Prediction of warfarin dose: why, when and how?

Prediction models are the key to individualized drug therapy. Warfarin is a typical example of where pharmacogenetics could help the individual patient by modeling the dose, based on clinical factors and genetic variation in CYP2C9 and VKORC1. Clinical studies aiming to show whether pharmacogenetic warfarin dose predictions are superior to conventional initiation of warfarin are now underway. This review provides a broad view over the field of warfarin pharmacogenetics from basic knowledge about the drug, how it is monitored, factors affecting dose requirement, prediction models in general and different types of prediction models for warfarin dosing.

KEYWORDS: dosing algorithm, human CYP2C9 protein, human VKORC1 protein, pharmacogenetics models, statistical regression analysis, warfarin

There is overwhelming evidence that the individual maintenance dose of warfarin can be predicted by pharmacogenetic dose models [1–4]. In 2007 the US FDA updated the label of warfarin to encourage, but not require, pharmacogenetic testing of patients initiating warfarin therapy [5]. In 2010 dosing recommendations according to genotypes of CYP2C9 and VKORC1 were added to the label (Table 1). Starting a patient’s treatment with an individually predicted warfarin dose would, in theory, lower the risk of early bleeding, which is highly related to the intensity of anticoagulation [6]. In spite of this, the translation of pharmacogenetic dose models into clinical practice has been slow. Clinical studies aiming to show whether pharmacogenetic warfarin dose predictions are superior to conventional initiation of warfarin are now underway.

This review provides an overview of the field of warfarin pharmacogenetics including basic information about the drug and how the anticoagulation effect is monitored, common problems encountered during treatment and the impact of genetic factors. Special focus is put on warfarin dose prediction models and how they are developed and evaluated.

Warfarin

Since the introduction of warfarin in the early 1950s, its use has steadily increased. It is now the most widely used anticoagulant in the world with annual prescriptions equaling 0.5–1.5% of the population [7]. Warfarin is a vitamin K-antagonist that targets the vitamin K cycle, thereby inhibiting the vitamin K-dependent coagulation factors II, VII, IX and X. It is administered as a racemic mixture of (R) and (S)-enantiomers with the (S)-isomer being three- to five-times more potent then the (R)-form [8].

Warfarin is mainly used for primary and secondary prevention of venous thromboembolism, and for prevention of systemic embolism in patients with prosthetic heart valves or atrial fibrillation [6]. Studies on atrial fibrillation patients show that the overall risk reduction of stroke is approximately 70% compared with placebo [6].

Monitoring of anticoagulation

One of the main problems with warfarin therapy is the large interindividual variation in the dose needed to reach adequate levels of anticoagulation. Dose requirements vary more than tenfold, ranging from <10 to >100 mg per week. The anticoagulant effect therefore needs to be monitored, especially during the initiation of therapy. This is done by measuring the prothrombin time (PT) International Normalized Ratio (INR), which is a measure of three of the four vitamin K–dependent coagulation factors: II, VII and X. The INR is determined by dividing the PT of a patient with the geometric mean of PT for at least 20 healthy subjects of both sexes with the same test system [9]. An INR of 1.0 is considered to be normal coagulation and an INR of 2.0 means that the clotting time has been doubled.

INR target ranges vary between countries and indications, but the most common target range is 2.0–3.0. For patients with a high risk of thrombosis, for example, patients with...
mechanical heart valves, the target range can be increased to 2.5–3.5 or even higher [10]. Patients receiving warfarin have frequent INR checks during the induction phase of therapy and once the target range is achieved the INR is measured once or twice a month.

A measure of how well a patient is anticoagulated during a specific time interval is the time in treatment range (TTR). The standard way to calculate TTR is by the Rosendaal method, which uses linear interpolation to calculate the percentage of time a patient is within treatment range (usually INR 2.0–3.0) [11]. TTR is associated with the efficacy of warfarin treatment, and a 10% increase in time spent outside therapeutic range relates to an augmented risk of mortality, ischemic stroke and other thromboembolic events [12,13].

**The induction phase**

There is no gold standard for how to initiate warfarin. The half-life of warfarin varies greatly between individuals, and is on average 36–42 h [6]. This means that it takes more than a week to reach a pharmacokinetic steady state when starting on a maintenance dose. Some apply a defensive strategy in patients not requiring rapid anticoagulation by starting with low doses and raising them over time with the guidance of frequent INR tests. This method is safe with respect to over-anticoagulation, which is related to bleeding, but patients requiring higher doses than average will be under-anticoagulated for a large part of the induction phase [10]. Loading doses are therefore often used to reach steady state more quickly. Commonly used initiation methods include giving 5–10 mg on the first 2 days or giving age-stratified initiation doses before switching to maintenance doses [14,15].

**Adverse effects**

The most common adverse effect of warfarin is bleeding and the risk is highly related to the intensity of anticoagulation [13]. The incidence of major bleeding during warfarin treatment is, according to the recent RE-LY trial, on average 3.36% per patient year, but varies between studies depending on inclusion criteria and quality of medical care in terms of INR control [16]. Studies have demonstrated that increasing the INR target range from 2.0–3.0 to 3.0–4.5 also increases the risk of clinically significant bleeding [6]. An interesting observation from these studies is that the mean increase in dose per day is ≈1 mg when changing from the low to the high target range.

If a patient is severely over-anticoagulated with high INR levels or bleeding, treatment is normally stopped and vitamin K is administered to reverse the effects of warfarin. In case of severe bleeding there is also an option to infuse fresh plasma or prothrombin concentrate [6].

**Dietary interactions**

Vitamin K is the natural antidote to warfarin. Vitamin K is found in food, and diet therefore imposes variability in warfarin response. Normal intake of vitamin K is in the range of 60–200 µg/day. Most dark green vegetables such as broccoli, Brussels sprouts and spinach contain high levels of vitamin K (>100 µg/2 dl), but also other common foodstuffs contain a fairly large amount of vitamin K [17]. It is estimated that an increase in vitamin K intake of 100 µg per day for 4 consecutive days lowers the INR by 0.2 and vitamin K intake has therefore been incorporated into some warfarin dose prediction models [18,19].

Studies on combining warfarin treatment with a vitamin K supplement have been performed with the aim to reduce the variability in drug response caused by a low or sporadic dietary intake [20]. These studies show varying results, but the overall conclusion is that vitamin K supplements do decrease the variation in drug response caused by dietary intake. The current recommendation for patients on warfarin treatment is to keep a constant intake of vitamin K through foodstuffs to minimize variation in warfarin response.

**Drug interactions**

The two warfarin isomers are metabolized by different pathways. The main enzyme involved in the metabolic elimination of (S)-warfarin is CYP2C9, while (R)-warfarin is eliminated by CYP1A1/CYP1A2/CYP3A4 [5]. Patients starting or stopping drugs that are known inducers or inhibitors of these enzymes should have extra INR tests, and their dose of warfarin adjusted

| Table 1. Ranges of recommended warfarin doses (mg/day) from the US FDA drug label. |
|---------------------------------|-----------------|
| VKORC1  | CYP2C9 (mg/day)  |
|        | *1/*1 | *1/*2 | *1/*3 | *2/*2 | *2/*3 | *3/*3 |
| GG      | 5–7  | 5–7  | 3–4  | 3–4  | 3–4  | 0.5–2 |
| AG      | 5–7  | 3–4  | 3–4  | 3–4  | 0.5–2 | 0.5–2 |
| AA      | 3–4  | 3–4  | 0.5–2 | 0.5–2 | 0.5–2 |

*Reproduced from the updated warfarin (Coumadin) product label.*

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Prediction of warfarin dose: why, when & how?

Developing & critically reviewing prediction models

A prediction model is any algorithm relating to a certain measure or measures of an outcome. In the field of medicine, it is most commonly the result of a regression model with estimated coefficients as weights of each variable. Although the process of prediction modeling can differ substantially between different projects/outcomes, there are still some general points to think of. These points are highlighted under Box 1, and are described in more detail here. Knowledge of certain important steps in prediction modeling can both help in future projects and when reading literature including prediction models.

First of all, it is of high importance that the data a prediction model is built on reflect the population that it will be used on. For instance, if we have a prediction model that includes age, then the data used to build the model should include the age interval of the future populations where predictions will be made. If a model has been built on data from a cohort aged 20–40 years and is used to predict in patients aged 60–80 years, there is a chance that the coefficient for age is wrong; the age-outcome relationship might for instance be of second order. This problem could arise if the cohort used to build the model is from a clinical study with narrow inclusion criteria for some key variables, while prediction in a more general population is desired.

Think of different validation techniques before any calculations are done. If there is no external data set available and the data at hand is fairly large, validation can be done by splitting the data into a training set and a validation set. This, however, requires that no calculations/assumptions/modeling have been done on the whole data. Data splitting could still give a positive bias, that is, the performance will appear better than it really is. Instead, it is recommended to separate the data before any calculations are done. The process of prediction modeling can differ substantially between different projects/outcomes, there are still some general points to think of. These points are highlighted under Box 1, and are described in more detail here. Knowledge of certain important steps in prediction modeling can both help in future projects and when reading literature including prediction models.

### Is data representative for the population the model will be used on?
- **Yes**
- **No**

### Validation of model; internal or external?
- **Yes**
- **No**

### Missing data? Imputation?
- **Yes**
- **No**

### Is the effect of continuous variables linear or should transformation be applied?
- **Linear**
- **Transformation required**

### Read the literature; gather as much information as possible from previous publications about important variables
- **Yes**
- **No**

### Before discarding any variables from the model, estimate univariate estimates (one estimate of variable effect against outcome at a time) and multiple estimates (all variables included in the model)
- **Yes**
- **No**

### If variable selection is necessary, decide which variables to drop by investigating estimates of effects with confidence intervals, not only p-values. Do not automatically discard variables with p > 0.05
- **Yes**
- **No**

### Collaboration between statistical and medical expertise is essential
- **Yes**
- **No**
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better than in real life, because the validation set is not entirely independent of the derivation set in terms of study wise definitions and design. Unbiased measures of performance are estimated by external validation on data totally independent from the modeling process.

Depending on study type, missing values could be a problem. A clinical study is most likely to have a low number of missing values, while data from registries often have more missing values. Missing values cause problems in the analysis, since patients with any missing value are discarded if nothing else is done. Most serious is the case of informative missing, that is, when the missing pattern has something to do with the outcome being analyzed. Various imputation techniques can be used to fill in missing values to reduce the amount of patients removed from the analysis.

If continuous predictors are used, ensure that the relationship to the outcome is linear by plotting Y against the predictor. If there is evidence of a nonlinear effect, then apply some kind of transformation (e.g., log or square root) to attain linearity. In the worst case it is possible to categorize the variable, but think twice before categorizing a continuous variable since this means loss of information.

Utilizing previous knowledge about predictors for the outcome will firstly lower the risk for spurious results by making it possible to drop some variables from the analysis before they are analyzed against the outcome. Second, it will help select variables for the modeling process.

As a first step in the modeling, estimating univariate effects is a good way to gain knowledge about the data. The next step would be to fit a full multiple model using all predictors. This model could be the final prediction model if there is no need for variable selection. However, the model is often improved, in terms of simplicity and clinical utility, by dropping some variables. For instance, do not keep a variable that, although significant, shows signs of being nonimportant (estimate close to zero) if it also costs money to produce in the clinic. Another reason for dropping variables is that a simple model is easier to interpret and use in a clinical setting than a more complex model.

In the process of variable selection, do not always obey the $p \leq 0.05$ significance rule that is inherited from clinical trials; point estimates and CIs for each coefficient include more information. Why is a variable with $p = 0.045$ more interesting than a variable with $p = 0.055$ or even $p = 0.15$? The effect of all three variables could actually be the same, but the precision of the estimate could be different due to one variable being infrequent in the cohort. As an example, if a known interacting drug is used seldom in your cohort and therefore has a low frequency, the precision will be low. This gives large CIs for the estimate, but the estimate itself may be of the same magnitude as in previous studies. In this case the p-value will probably be $>0.05$, but given the previous knowledge the variable should be kept in the model.

The most common performance measure of linear regression models is the coefficient of determination, $R^2$. It measures the percentage (or proportion) of variability of the outcome that is explained by the model. While the $R^2$ value gives a point estimate for the predictive ability of the model, examining scatter plots of observed values versus predicted values gives more information about performance across the whole range of outcome values.

If external validation of the final model is used, and it includes or only consists of another ethnicity, the estimates of performance might be lower than expected. This could be due to the ethnicities having different allele frequencies of the genetic markers included in the model. This is truly the case for warfarin (see section on ‘Stable warfarin maintenance dose’).

For further in depth details about prediction modeling, the authors recommend Regression Modeling Strategies by Frank E Harrell Jr [39].

Why predict warfarin dose?

The ultimate goal of pharmacogenetics is to individualize therapy and warfarin is a good example of where pharmacogenetics could help tailor the dose from the beginning of treatment. The narrow therapeutic index of warfarin and high interindividual variation in dose needed is a challenge in clinical care. Complications from inappropriate warfarin dosing remain as one of the most common reasons for emergency room visits in the USA [7,40]. Unintentional overdosing of warfarin is the most common cause of hospitalization due to adverse reactions in the USA, and the second most common cause in the UK [41,42]. There is evidence of a pronounced risk of bleeding during the initiation phase (1–3 months), which a genotype-guided dose has the potential to reduce [6,43]. For instance, a study by Wadelius et al. showed that the risk of over anticoagulation (INR $>4$) is highly related to CYP2C9 and VKORC1 genotypes [4]. A small prospective cohort study by Gong et al. indicated that the genotypic risk could be removed.
by using a pharmacogenetics-guided initiation dosing protocol [44].

**When to predict warfarin dose**

In the following sections three different types of prediction model are discussed. The first is the prediction of stable warfarin maintenance dose, which is the most studied outcome in warfarin pharmacogenetics. However, maintenance dose prediction models do not tell us how to initiate treatment in the best way, which leads us to the second type of model: prediction of starting doses. The third type of prediction model is the dose refinement model that also includes INR response after the initial doses. For the different types of models examples from the largest international studies are shown and discussed.

### Stable warfarin maintenance dose

The first prediction models for stable warfarin dose were estimated on relatively small populations mainly consisting of Caucasian patients included from one area or clinic [23]. While these models might predict the dose within the same catchment area, they are probably not portable to a more broad population from other regions or countries [46]. To address the problem of generalizability, the International Warfarin Pharmacogenetics Consortium (IWPC) was established with the primary aim to develop an algorithm for warfarin dose prediction. The IWPC includes 21 research groups from nine countries that have contributed clinical and genetic data for 5700 patients who were treated with warfarin [1]. The resulting pharmacogenetic model was shown to predict patients requiring low doses (≤21 mg/week) or high doses (≥49 mg/week) significantly better than a clinical model. In the validation data the pharmacogenetic algorithm explained 43% of the variance in warfarin dose ($R^2 = 43\%$), while the clinical algorithm had an $R^2$ of 26%. The IWPC model includes the variables $VKORC1$ (rs9923231), $CYP2C9$ (*2 and *3), age, height, weight, race and the following interacting drugs: enzyme inducers and amiodarone (Table 2) [1]. Several studies have externally validated the IWPC model together with other models, and the overall conclusion is that the IWPC model performs well; however, the sample size has been small in these studies [46-48]. In 2010 the US FDA updated their Coumadin label with a table giving stable dosing recommendations per genotype of $CYP2C9$ and $VKORC1$ (Table 1). A dose nomogram for the IWPC model illustrating the effect of $VKORC1$, $CYP2C9$ and age on week dose (mg) for a Caucasian patient with no interacting drugs, height 175 cm and weight 75 kg is given in Figure 1. The FDA’s recommended dosing recommendations from the Coumadin label are shown for comparison (shaded areas).

A pharmacogenetic dosing algorithm has been shown to be superior to the FDA table on dosing recommendations [49].

The SNPs commonly used in warfarin maintenance dose prediction models are presented in Table 3 together with the estimates of minor allele frequencies (MAF) from Scott et al. and Limdi et al. [1,2,4,50,51,52]. Notable is the difference in MAF between ethnicities, with more genetic variation among individuals of European descent than in individuals of African and Asian descent. For the $VKORC1$ SNP rs9923231 there is also a shift in minor allele between Europeans, Asians and Africans with the minor allele being A for Europeans and Africans and G for Asians. The differences in MAF affects how well a prediction model containing this variable, in terms of $R^2$, performs in the population [53]. The second study by IWPC showed that the reason for the lower performance of the IWPC algorithm in African Americans and Asians ($R^2 \approx 24\%$) than in Caucasians ($R^2 = 40\%$) was mainly due to less variation in the $VKORC1$ SNP rs9923231 in African–Americans and Asians. By simulations the IWPC showed that given the population MAF, the $R^2$ for the $VKORC1$ SNP rs9923231 was in the range of what would be expected if the genotype effect was the same across ethnicities [52]. The conclusion was that non-Caucasian patients with variant alleles have similar benefit of genetic-based dosing with the IWPC model, even if the performance of the algorithm is lower on a population level. In other words genetic based dosing for a single patient will work as well for African–Americans and Asians as it does for Caucasians but on a population level Caucasians, with most variation in $VKORC1$, will benefit most from genetic dosing.

### Starting doses

The time to reach steady-state concentrations of a drug depends on how quickly it is cleared from the system. If you initiate a drug with the maintenance dose, it takes approximately five-times the half-life of the drug to reach steady state. Compared with a person without $CYP2C9$ variant alleles, the half-life of the more potent enantiomer (S)–warfarin is doubled in a *2/*2 person and increased three- to six-fold.
in a *3/*3 person. Hence, if treatment is initiated with the predicted maintenance dose, the time to reach steady state is prolonged two- to-six fold in patients with only *CYP2C9* variant alleles. Recent work by Hamberg et al. has described these pharmacokinetic differences in more detail [54,55]. The time to reach steady state can be optimized by combining a stable dose prediction algorithm with information on the estimated clearance of *CYP2C9* genotypes [25]. This individualized pharmacogenomics-based warfarin initiation dose regimen is especially beneficial in patients with *CYP2C9* variant alleles. A table of pharmacogenetic starting doses calculated using the formulas by Avery et al. (see formula in Table 2) is given in Table 4 for an example patient with six different combinations of *CYP2C9* and *VKORC1* [25]. The predicted maintenance doses are taken from the dose nomogram in Figure 1. These predicted loading doses show that for a Caucasian patient at the age of 60 years, height 175 cm, weight 75 kg, with no interacting drugs and the most common combination of genotypes for Caucasians (*CYP2C9* *1/*1 and *VKORC1* rs9923231 A/G) the predicted maintenance dose would be 4.71 mg per day and the loading doses would be 7.6 mg on day one, 6.61 mg on day two and 5.66 mg on day three. If a patient with the same clinical and demographic variables had impaired metabolism (*CYP2C9* *3/*3) and required a low dose according to *VKORC1* (rs9923231 A/A) the predicted maintenance dose would be 1.00 mg and the loading.

### Table 2. Three different prediction models for warfarin dosing.

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of model</th>
<th>Algorithm</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klein et al., IWPC</td>
<td>Maintenance dose</td>
<td>(\text{Dose (mg/week)} = 5.6044 - 0.2614 \times \text{age (in decades)} + 0.0087 \times \text{height (cm)} + 0.0128 \times \text{weight (kg)} - 0.8677 \times \text{VKORC1 A/G} - 1.6974 \times \text{VKORC1 A/A} - 0.4854 \times \text{VKORC1 genotype unknown} - 0.5211 \times \text{CYP2C9 *1/*2} - 0.9257 \times \text{CYP2C9 *1/*3} - 1.0616 \times \text{CYP2C9 *2/*2} - 1.9206 \times \text{CYP2C9 *2/*3} - 2.3312 \times \text{CYP2C9 *3/*3} - 0.2188 \times \text{CYP2C9 genotype unknown} - 0.1902 \times \text{Asian race} - 0.2760 \times \text{Black or African–American} - 0.1632 \times \text{missing or mixed race} + 1.1816 \times \text{enzyme-inducer status} - 0.5503 \times \text{amiodarone status} ) The result is Dose (mg/week) and should be squared to calculate the weekly warfarin dose in mg. Age in decades should be entered as 1 for 10–19 years, 2 for 20–29 years, 3 for 30–39 years, 4 for 40–49 years, 5 for 50–59 years, 6 for 60+ years. for African origin, stroke, diabetes, amiodarone use and fluvastatin use, enter 1 if present, otherwise 0. For example, if the patient is <em>VKORC1</em> A/G then the coefficient for <em>VKORC1</em> is -0.8677 \times 1</td>
<td>[1]</td>
</tr>
<tr>
<td>Avery et al.</td>
<td>Initial doses</td>
<td>[x = MD + \frac{\frac{1}{1 + e^{k(t-\tau)}} \times (1 - e^{k(t-\tau)} - e^{k(t-\tau)})}{\frac{1}{3} + 2 \times e^{k(t-\tau)} + e^{2k(t-\tau)}}]^{\frac{1}{2}}] Where MD is the calculated maintenance dose per day in mg. Could be calculated by the IWPC model above (note that the IWPC model gives weekly dose, divide by seven to get the daily dose) (k) is the elimination rate constant for the <em>CYP2C9</em> genotypes: *1/*1 (= 0.0189\ h^{-1}), *1/*2 (= 0.0158\ h^{-1}), *1/*3 (= 0.0132\ h^{-1}), *2/*2 (= 0.0130\ h^{-1}), *2/*3 (= 0.009\ h^{-1}) and *3/*3 (= 0.0075\ h^{-1}) (\tau) is the warfarin-dosing interval, use 24 (24 h) The initial doses on days one to three are then calculated as: day one = MD + x, day two = MD + 2x/3 and day three = MD + x/3</td>
<td>[25]</td>
</tr>
<tr>
<td>Lenzini et al., IWDRC</td>
<td>Dose revision</td>
<td>Dose (mg/week) = EXP (3.10894 - 0.00767 \times \text{age} + 0.51611 \times \ln(\text{INR}) - 0.23032 \times \text{VKORC1-1639 G&gt;A} + 0.14745 \times \text{CYP2C9<em>2} - 0.3077 \times \text{CYP2C9</em>3} + 0.24597 \times \text{BSA} + 0.26729 \times \text{target INR} - 0.09644 \times \text{African origin} - 0.2059 \times \text{stroke} - 0.11216 \times \text{diabetes} - 0.1035 \times \text{amiodarone use} - 0.19275 \times \text{fluvastatin use} + 0.0169 \times \text{dose}<em>{2,3} + 0.02018 \times \text{dose}</em>{2,3} + 0.01605 \times \text{dose}<em>{2,3}) ) Where INR is the INR on days four or five <em>VKORC1</em>-1639 G&gt;A should be entered as 0 for homozygous G/G, 1 for heterozygous and 2 for homozygous A/A <em>CYP2C9</em> <em>2</em> and <em>CYP2C9</em> <em>3</em> should be entered as 0 if absent, 1 if heterozygous and 2 if homozygous Dose (</em>{2,3}) refers to dose given 2, 3 and 4 days before INR is measured BSA is calculated according to the formula by Dubois and Dubois where BSA = (weight (\times \text{height}^2)) / 139.2 For African origin, stroke, diabetes, amiodarone use and fluvastatin use, enter 1 if present, otherwise 0</td>
<td>[56]</td>
</tr>
</tbody>
</table>

**BSA:** Body surface area; **INR:** International Normalized Ratio; **IWDRC:** International Warfarin Dose Refinement Collaboration; **IWPC:** International Warfarin Pharmacogenetics Consortium; **MD:** Maintenance dose.
doses would be 3.23 mg on day one, 2.49 mg on day two and 1.74 mg on day three. This shows that the loading doses are less than half and the maintenance dose less than a quarter for a patient with CYP2C9 *3/*3 and VKORC1 A/A compared with a CYP2C9 *1/*1 and VKORC1 A/G patient.

**Dose refinement**

A dose refinement model is used after the first doses of therapy and incorporates knowledge about given doses and the INR response. The International Warfarin Dose Refinement Collaboration investigated if genetic information adds to dose prediction after initiating treatment by deriving both pharmacogenetic and clinical dose refinement models [56]. These models that can be used on days four to five were derived on 969 patients and internally validated in 20% of the data that were left out of the modeling. In a final step the model parameters were refitted on the pooled derivation and internal validation cohorts (n = 1213) and evaluated in an external validation cohort (see prediction model in Table 2). Results of internal validation were $R^2 \approx 60\%$ for the pharmacogenetic model on days four and five and for the clinical model $R^2 \approx 44\%$ on days four and five. External validation gave somewhat lower accuracy of prediction with $R^2 \approx 42\%$ for the pharmacogenetic model on days four and five and for the clinical model $R^2 \approx 28\%$ on days four and five. The overall conclusion was that the pharmacogenetic dose refinement model significantly improved the $R^2$ by 12–17% compared with a clinical dose refinement model. This indicates that knowledge of CYP2C9 and VKORC1 genotypes still adds information 4–5 days into warfarin therapy.

**How the prediction models could be used**

All three types of prediction models can be used in all patients. If possible, use a model derived in a population with similar characteristics to the patients it will be used on, for example, age, ethnicity and diet. Imagine a patient that is to be treated with warfarin due to atrial fibrillation and additional risk factors for stroke with a target INR of 2.5. The patient has the following characteristics; Caucasian, 60 years of age, height 175 cm, weight 75 kg, no interacting drugs and the genotypes CYP2C9 *3/*3 and VKORC1 A/G. To predict the initial doses for this patient first use a pharmacogenetic maintenance dose model, such as the IWPC model.
then use the formulas by Avery et al. to calculate the initial doses (Tables 2 & 4) [1,25]. This patient would be predicted a maintenance dose of 11 mg/week (1.6 mg/day) and initial doses of 5.1, 3.9 and 2.7 mg on days one to three, respectively. On day four the patient comes back for an INR test and has an INR of 2.0. At this point a dose revision model could be used, such as the model by Lenzini et al. (Table 2) which would predict a dose of 15.6 mg/week (2.2 mg/day) [56]. Preferably prediction models should be used with the VKORC1 SNP they were derived for, but due to the haplotype structure of VKORC1 it is possible to use other SNPs in linkage disequilibrium (LD) as surrogate markers [32]. Four SNPs are in high LD ($r^2 > 0.9$) in Caucasians and Asians (rs9923231, rs2359612, rs9934438 and rs8050894) whereas the LD is lower in people of African–American ancestry. This LD information was used by the IWPC group when rs9923231 was imputed, more information on the imputation of rs9923231 is in the section S4 of the supplementary information to the IWPC paper [1,101].

All of the discussed prediction models have been derived on datasets of adult patients. The first bullet point under Box 1, “Is the data representative for the population the model will be used on”, highlights that these models probably would not work in children aged 0–18 years. At the present time many research groups are evaluating genetic effects on warfarin dose in children and a recent paper by Biss et al. showed the current IWPC model constantly overestimates the warfarin dose in children by an average of 1.5 mg/day [57]. They however showed that a similar proportion of the variation in dose was explained by VKORC1 and CYP2C9 in children as in adult patients.

### Randomized trials
The clinical benefit of pharmacogenetic dosing is still to be shown. At least five clinical trials of pharmacogenetic dosing are underway [102]. Three of the largest are the COAG and the GIFT trials in the USA, and the European EU-PACT trial [58–60].

The COAG study is a two-armed, double-blinded, randomized controlled trial that compares genotype-based dosing with clinical guided dosing. Both treatment arms use a baseline dose-initiation model [2] and a dose refinement model after four or five days of warfarin therapy [56]. The aim is to include over 1200 patients that are expected to be on warfarin therapy for at least 3 months. The primary outcome of the study is TTR within the first 4 weeks of therapy.

The GIFT trial is a 2 × 2 factorial-design, randomized control trial. It has two aims where the first aim is to compare pharmacogenetic dosing with clinical dosing and the second aim is to compare a target range of INR 2.0–3.0 with a

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**Table 3. SNPs included in current prediction models.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Descent/ethnicity†</th>
<th>n used to estimate MAF</th>
<th>MAF‡ (%)</th>
<th>Alleles</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9</td>
<td>rs1799853 (*2)</td>
<td>White§</td>
<td>3062</td>
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<td>The T allele is associated with warfarin clearance; patients require lower warfarin doses</td>
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<tr>
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<td></td>
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<td>A&gt;C</td>
<td>The C allele is associated with warfarin clearance; patients require lower warfarin doses</td>
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<td>The A allele is associated with decreased warfarin dose</td>
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<td>African–American¶</td>
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</table>

†The descent/ethnicity is kept with the original wording from the cited papers.
‡MAF is for the minor allele positioned to the right of the ‘>’ sign under the ‘Alleles’ column.
§Estimates according to Limdi et al. [52].
¶Estimates according to Scott et al. [51].
MAF: Minor allele frequency.
lower target range of INR 1.5–2.1. Dosing will be guided by the website WarfarinDosing.org for a minimum of the first 11 days of treatment [103]. The primary study outcome for aim one is the composite of venous thromboembolism, major hemorrhage, INR ≥ 4 or death and for aim two the primary outcome is the composite of nonfatal venous thromboembolism or death. The plan is to include 1600 patients undergoing elective total hip or knee replacement surgery.

The EU-PACT study is a two-armed, single-blinded, randomized controlled trial aiming to recruit almost 3000 patients commencing anticoagulation therapy with warfarin, acenocoumarol or phenprocoumon. Patients in the intervention arm are dosed according to a drug-specific dosing algorithm, which is based on genetic information and clinical data.

For warfarin, three to four starting doses are calculated by combining the IWPC pharmacogenetic prediction model with an estimated accumulation index that is based on the clearance of warfarin in different CYP2C9 genotypes [1,25,55]. Thereafter a pharmacogenetic dose revision algorithm is used [56]. Patients in the control arm are dosed without information about genotype. The follow-up period is 3 months. The primary aim of the study is to show improved anticoagulation therapy by increased TTR during the first 3 months of treatment.

**Future perspective**

The dose of warfarin that is required for therapeutic INR levels is strongly related to SNPs in CYP2C9 and VKORC1. Together with clinical factors, these genetic markers explain a large amount of the variability in dose. The variability in dose requirements between ethnicities is largely caused by different allele frequencies of the SNPs in VKORC1. Compared with dosing according to a clinical algorithm, the IWPC pharmacogenetic model performs better, especially in the high- and low-dose groups.

**New alternatives to warfarin**

New alternatives to warfarin therapy have recently been investigated. They include the direct thrombin inhibitor dabigatran (RE-LY study) [16] and the factor Xa inhibitors rivoxaban (ROCKET AF study) [61] and apixaban (ARISTOTLE study) [62]. These new oral anticoagulants are proven noninferior or even superior to warfarin; however, the TTR for the warfarin arms of these three studies was low with 64% in the RE-LY study, 55% in the ROCKET study and 62% in the ARISTOTLE study. The cost-effectiveness of dabigatran in atrial fibrillation compared with warfarin was studied in a recent paper by Shah et al. [63]. The results were that for atrial fibrillation patients dabigatran is more cost effective in patients with poor INR control (TTR <57.1%) whereas warfarin is more cost effective in patients with excellent INR control (TTR >72.6). Another aspect of these new drugs is that there is no natural antidote, and agents to reverse the effect are still under development [64].

**Table 4. Pharmacogenetic loading doses according to Avery et al.**

<table>
<thead>
<tr>
<th>CYP2C9</th>
<th>VKORC1</th>
<th>IWPC* (dose/week in mg)</th>
<th>IWPC* (dose/day in mg)</th>
<th>Loading doses‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day one (mg)</td>
<td>Day two (mg)</td>
<td>Day three (mg)</td>
</tr>
<tr>
<td>*1/*1</td>
<td>A/A</td>
<td>24</td>
<td>3.43</td>
<td>5.51</td>
</tr>
<tr>
<td>*3/*3</td>
<td>A/A</td>
<td>7</td>
<td>1.00</td>
<td>3.23</td>
</tr>
<tr>
<td>*1/*1</td>
<td>A/G</td>
<td>33</td>
<td>4.71</td>
<td>7.56</td>
</tr>
<tr>
<td>*3/*3</td>
<td>A/G</td>
<td>11</td>
<td>1.57</td>
<td>5.07</td>
</tr>
<tr>
<td>*1/*1</td>
<td>G/G</td>
<td>44</td>
<td>6.29</td>
<td>10.1</td>
</tr>
<tr>
<td>*3/*3</td>
<td>G/G</td>
<td>18</td>
<td>2.57</td>
<td>8.3</td>
</tr>
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</table>

*Approximate predictions are from the example nomogram in Figure 1 for a Caucasian patient with no interacting drugs, age 60 years, height 175 cm and weight 75 kg [1].

*Prediction of loading doses were made using k (elimination rate constant) of 0.0189 for *1/*1 and 0.0075 for *3/*3 as in Avery et al. [25].

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Review
Eriksson & Wadelius

Executive summary

Warfarin

- Warfarin is monitored by the International Normalized Ratio (INR), which is a measure of the clotting ability of blood. A level of 1 is associated with normal coagulation and 2 indicates doubled coagulation time compared with normal.
- The optimal effect for most patients is in the target range of INR 2.0–3.0 and the required dose to reach such levels varies between <10 mg per week to >100 mg per week.

Adverse effects

- The most common adverse effect of warfarin is bleeding and the risk is highly related to the intensity of anticoagulation.

Genetic effects

- CYP2C9 is associated with the clearance of warfarin. Patients with normal metabolism (CYP2C9 *1/*1), have an estimated half-life of 30–37 h whereas those with severely impaired metabolism (CYP2C9 *3/*3) have an estimated half-life of 92–203 h.
- VKORC1 codes for the enzyme vitamin K epoxide reductase, the target of warfarin.

Why predict warfarin dose?

- Unintentional overdosing of warfarin remains as one of the most common causes of hospitalization due to adverse reactions in the USA and the UK.
- Genotype-guided dose has the potential to reduce the risk of bleeding during the initiation phase (1–3 months) of warfarin.

When to predict warfarin dose

- Three different kinds of pharmacogenetic prediction models exist:
  - Prediction of stable maintenance dose.
  - Prediction of loading doses to be used during the initiation of warfarin.
  - Dose revision models including the INR response on day 4 and later.

Randomized trials

- Randomized trials incorporating pharmacogenetic dosing of warfarin have to date been too small to draw solid conclusion about the value of genotyping before drug initiation.
- At least three large trials are ongoing; the COAG and the GIFT trials in the USA, and the European EU-PACT trial.

Regardless of this, the future of pharmacogenetic warfarin dosing relies on the results of the ongoing clinical trials.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest


5 Extensive study investigating the effect on warfarin dose of genes in the warfarin pathway.

6 Largest study to date used to derive a prediction model. Used in the EU-PACT trial.
Prediction of warfarin dose: why, when & how?


**Pharmacogenetic prediction of loading doses. Used in the EU-PACT trial.**


33 Wadelius M, Chen LY, Downes K et al., Common VKORC1 and GGCX polymorphisms associated with warfarin dose. Pharmacogenomics J. 5(4), 262–270 (2005).


et al. (2011).

Websites

Section S4 of the supplementary information to the International Warfarin Pharmacogenetics Consortium paper [1].

Clinical Trials.

www.clinicaltrials.gov

WarfarinDosing.

www.WarfarinDosing.org