不要做华法林基因测试。所以我现在要尝试一个简短的版本，看看那里需要改进。作出选择之前你会要先比较一些东西，而这主要就是效益和花费。

23) 在这 5 个当中，你会选择哪 1 或 2 个来代表效益？换句话，哪个对你最重要或相应？

24) 为什么?

25) 你可以解释为什么没有选这个？

在此我用从开始到剂量稳定时 INR 检测的次数和严重副作用的风险来代表效益。
[Show blank DCE format]
这选择实验的目的是要看你在效益和花费之间怎么取舍，而从中推断病人愿意支付的价格。所以我会给你 5 个选择组，而每组这些数字都会不同。[show 2 examples] 假设你是个新的华法林病人，基因测试已经可得，而且是可选的。请把每个选择组当真实情形来评估。

让我解释这三个考虑范围的意思。
1）INR 检测的次数反映 warfarin 基因测试给你开始剂量的预测有多准确。这是从开始到剂量稳定是平均需要的 INR 检测数次。

2）在初期你的 INR 需要一些时间来稳定，所以初期流血或凝血副作用的风险比较高。我们指的是头 3 个月内严重副作用的风险，比如大出血，凝血（静脉血栓形成或肺动脉栓塞）以及维他命 K 的使用。

3）这是头 6 个月的花费。这包括 INR 测试，看药剂师，药物和基因测试的总花费。

有什么问题吗？

<u>Post-DCE Evaluation</u>
26) Do you think you can understand the choice experiment?
你觉得你能了解这个选择试验吗？

27) What problems do you have while doing the choice experiment?
在做选择试验时有什么问题吗？

I have shown you several possible ways the warfarin PGT may benefit a patient. [Show warfarin PGT slide 3] Do you think these 2 are the best representations of its benefits? In other words are these important or relevant to you?

刚才我给你看了华法林基因测试可能帮助新病人的几种方式。你觉得这两个是华法林基因测试效益的最好代表吗？换句话，这两个对你重要或相应吗？

If not, which of these other benefits would you want to consider instead of these, if you have to make such a choice?

如果这两个不是最好代表，你会要考虑哪个，如果你得选择做不做华法林基因测试？

28) Do these 2 seem independent to you? [INR tests & ADR]

**这两个对你来说是各自独立的吗？也就是说，它们之间有关系吗？**

29) I have 7 choice sets for you here, do you think you can answer all of them accurately?

这里我们有 7 个选择组。你觉得你可以准确地回答全部吗？

Efficacy Attributes

These [show slide 3] are different ways that the warfarin PGT may benefit a new patient. In the next part of this study I'll be doing a choice experiment where I'll ask patients to choose between having and not having the warfarin PGT. To make the choice, they would need to compare a few things, for example out of the 2 options one may be more effective but more expensive, and the other less effective but cheaper. Therefore, we hope to find out what attributes are most important or relevant to you when you make such choices. There are a few ways to express effectiveness here.. [show cards on possible attributes]

这些是华法林基因测试可能帮助新病人的几种方式。在这研究的下一步我将会做个选择实验，要求病人选择要不要做华法林基因测试。作出选择之前需要先比较一些东西，比如两个选项中有一个可能比较有效却同时也比较贵，另一个则比较便宜可是却没那么有效。因此，我们希望知道你做这种选择时最终要或相关的考虑范围。这里有几个可表达有效性的方法。

23) Which of these are most relevant or important to you? In other words, if you have to make choices between having and not having warfarin PGT, which of these do you want to consider? You may choose 1 or 2.

这些当中哪个对你最重要或最相关？换句话，假设我要你决定你是否要做华法林基因测试，你会考虑以上的几个范围？你可以选一个或两个。

24) Why?

为什么?

25) Can you explain why you didn't pick this? [choose 1 or 2 other attributes]

你可以解释为什么没有选这个？

*If >1 attribute chosen:*

26) Do they seem independent to you?

**这些考虑范围对你来说是各自独立的吗？也就是说，它们之间有关系吗？**

Cost

This warfarin PGT is already available in the US and it costs about US$200-600 (S$260-780). Imagine that the test is available in Singapore and taking it is voluntary.

这个华法林基因测试已经在美国推出了。价格是在 200- 600 美元之间（那是新币 260-780 元之间）。现在，请你假设这个测试也来到了新加坡，而且测试是自愿的。

27) What is the maximum price you will be willing to pay for it? (assuming no subsidy, out-of-pocket payment) Your answer does not have to be in the price range I mentioned.

你愿意为它付出的最高价格是什么？（假设没有津贴）你的回答不必在我刚才提到的价格范围之内。

[Optional]

B8) If there's subsidy, how much subsidy would you need before you agree to take the test, assuming the price is $500?

如果有津贴，你需要多少津贴你才会同意测试，假设价格是 500 元？

**CLOSING**

We have come to the end of our discussion. Thank you so much for your participation.
我们已经来到了访谈的尾声。非常谢谢您的参与。

## Appendix 10. Study 4 Pilot 1 Show Cards

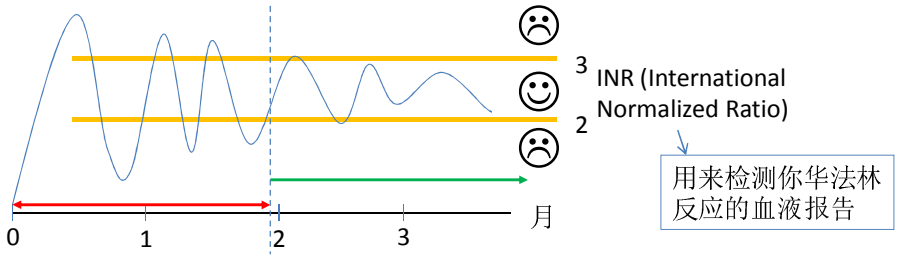
Show card 1

**华法林 (Warfarin)**

- 您正在服用防止血液凝固的药物

**华法林 (Warfarin) 的问题**

- 你需要正确的剂量以防止凝血或出血




- 许多因素会影响一个人所需要的华法林剂量


Wafarin PGT Slide 1

Show card 2

**华法林目前的治疗管理**

- 你从标准剂量开始，需要通过频密的验血来寻找适合你的剂量

**华法林(warfarin)基因测试**

- 一个从一开始就可以帮助医生预测你需要的剂量的基因测试

- 一次的验血
  - 需要5 毫升的血液

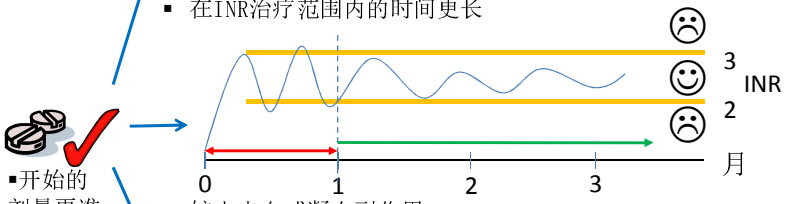
Warfarin PGT Slide 2

Show card 3

## 华法林(warfarin)基因测试可能带来的好处

- 较少的剂量调整
- 较少的 INR 验血检测

- 较短的时间内稳定剂量
- 较短的时间内达到 INR 治疗范围
- 在INR治疗范围内的时间更长




▪开始的剂量更准确

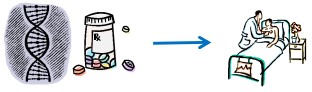
- 较少出血或凝血副作用
- 较少因副作用而住院的次数

Warfarin PGT Slide 3

Show card 4

## 华法林(warfarin)基因测试可能带来的风险

- 焦虑
- 被附上名称的可能性，例如：“敏感”，“耐药”，“高风险”，或“没反应者”
  - 虽然可能性很小，但可能会影响你对自己的感想或
  - 可能会影响买保险或就职的能力



- 而且虽然现在不是这样，未来的研究有可能使人们能以华法林(warfarin)基因测试结果揭示其他疾病的风险

Warfarin PGT slide 4

Show card 5

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **效益** | | |
| **花费** | | |
| 我选择： | | |

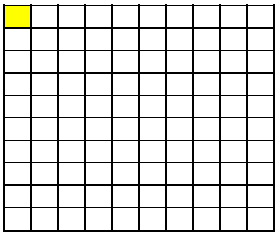
Show card 6

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | | |
| **头3个月内严重副作用的风险** | | |
| **头6个月内的总花费** | | |
| 我选择： | | |

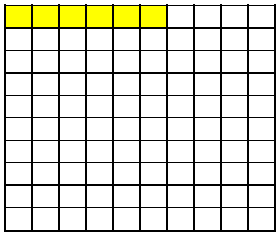
Show card 7

## 头3个月内严重副作用的风险

- 1%

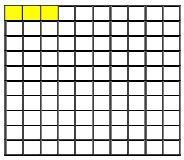
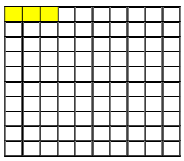


- 6%



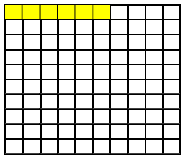
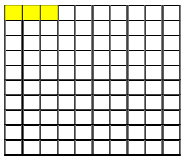
Show card 8

## 例子1

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 11 | 8 |
| **头3个月内严重副作用的风险** | 3%  | 3%  |
| **头6个月内的总花费** | $650 | $950 |
| 我选择： | | |

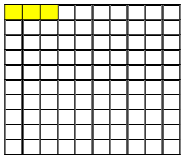
Show card 9

## 例子2

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 11 | 5 |
| **头3个月内严重副作用的风险** | 6%  | 3%  |
| **头6个月内的总花费** | $350 | $650 |
| 我选择： | | |

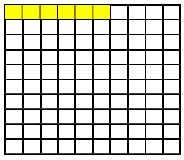
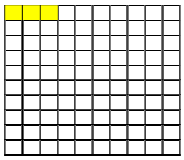
Show card 10

## 选择组1

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 11 | 5 |
| **头3个月内严重副作用的风险** | 3%  | 3%  |
| **头6个月内的总花费** | $650 | $950 |
| 我选择： | | |

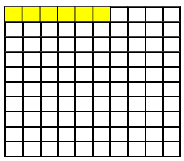
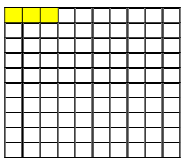
Show card 11

## 选择组2

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 8 | 8 |
| **头3个月内严重副作用的风险** | 6%  | 3%  |
| **头6个月内的总花费** | $350 | $650 |
| 我选择： | | |

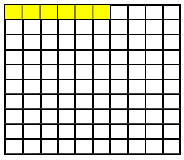
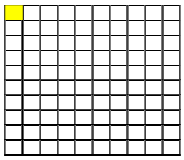
Show card 12

## 选择组3

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 11 | 5 |
| **头3个月内严重副作用的风险** | 6%  | 3%  |
| **头6个月内的总花费** | $650 | $950 |
| 我选择： | | |

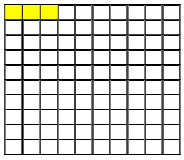
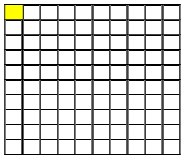
Show card 13

## 选择组4

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 11 | 5 |
| **头3个月内严重副作用的风险** | 6%  | 1%  |
| **头6个月内的总花费** | $650 | $1250 |
| 我选择： | | |

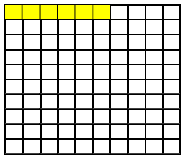
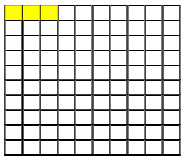
Show card 14

## 选择组5

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 8 | 5 |
| **头3个月内严重副作用的风险** | 3%  | 1%  |
| **头6个月内的总花费** | $650 | $650 |
| 我选择： | | |

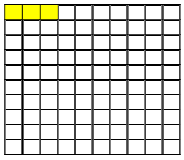
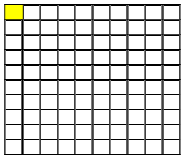
Show card 15

## 选择组6

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 11 | 5 |
| **头3个月内严重副作用的风险** | 6%  | 3%  |
| **头6个月内的总花费** | $950 | $1250 |
| 我选择： | | |

Show card 16

## 选择组7

| | **没有**华法林(warfarin)基因测试 | **有**华法林(warfarin)基因测试 |
|---|---|---|
| **剂量稳定为止 INR 测试次数** | 8 | 5 |
| **头3个月内严重副作用的风险** | 3%  | 1%  |
| **头6个月内的总花费** | $350 | $950 |
| 我选择： | | |

## Appendix 11. Study 4 Pilot 1 Supplementary Methods

Development of efficacy attributes

Efficacy of WPGT can be expressed in terms of various clinical outcomes. These outcomes are intrinsically correlated with one another, but more than 1 may be chosen if patients view them as independent. While it is ideal that the selection of an efficacy attribute(s) and their levels are guided by evidence, relevance and accessibility (ease of being understood) of the attribute to the patient may be more important. Supplementary Table 1 summarizes the existing evidence of the effect of WPGT on various clinical outcomes. Follow up time in the clinical studies therein were used as guides to put a time period to define the efficacy attributes and their levels.

| Outcome | Measure | Results (WPGT vs. standard) | Sample size | Duration of follow up | Ref |
|---|---|---|---|---|---|
| Therapeutic range | Time within range (%) | 41.7% vs. 41.5% | 38 | 28 days | [229] |
| | | 45.4% vs. 24.5%* | 185 | Till stabilization | [230] |
| | | 69.7% vs. 68.6% | 200 | 3 months | [231] |
| | | 43.3% vs. 44.9% | 229 | 90 days | [233] |
| | Time within range (mean days) | 18.5 vs. 19.6 | 121 | 50 days | [234] |
| | Time below range | 2.01 vs. 8.00* (days) | 185 | Till stabilization | [230] |
| | | 48.0% vs. 47.1% (% time) | 229 | 90 days | [233] |
| | Time above range | 1.77 vs. 6.58* (days) | 185 | Till stabilization | [230] |
| | | 8.7% vs. 8.0% (% time) | 229 | 90 days | [233] |
| | % time out of range | 30.7% vs. 33.1% | 200 | 3 months | [231] |
| | Time to 1st therapeutic range | HR 2.89 (95% CI: 2.11–3.97)* | 185 | Till stabilization | [230] |
| | | 5.4 vs. 4.8 (mean no. of doses) | 229 | 90 days | [233] |
| | Time to 1st supra-therapeutic INR/ vitamin K use | 53.4 vs. 47.1 (mean days) | 200 | 3 months | [231] |
| Stable dose | Time to stable dose | 14.1 vs. 32.2* (mean days) | 185 | Till stabilization | [230] |
| | | 24 vs. 35* (median days) (HR 0.52) | 121 | 50 days | [234] |
| | % with stable dose | 82.0% vs. 61.7%* | | | |
| INR tests | Total no. (mean) | 4.9 vs. 10.7* | 185 | Till stabilization | [230] |
| | | 7.2 vs. 8.1 | 200 | 3 months | [231] |
| Dose changes | Mean no. of changes | 3.0 vs. 3.6* (p=0.035) | | | |
| | | 8.4 vs. 7.0 | 229 | 90 days | [233] |
| | Time to 1st dose change | 3.1 vs. 3.6 (No. of doses) | | | |
| | % with dose changes | 83.5% vs. 78% | | | |
| ADR | Rate of major bleeding | RR 0.69 (95% CI: 0.16-2.9) | | | [377] † |
| | % of minor bleeding | 3.2% vs. 12.5%* | 185 | Till stabilization | [230] |
| | % with ADR | 34.7% vs. 42.4% | 200 | 3 months | [231] |
| | | 11.5% vs. 13.3% | 121 | 50 days | [234] |
| | % with serious ADR | 4% vs. 5.1% | 200 | 3 months | [231] |
| | | 1.75% vs. 3.47% | 229 | 90 days | [233] |
| Hospitalization | All cause | 18.5% vs. 25.5%* | 3584 | 6 months | [235] |
| | Due to bleeding/ thromboembolism | 6.0% vs. 8.1%* | | | |

Ref: reference, HR: hazard ratio, * statistically significant, † Meta-analysis of studies [229-231]

**Supplementary Table 1. Summary of Improvements in Clinical Outcomes with WPGT in Prospective Studies**

While several clinical trials are currently ongoing to clarify the clinical benefit of WPGT, evaluations of dose prediction accuracy of published dosing algorithms in several populations, including the Singapore multiethnic population, have consistently demonstrated substantial improvement over clinical and fixed-dose models [134,221,225-227]. Dose prediction accuracy is expressed as the MAE, prediction $r^2$ or proportion of patients with predicted dose within acceptable limits of their actual dose (±20% or ±1mg/day) and the results are summarized in Supplementary Table 2. As an efficacy attribute, the third measure (proportion of patients with predicted dose within acceptable limits of their actual dose) can be expressed as the chance of having an accurate starting dose. This is also the most likely measure out of the 3 to be understood by patients.

| Reference | Definition of accuracy | Results (% of patients) | | | | Remarks |
|---|---|---|---|---|---|---|
| IWPC 2009 [225] | Predicted dose within ±20% actual dose | | P | C | F | |
| | | <21mg/wk | 33 | 25.9 | 0 | |
| | | 21-49mg/wk | 54.6 | 53.6 | 51.6 | |
| | | >49mg/wk | 36.8 | 9.6 | 0 | |
| | | overall | 45.5 | 39.3 | 28.6 | |
| Roper *et al.* 2010 [221] | | 45.9 (IWPC algorithm, best performing) | | | | 5 models compared |
| Chan *et al.* [328] | | 46.8 (P) vs. 40.0 (RS) vs. 25.1 (F) | | | | |
| Sagreiya *et al.* 2010 [134] | Predicted dose within ±1mg/day of actual dose | 63 (P) vs. 54 (C) vs. 38 (F) | | | | |
| Shaw *et al.* 2010 [226] | | 31 – 41 (P) vs. 24 (F) | | | | 6 models compared |
| Takeuchi *et al.* 2010 [227] | | | P | C | | |
| | | ≤10.5mg/wk | 68 | 36 | | |
| | | >10.5-<31.5mg/wk | 80 | 79 | | |
| | | ≥31.5mg/wk | 21 | 0 | | |
| | | overall | 71.4 | 64.1 | | |

P: pharmacogenetic model, C: clinical model, F: fixed dose (5mg), RS: race specific dose (3mg for Chinese & Malays, 5mg for Indians), wk: week
* In the Singapore Asian population

**Supplementary Table 2. Summary of Studies on Accuracy of Dose Prediction**

The list of possible clinical outcomes that may be used as efficacy attributes were then trimmed down to 5, which were deemed to have more clinical relevance to

patients than the others. The main intention of reducing the number of attributes was to facilitate the selection process for patients. The 5 shortlisted attributes were:

i) Chance of having accurate starting dose (accurate dose defined as ±20% maintenance dose),

ii) Time to stable dose,

iii) Number of INR tests till stabilization,

iv) Risk of serious ADR (thrombosis, bleeding or use of vitamin K) in the first 3 months, and

v) Risk of hospitalization due to serious ADR in first 6 months.

Design of mock DCE

To generate a mock DCE, relevant attributes and their levels have to be chosen and defined. Two efficacy attributes (Number of INR tests till stabilization and risk of serious ADR in the first 3 months) were chosen as these were thought to be the 2 most relevant. The levels of these 2 attributes were chosen to cover the reported ranges in existing studies (Supplementary Table 1) and are given below:

- Number of INR tests till stabilization: 5, 8, 11
- Risk of serious ADR in first 3 months: 1%, 3%, 6%

The cost attribute was expressed as total expenditure on warfarin management in the first 6 months. This included the cost of INR tests, ACC visits, drugs (warfarin only) and WPGT (if applicable). WPGT is not yet available in clinical practice in Singapore but reported costs of genotyping *CYP2C9* and *VKORC1* in the US range from US$200-600 (S$260 – 780, based on Dec 2010 exchange rates) [267,341-343], so it was decided to explore a range of $100-$600 for WPGT alone. Using subsidized rates the cost incurred for INR tests, ACC visits and drugs in the first 6 months were

estimated to be between $365 and $635, and therefore cost levels including WPGT were set at $350, $650, $950 and $1250. Detailed calculations are given in Supplementary Table 3.

| Time | Estimated no. of INR tests | Estimated cost for INR tests & ACC visits* | Total in 6 months including drug* | Total cost in 6 months including WPGT | |
|---|---|---|---|---|---|
| | | | | Lower end (+ $100) | Upper end (+ $600) |
| Week 1 | 4 – 7 | $100 - $167.50 | $365 - $635 | $465 | $1235 |
| Week 2 | 2 – 4 | $45 - $90 | | | |
| Week 3 | 1 – 2 | $22.50 - $45 | | | |
| Week 4 | 1 – 2 | $22.50 - $45 | | | |
| Month 2 – 3 | 4 – 8 | $90 - $180 | | | |
| Month 4 – 6 | 2 – 3 | $45 - $67.50 | | | |

* No. of INR tests assumed to be equal to no. of ACC visits. Prices used for calculations are: INR test: $12.50, ACC visit: $20 for first visit, $10 for subsequent visits, drug: $1.40 per week.

**Supplementary Table 3. Calculation of Cost Attribute**

Choice sets were designed to present 2 alternatives at once, 1 without WPGT (current management) and the other with WPGT, as this was thought to be the most likely scenario confronting patients should WPGT become available. 5 – 7 choice sets were then created by manually varying the attribute levels, not according to any statistical design, as the aim here was not to analyze it but to assess if patients can understand the DCE and complete a sufficient number of tasks. However, in the set of 7 choice tasks, 1 task was purposely designed to be dominant (i.e. 1 alternative is logically preferred) as an additional assessment if patients understand the exercise.

## Appendix 12. Study 4 Pilot 2 and 3 Debrief Questions

1. Are there any words or things that are difficult in the questionnaire?

2. Do you understand what is warfarin?

3. Check answers to section 1 question 1.

If wrong: Why did you chose this answer?

Do you think this question (question after section 1) is a good test of your knowledge of the information we gave?

4. Check answers to section 2 questions 5 & 6. If wrong: Why did you choose this answer?

5. Do you have any problems with the choice experiment? (Probe further as appropriate)

6. Do you understand what is WPGT?

Check answers to section 3 question 1. If wrong: Why did you choose this answer?

7. How did you make your choice for this question? (Pick at a few, especially section 4)

8. What were you thinking when you made the choice? (Pick at a few questions, especially section 4)

9. Do you have any other comments on the questionnaire?

**Appendix 13. Summary of Study 4 Pilot 2 and 3 Results**

Pilot 2

Eleven patients consented to participate but 1 withdrew, leaving 10 patients who completed pilot 2, 4 in English and 6 in Chinese. There were 5 males and females each and the mean age was 52. All questions in section 1 were answered correctly except for 1 by 1 patient, but half of the patients had at least 1 wrong answer to section 2 questions 5 and 6, which were designed to test attribute knowledge. Four patients also had problems with the DCE and did not complete it initially. One of them could not complete the rest of the questionnaire. For the rest, most questions in section 3 were correctly answered. In the last section, 2 patients indicated 'already have it' or 'don't know' for all items in question 5, thus providing no information on self-perception of disease risk. One found it offensive while the other found it difficult to put down a risk.

Pilot 3

Eight participants completed the questionnaire on paper (6 females, 2 males; 7 in English and 1 in Chinese; mean age 52 years) and 4 completed it online (all female, 1 in English and 3 in Chinese, mean age 47 years). Most participants found the survey lengthy and technical and had to take some time to understand the information. There was generally no problem with sections 1 and 3, but 3 participants had problems understanding the DCE. One participant did not understand 1 item on the perceived benefits scale (Section 4 question 2c) and was confused by the negative direction of the statement and another participant found section 5 question 10 (self-perception of disease risk) a bit taboo.

**Appendix 14. Study 4 Main Survey Patient Questionnaire Sample**

Participant No.: _____ DCE Version: 1

Date: _____ Time: _____

## QUESTIONNAIRE ON ATTITUDES, WILLINGNESS TO PAY AND PREFERENCES FOR WARFARIN PHARMACOGENETIC TESTING

## SECTION 1: INFORMATION ON WARFARIN

Thank you for taking part in this survey. First, here is some basic information about warfarin so that you will be able to answer the subsequent questions.

**Warfarin**

- A medicine for preventing abnormal blood clots in our blood vessels by making blood less likely to clot.

**The problem with warfarin**

- It is a difficult drug to manage because the dose every patient needs to achieve the same anti-clotting effect can vary a lot.
- The dose of warfarin is adjusted, based on the results of periodic blood tests called INR test.
- The INR test measures how likely blood clots and we want to maintain the INR value within a target range. The most suitable dose maintains the INR within the target range.
- If the dose is too low, the risk of blood clots increases and if the dose is too high, the risk of bleeding increases.
- When warfarin is first started, a 'standard' dose is usually given and then adjusted until the target range is reached (i.e. trial & error), so INR tests are more frequently needed in the initial stabilization period.
- Warfarin control is ideal when a patient reaches target INR range quickly and remains there. The risks of bleeding or clotting side effects are the lowest in this situation.
- However, this is not easy to achieve because many factors such as age, weight, diet, certain medications and diseases, and genes* can affect the dose a person needs.

*Genes are the hereditary materials passed from parents to children, and are what makes one person different from another (for e.g. the colour of our eyes)

We now have a few questions to check that you now have some idea about warfarin.

1. Please indicate if the statements below are TRUE or FALSE, based on the information provided on the previous page. (Circle one option each)

| | | | |
|---|---|---|---|
| a) | Warfarin dose is adjusted according to INR test results. | TRUE | FALSE |
| b) | The most suitable dose is the one that maintains a patient within the target range. | TRUE | FALSE |
| c) | The risks of side effects are higher when the patient is NOT in target range. | TRUE | FALSE |
| d) | The dose each patient needs may be different and currently this dose is determined by trial and error. | TRUE | FALSE |

## SECTION 2: CHOICE EXPERIMENT

You have seen the problems with using the drug warfarin. Imagine there is a new blood test (which is different from an INR test) that can help to improve the anti-clotting control and therefore safety of the drug. We will describe 4 attributes of this new test and then ask you a series of choice questions on which test you would prefer. This is called a choice experiment and the purpose is to learn about your preferences (i.e. what you like more or like less) through the choices you make.

Let's first go through the 4 attributes as it is important that you understand them before doing the choice experiment.

### Attributes of Hypothetical New Test

The new test is a one-time blood test done just before starting the drug Warfarin and it can be described by the following attributes. The first attribute describes what type of test it is, the second and third attributes represent how good the test is in improving the management of warfarin, and the fourth is cost.

| | Attribute | Levels in choice experiment | Explanation |
|---|---|---|---|
| 1 | Nature of test | Genetic or non-genetic | If it's genetic it means the test works by getting information about your genes. |
| 2 | Number of INR tests needed till dose stabilization | 5 to 21 | ▪ Warfarin dose is adjusted based on INR test results.<br>▪ The closer your starting dose is to your actual dose, the fewer INR tests will be needed.<br>▪ Each INR test is usually accompanied by a visit to the pharmacist, who will review the results and adjust the dose if necessary. The whole visit can take several hours.<br>▪ Dose stabilization is defined as the time when 2 consecutive INR readings at least 2 weeks apart are within target range and no dose changes are made. |
| 3 | Risk of serious side effects (major bleeding or clotting) | 1% per year to 9% per year | ▪ Poor warfarin control is linked to a higher risk of side effects.<br>▪ Better control means the risk of serious side effects will be lower.<br>▪ A risk of 1% per year means every year 1 in a 100 patients will experience a serious side effect. |
| 4 | Cost of test | $100 - $600 | This will be a one-time out-of-pocket payment. There will not be any subsidy and you CANNOT pay for this using Medisave or any insurance. |

Before proceeding to the choice experiment, here are some questions to further help you familiarize with the attributes, especially the second & third attributes.

1. How long have you been on warfarin therapy? (Tick one)

- [ ] 1 week or less
- [ ] >1 week to <3 months
- [ ] 3 to 6 months
- [ ] 7 to 12 months [skip to question 3]
- [ ] >1 to 3 years [skip to question 3]
- [ ] > 3 years [skip to question 3]
- [ ] Don't know/Not sure

2. Is your dose stable now? (Stable dose is defined as the time when 2 consecutive INR readings at least 2 weeks apart are within target range and no dose changes are made.) (Tick one)

- [ ] Yes
- [ ] No
- [ ] Don't know/ Not sure

3. How many INR tests did you have from beginning until your dose was stable or until now if dose is not stable? (Stable dose is defined as the time when 2 consecutive INR readings at least 2 weeks apart are within target range and no dose changes are made.) (Tick one)

- [ ] <5
- [ ] 5 – 9
- [ ] 10 – 14
- [ ] 15 – 20
- [ ] ≥ 21
- [ ] Don't know/Not sure

4. Have you experienced any side effects (for eg., clotting, bleeding or use of vitamin K)? (Tick all that applies)

- [ ] Yes, clotting
- [ ] Yes, bleeding
- [ ] Yes, used Vitamin K
- [ ] Yes, but not sure of the details
- [ ] No
- [ ] Don't know/Not sure

5. **IF** patient A needs 12 INR tests before his warfarin dose is stable while patient B only needs 5, which of the following is correct? (Tick one)

- [ ] Patient A has better warfarin control
- [ ] Patient B has better warfarin control
- [ ] Don't know/Not sure

6. **IF** the risk of a bleeding or clotting side effect is 2% per year WITH the new test and 5% per year WITHOUT the new test, which of the following is correct? (Tick one)

- ☐ Warfarin control is BETTER with the new test
- ☐ Warfarin control is THE SAME with the new test
- ☐ Warfarin control is WORSE with the new test
- ☐ Don't know/Not sure

---

Now we will ask you a series of choice questions. In each question, we will show you 2 hypothetical new tests at a time and ask you to choose the one you prefer. An example of a choice question looks like this:

**Choice Experiment Example**
(You DO NOT need to answer this)

| | Test A | Test B |
|---|---|---|
| Nature of test | Genetic | Non-genetic |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 5% per year |
| Cost of test | $375 | $225 |
| If these were the only 2 choices, I would choose: | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really have taken it? | | |

Here are some <u>important points</u> to note for the choice experiment:

- This new one-time blood test is most useful for new patients, just before starting on the drug. When doing the choice experiment, **imagine the time just before you started on warfarin.**
- Please study the 2 options carefully and consider them as though they are real choices before making your choice. They may look very similar to you but their attribute levels will differ.
- When making decisions we ask that you think about what you would prefer, NOT what you think would be best for your family or your friends.
- **There are no right or wrong answers**. We understand that everyone may make different choices. We want to know what you prefer.

Here are the <u>instructions</u> for the choice experiment:

- Please indicate the test you would **prefer**.
- There are 2 parts to each choice question. Please answer both parts.

We will now begin the series of choice questions.

**Choice Set 1**

| | Test A | Test B |
|---|---|---|
| Nature of test | Genetic | Non-genetic |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 21 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 1% per year |
| Cost of test | $375 | $225 |
| If these were the only 2 choices, I would choose (tick one): | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really have taken it? (tick one) | | |

---

**Choice Set 2**

| | Test A | Test B |
|---|---|---|
| Nature of test | Genetic | Non-genetic |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 1% per year | 9% per year |
| Cost of test | $600 | $100 |
| If these were the only 2 choices, I would choose (tick one): | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really it? (tick one) | | |

**Choice Set 3**

| | Test A | Test B |
|---|---|---|
| Nature of test | Genetic | Non-genetic |
| Number of INR tests needed before warfarin dose stabilizes | 21 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 5% per year |
| Cost of test | $600 | $225 |
| If these were the only 2 choices, I would choose (tick one): | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really have taken it? (tick one) | | |

---

**Choice Set 4**

| | Test A | Test B |
|---|---|---|
| Nature of test | Genetic | Non-genetic |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 21 |
| Risk of serious side effects (Major bleeding or clotting) | 9% per year | 1% per year |
| Cost of test | $100 | $100 |
| If these were the only 2 choices, I would choose (tick one): | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really have taken it? (tick one) | | |

**Choice Set 5**

| | Test A | Test B |
|---|---|---|
| Nature of test | Genetic | Non-genetic |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 1% per year | 5% per year |
| Cost of test | $225 | $375 |
| If these were the only 2 choices, I would choose (tick one): | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really have taken it? (tick one) | | |

---

**Choice Set 6**

| | Test A | Test B |
|---|---|---|
| Nature of test | Non-genetic | Genetic |
| Number of INR tests needed before warfarin dose stabilizes | 13 | 21 |
| Risk of serious side effects (Major bleeding or clotting) | 1% per year | 9% per year |
| Cost of test | $600 | $375 |
| If these were the only 2 choices, I would choose (tick one): | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really have taken it? (tick one) | | |

**Choice Set 7**

| | Test A | Test B |
|---|---|---|
| Nature of test | Genetic | Genetic |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 1% per year |
| Cost of test | $375 | $100 |
| If these were the only 2 choices, I would choose (tick one): | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really have taken it? (tick one) | | |

---

**Choice Set 8**

| | Test A | Test B |
|---|---|---|
| Nature of test | Non-genetic | Genetic |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 1% per year |
| Cost of test | $375 | $225 |
| If these were the only 2 choices, I would choose (tick one): | | |

| | Yes | No |
|---|---|---|
| If your chosen test is now available and is not compulsory, would you really have taken it? (tick one) | | |

---

If you indicated that you would NOT really take the option you chosen for ALL 8 choice sets, can you please share with us why?

_____

_____

## SECTION 3: INFORMATION ON WARFARIN PHARMACOGENETIC TESTING

After the choice experiment I believe you are quite familiar with the attributes of the new test already. There is such a new test which is currently still under research and may be introduced into clinical use in the near future. We will briefly describe it here as we would like to ask you some questions about how you feel about such a test in the next section. This new test is called warfarin pharmacogenetic test (WPGT).

**Warfarin Pharmacogenetic Test (WPGT)**

- One-time blood test done just before starting warfarin
- It uses your genes (DNA) to predict the dose you'll need

**Possible benefits**

- More accurate starting dose, which theoretically would translate to:
  - Fewer dose adjustments
  - Fewer INR tests (during initial stabilization phase)
  - Shorter time to stable dose
  - Shorter time to reach target INR range
  - Longer time spent in target INR range
  - Lower risk of bleeding or clotting side effects
  - Lower risk of hospitalization due to side effects
- As a result of these possible benefits, you MAY save some money

**Possible risks**

These are some theoretical risks of WPGT. You may or may not find them a problem.

- Anxiety
- Possibility of labels attached to people, for eg, "sensitive", "resistant", "high risk" or "non-responder"
  - May affect self perception
  - May affect even ability to obtain insurance or employment
- Although not the case now, future research may make it possible to indicate risk of other diseases based on the information obtained from the WPGT

We now have a few questions to check that you now know about WPGT before we proceed to the next section.

1. Please indicate if the statements about WPGT below are TRUE or FALSE, based on the information provided on the previous page. (Circle one option each)

| | | | |
|---|---|---|---|
| a) | It is a genetic test. | TRUE | FALSE |
| b) | It works by predicting the dose you need. | TRUE | FALSE |
| c) | While it is not perfect, warfarin treatment is expected to be safer and more effective with WPGT. | TRUE | FALSE |
| d) | WPGT is different from an INR test. | TRUE | FALSE |

## SECTION 4: ATTITUDES TOWARDS WPGT

We'll now ask you some questions on how you feel about WPGT.

- Imagine that your doctor has just prescribed warfarin for you and has given you all the information above about WPGT.
- Imagine that the WPGT is available and is not compulsory.

1. On this scale below, how willing are you to take the WPGT before starting on warfarin therapy? (Please circle one option)

| Very unwilling | Somewhat unwilling | Neutral | Somewhat willing | Very willing |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

If you indicated 'very unwilling', what is the reason? (Tick all that applies)

- ☐ It's too costly
- ☐ I'm uncomfortable with a genetic test
- ☐ I don't think it will benefit me
- ☐ Others (pls specify): _____

2. I'll now ask you to rate your agreement to statements using these 5 levels of agreement. (Please circle one option each)

| **With regards to the WPGT,** | Strongly disagree | Disagree | Neutral | Agree | Strongly agree |
|---|---|---|---|---|---|
| a) I am hopeful that it can detect which dose works best | 1 | 2 | 3 | 4 | 5 |
| b) If it reveals that I need a very low or very high dose, I would feel anxious | 1 | 2 | 3 | 4 | 5 |
| c) I don't think it will lower my risk of warfarin side effects. | 1 | 2 | 3 | 4 | 5 |
| d) I am worried that it may subsequently reveal that I possess additional risk factors for another disease that I was unaware of | 1 | 2 | 3 | 4 | 5 |
| e) I think it can predict a more suitable starting dose for me. | 1 | 2 | 3 | 4 | 5 |
| f) I am hopeful that there may be less trial and error in finding my warfarin dose | 1 | 2 | 3 | 4 | 5 |
| g) I am worried that the results may be passed onto unauthorized persons | 1 | 2 | 3 | 4 | 5 |

For items h) and i):
Apart from the fact that I'm taking warfarin or have a pre-existing condition, if it reveals that I need a very low or very high dose,

| | Strongly disagree | Disagree | Neutral | Agree | Strongly agree | |
|---|---|---|---|---|---|---|
| h) I may be additionally disadvantaged when buying health insurance | 1 | 2 | 3 | 4 | 5 | N/A |
| i) I may be additionally treated unfairly at work or job-seeking | 1 | 2 | 3 | 4 | 5 | N/A |

3. Are there any other concerns about WPGT that we have not captured in the statements above? If yes, please kindly share with us.

_____
_____

## SECTION 5: DEMOGRAPHICS, BACKGROUND INFO, QUALITY OF SURVEY

Thank you for completing the survey till this point. This is the last section and we will now collect some personal information about you to help us have a better understanding of your responses compared to other participants.

1. Age: _____

2. Gender:
- ☐ Male
- ☐ Female

3. Religion:
- ☐ Christianity
- ☐ Buddhism
- ☐ Taoism
- ☐ Islam
- ☐ Hinduism
- ☐ Free thinker
- ☐ Others (pls specify): _____

4. Marital status:
- ☐ Single
- ☐ Married
- ☐ Divorced/ Separated
- ☐ Widowed

5. Highest Educational level attained:
- ☐ No qualification / lower primary
- ☐ Primary (PSLE)
- ☐ Secondary ('O'/'N' level)
- ☐ Upper secondary ('A' level/vocational)
- ☐ Diploma
- ☐ Degree

6. Housing type:
- ☐ 1-2 room HDB
- ☐ 3-room HDB
- ☐ 4-room HDB
- ☐ 5-room HDB or Executive
- ☐ Private condominium
- ☐ Landed

7. Which language are you more comfortable with?
- ☐ English
- ☐ Mandarin
- ☐ Both English and Mandarin

8. Are you enrolled in any warfarin PGT clinical trial?

☐ Yes
☐ No

9. Have you ever taken any genetic test in the past?

☐ Yes
☐ No

If yes, what was the genetic test? _____

10. What do you think is your chance of developing the following diseases in your lifetime? We would like to know your personal perception of these chances. You can base them on your lifestyle, family history and any other relevant information. (Please circle one option each)

| | Not at all | Very unlikely | Somewhat unlikely | Somewhat likely | Very likely | Definitely | Already have it | Don't know |
|---|---|---|---|---|---|---|---|---|
| a) Stroke | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| b) Heart attack | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| c) Diabetes | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| d) Cancer | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| e) H1N1 infection | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| f) Hepatitis B | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

11. In your opinion, on a scale of 0 to 10, how easy is it to <u>understand</u> the instructions in the survey that you have just completed? (Please circle one option)

| Least easy | | | | | | | | | | Most easy |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

12. In your opinion, on a scale of 0 to 10, how hard do you need to <u>concentrate</u> during the survey that you have just completed? (Please circle one option)

| Very hard | | | | | | | | | | Not at all |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

13. In your opinion, on a scale of 0 to 10, how <u>offensive</u> do you find the survey that you have just completed? (Please circle one option)

| Most offensive | | | | | | | | | | Least offensive |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

14. Was there anything about this questionnaire that you found difficult to understand?

☐ Yes
☐ No

If Yes, please specify: _____

We have come to the end of the survey. Thank you very much for your participation.

## Appendix 15. Study 4 Main Survey Public Questionnaire Sample (Screenshots)

**SURVEY ON ATTITUDES, WILLINGNESS TO PAY AND PREFERENCES FOR WARFARIN PHARMACOGENETIC TESTING**
**对华法林(Warfarin)基因测试的态度，愿付额和偏好问卷**

**Welcome! You are invited to take part in a research study.**
**欢迎！您被邀请参加一项研究。**

By coming to this survey, you have confirmed that you have read the Participant Information Sheet (attached in the email advertisement sent to NUS students) and agreed to participate in this research study.
您来到这调查网页代表您确认您已经阅读了研究信息表(附着在发给国大学生的电子邮件广告里)，而且答应参加这项研究。

If you would like to read the Participant Information Sheet again, please click
如果您想再次阅读参与者信息表，请点击here 这里

The principal investigator of this study is:
Dr Wee Hwee Lin
Assistant Professor
Department of Pharmacy, NUS
这项研究的首席研究员是:
黄慧琳助理教授
新加坡国立大学药剂系

You may contact Sze Ling (graduate student in charge of this study) at email g0801729@nus.edu.sg) for all research-related matters.
如果您对此项研究有问题，您可以以电邮(g0801729@nus.edu.sg)联络思玲(负责这项研究的研究生)。

---

Before we start, we need your postal code and house number to check that the number of respondents per household do not exceed our pre-set limits and also for postage of your reimbursement after you successfully complete the survey.

The following information also serves as passwords so you may complete the survey only ONCE using the same combination. If you stop halfway, you may log in again using the same combination to continue the survey at a later time. However, if you do not complete the survey for any reason, this information will be discarded.

在我们开始之前，我们需要您的邮政编码和门牌号码来确保每户的受访者人数不超过预先设定的限制和让我们在您成功地完成调查后把报销邮寄给您。

以下的信息也作为密码，所以您只可用相同的组合完成调查**一次**。如果您中途停止，您可以使用相同的组合在以后的时间再次登入网站继续调查。不过，如果您因任何原因没有完成调查，此信息将被丢弃。

Please enter your postal code:
请输入您的邮政编码:

Please enter your house or unit no.:
请输入您的门牌号码:

Please enter the NUS email of the student who referred you to this survey:
请输入介绍您来做这项调查的学生的大学电邮地址:

**Please choose your preferred language for this survey:**
**请选择您的语言:**

English 英文  Mandarin 华文

**Before we begin, we would first like to ask you several questions to ascertain your eligibility.**

1. What is your age?

2. What is your ethnicity?

- ◯ Chinese
- ◯ Malay
- ◯ Indian
- ◯ Others

3. Have you ever taken the drug called Warfarin before?

- ◯ Yes
- ◯ No

0% 100%

**SECTION 1: INFORMATION ON WARFARIN**

Thank you for taking part in this survey. First, here is some basic information about warfarin so that you will be able to answer the subsequent questions.

**Warfarin**

- A medicine for preventing abnormal blood clots in our blood vessels by making blood less likely to clot.

**The problem with warfarin**

- It is a difficult drug to manage because the dose every patient needs to achieve the same anti-clotting effect can vary a lot.
- The dose of warfarin is adjusted, based on the results of periodic blood tests called INR (International Normalized Ratio) test.
- The INR test measures how likely blood clots and we want to maintain the INR value within a target range. The most suitable dose maintains the INR within the target range.
- If the dose is too low, the risk of blood clots increases and if the dose is too high, the risk of bleeding increases.
- When warfarin is first started, a 'standard' dose is usually given and then adjusted until the target range is reached (ie. trial and error), so INR tests are more frequently needed in the initial stabilization period.
- Warfarin control is ideal when a patient reaches target INR range quickly and remains there. The risks of bleeding or clotting side effects are the lowest in this situation.
- However, this is not easy to achieve because many factors such as age, weight, diet, certain medications and diseases, and genes* can affect the dose a person needs.

*Genes are the hereditary materials passed from parents to children, and are what makes one person different from another (for eg. the colour of our eyes)

0% 100%

**SECTION 2: Choice Experiment**

You have seen the problems with using the drug warfarin. Imagine there is a **new blood test** (which is **different from an INR test**) that can help to improve the anti-clotting control and therefore safety of the drug. We will describe 4 attributes of this new test and then ask you a series of questions on which test you would prefer. This is called a choice experiment and the purpose is to learn about your preferences (ie. what you like more or like less) through the choices you make.

Let's first go through the 4 attributes as it is important that you understand them before doing the choice experiment.

**Attributes of Hypothetical New Test**

The new test is a one-time blood test done just before starting the drug Warfarin and it can be described by the following attributes. The first attribute describes what type of test it is, the second and third attributes represent how good the test is in improving the management of warfarin, and the fourth is cost.

| | Attribute | Levels in choice experiment | Explanation |
|---|---|---|---|
| 1 | Nature | Genetic or non-genetic | If it's genetic it means the test works by getting information about your genes. |
| 2 | Number of INR tests needed till dose stabilization | 5 to 21 | ▪ Warfarin dose is adjusted based on INR test results.<br>▪ The closer your starting dose is to your actual dose, the fewer INR tests will be needed.<br>▪ Each INR test is usually accompanied by a visit to the pharmacist, who will review the results and adjust the dose if necessary. The whole visit can take several hours.<br>▪ Dose stabilization is defined as the time when 2 consecutive INR readings at least 2 weeks apart are within target range and no dose changes are made. |
| 3 | Risk of serious side effects (major bleeding or clotting) | 1% per year to 9% per year | ▪ Poor anti-clotting control is also linked to a higher risk of side effects.<br>▪ Better control means the risk of serious side effects will be lower.<br>▪ A risk of 1% per year means every year 1 in a 100 patients will experience a serious side effect. |
| 4 | Cost of test | $100 - $600 | This will be a one-time out-of-pocket payment. There will not be any subsidy and you CANNOT pay for this using Medisave or any insurance. |

**Before proceeding to the choice experiment, here are some questions to further help you familiarize with the attributes, especially the second & third attributes.**

1. If you need repeated blood tests to monitor a medical condition, how would it affect you? (Select all that applies)

- ☐ It would take a lot of time
- ☐ It is a hassle to travel to the hospital
- ☐ It would be agonizing because I'm afraid of needles
- ☐ I would feel more assured
- ☐ It would cost a lot of money
- ☐ Others (pls specify):

2. If you need to take warfarin, how much would you value a new test that can potentially decrease the number of INR tests needed?

○ Not at all ○ A little ○ Somewhat more ○ Quite a lot ○ Very much

3. Have you ever experienced a serious side effect from taking a drug before?

- ○ Yes
- ○ No
- ○ Don't know

4. Do you know of a friend or family member who has suffered a serious side effect from taking a drug before?

- ○ Yes
- ○ No

If you would like to review the information on Warfarin again, please click here

5. **IF** patient A needs 12 INR tests before his warfarin dose is stable while patient B only needs 5, which of the following is correct?

- ○ Patient **A** has better warfarin control
- ○ Patient **B** has better warfarin control
- ○ Don't know/Not sure

6. **IF** the risk of a bleeding or clotting side effect is 2% per year with the new test and 5% per year without the new test, which of the following is correct?

- ○ Warfarin control is **BETTER** with the new test
- ○ Warfarin control is **THE SAME** with the new test
- ○ Warfarin control is **WORSE** with the new test
- ○ Don't know/Not sure

0% 100%

Now we will ask you a series of choice questions. In each question, we will show you 2 hypothetical new tests at a time and ask you to choose the one you prefer. An example of a choice question looks like this:

**Choice Experiment Example**
(You DO NOT need to answer this)

**If these were the only 2 choices, I would choose:**

| | Genetic | Non-genetic |
|---|---|---|
| Nature of test | | |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 5% per year |
| Cost of test | $375 | $225 |
| | ○ | ○ |

If your chosen test is now available and is not compulsory, would you really have taken it?

○ Yes
○ No

---

If you would like to review the explanations of the attributes again, please click here

Here are some **important points** to note for the choice experiment:

- This **new one-time blood test** is most useful for new patients, just before starting on the drug. When doing the choice experiment, **imagine that your doctor has just prescribed warfarin for you.**
- Please study the 2 options carefully and consider them as though they are real choices before making your choice. They may look very similar to you but their attribute levels will differ.
- When making decisions we ask that you think about what you would prefer, NOT what you think would be best for your family or your friends.
- **There are no right or wrong answers.** We understand that everyone may make different choices. We want to know what you prefer.

---

Here are the **instructions** for the choice experiment:

- Please indicate the test you would **prefer**.
- There are 2 parts to each choice question. Please answer both parts.

We will now begin the series of choice questions.

0% 100%

**If these were the only 2 choices, I would choose: [Click one circle]**

| Nature of test | Genetic | Non-genetic |
|---|---|---|
| Number of INR tests needed before warfarin dose stabilizes | 13 | 21 |
| Risk of serious side effects (Major bleeding or clotting) | 9% per year | 5% per year |
| Cost of test | $100 | $225 |

If your chosen test is now available and is not compulsory, would you really have taken it?

- Yes
- No

0% 100%

**If these were the only 2 choices, I would choose: [Click one circle]**

| Nature of test | Genetic | Non-genetic |
|---|---|---|
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 1% per year |
| Cost of test | $600 | $375 |

If your chosen test is now available and is not compulsory, would you really have taken it?

- Yes
- No

0% 100%

**If these were the only 2 choices, I would choose: [Click one circle]**

| Nature of test | Non-genetic | Genetic |
|---|---|---|
| Number of INR tests needed before warfarin dose stabilizes | 13 | 5 |
| Risk of serious side effects (Major bleeding or clotting) | 1% per year | 9% per year |
| Cost of test | $225 | $100 |

If your chosen test is now available and is not compulsory, would you really have taken it?

- Yes
- No

0% 100%

**If these were the only 2 choices, I would choose: [Click one circle]**

| Nature of test | Genetic | Non-genetic |
|---|---|---|
| Number of INR tests needed before warfarin dose stabilizes | 5 | 21 |
| Risk of serious side effects (Major bleeding or clotting) | 1% per year | 5% per year |
| Cost of test | $375 | $600 |

If your chosen test is now available and is not compulsory, would you really have taken it?

- Yes
- No

0% 100%

**If these were the only 2 choices, I would choose: [Click one circle]**

| Nature of test | Genetic | Non-genetic |
|---|---|---|
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 1% per year | 5% per year |
| Cost of test | $225 | $375 |
| | ○ | ○ |

If your chosen test is now available and is not compulsory, would you really have taken it?

○ Yes
○ No

0% 100%

**If these were the only 2 choices, I would choose: [Click one circle]**

| Nature of test | Genetic | Non-genetic |
|---|---|---|
| Number of INR tests needed before warfarin dose stabilizes | 21 | 5 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 9% per year |
| Cost of test | $225 | $100 |
| | ○ | ○ |

If your chosen test is now available and is not compulsory, would you really have taken it?

○ Yes
○ No

0% 100%

**If these were the only 2 choices, I would choose: [Click one circle]**

| | | |
|---|---|---|
| Nature of test | Genetic | Genetic |
| Number of INR tests needed before warfarin dose stabilizes | 5 | 13 |
| Risk of serious side effects (Major bleeding or clotting) | 5% per year | 1% per year |
| Cost of test | $375 | $100 |
| | ○ | ○ |

If your chosen test is now available and is not compulsory, would you really have taken it?

- ○ Yes
- ○ No

0% 100%

**If these were the only 2 choices, I would choose: [Click one circle]**

| | | |
|---|---|---|
| Nature of test | Genetic | Non-genetic |
| Number of INR tests needed before warfarin dose stabilizes | 13 | 21 |
| Risk of serious side effects (Major bleeding or clotting) | 9% per year | 1% per year |
| Cost of test | $600 | $600 |
| | ○ | ○ |

If your chosen test is now available and is not compulsory, would you really have taken it?

- ○ Yes
- ○ No

0% 100%

**IF** you indicated that you would **NOT** really take the option you chosen for **ALL 8 choice sets**, can you please share with us why?

Do you have any problems completing section?

- Not at all
- A little
- A lot

0% 100%

**SECTION 3: INFORMATION ON WARFARIN PHARMACOGENETIC TESTING**

After the choice experiment I believe you are quite familiar with the attributes of the new test already. There is such a new test which is currently still under research and may be introduced into clinical use in the near future. We will briefly describe it here as we would like to ask you some questions about how you feel about such a test in the next section. This new test is called warfarin pharmacogenetic test (WPGT).

If you would like to review the information on Warfarin again, please click here

**Warfarin Pharmacogenetic Test (WPGT)**

- One-time blood test done just before starting warfarin
- It uses your genes (DNA) to predict the dose you'll need

**Possible benefits**

- More accurate starting dose, which theoretically would translate to:
  - Fewer dose adjustments
  - Fewer INR tests (during initial stabilization phase)
  - Shorter time to stable dose
  - Shorter time to target INR range
  - Longer time in target INR range
  - Lower risk of bleeding or clotting side effects
  - Lower risk of hospitalization due to side effects
- As a result of these possible benefits, you MAY save some money

**Possible risks**

These are some theoretical risks of WPGT. You may or may not find them a problem.

- Anxiety
- Possibility of labels attached to people, for eg, "sensitive", "resistant", "high risk" or "non-responder"
  - May affect self perception
  - May affect even ability to obtain insurance or employment
- Although not the case now, future research may make it possible to indicate risk of other diseases based on the information obtained from the WPGT

0% 100%

**We now have a few questions to check that you now know about WPGT before we proceed to the next section.**

1. Please indicate if the statements about WPGT below are TRUE or FALSE, based on the information provided so far.

| | True | False |
|---|---|---|
| a) It is a genetic test. | ○ | ○ |
| b) It works by predicting the dose you need. | ○ | ○ |
| c) While it is not perfect, warfarin treatment is expected to be safer and more effective with WPGT. | ○ | ○ |
| d) WPGT is different from an INR test. | ○ | ○ |

0% 100%

**SECTION 4: ATTITUDES TOWARDS WPGT**

We'll now ask you some questions on how you feel about WPGT.

- Imagine that your doctor has just prescribed warfarin for you and has given you all the information above about WPGT.
- Imagine that the WPGT is available and is not compulsory.

If you would like to review the information on WPGT again, please click here

1. On this scale below, how willing are you to take the WPGT before starting on warfarin therapy?

○ Very unwilling ○ Somewhat unwilling ○ Neutral ○ Somewhat willing ○ Very willing

0% 100%

You indicated '**very unwilling**', what is the reason? (Choose all that applies)

- ☐ It's too costly
- ☐ I'm uncomfortable with a genetic test
- ☐ I don't think it will benefit me
- ☐ Others (pls specify):

0% 100%

**2. I'll now ask you to rate your agreement to statements using these 5 levels of agreement.**

With regards to the WPGT,

| | Strongly disagree | Disagree | Neutral | Agree | Strongly Agree |
|---|---|---|---|---|---|
| a) I am hopeful that it can detect which dose works best | ○ | ○ | ○ | ○ | ○ |
| b) If it reveals that I need a very low or very high dose, I would feel anxious | ○ | ○ | ○ | ○ | ○ |
| c) I think it will lower my risk of warfarin side effects. | ○ | ○ | ○ | ○ | ○ |
| d) I am worried that it may subsequently reveal that I possess additional risk factors for another disease that I was unaware of | ○ | ○ | ○ | ○ | ○ |
| e) I am hopeful that there may be less trial and error in finding my warfarin dose | ○ | ○ | ○ | ○ | ○ |
| f) I am worried that the results may be passed onto unauthorized persons | ○ | ○ | ○ | ○ | ○ |

For items g) and h):
Apart from the fact that I'm taking warfarin or have a pre-existing condition,

| | Strongly disagree | Disagree | Neutral | Agree | Strongly Agree | N/A |
|---|---|---|---|---|---|---|
| g) if it reveals that I need a very low or very high dose, I may be **additionally** disadvantaged when buying health insurance | ○ | ○ | ○ | ○ | ○ | ○ |
| h) if it reveals that I need a very low or very high dose, I may be **additionally** treated unfairly at work or job-seeking | ○ | ○ | ○ | ○ | ○ | ○ |

0% 100%

1. Gender:

- ◯ Male
- ◯ Female

2. Religion:

- ◯ Christianity
- ◯ Buddhism
- ◯ Taoism
- ◯ Islam
- ◯ Hinduism
- ◯ Free thinker
- ◯ Others (please specify):

3. Marital status:

- ◯ Single
- ◯ Married
- ◯ Divorced/ Separated
- ◯ Widowed

4. Highest Educational level attained:

- ◯ No qualification / lower primary
- ◯ Primary (PSLE)
- ◯ Secondary ('O'/'N' level)
- ◯ Upper secondary ('A' level/vocational)
- ◯ Diploma
- ◯ Degree

5. Housing type:

- ◯ 1-2 room HDB
- ◯ 3-room HDB
- ◯ 4-room HDB
- ◯ 5-room HDB or Executive
- ◯ Private condominium
- ◯ Landed

0% 100%

6. Do you know any friends or relatives who are taking warfarin?

- Yes
- No

7. Have you ever taken any genetic test in the past?

- Yes (please specify):
- No

8. What do you think is your chance of developing the following diseases in your lifetime? We would like to know your personal perception of these chances. You can base them on your lifestyle, family history and any other relevant information.

| | Very unlikely | Somewhat unlikely | Somewhat likely | Very likely | Already have it |
|---|---|---|---|---|---|
| a) Stroke | | | | | |
| b) Heart attack | | | | | |
| c) Diabetes | | | | | |
| d) Cancer | | | | | |

0% 100%

As a token of appreciation for taking time to complete the survey, you will be reimbursed $10.

To allow us to send the reimbursement to you, please provide your name or initials:

Reimbursements will be sent out within 1 month of the end of the survey. Please contact Sze Ling at g0801729@nus.edu.sg if you do not receive it by then.

0% 100%

**We have come to the end of the survey. Thank you very much for your participation.**
You may close the browser now.

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0% 100%