VKORC1: warfarin 6235/6236

95% CI = 95% confidence interval, AA = homozygous for the variant allele (= -1639 AA = 1173 TT), GA = heterozygous (= -1639 GA = 1173 CT), GG = homozygous for the wild type allele (= -1639 GG = 1173 CC), HR = hazard ratio, INR = international normalised ratio, NS = not significant, RR = risk ratio, S = significant, VKORC1 = vitamin K epoxide reductase, subunit 1

Disclaimer: The Pharmacogenetics Working Group of the KNMP formulates the optimal recommendations for each phenotype group based on the available evidence. If this optimal recommendation cannot be followed due to practical restrictions, e.g. therapeutic drug monitoring or a lower dose is not available, the health care professional should consider the next best option.

Concise summary and justification of choices:
Warfarin exerts its anti-coagulant effect by inhibiting the activity of the VKORC1 enzyme. Variations in the VKORC1 gene can result in reduced expression of the enzyme. In patients with these gene variations, the warfarin dose required to attain the therapeutic INR is lower.
Four meta-analyses showed a clear effect of the homozygous variant genotype AA on the required warfarin dose. There was only one meta-analysis of the clinical effects. This meta-analysis found a 2-fold increase in the risk for INR > 4 (2.5-fold in the first month of therapy). However, the meta-analysis did not find a significant increase in bleeding risk. Despite direct evidence for an increase in bleeding risk is lacking, we consider the effect of the gene-drug interaction on the dose and risk for overanticoagulation strong enough to recommend adjustment of therapy by starting with a lower warfarin dose (yes/yes-interaction).
The effect of heterozygous variant genotype GA on the required warfarin dose and risk of overanticoagulation is smaller. The risk for INR > 4 was increased 1.5-fold (1.9-fold in the first month of therapy). In addition, GA is the most frequent genotype in the European/Caucasian population, so dosing will be based mainly on this genotype. For these reasons, we do not recommend adjustment of therapy for this gene-drug interaction (yes/no-interaction).
You can find an overview of the effects per genotype in the background information text of the corresponding genotype-drug interaction on the KNMP Kennisbank. You might also have access to this background information text via your pharmacy or physician electronic decision support system.
The justification of the dose recommendation for AA and a note on the meta-analyses comparing genotype-guided with non-genotype-guided dosing you will find below.
Dose recommendation for AA
Dose reductions in comparison with GG vary from 49-50% for all studies and 50-52% for studies in Caucasians. Dose reductions in comparison with GA, the most prevalent genotype in Caucasians, vary from 31-34% both in all studies and in studies in Caucasians. Dose reductions in comparison with GA+GG vary from 39% for all studies and 42-44% for studies in Caucasians. Because dosing in Caucasians will be mainly based on the groups with genotypes GA and GG, we recommend to adjust the therapy for AA by starting with 60% of the normal (i.e. not genotype guided) dose. The dosing algorithm from the EU-PACT study (Avery 2011) is added to allow a more accurate dose calculation.
Note
The meta-analyses of studies comparing genotype-guided with not genotype-guided dosing showed little or no improvement of clinical outcomes by genotype-guided dosing. However, these studies considered only data for the whole group, including the patients without variant alleles. The absence of an effect for the whole group does not preclude an effect for the patients with the expected most benefit of genotype-guided dosing, i.e. the patients homozygous for variant alleles. In addition, in several of the included studies the initial dose was fixed and not genotype-guided. In patients with known genotypes, also the initial dose can be adapted.

The table below follows the KNMP nomenclature for the VKORC1 polymorphism and genotypes. The nomenclature used in the table below may therefore differ from the nomenclature used by the authors in the article.

Source	Code	Effect	Comments
ref. 1 Zhang J et al. The influence of VKORC1 gene polymorphism on warfa-	4	Meta-analysis of 7 studies with 562 pediatric patients. Patients were predominantly Caucasian. Studies differed in target INR and mean age. In one study warfarin dose was corrected for body weight.	Author's conclusion: 'We found that VKORC1−1639 gene polymor-

<table>
<tr>
<td>rin maintenance dosage in pediatric patients: A systematic review and meta-analysis.
Thromb Res 2015;136:955-61.
PubMed PMID: 26433837.

ref. 1, continuation</td>
<td>GA: A
AA: A</td>
<td>Results:
<table>
<tr><td colspan="4">Dose in comparison with GG (mean dose per study varied from 3.8-5.0 mg/day and 0.22 mg/kg per day):</td></tr>
<tr><td>genotype</td><td>polymorphism determined</td><td>mean difference</td><td>95% CI</td></tr>
<tr><td>GA</td><td>-1639 G>A</td><td>-26%</td><td>-22 - -31</td></tr>
<tr><td>AA</td><td>-1639 G>A</td><td>-50%</td><td>-34 - -65</td></tr>
<tr><td>AA</td><td>1173 C>T</td><td>NS</td><td></td></tr>
<tr><td colspan="4">Number of studies and patients per genotype for each comparison:
-1639 G>A determined: 6 studies, n = 484: 239 GA, 80 AA and 165 GG
1173 C>T determined: 2 studies, n = 38: 20 AA and 18 GG</td></tr>
<tr><td colspan="4">The heterogeneity between the studies was high and significant for:
- AA, -1639 G>A determined
- AA, 1173 C>T determined
The heterogeneity disappeared in both cases after excluding the only study that did not show a significant difference between GG and AA.</td></tr>
<tr><td colspan="4">There were no indications for publication bias.</td></tr>
</table></td>
<td>phisms have a moderate effect on warfarin dosage in pediatric patients.’</td>
</tr>
<tr>
<td>ref. 2
Liao Z et al.
Meta-analysis of randomized controlled trials reveals an improved clinical outcome of using genotype plus clinical algorithm for warfarin dosing.
J Thromb Thrombolysis 2015;39:228-34.
PubMed PMID: 24962733.</td>
<td>4

genotype-guided versus not genotype-guided therapy: AA#</td>
<td>Meta-analysis of 7 randomized controlled trials with 1,910 patients comparing genotype-guided dosing (n = 960) with not genotype-guided dosing (n = 950). The number of patients per trial varied from 38 to 1,015. The follow-up duration was not reported for one trial and varied from 46 to 180 days for the other six. In 4 trials, initial dose in the not-genotype-guided group was fixed. In the other 3 trials, it was calculated with a clinical algorithm. In all trials, genotype-guided dose was calculated with an algorithm including clinical parameters. Except for the smallest trial, genotype-guided dosing was based on the genotypes of both CYP2C9 and VKORC1.
The therapeutic INR range differed between the trials.
Adverse events were defined as major bleeding, thromboembolism, myocardial infarction, death from any cause, any clinical relevant non-major bleeding event or other conditions requiring emergency medical management, INR > 4 (3 trials) or INR > 3,5 (1 trial).
A fixed-effects model was used in all analyses, also if there was heterogeneity between the studies.

Results:
<table>
<tr><td colspan="4">Genotype-guided in comparison with not-genotype-guided therapy:</td></tr>
<tr><td></td><td></td><td></td><td>% of patients in not-genotype-guided group</td></tr>
<tr><td rowspan="2">% of time within the therapeutic INR range (standardized mean difference)</td><td>all (5 trials, n = 1,729)</td><td>NS</td><td rowspan="2"></td></tr>
<tr><td>fixed initial dose (3 trials, n < 700)</td><td>+0.24 (95% CI: 0.09-0.40) (S)</td></tr>
<tr><td>Adverse events</td><td>4 trials, n = 1,763</td><td>NS</td><td>38%</td></tr>
<tr><td>Death</td><td>3 trials, n = 1,571</td><td>NS</td><td>0.64%</td></tr>
<tr><td colspan="4">Heterogeneity between the trials was only significant and moderate for:
- percentage of time within the therapeutic INR range, all trials</td></tr>
<tr><td colspan="4">Indications for publication bias were not found.</td></tr>
</table></td>
<td>Author’s conclusion:
‘Allocation to genotype plus clinical algorithm may be associated with a significant improvement of the percentage of time within the therapeutic INR range for patients adopting fixed dose of warfarin. The incidence of total adverse events and death rates did not differ between these two groups.’</td>
</tr>
</table>

<table>
<tr><td>ref. 2, continuation</td><td></td><td>NOTE: The authors do not state whether the polymorphism determined for VKORC1 in the included trials was -1639 G>A or 1173C>T.</td><td></td></tr>
<tr><td>ref. 3
Xu H et al.
Meta-analysis of efficacy and safety of genotype-guided pharmacogenetic dosing of warfarin.
Int J Cardiol 2014;177:654-7.
PubMed PMID: 25449474.</td><td>4

genotype-guided versus not genotype-guided therapy: AA#</td><td>Meta-analysis of 8 randomized controlled trials with 2,158 patients comparing genotype-guided dosing (n = 1,084) with not genotype-guided dosing (n = 1,074). The number of patients per trial varied from 26 to 955. The follow-up duration varied from 28 to 90 days. In 5 trials, initial dose in the not-genotype-guided group was fixed. In the other 3 trials, it was calculated with a clinical algorithm. In 6 trials, genotype-guided dosing was based on the genotypes of CYP2C9 and VKORC1. In 1 trial it was based only on the CYP2C9 genotype and in 1 trial on the genotypes of CYP2C9, VKORC1 and CYP4F2.
The therapeutic INR range differed between the trials.
5 of the trials in this meta-analysis were also included in the meta-analysis of Liao 2015.

Results:
<table>
<tr><td colspan="4">Genotype-guided in comparison with not-genotype-guided therapy:</td></tr>
<tr><td></td><td></td><td></td><td>% in not-genotype-guided group</td></tr>
<tr><td rowspan="5">% of time within the therapeutic INR range (standardized mean difference)</td><td>all (8 trials)</td><td>Not determined due to high heterogeneity</td><td rowspan="5"></td></tr>
<tr><td>fixed initial dose (5 trials, n = 812)</td><td>+0.30 (95% CI: 0.08-0.53) (S)</td></tr>
<tr><td>not fixed initial dose (3 trials, n = 1,286)</td><td>NS</td></tr>
<tr><td>follow-up ≤ 28 days (3 trials, n = 1,218)</td><td>NS</td></tr>
<tr><td>follow-up > 28 days (5 trials, n = 880)</td><td>+0.25 (95% CI: -0.00-0.51) (NS, trend)</td></tr>
<tr><td>Major bleeding</td><td>8 trials</td><td>NS</td><td>2.6%</td></tr>
<tr><td>Minor bleeding</td><td></td><td>NS</td><td></td></tr>
<tr><td>Thrombo-embolic events</td><td></td><td>NS</td><td></td></tr>
<tr><td>INR > 4</td><td></td><td>NS</td><td></td></tr>
<tr><td colspan="4">Heterogeneity between the trials was only significant for:
- percentage of time within the therapeutic INR range, all trials
- percentage of time within the therapeutic INR range, follow-up > 28 days</td></tr>
<tr><td colspan="4">Indications for publication bias were not found.</td></tr>
</table>
NOTE: The authors do not state whether the polymorphism determined for VKORC1 in the included trials was -1639 G>A or 1173C>T.</td><td>Author’s conclusion:
‘We found that time in the therapeutic INR range of the genotype-guided group was increased compared with the control group in the RCTs when the initial warfarin dose was fixed. However, the genotype-guided group failed to exhibit statistically significant outcome compared to the control group in the studies using equation initial dose. Only a limited recommendation can be made that genotype-guided pharmacogenetic algorithm should be applied to guide warfarin dose rather than fixed dose. …..
The results from our meta-analysis indicated that there was no benefit of genotype-guided dosing of warfarin with respect to hemorrhagic events. But this should be interpreted with caution because of the number of events was relatively small.’</td></tr>
<tr><td>ref. 4
Franchini M et al.</td><td>4</td><td>Meta-analysis of 9 randomized controlled trials with 2,812 patients comparing genotype-guided dosing (n = 1,411)</td><td>Author’s conclusion:</td></tr>
</table>

<table>
<tr>
<td>Effects on bleeding complications of pharmacogenetic testing for initial dosing of vitamin K antagonists: a systematic review and meta-analysis. J Thromb Haemost 2014;12:1480-7. PubMed PMID: 25040440.

ref. 4, continuation</td>
<td>genotype-guided versus not genotype-guided therapy: AA#</td>
<td>with not genotype-guided dosing (n = 1,401). The number of patients per trial varied from 26 to 1015. The mean follow-up period varied from 22 to 90 days. When the information was available, clinical endpoints were referred to a 30-day follow-up period. In 7 trials, genotype-guided dosing was based on the genotypes of CYP2C9 and VKORC1. In 2 trials it was based only on the CYP2C9 genotype.
One of the included trials (Verhoef 2013; n = 548) studied acenoumarol and phenprocoumon instead of warfarin. The largest trial (Kimmel 2013) included a significant number of African American patients.
Of the 9 trials in this meta-analysis, 6 were included in Liao 2015 and 7 in Xu 2014.

Results:
<table>
<tr><td colspan="4">Genotype-guided in comparison with not-genotype-guided therapy:</td></tr>
<tr><td></td><td></td><td></td><td>% in not-genotype-guided group</td></tr>
<tr><td rowspan="2">Major bleeding</td><td>6 trials, n = 2,131</td><td>RR = 0.47 (95% CI: 0.23-0.96) (S)</td><td>2.2%</td></tr>
<tr><td colspan="3">2 trials with 0 major bleeds were excluded from the analysis (n = 481)</td></tr>
<tr><td>Thrombo-embolic events</td><td></td><td>NS</td><td></td></tr>
<tr><td>Deaths</td><td></td><td>NS</td><td></td></tr>
<tr><td>INR > 4</td><td></td><td>NS</td><td></td></tr>
<tr><td rowspan="2">% of time within the therapeutic INR range</td><td>weighted mean difference</td><td>NS</td><td rowspan="2">46.8%</td></tr>
<tr><td>standardized mean difference</td><td>NS</td></tr>
<tr><td colspan="4">Heterogeneity was very high for the percentage of time within the therapeutic INR range. There was no heterogeneity for the other outcomes.</td></tr>
</table>
There were not enough data to perform meta-analysis for the following outcomes: time to first therapeutic INR, time to stable warfarin dose, number of patients with INR < 2 and number of days in hospital.

NOTE: The authors do not state whether the polymorphism determined for VKORC1 in the included trials was -1639 G>A or 1173C>T.</td>
<td>‘The results of this meta-analysis show that genotype-guided initial VKA dosing is able to reduce serious bleeding events by approximately 50% compared with clinically-guided dosing approaches.’</td>
</tr>
<tr>
<td>ref. 5
Stergiopoulos K et al. Genotype-guided vs clinical dosing of warfarin and its analogues: meta-analysis of randomized clinical trials. JAMA Intern Med 2014;174:1330-8. PubMed PMID: 24935087.</td>
<td>4</td>
<td>Meta-analysis of 9 randomized controlled trials with 2,812 patients comparing genotype-guided dosing (n = 1,411) with not genotype-guided dosing (n = 1,401). The number of patients per trial varied from 26 to 1015. Patients were from the United States, Europe or Israel. The follow-up period varied from 4 weeks to 6 months (median 12 weeks). In 6 trials, genotype-guided dosing was based on the genotypes of CYP2C9 and VKORC1. In 2 trials it was based only on the CYP2C9 genotype and in 1 trial on the genotypes of CYP2C9, VKORC1 and CYP4F2.
One of the included trials (Verhoef 2013; n = 548) studied acenoumarol and phenprocoumon instead of warfarin.
The therapeutic INR range differed between the trials.
The 9 trials in this meta-analysis are the same as those in Franchini 2014.</td>
<td>Author’s conclusion:
‘In this meta-analysis of randomized clinical trials, a genotype-guided dosing strategy did not result in a greater percentage of time that the INR was within the therapeutic range, fewer patients with an INR greater than 4, or a reduction in major blee-</td>
</tr>
</table>

<table>
<tr>
<td>ref. 5, continuation</td>
<td>genotype-guided versus not genotype-guided therapy: AA</td>
<td>Results:
<table>
<tr><td colspan="4">Genotype-guided in comparison with not-genotype-guided therapy:</td></tr>
<tr><td></td><td></td><td></td><td>% in not-genotype-guided group</td></tr>
<tr><td>% of time within the therapeutic INR range (standardized mean difference)</td><td>9 trials</td><td>NS</td><td></td></tr>
<tr><td>INR > 4</td><td>8 trials, n = 2,621</td><td>NS</td><td>28%</td></tr>
<tr><td>Major bleeding</td><td>7 trials, n = 2,586</td><td>NS</td><td>1.6%</td></tr>
<tr><td>Thromboembolic events</td><td>7 trials, n = 2,586</td><td>NS</td><td>1.2%</td></tr>
<tr><td colspan="4">Analyses of subgroups based on study quality, study location or sample size did not result in significantly different outcomes. Neither did exclusion of any single trial from the analyses.</td></tr>
<tr><td colspan="4">Heterogeneity was high for the percentage of time within the therapeutic INR range. There was no heterogeneity for the other outcomes.</td></tr>
<tr><td colspan="4">Indications for publication bias were not found.</td></tr>
</table>
NOTE: The authors do not state whether the polymorphism determined for VKORC1 in the included trials was -1639 G>A or 1173C>T.</td>
<td>ding or thromboembolic events compared with clinical dosing algorithms.’</td>
</tr>
<tr>
<td>ref. 6
Jin B et al.
The impact of VKORC1-1639G > A genetic polymorphism upon warfarin dose requirement in different ethnic populations.
Curr Med Res Opin 2014;30:1505-11.
PubMed PMID: 24708259.</td>
<td>4

GA: A

AA: A</td>
<td>Meta-analysis of 24 prospective clinical trials with 3932 patients. Patients were Caucasian in 16 studies, Asian in 6 studies and African in 2 studies. Studies differed in the target INR.

Results:
<table>
<tr><td colspan="4">Dose in comparison with GG:</td></tr>
<tr><td>genotype</td><td></td><td>weighted mean difference (mg/day)</td><td>95% CI</td></tr>
<tr><td>GA</td><td></td><td>-1.32</td><td>-1.67 - -0.96</td></tr>
<tr><td></td><td>Caucasian</td><td>-1.17</td><td>-1.55 - -0.80</td></tr>
<tr><td></td><td>Asian</td><td>-1.72</td><td>-2.96 - -0.48</td></tr>
<tr><td></td><td>African</td><td>-1.40</td><td>-2.04 - -0.77</td></tr>
<tr><td>AA</td><td></td><td>-2.62</td><td>-3.10 - -2.14</td></tr>
<tr><td></td><td>Caucasian</td><td>-2.47</td><td>-2.92 - -2.03</td></tr>
<tr><td></td><td>Asian</td><td>-2.84</td><td>-4.57 - -1.11</td></tr>
<tr><td></td><td>African</td><td>-2.76</td><td>-3.54 - -1.99</td></tr>
<tr><td colspan="4">Total number of patients and patients per genotype for each comparison:
All ethnicities: n = 3932: 1437 GA, 1221 AA, 1274 GG
Caucasian: n = 2529: 1110 GA, 393 AA, 1026 GG
Asian: n = 1091: 226 GA, 799 AA, 66 GG
African: n = 312: 101 GA, 29 AA, 182 GG</td></tr>
<tr><td colspan="4">The heterogeneity between the studies was significant for all comparisons, except for the African subgroup.</td></tr>
<tr><td colspan="4">There were no indications for publication bias.</td></tr>
<tr><td colspan="4">The results were not influenced by the omission of any individual study from the analyses.</td></tr>
</table>
<table>
<tr><td colspan="4">Dose in comparison with GA:</td></tr>
<tr><td>genotype</td><td></td><td>weighted mean difference (mg/day)</td><td>95% CI</td></tr>
<tr><td>AA</td><td>Caucasian</td><td>-1.27</td><td>-1.51 - -1.02</td></tr>
</table></td>
<td>Author’s conclusion:
‘This meta-analysis indicated that the VKORC1-1639 G>A genetic polymorphism is associated with the variation of inter-individual warfarin dose requirement in different ethnic populations.’</td>
</tr>
</table>

<table>
<tr>
<td>ref. 6, continuation</td>
<td></td>
<td>
<table>
<tr><td>Total number of patients and patients per genotype:
n = 1503: 1110 GA, 393 AA</td></tr>
<tr><td>The heterogeneity between the studies was significant.</td></tr>
<tr><td>There were no indications for publication bias.</td></tr>
<tr><td>The results were not influenced by the omission of any individual study from the analysis.</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 7
Yang J et al.
Influence of CYP2C9 and VKORC1 genotypes on the risk of hemorrhagic complications in warfarin-treated patients: a systematic review and meta-analysis.
Int J Cardiol 2013;168:4234-43.
PubMed PMID: 23932037.</td>
<td>4

GA: A

AA: A</td>
<td>
Meta-analysis of 10 studies with 4361 patients. Patients were Caucasian in 6 studies, Caucasian or African-American in 3 studies and Asian in 1 study. The follow-up periods for bleeding or over-anticoagulation varied from 3 weeks to 6 years.
Of the 10 studies in this meta-analysis, none were included in the meta-analysis of Jin 2014.

Results:
<table>
<tr><td colspan="5">GA in comparison with GG:</td></tr>
<tr><td></td><td></td><td>HR</td><td>95% CI</td><td>Incidence for GG</td></tr>
<tr><td rowspan="3">INR > 4
(6 studies, n = 2,486)</td><td></td><td>1.49</td><td>1.15-1.92</td><td rowspan="3">23%</td></tr>
<tr><td>0-30 days</td><td>1.90</td><td>1.44-2.52</td></tr>
<tr><td>> 30 days</td><td>NS</td><td></td></tr>
<tr><td rowspan="3">Total bleeding
(4 studies, n = 504)</td><td></td><td>trend</td><td>0.98-2.99</td><td rowspan="3">15%</td></tr>
<tr><td>Caucasian</td><td>NS</td><td></td></tr>
<tr><td>Asian</td><td>-</td><td></td></tr>
<tr><td colspan="5">There was no significant heterogeneity between the studies.</td></tr>
<tr><td colspan="5">Publication bias could not be excluded for total bleeding.</td></tr>
<tr><td colspan="5">The HR's were not influenced by the omission of any individual study from the analyses.</td></tr>
</table>

<table>
<tr><td colspan="5">AA in comparison with GG:</td></tr>
<tr><td></td><td></td><td>HR</td><td>95% CI</td><td>Incidence for GG</td></tr>
<tr><td rowspan="3">INR > 4
(6 studies, n = 1,745)</td><td></td><td>2.18</td><td>1.03-4.63</td><td rowspan="3">23%</td></tr>
<tr><td>0-30 days</td><td>2.59</td><td>1.96-3.43</td></tr>
<tr><td>> 30 days</td><td>NS</td><td></td></tr>
<tr><td rowspan="3">Total bleeding
(4 studies, n = 555)</td><td></td><td>NS</td><td></td><td rowspan="3">15%</td></tr>
<tr><td>Caucasian</td><td>NS</td><td></td></tr>
<tr><td>Asian</td><td>-</td><td></td></tr>
<tr><td colspan="5">The heterogeneity between the studies was high and significant for INR > 4.</td></tr>
<tr><td colspan="5">Publication bias could not be excluded for total bleeding.</td></tr>
<tr><td colspan="5">Except for INR > 4, the HR's were not influenced by the omission of any individual study from the analyses.
For INR > 4, significance was lost after exclusion of either Lund 2012 or Wadelius 2009 from the analysis. These were the largest studies with respectively 557 and 1496 Caucasian patients.</td></tr>
</table>

<table>
<tr><td colspan="5">GA+AA in comparison with GG:</td></tr>
<tr><td></td><td></td><td>HR</td><td>95% CI</td><td>Incidence for GG</td></tr>
<tr><td rowspan="3">INR > 4
(7 studies, n = 3,691)</td><td></td><td>1.93</td><td>1.24-2.99</td><td rowspan="3">22%</td></tr>
<tr><td>0-30 days</td><td>2.14</td><td>1.75-2.62</td></tr>
<tr><td>> 30 days</td><td>NS</td><td></td></tr>
<tr><td rowspan="2">Total bleeding</td><td></td><td>NS</td><td></td><td rowspan="2">25%</td></tr>
<tr><td>Caucasian</td><td>NS</td><td></td></tr>
</table>
</td>
<td>Author's conclusion:
'Both CYP2C9 and VKORC1 genotypes are associated with an increased risk for warfarin over-anticoagulation, with VKORC1 c. −1639 G > A more sensitive early in the course of anticoagulation.'</td>
</tr>
</table>

<table>
<tr><td>ref. 7, continuation</td><td></td><td>
<table>
<tr><td>(5 studies, n = 1,278)</td><td>Asian</td><td>-</td><td></td><td></td></tr>
<tr><td colspan="2">Major bleeding (2 studies, n = 753)</td><td>NS</td><td></td><td>7,9%</td></tr>
<tr><td colspan="5">The heterogeneity between the studies was high and significant for INR > 4.</td></tr>
<tr><td colspan="5">Publication bias could not be excluded for total and major bleeding.</td></tr>
<tr><td colspan="5">The HR’s were not influenced by the omission of any individual study from the analyses.</td></tr>
</table>
</td><td></td></tr>
<tr><td>ref. 8
Jorgensen AL et al.
Influence of CYP2C9 and VKORC1 on patient response to warfarin: a systematic review and meta-analysis.
PLoS One 2012;7:e44064.
PubMed PMID: 22952875.</td><td>4

GA: A

AA: A</td><td>Meta-analysis of 16 studies. Most studies were retrospective cohorts.
The authors do not state which allele they consider to be the mutant allele. There are indications in the text and results that the definition of the mutant allele differs between the polymorphisms determined (1173 C>T or -1639 G>A) and between Caucasians and Asians. For the summary below, the mutant allele according to our definition was identified based on the direction of the effect.
Of the 16 studies in this meta-analysis, 3 were included in the meta-analysis of Jin 2014 and 0 in the meta-analysis of Yang 2013.

Results:
<table>
<tr><td colspan="5">Dose in comparison with GG:</td></tr>
<tr><td>genotype</td><td>polymorphism determined</td><td>ethnicity</td><td>difference in means (mg/day)</td><td>95% CI</td></tr>
<tr><td>GA</td><td>1173 C>T</td><td>Caucasian</td><td>-1.68</td><td>-0.51 - -2.85</td></tr>
<tr><td></td><td></td><td>Japanese</td><td>-0.81</td><td>-0.37 - -1.25</td></tr>
<tr><td></td><td>-1639 G>A</td><td>Caucasian</td><td>-1.45</td><td>-0.72 - -2.18</td></tr>
<tr><td></td><td></td><td>Chinese</td><td>-1.45</td><td>-1.13 - -1.77</td></tr>
<tr><td></td><td></td><td>Japanese</td><td>NS</td><td></td></tr>
<tr><td>AA</td><td>1173 C>T</td><td>Caucasian</td><td>-3.14</td><td>-2.67 - -3.61</td></tr>
<tr><td></td><td>-1639 G>A</td><td>Caucasian</td><td>-2.86</td><td>-2.49 - -3.23</td></tr>
<tr><td colspan="5">Number of studies for each comparison:
1173 C>T determined: 6 studies (2 Caucasian)
-1639 G>A determined: 10 studies (6 Caucasian)</td></tr>
<tr><td colspan="5">The heterogeneity between the studies was significant for:
- GA and AA, Caucasian, -1639 G>A determined
- GA, Japanese, -1639 G>A determined</td></tr>
</table>
There were not enough studies for meta-analyses of time to stable dose and of bleeding (2 studies (1 North-American and 1 Asian) in both cases).</td><td>Author’s conclusion:
‘Effect estimates were significant for VKORC1 and stable dose for most ethnicities, although direction differed between asians and non-asians (mutant types requiring more in asians and less in non-asians).’</td></tr>
<tr><td>ref. 9
Yang L et al.
Impact of VKORC1 gene polymorphism on interindividual and interethnic warfarin dosage requirement--a systematic review and meta analysis.
Thromb Res 2010;125:e159-66.
PubMed PMID: 19942260.</td><td>4

AA: A</td><td>Meta-analysis of 19 studies with 3,175 Caucasian, 1,021 Asian and 187 African patients. Studies differed in the target INR.
Of the 19 studies in this meta-analysis, 6 were included in the meta-analysis of Jin 2014, 2 in the meta-analysis of Yang 2013 and 7 in the meta-analysis of Jorgensen 2012.

Results:
<table>
<tr><td colspan="4">Weighted mean difference in the dose for AA in comparison with GG:</td></tr>
<tr><td>polymorphism determined</td><td></td><td>mean difference</td><td>95% CI</td></tr>
<tr><td>1173 C>T</td><td></td><td>-49%</td><td>-42 - -55</td></tr>
<tr><td></td><td>Caucasian</td><td>-52%</td><td>-44 - -57</td></tr>
</table></td><td>Author’s conclusion:
‘Our studies showed that gene polymorphisms of VKORC1 significantly associated with the variation of interindividual warfarin dose requirement variation, and the effects are different in ethnicities.’</td></tr>
</table>

<table>
<tr>
<td>ref. 9, continuation</td>
<td></td>
<td>
<table>
<tr><td></td><td>Asian</td><td>-37%</td><td>-35 - -45</td></tr>
<tr><td>-1639 G>A</td><td></td><td>-50%</td><td>-46 - -54</td></tr>
<tr><td></td><td>Caucasian</td><td>-50%</td><td>-45 - -55</td></tr>
<tr><td></td><td>Asian</td><td>-38%</td><td>-5 - -53</td></tr>
<tr><td colspan="4">Number of studies, total number of patients and patients per genotype for each comparison:
1173 C>T determined: 6 studies, n = 571, 233 AA, 338 GG
-1639 G>A determined: 8 studies, n = 1837, 687 AA, 1150 GG</td></tr>
<tr><td colspan="4">The heterogeneity between the studies was significant and high.</td></tr>
<tr><td colspan="4">There were no indications for publication bias.</td></tr>
<tr><td colspan="4">The mean differences were not influenced by the omission of any individual study from the analysis.</td></tr>
</table>

<table>
<tr><td colspan="4">Weighted mean difference in the dose for AA in comparison with GG+GA:</td></tr>
<tr><td>polymorphism determined</td><td></td><td>mean difference</td><td>95% CI</td></tr>
<tr><td>1173 C>T</td><td></td><td>-39%</td><td>-32 - -44</td></tr>
<tr><td></td><td>Caucasian</td><td>-44%</td><td>-35 - -51</td></tr>
<tr><td></td><td>Asian</td><td>-31%</td><td>-22 - -38</td></tr>
<tr><td>-1639 G>A</td><td></td><td>-39%</td><td>-35 - -43</td></tr>
<tr><td></td><td>Caucasian</td><td>-42%</td><td>-35 - -47</td></tr>
<tr><td></td><td>Asian</td><td>-32%</td><td>-21 - -41</td></tr>
<tr><td colspan="4">Number of studies, total number of patients and patients per genotype for each comparison:
1173 C>T determined: 9 studies, n = 1141, 395 AA, 746 GG+GA
-1639 G>A determined: 12 studies, n = 3751, 1057 AA, 2694 GG+GA</td></tr>
<tr><td colspan="4">The heterogeneity between the studies was significant and high.</td></tr>
<tr><td colspan="4">There were no indications for publication bias.</td></tr>
<tr><td colspan="4">The mean differences were not influenced by the omission of any individual study from the analysis.</td></tr>
</table>

<table>
<tr><td colspan="4">Weighted mean difference in the dose for AA in comparison with GA:</td></tr>
<tr><td>polymorphism determined</td><td></td><td>mean difference</td><td>95% CI</td></tr>
<tr><td>1173 C>T</td><td></td><td>-31%</td><td>-24 - -36</td></tr>
<tr><td></td><td>Caucasian</td><td>-31%</td><td>-25 - -36</td></tr>
<tr><td></td><td>Asian</td><td>-27%</td><td>-19 - -33</td></tr>
<tr><td>-1639 G>A</td><td></td><td>-34%</td><td>-29 - -39</td></tr>
<tr><td></td><td>Caucasian</td><td>-34%</td><td>-26 - -40</td></tr>
<tr><td></td><td>Asian</td><td>-28%</td><td>-13 - -38</td></tr>
<tr><td colspan="4">Number of studies, total number of patients and patients per genotype for each comparison:
1173 C>T determined: 8 studies, n = 701, 312 AA, 389 GA
-1639 G>A determined: 10 studies, n = 2321, 825 AA, 1496 GA</td></tr>
<tr><td colspan="4">The heterogeneity between the studies was significant and high for the comparison based on determination of -1639 G>A and significant and moderate for the comparison based on determination of 1173 C>T.</td></tr>
<tr><td colspan="4">There were no indications for publication bias.</td></tr>
<tr><td colspan="4">The mean differences were not influenced by the omission of any individual study from the analysis.</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 10
SPC Coumadin (warfarin), USA, 28-</td>
<td>0</td>
<td><u>Dosing</u>:
The initial dose is influenced by:
• Clinical factors including age, race, body weight, sex,</td>
<td></td>
</tr>
</table>

10-15.

ref. 10, continuation

GA: AA
AA: A

concomitant medications, and comorbidities
• Genetic factors (CYP2C9 and VKORC1 genotypes)
Dosing Recommendations with Consideration of Genotype
Table 1 displays three ranges of expected maintenance Coumadin doses observed in subgroups of patients having different combinations of CYP2C9 and VKORC1 gene variants. If the patient's CYP2C9 and/or VKORC1 genotype are known, consider these ranges in choosing the initial dose.

Table 1: Three ranges of expected maintenance Coumadin daily doses (in mg) based on CYP2C9 and VKORC1 genotypes†

VKORC1	CYP2C9					
	*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	0.5-2

†Ranges are derived from multiple published clinical studies. VKORC1 −1639G>A (rs9923231) variant is used in this table. Other co-inherited VKORC1 variants may also be important determinants of warfarin dose.

Clinical Pharmacology:
Pharmacogenomics
Warfarin reduces the regeneration of vitamin K from vitamin K epoxide in the vitamin K cycle through inhibition of VKOR, a multiprotein enzyme complex. Certain single nucleotide polymorphisms in the VKORC1 gene (e.g., -1639G>A) have been associated with variable warfarin dose requirements. VKORC1 and CYP2C9 gene variants generally explain the largest proportion of known variability in warfarin dose requirements.
CYP2C9 and VKORC1 genotype information, when available, can assist in selection of the initial dose of warfarin.

Risk group	CYP2C9 polymorphism, use of CYP2C9 inhibitors

Comments:

- We only included meta-analyses in the risk analysis. Separate studies did not contribute enough to the evidence to be included.
- Dosing algorithms:
  The size of the effect of VKORC1 variants on warfarin dose differs between ethnicities. The proportional decrease was higher among Caucasians compared with Asians (Yang 2010) and among European Americans compared with African Americans (Limdi NA et al. Race influences warfarin dose changes associated with genetic factors. Blood 2015;126:539-45. PubMed PMID: 26024874). For this reason, only algorithms based on a predominantly Caucasian population are included. For patients with an African or (East-)Asian background, other algorithms might give better results. For patients with an African background, additional CYP2C9 alleles, such as *6, *8 and *11, should be taken into account. Examples of algorithms for patients with an African background can be found in Alzubiedi S et al, Pharmacogenetic-guided warfarin dosing algorithm in African-Americans, J Cardiovasc Pharmacol 2016;67:86-92, PubMed PMID: 26355760 and Hernandez W et al, Ethnicity-specific pharmacogenetics: the case of warfarin in African Americans, Pharmacogenomics J 2014;14:223-8, PubMed PMID: 24018621). The algorithm of Ramirez 2012 below also includes data on African-Americans. Examples of algorithms for patients with an East-Asian background can be found in Chen J et al, A pharmacogenetics-based warfarin maintenance dosing algorithm from Northern Chinese patients, PLoS One 2014;9:e105250, PubMed PMID: 25126975 and Choi JR et al, Proposal of pharmacogenetics-based warfarin dosing algorithm in Korean patients, J Hum Genet 2011;56:290-5, PubMed PMID: 21326313.
  In addition, only algorithms based on at least 150 patients are included. Furthermore, only articles are included in which the equation describing the developed algorithm is given.
  - Pirmohamed M et al. A randomized trial of genotype-guided dosing of warfarin. N Engl J Med 2013;369: 2294-303. PubMed PMID: 24251363.
    227 patients were randomly assigned to genotype-guided warfarin dosing and 228 patients to the control group. The control group received loading doses of 10-5-5 mg (≤ 75 years of age) or 5-5-5 mg (> 75 years of age). The mean percentage of time in the therapeutic range was significantly higher in the genotype-guided

group than in the control group (67.4% and 60.3% respectively). There were significantly fewer incidences of excessive anticoagulation (INR ≥4.0) in the genotype-guided group. The median time to reach a therapeutic INR was significantly shorter in the genotype-guided group than in the control group (21 days and 29 days respectively).

The genotype-guided algorithms employed were:

- Maintenance dose, slightly modified IWPC model (IWPC, 2009):

√ Dose (mg/week) = 5.6044 – 0.02614 × Age [in years] + 0.0087 × Height [cm] + 0.0128 × Weight [kg] – 0.8677 × VKORC1 -1639 GA – 1.6974 × VKORC1 -1639 AA – 0.5211 × CYP2C9 *1/*2 – 0.9357 × CYP2C9 *1/*3 – 1.0616 × CYP2C9 *2/*2 – 1.9206 × CYP2C9 *2/*3 – 2.3312 × CYP2C9 *3/*3 – 0.5503 × amiodarone

- Initial doses (Avery et al, 2011):

Loading over three days was calculated as followed: LD3=D/(1- exp(-24κ))(1+ exp(-24κ)+ exp(-48κ)).

Where D was the IWPC predicted maintenance dose per day in mg.

k is the elimination rate constant for the CYP2C9 genotypes:

*1/*1 = 0.0189h-1
*1/*2 = 0.0158h-1
*1/*3 = 0.0132h-1
*2/*2 = 0.0130h-1
*2/*3 = 0.009h-1
*3/*3 = 0.0075h-1

A loading dose regimen was used where the loading dose was gradually reduced, i.e. Day 1 dose > Day 2 dose > Day 3 dose. This was derived from the difference between the predicted daily dose (D) and the 3-day loading dose (LD3) according to the following:

Loading on Day 1: (LD3-D) x 1.5 + D
Loading on Day 2: ((LD3-D) x 1 + D
Loading on Day 3: (LD3-D) x 0.5 + D

- Dose revision on day 4 or 5 (slightly modified model by Lenzini et al, 2010):

Dose (mg/week) = EXP (3.10894 − 0.00767 × age − 0.51611 × ln(INR) − 0.23032 × VKORC1 -1639A − 0.14745 × CYP2C9*2 − 0.3077 × CYP2C9*3 + 0.24597 × BSA + 0.26729 × Target INR − 0.1035 × amiodarone + 0.0169 × $dose_{-2}$ + 0.02018 × $dose_{-3}$ + 0.01065 × $dose_{-4}$).

Where INR is the INR on day 4 or 5.

$Dose_{-2}$ is the dose given 2 days before the INR is measured, $Dose_{-3}$ is the dose given 3 days before the INR is measured, and $Dose_{-4}$ is the dose given 4 days before the INR is measured. $Dose_{-4}$ is only available if the INR is measured on the 5th day.

Body surface area (BSA) is calculated according to the formula by Dubois & Dubois where BSA = (weight $(kg)^{0.425}$ × height $(cm)^{0.725}$)/139.2.

- Ramirez AH et al. Predicting warfarin dosage in European-Americans and African-Americans using DNA samples linked to an electronic health record. Pharmacogenomics 2012;13:407-18. PubMed PMID: 22329724.

An algorithm for the maintenance dose of warfarin was developed based on data from 1022 European-Americans and 145 African–Americans. The algorithm explained 53% of dose variation in European-Americans and 41% of dose variation in African–Americans.

The algorithm is:

log[weekly warfarin dose] = 5.9487517 - 0.0073436353 * race (AA=0,EA=1) - 0.025161445 * age (in years) + 0.058138499 * sex (F=0,M=1) + 1.1848957 * bsa (kg/m^2) + 0.068020571 * smoking status (nonsmoker=0, smoker=1) + 0.058578086 * VTE indication (no=0,yes=1) - 0.10646416 * atrial fibrillation indication (no=0, yes=1) - 0.8142521 * amiodarone use (no=0,yes=1) - 0.64877338 * CYP2C9*2 (wt=0,heterozygote=1, homozygote=2) - 1.0601067 * CYP2C9*3 (wt=0,heterozygote=1,homozygote=2) - 1.9737831 * CYP2C9*6 (wt=0, heterozygote=1,homozygote=2) - 1.0622944 * CYP2C9*8 (wt=0,heterozygote=1,homozygote=2) + 0.24749973 * CYP4F2 (wt=0,heterozygote=1,homozygote=2) - 0.31996754 * CALU (wt=0,heterozygote=1, homozygote=2) - 0.87262446 * VKORC1 (wt=0,heterozygote=1,homozygote=2)

- Horne BD et al. Pharmacogenetic warfarin dose refinements remain significantly influenced by genetic factors after one week of therapy. Thromb Haemost 2012;107:232-40. PubMed PMID: 22186998.

An algorithm for the warfarin dose on day 6-11 of treatment was developed based on data from 1342 patients. The algorithm was validated in a separate retrospective cohort of 342 patients. The algorithm explained 69% of dose variation after about one week of therapy.

The algorithm is:

dose (mg/week) = EXP (2.59853 − 0.47578 × Treatment Response Index − 0.17132 × VKORC1 − 0.23385 × CYP2C9*3 − 0.10696 × CYP2C9*2 − 0.00549 × Age in years + 0.16491 × BSA − 0.09091 × Simvastatin Use − 0.251 × Fluvastatin Use − 0.11994 × Amiodarone Use + 0.3319 × Inducer Use + 0.08796 × Target INR − 0.13902 × Stroke + 0.01028 × Day of Therapy)

BSA = body surface area

treatment response index = ln(INR/effective dose), with the effective dose calculated by summing weighted prior doses. The relative weights of doses that were prescribed 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days ago in the

effective dose calculation were 0.306, 0.804, 0.555, 0.357, 0.229, 0.149, 0.099, 0.067, 0.047, and 0.033, respectively.

- Avery PJ et al. A proposal for an individualized pharmacogenetics-based warfarin initiation dose regimen for patients commencing anticoagulation therapy. Clin Pharmacol Ther 2011;90:701-6. PubMed PMID: 22012312.
  The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) study group developed an algorithm for the initiation dose of warfarin based on data from 671 patients. The initiation dose was calculated based on the half-life of S-warfarin for the different CYP2C9 genotypes (maintenance dose = initiation dose/(1-e-^{k*t}). The initiation dose was divided over 3 days to minimize the risk of INR exceeding the therapeutic range. Based on simulation, the initiation dose reduces the time to stable, therapeutic INR for patients with variant CYP2C9 alleles. According to the simulation, the time to stable, therapeutic INR was reduced for *3/*3 from approximately 30 days to 12.5 days, the latter being equal to the time to stable, therapeutic INR for *1/*1.
  The maintenance dose was calculated with a modification of the algorithm of the International Warfarin Pharmacogenetics Consortium (IWPC). The modified IWPC algorithm was adapted to the mainly Caucasian population in Europe by removing the ethnicity variable. In addition, drugs that induce warfarin metabolism were also omitted from the IWPC algorithm, because very few patients are treated with potent inducers. In addition, their inclusion in the algorithm would require information on the duration of treatment, because it takes at least 2–3 weeks to induce enzymes.
  The modified IWPC algorithm is:
  √ Predicted maintenance dose (mg/week) = [5.6044 − 0.02614 × age (years) + 0.0087 × height (cm) + 0.0128 × weight (kg) − 0.8677 (VKORC1 -1639 GA) − 1.6974 (VKORC1 -1639 AA) − 0.5211 (CYP2C9 *1/*2) − 0.9357 (CYP2C9 *1/*3) − 1.0616 (CYP2C9*2/*2) − 1.9206 (CYP2C9*2/*3) − 2.3312 (CYP2C9*3/*3) − 0.5503 (amiodarone)].
  The developed EU-PACT algorithm for the initiation dose is:
  MD + x (day 1), MD + 2x/3 (day 2), MD + x/3 (day 3), in which:
  x = MD*[1/(1-e-^{k*24})-1- e-^{k*24}- e-^{k*48}]/[1/3+2/3*e-^{k*24}+ e-^{k*48}]
  MD = maintenance dose, k = elimination rate constant
  *1/*1: k = 0.0189
  *1/*2: k = 0.0158
  *1/*3: k = 0.0132
  *2/*2: k = 0.0130
  *2/*3: k = 0.009
  *3/*3: k = 0.0075
- Zambon CF et al. VKORC1, CYP2C9 and CYP4F2 genetic-based algorithm for warfarin dosing: an Italian retrospective study. Pharmacogenomics 2011;12:15-25. PubMed PMID: 21174619.
  An algorithm for the maintenance dose of warfarin was developed based on data from 274 patients. The algorithm was validated in a separate set of 91 patients. The algorithm explained 56-65% of dose variation, which was comparable to the performance of the algorithm of the International Warfarin Pharmacogenetics Consortium (57-63% of dose variation) in these patient groups.
  The algorithm is:
  √ maintenance dose (mg/week) = 7.39764 - 0.02734 x age (years) + 1.06287 x BSA (m^2) - 1.04468 (VKORC1 -1639 GA) - 2.12117 (VKORC1 -1639 AA) - 0.78983 (CYP2C9*1*2) - 1.17138 (CYP2C9*1*3) - 1.81292 (CYP2C9*2*2, *2*3 or *3*3) - 0.46723 (CYP4F2*1*3) - 0.71528 (CYP4F2*1*1)
  BSA = body surface area
- Wells PS et al. A regression model to predict warfarin dose from clinical variables and polymorphisms in CYP2C9, CYP4F2, and VKORC1: Derivation in a sample with predominantly a history of venous thromboembolism. Thromb Res 2010;125:e259-64. PubMed PMID: 20421126.
  An algorithm for the maintenance dose of warfarin was developed based on data from 249 patients. The algorithm explained 58% of the dose variation.
  The algorithm is:
  Dose = 1.85 - 0.048 x Age (in years) + 0.041 x BMI + 0.05 x Height (in cm) - 0.73 (Less Exercise) - 1.13 (2C9*2 Hetero) - 2.09 (2C9*2 Homo) - 1.51 (2C9*3 Hetero) - 1.43 (VKORC1 -1639 GA) - 2.86 (VKORC1 -1639 AA) - 1.33 (4F2 CC) - 1.24 (4F2 CT) - 1.46 (Angiotensin II Receptor Antagonist) - 0.84 (β-Blocker).
  Less exercise means that a patient's self-rated physical activity level is less than or much less than those of a similar age.
  In the investigated patient population ACE inhibitors included losartan and candesartan and β-blockers included metoprolol, atenolol and bisaprolol.
- Lenzini P et al. Integration of genetic, clinical, and INR data to refine warfarin dosing. Clin Pharmacol Ther 2010;87:572-8. PubMed PMID: 20375999.
  An algorithm for the warfarin dose after day 4-5 of treatment was developed based on data from 969 patients. The algorithm was validated in a separate set of 616 patients. The algorithm explained 42-63% of dose variation and performed better than an algorithm without genotypes (26-48% of dose variation).
  The algorithm is:

Maintenance dose (mg/week) = EXP [3.10894 - 0.00767 × Age (in years) - 0.51611 × ln(INR) - 0.23032 × VKORC1-1639 G>A - 0.14745 × CYP2C9*2 - 0.3077 × CYP2C9*3 + 0.24597 × BSA + 0.26729 × Target INR - 0.09644 (African Origin) - 0.2059 (Stroke) - 0.11216 (Diabetes) - 0.1035 (Amiodarone Use) - 0.19275 (Fluvastatin Use) + 0.0169 × $Dose_{-2}$ + 0.02018 × $Dose_{-3}$ + 0.01065 × $Dose_{-4}$].

- International Warfarin Pharmacogenetics Consortium. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009;360:753-64. PubMed PMID: 19228618.
  An algorithm for the maintenance dose of warfarin was developed based on data from 4043 patients. The algorithm was validated in a separate set of 1009 patients. The algorithm explained 43-47% of dose variation.
  The International Warfarin Pharmacogenetics Consortium (IWPC) algorithm is:
  √ maintenance dose (mg/week) = 5.6044 - 0.2614 x Age (in decades) + 0.0087 x Height (in cm) + 0.0128 x Weight (in kg) - 0.8677 (VKORC1 -1639 A/G) - 1.6974 (VKORC1 -1639 A/A) - 0.4854 (VKORC1 genotype unknown) - 0.5211 (CYP2C9 *1/*2) - 0.9357 (CYP2C9 *1/*3) - 1.0616 (CYP2C9 *2/*2) - 1.9206 (CYP2C9 *2/*3) - 2.3312 (CYP2C9 *3/*3) - 0.2188 (CYP2C9 genotype unknown) - 0.1092 (Asian race) - 0.2760 (Black or African American) - 0.1032 (Missing or Mixed race) + 1.1816 (Enzyme inducer) - 0.5503 (Amiodarone)
  Use of an enzyme inducer means use of carbamazepine, phenytoin, rifampin or rifampicin.
- Wadelius M et al. The largest prospective warfarin-treated cohort supports genetic forecasting. Blood 2009;113:784-92. PubMed PMID: 18574025.
  An algorithm for the maintenance dose of warfarin was developed based on data from 1523 patients. The algorithm was validated in a separate set of 181 patients. The algorithm explained 53-59% of dose variation. Body weight and height were not available in the cohort. Instead of these parameters sex is included in the algorithm as a substitute. In addition, drugs that decrease the effect of warfarin are not included in the algorithm, because too few were present in the cohort to get reliable results.
  The Swedish Warfarin Genetics cohort (WARG) algorithm is:
  √ maintenance dose (mg/week) = 9.46832 - 0.90112 (VKORC1 -1639 A/G) **-** 2.01863 (VKORC1 -1639 A/A) - 0.50836 (CYP2C9 *1/*2) - 0.97546 (CYP2C9 *1/*3) - 1.10204 (CYP2C9 *2/*2) - 1.74761 (CYP2C9 *2/*3) - 3.40061 (CYP2C9 *3/*3) - 0.03686 x Age (in years) - 0.27698 (Female sex) - 0.06992 x number of drugs that increase INR.
- Gage BF et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin Pharmacol Ther 2008;84:326-31. PubMed PMID: 18305455.
  An algorithm for the maintenance dose of warfarin was developed based on data from 1015 patients. The algorithm was validated in a separate set of 292 patients. The algorithm explained 53-54% of dose variation (31% in African Americans).
  The Warfarin Dose Refinement Collaboration algorithm is:
  warfarin dose (mg/day) = exp [0.9751 − 0.3238 × VKOR3673G>A + 0.4317 × BSA − 0.4008 × CYP2C9*3− 0.00745 × age − 0.2066 × CYP2C9*2 + 0.2029 × target INR − 0.2538 (amiodarone) + 0.0922 (smoker) − 0.0901 (African-American race) + 0.0664 (DVT/PE)]
  DVT/PE = deep venous thrombosis or pulmonary embolism
- Sconce EA et al. The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. Blood 2005;106:2329-33. PubMed PMID: 15947090.
  An algorithm for the maintenance dose of warfarin was developed based on data from 297 patients. The algorithm was validated in a separate set of 38 patients. The algorithm explained 54% of dose variation.
  The algorithm is:
  √ Dose = 0.628 - 0.0135 x Age (in years) - 0.240 x CYP*2 - 0.370 x CYP*3 - 0.241 (VKORC1 -1639 GG) – 0.482 (VKORC1 -1639 GA) - 0.723 (VKORC1 -1639 AA) + 0.0162 x Height (in cm).

- <u>Cost effectiveness</u>:
  A cost effectiveness analysis in Thai patients was not included, because it does not add enough information about the cost effectiveness in a mainly Caucasian population.
  QALY = quality adjusted life year
  - Verhoef TI et al. Cost-effectiveness of pharmacogenetic-guided dosing of warfarin in the United Kingdom and Sweden. Pharmacogenomics J 2016 Jun 7. [Epub ahead of print] PubMed PMID: 27272045.
    For patients with atrial fibrillation from the UK and Sweden with mean ages of respectively 70.9 and 72.5 years, genotype guided warfarin therapy was more cost effective than standard care. The costs were £ 6,702 and 253,848 Swedish Kronor (SEK) per QALY gained, which is less than the thresholds of £ 20,000 and 500,000 SEK per QALY gained. The Swedish threshold of 500,000 SEK is approximately £ 40,000. In 93% of the simulations in the United Kingdom and 67% of the simulations in Sweden, the costs were lower than the thresholds.
    Lifelong medical costs were calculated. The calculations were based on a price of INR monitoring and visit to an anticoagulation clinic of £ 24.20 and 221 SEK, a price of warfarin of £ 3.20 and 45 SEK per month, a price of aspirin of £ 1.72 and 21 SEK per month, and costs for genotyping of £ 35.03 and 440 SEK. The percentage time within different INR ranges (<2.0, 2.0–3.0, 3.0–5.0 and >5.0) was derived from the EU-PACT trial (Pirmohamed M et al. A randomized trial of genotype-guided dosing of warfarin. N Engl J Med 2013;369: 2294-303). The risks of adverse events associated with each of the four INR ranges were derived from a meta-analysis of 19 randomized trials and observational studies of coumarin anticoagulants (Oake N et al. Anticoagulation intensity and outcomes among patients prescribed oral anticoagulant therapy: a systematic

review and meta-analysis. CMAJ 2008; 179: 235–244). Thromboembolic events consisted mainly of ischaemic strokes, but 28% were assumed to be transient ischaemic attacks. Patients with a stroke were assumed to have a 10% chance of dying and a 47% chance of disability, while patients with a transient ischaemic attack were assumed to fully recover. The majority of haemorrhagic events (80%) were assumed to be extracranial haemorrhage. The risk that an intracranial haemorrhage would result in permanent disability was assumed to be 50% and the chance that it would be fatal 45%; these values were assumed to be zero for an extra-cranial haemorrhage. Patients were assumed to switch to aspirin after an intracranial haemorrhage. Event rates after 3 months were assumed to be the same in both types of warfarin therapy. The number of INR measurements in the first three months was derived from the EU-PACT trial. The authors assumed one measurement per month thereafter.
Pharmacogenetic-guided dosing was cost-effective if genotyping costs would be no higher than £86 in the United Kingdom (given a cost-effectiveness threshold of £ 20,000 per QALY gained) or 809 SEK (approximately £64) in Sweden (given a threshold of 500 000 SEK).

- You JH. Universal versus genotype-guided use of direct oral anticoagulants in atrial fibrillation patients: a decision analysis. Pharmacogenomics 2015;16:1089-100.PubMed PMID: 26230572.
For 65-year-old newly diagnosed atrial fibrillation patients with a high risk for stroke, a genotype guided therapy was more cost effective than direct oral anticoagulants for all patients (costs were 314,129 USD less per QALY gained, which is higher than the threshold of 50,000 USD/QALY). Genotype guided therapy consisted of warfarin for patients with normal warfarin sensitivity and a direct oral anticoagulant for patients with a genotype leading to high warfarin sensitivity (CYP2C9 *2 or *3 variants or VKORC1 -1639 A/A) or low warfarin sensitivity (VKORC1 -1639 G/G in combination with CYP2C9 *1/*1).
Medical costs were calculated for a period of 35 years. For the genotype guided therapy the calculated costs were 124,568 USD and the calculated QALYs 10.6087. The calculation was based on a price of warfarin management (anticoagulation care with INR monitored at least monthly) of USD 34 per month, a price of warfarin (dosage to be determined by the International Warfarin Pharmacogenetics Consortium (IWPC) algorithm) of USD 10.5 per month, a price of direct oral anticoagulants (dabigatran 150 mg twice daily, rivaroxaban 20 mg daily or apixaban 5 mg twice daily) of USD 173 per month, and genotyping costs of USD 80. Risk for clinical events and costs of genotyping were retrieved from literature. In the model, patients who survived a major haemorrhagic event would stop the current anticoagulation therapy and start on aspirin alone. Patient who survived a major thromboembolic event while receiving a direct oral anticoagulant would resume the initial anticoagulation therapy. Those who experienced a major thromboembolic event on warfarin would change to a direct oral anticoagulant. Out-of-range INR in patients receiving warfarin was defined as <1.8 or >3.2. A focused warfarin care system, leading to 81% of time within the therapeutic range was assumed.
Universal use of direct oral anticoagulants would become cost-effective if the monthly drug cost of direct oral anticoagulants was less than USD 30 (lower than the monthly drug cost and management cost of warfarin of USD 40) or if time in the therapeutic range of warfarin therapy was less than 67.8%. The two-way sensitivity analysis on these two variables demonstrated that if the time in the therapeutic range of warfarin was similar to the average anticoagulation control reported in clinical trials of direct oral anticoagulants versus warfarin (60% of time in the therapeutic range), the genotype guided therapy would remain the preferred strategy when direct oral anticoagulants cost more than USD 250 per month. When the monthly cost of direct oral anticoagulants declined to USD 30, a high percentage of time in the therapeutic range (81%) of warfarin should be achieved in order for the genotype guided therapy to be the preferred option. Extended sensitivity analysis on prevalence of normal warfarin sensitivity showed genotype guided therapy to be the preferred strategy in ethnic groups with prevalence of normal warfarin sensitivity >0.56%.
- Pink J et al. Cost-effectiveness of pharmacogenetics-guided warfarin therapy vs. alternative anticoagulation in atrial fibrillation. Clin Pharmacol Ther 2014;95:199-207. PubMed PMID: 24067746.
For the UK population of atrial fibrillation patients, apixaban was more cost effective than genotype guided warfarin therapy (costs were £ 19,858 per QALY gained, which is lower than the threshold of £ 20,000-30,000/QALY). Genotype guided warfarin therapy was more cost effective than not genotype guided warfarin therapy (costs were £ 13,226 per QALY gained). For both therapies, the initial warfarin doses were fixed (10 mg on day 1, 10 mg on day 2 and 5 mg on day 3) and IWPC algorithms were used. Neither dabigatran nor rivaroxaban were cost-effective options.
Lifelong medical costs were calculated. For the genotype guided therapy the calculated costs were £ 5,921 and the calculated QALYs 5.724. The calculation was based on a price of warfarin monitoring of £ 198.39 per year, a price of warfarin of £ 41.23 per year, a price of dabigatran (150 mg twice daily) of £ 919.80 per year, a price of rivaroxaban (20 mg daily) of £ 766.50 per year, a price of apixaban (5 mg twice daily) of £ 802.25 per year, a price of aspirin of £ 7.39 per year, and genotyping costs of £ 20.00. Drug costs were taken from the British National Formulary. Risk for clinical events were calculated from an indirect comparison of the trials of warfarin versus the direct oral anticoagulants (RE-LY, ROCKET-AF and ARISTOTLE). As indicated by You 2015, average anticoagulation control with warfarin in these trials is relatively low (60% of time in the therapeutic range). Event rates for genotype guided versus not genotype guided warfarin therapy were derived from literature. Event rates after 3 months were assumed to be the same in both types of warfarin therapy. In the model, patients who discontinued dabigatran, rivaroxaban, or apixaban because of a bleed or who discontinued warfarin for any reason were assumed to be switched to aspirin. Other discontinuing patients were switched to warfarin.

- Nshimyumukiza L et al. Dabigatran versus warfarin under standard or pharmacogenetic-guided management for the prevention of stroke and systemic thromboembolism in patients with atrial fibrillation: a cost/utility analysis using an analytic decision model. Thromb J 2013;11:14. PubMed PMID: 23866305.
  For newly diagnosed atrial fibrillation patients with a mean age of 64 years, who never had a previous stroke, genotype guided warfarin therapy was less cost effective than standard warfarin therapy (costs were CAD$ (Canadian dollar) 54,118 per QALY gained, which is higher than the threshold of CAD$ 50,000 per QALY gained). Dabigatran 150 mg twice daily was more cost effective than standard warfarin therapy (costs were CAD$ 4,765 per QALY gained).
  Medical costs were calculated for the first 5 years of therapy. For the genotype guided therapy the calculated costs were CAD$ 7,749 and the calculated QALYs 3.5453. The calculation was based on a price of INR monitoring in the first year of CAD$ 8.06 per month for standard warfarin therapy and CAD$ 5 per month for genotype guided warfarin therapy, a price of INR monitoring in subsequent years of CAD$ 4.03 per month, a price of warfarin 5 mg/day of CAD$ 0.074 per day, a price of dabigatran of CAD$ 3.20 per day, and genotyping costs of CAD$ 615. For the standard warfarin therapy, the time spent in each INR category (below, within or above the therapeutic range) was based on the results of the RE-LY clinical trial comparing dabigatran and warfarin (Connolly 2009), while for the genotype guided warfarin therapy, it was calculated using data from the Couma-Gen trial, which compares genotype guided therapy with standard therapy (Anderson JL et al. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. Circulation 2007;116:2563-70). This means that the data for standard warfarin therapy and the relative effect of genotype guided warfarin therapy were derived from different trials and patient groups. In the RE-LY trial, patients from 44 different countries participated. The Couma-Gen trial studied only patients from the United States. It was assumed that after one year 100% of individuals, whatever the warfarin group they belong to, had reached a stable maintenance dose.
  Genotype guided warfarin therapy would be more cost effective than standard warfarin therapy and dabigatran, if it would change the average patient time in the therapeutic range in the first year from 66.6% to 76.8%, i.e. an increase with 20%. In this case, the costs per QALY gained for genotype guided warfarin therapy would be CAD$ 3,250. In the Couma-Gen trial, genotype guided therapy increased the time in the therapeutic range with 7.3%.
  With a threshold of CAD$ 50,000 per QALY gained, dabigatran was more cost-effective than standard warfarin therapy in 99.75% of simulations.
- Patrick AR et al. Cost-effectiveness of genotype-guided warfarin dosing for patients with atrial fibrillation. Circ Cardiovasc Qual Outcomes 2009;2:429-36. PubMed PMID: 20031873.
  For 70-year-old patients with newly-diagnosed atrial fibrillation, genotype guided warfarin therapy was more cost effective than usual care only if it reduces out-of-range international normalized ratio values by more than 5 to 9 percentage points (costs respectively less than 100,000 and 50,000 USD per QALY gained). The midpoint of the result of 2 published randomized controlled trials of genetically-guided dosing was 8.5% more time spent in the therapeutic range.
  Medical costs were calculated for the life expectancy of 11.42 years. The calculations were based on a price of INR monitoring (assumed: 8 INR tests during the first month of treatment and 1 in each subsequent month) of USD 29, a price of warfarin (average dose of 5 mg/day) of USD 71 per 3 months, and costs for genotyping and blood sample collection of USD 575. Risks for clinical events were retrieved from literature. Event rates after 3 months were assumed to be the same in both types of warfarin therapy.
  Variation of input data resulted in 42% of scenarios being cost effective at a threshold of 50,000 USD/QALY and 70% at a threshold of 100,000 USD/QALY. Genotyping was more cost-effective in younger patients, with the costs increasing from USD 29,000 per QALY in 50-year-old patients to USD 120,000 per QALY in 85-year-old patients. Test costs and assumptions about the rate of major bleeding during treatment initiation also influenced the results, because this parameter affected the number of bleeding events that could be averted through genotyping. In addition, the amount of patient time shifted from high INR to therapeutic INR influenced the cost effectiveness.
- Meckley LM et al. A policy model to evaluate the benefits, risks and costs of warfarin pharmacogenomics testing. Pharmacoeconomics 2010;28:61-74. PubMed PMID: 20014877.
  For 65-year-old atrial fibrillation patients starting with warfarin, genotype guided therapy was less cost effective than usual care (costs were 60,725 USD more per QALY gained, which is higher than the threshold of 50,000 USD/QALY). Varying of the input parameters resulted in 46% of simulations with additional costs for genotype guided therapy of less than 50,000 USD/QALY.
  Lifelong medical costs were calculated. For the genotype guided therapy the calculated costs were 46,970 USD and the calculated QALYs 12.0851. The calculation was based on a price of warfarin therapy of USD 63 per year and genotyping costs of USD 175. Risk for clinical events were mostly based on INR differences observed in the Couma-Gen trial in which the use of a pharmacogenetic maintenance dose algorithm to select initial warfarin dose was compared with standard care via an anticoagulation specialist pharmacist (n = 200). The effect of CYP2C9 on bleeding risk was retrieved from the meta-analysis of Sanderson 2005. Event rates after 6 months were assumed to be the same in both types of warfarin therapy.
  Results were most sensitive to the cost of genotyping and the effect of genotyping. If the genotyping test costs less than USD 13, then pharmacogenomic testing would be a cost-saving strategy. If data from the Caraco trial (n = 283) were used instead of data from the Couma-Gen trial, or pharmacogenomic initiation further reduced bleeds in the CYP2C9 variant patients, pharmacogenomic warfarin initiation would be cost

saving. Changes to some key structural assumptions increased the additional costs to more than USD 100,000 per QALY.
The costs and benefits of genotype guided therapy were not evenly distributed between the different genotypes. For patients that were both VKORC1 and CYP2C9 wild-type, the costs increased with USD 13,500/QALY due to a reduction in bleeds (despite higher doses), thromboembolism and deaths. For CYP2C9 variant patients the reduction in bleeds was offset by the increased number of thromboembolic events, resulting in reduced QALYs. These results imply that CYP2C9 variant patients may be more challenging to manage, even once their genetic status is known. This is potentially the result of a significantly slower therapeutic response in these patients due to the decreased rate of drug metabolism.

- You JH et al. Potential clinical and economic outcomes of CYP2C9 and VKORC1 genotype-guided dosing in patients starting warfarin therapy. Clin Pharmacol Ther 2009;86:540-7. PubMed PMID: 19571807.
For patients starting with warfarin, genotype guided therapy was less cost effective than usual care (costs were 347,059 USD more per QALY gained, which is higher than the threshold of 50,000 USD/QALY). Varying of the input parameters resulted in 48% of simulations with additional costs for genotype guided therapy of less than 50,000 USD/QALY.
Medical costs for the first year of treatment were calculated. The calculation was based on a price of anticoagulation clinic care of USD 303 per year and genotyping costs of USD 200. Risk for clinical events were based on INR differences observed in the Couma-Gen trial in which the use of a pharmacogenetic maintenance dose algorithm to select initial warfarin dose was compared with standard care via an anticoagulation specialist pharmacist (n = 200).
Results were most sensitive to the cost of genotyping, the relative percentage reduction in out-of-range INRs in the genotype guided dosing group, and the percentage of out-of-range INRs in the standard dosing group.
- Eckman MH et al. Cost-effectiveness of using pharmacogenetic information in warfarin dosing for patients with nonvalvular atrial fibrillation. Ann Intern Med 2009;150:73-83. PubMed PMID: 19153410.
For male patients aged 69 years with nonvalvular atrial fibrillation at average risk for stroke and without specific risk factors for bleeding, genotype guided dosing was less cost effective than usual care (costs were 171,800 USD more per QALY gained, which is higher than the threshold of 50,000 USD/QALY). Varying of the input parameters resulted in 10% of simulations with additional costs for genotype guided therapy of less than 50,000 USD/QALY. For genetic testing to cost less than USD 50,000 per QALY, it would have to be restricted to patients at high risk for haemorrhage or meet the following criteria: prevent greater than 32% of major bleeding events, be available within 24 hours, and cost less than $200.
Life-long medical costs were calculated. For the genotype guided therapy the calculated costs were 19,684 USD and the calculated QALYs 7.5780. The calculation was based on a price of warfarin of USD 36.99 per month, genotyping costs of USD 400 and a 3-day delay in initiating warfarin therapy due to genotyping. The relative risk for major bleeding in the genotype guided versus standard warfarin therapy (0.68; 95% CI: 0.22-2.06) was calculated from a meta-analysis of the 3 trials comparing both types of therapy (n_{total} = 429). Major bleeding rates after 1 month were assumed to be the same in both types of warfarin therapy.
Results were most sensitive to the cost of genotyping and the delay in warfarin therapy due to genotyping. If the cost of genotyping was less than USD 140, genotype guided dosing would be cost effective (additional costs less than USD 50,000/QALY). If the cost of genotyping was less than USD 40, genotype guided dosing would be cost saving. If there were no delay in initiating treatment, the additional costs would be USD 116,000/QALY. In the scenario where in-hospital genotyping would be possible, with cost of genotyping of USD 200 and the results available in 24 hours, allowing immediate initiation of warfarin therapy, the additional costs of genotype-guided dosing would be USD 51,000/QALY.

- Existing guideline:
Johnson JA et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. Clin Pharmacol Ther 2011;90:625-9. PubMed PMID: 21900891.
CPIC uses the same definition for the VKORC1 genotypes as we do. They also state that this genotype can both be reported by the -1639G>A polymorphism as by the linked 1173C>T polymorphism. However, CPIC itself uses the -1639G>A polymorphism for reporting. CPIC also states that inclusion of other common VKORC1 SNP's in dosing algorithms does not further improve warfarin dose prediction (Limdi 2010 and Gage 2008).
CPIC classifies the recommendations for warfarin dosing based on genotype in this guideline as strong. However, CPIC indicates that there are limited prospective data from randomized trials on the use of genetic information to guide warfarin dosing, and the impact on clinical outcomes is unknown, although several such studies were ongoing in 2011. (Note: Two of these studies were published in 2013 and are included in the meta-analyses of Liao 2015, Xu 2014, Franchini 2014 and Stergiopoulos 2014.)
CPIC indicates that dosing algorithms using genetics outperform nongenetic clinical algorithms and fixed-dose approaches in dose prediction (Gage 2008 and International Warfarin Pharmacogenetics Consortium 2009). They also predict the warfarin dose better than the FDA-approved warfarin label table (Finkelman 2011). CPIC indicates that two algorithms perform well in estimating stable warfarin dose across different ethnic populations (Gage 2008 and International Warfarin Pharmacogenetics Consortium 2009); these were created using more than 5,000 subjects.
The recommendation is to use pharmacogenetic algorithm based dosing when possible, although if electronic means for such dosing are not available, the table based dosing approaches are suggested.
CPIC indicates that the algorithms available on http://www.warfarindosing.org are the best way to estimate the anticipated stable dose of warfarin. The algorithms on this website are from The Warfarin Dose Refinement Collaboration (Gage 2008) and International Warfarin Pharmacogenetics Consortium (International Warfarin Pharma-

cogenetics Consortium 2009). They compute the anticipated stable daily warfarin dose to one decimal, and the clinician must then prescribe a regimen (e.g. an estimate of 4.3 mg/day might be given as 4 mg daily except 5 mg two days per week). When using the FDA-approved warfarin label table, the specific dose selected within the given range should take into account other important variables, such as patient age, body size, and interacting drugs. (Note: the warfarin table of the FDA is included in the last row of the table in this risk analysis).
The recommendations above are still the same after the last update on 19-6-2014 on the PharmGKB-site.

Date of the literature search: 29 June 2016.

	Genotype	Code	Gene-drug interaction	Action	Date
Project group decision	GA	4 A	yes	no	24 August 2016
	AA	4 A	yes	yes	

Mechanism:
Coumarins exert their anticoagulant effect by inhibiting the enzyme activity of the vitamin K 2,3-epoxide reductase complex, subunit 1 (VKORC1). Variations in the VKORC1 gene can result in a decreased expression of the VKORC1 protein. As a consequence, lower doses of coumarins are required for inhibition of this protein.
VKORC1 converts reduced vitamin K (vitamin K 2,3-epoxide) back into the active oxidized form (vitamin K hydroquinone). Vitamin K is an essential cofactor for the carboxylation of glutamic acid residues of blood coagulation factors II, VII, IX and X and the anti-coagulation proteins C, S and Z. For this reason, inhibition of VKORC1 diminishes coagulation.