CYP2C9: warfarin 6228 t/m 6234

*2 = CYP2C9 allele with decreased activity, *3 = CYP2C9 allele with strongly decreased activity, 95% CI = 95% confidence interval, EM = extensive metaboliser (*1/*1) (normal CYP2C9 enzyme activity), HR = hazard ratio, IM = intermediate metaboliser, other genotype (decreased CYP2C9 enzyme activity due to an allele with decreased activity other than *2 or *3), INR = international normalised ratio, NS = not significant, OR = odds ratio, PM = poor metaboliser, other genotype (strongly decreased CYP2C9 enzyme activity involving one or two alleles with decreased activity other than *2 or *3), 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, then the health care professional should consider the next best option.

Brief summary and justification of choices:
The most potent enantiomer of warfarin, S-warfarin, is predominantly metabolised into inactive metabolites by CYP2C9. Gene variants leading to an enzyme with reduced activity might therefore diminish the dose required. This was confirmed by three meta-analyses investigating the association between warfarin dose and gene variants with reduced activity.
Two meta-analyses found an increased bleeding risk in patients with one or two alleles with reduced activity. A third meta-analysis found a significant effect only for *3/*3. For the separate genotypes, a significantly increased bleeding risk was found in the largest meta-analysis for *1/*3 and *3/*3. For the other genotypes, a significant effect was only found after grouping them with other genotypes. As expected based on predicted enzyme activity, Lindh 2009 found a larger dose reduction for *2/*3 than for *1/*3. Both Lindh 2009 and Jorgensen 2012 found a similar dose reduction for *2/*2 as for *1/*3. Based on these results, it can be expected that *2/*2 and *2/*3 also result in an enhanced bleeding risk. For this reason, for these four genotypes, the gene-drug interaction leads to the necessity to adjust therapy by using lower initial doses (guideline with therapeutic recommendations).
For *1/*2, there is only evidence of an increased bleeding risk when analysed together with genotypes with a more greatly reduced CYP2C9 activity. In addition, the required dose reduction is small (approximately 20%, which is 1.5 times smaller than the required dose reduction for *1/*3 and *2/*2). Therefore, if *1/*2 has an increased bleeding risk, the increase in risk is probably small. For this reason, we consider the evidence insufficient to recommend therapy adjustment for this gene-drug interaction (guideline without therapeutic recommendations).
There are no data for IM and PM, the genotypes involving one or two alleles with reduced activity other than *2 and *3. Because the *6 allele has no activity, these phenotypes include genotypes with an effect size equal to or larger than *1/*3 and *3/*3. Therefore, lowering of the initial doses is also recommended for these genotypes (guideline with therapeutic recommendations).
You can find an overview of the effects per genotype in the background information text of the corresponding genotype-drug interaction on the KNMP Knowledge Bank. You might also have access to this background information text via your pharmacy or physician electronic decision support system.
You will find the source of the dose recommendations and a note on the meta-analyses comparing genotype-guided with non-genotype-guided dosing below.
Dose recommendations
Dose reductions are derived from the meta-analysis of Lindh 2009. This is the largest meta-analysis on the effect of CYP2C9 genotype on dose. To facilitate the use, the recommended percentages of the standard initial doses are rounded off to multiples of 5%.
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 non-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 who are expected to benefit most from genotype-guided dosing, i.e. the patients with variant alleles. In addition, in several of the included studies, the initial dose was fixed and not genotype-guided. Also, in patients with known genotypes, the initial dose can be adapted.

<table>
<tr><th>Source</th><th>Code</th><th>Effect</th><th>Comments</th></tr>
<tr><td>ref. 1
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</td><td>Meta-analysis of 7 randomised controlled trials with 1,910 patients comparing genotype-guided dosing (n = 960) to non-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 non-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 clinically 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:
Genotype-guided in comparison to non-genotype-guided therapy:<table><tr><td></td><td></td><td></td><td>% of patients in non-genotype-guided group</td></tr><tr><td>% of time within the therapeutic INR range (standardised mean difference)</td><td>all (5 trials, n = 1,729)</td><td>NS</td><td></td></tr><tr><td></td><td>fixed initial dose (3 trials, n < 700)</td><td>+0.24 (95% CI: 0.09-0.40) (S)</td><td></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>
<tr><td>ref. 2
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</td><td>Meta-analysis of 8 randomised controlled trials with 2,158 patients comparing genotype-guided dosing (n = 1,084) to non-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 non-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:
Genotype-guided in comparison to non-genotype-guided therapy:<table><tr><td></td><td></td><td></td><td>% in non-ge-</td></tr></table></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 stu-</td></tr>
</table>

<table>
<tr><td>ref. 2, continuation</td><td></td><td>
<table>
<tr><td></td><td></td><td></td><td>notype-guided group</td></tr>
<tr><td>% of time within the therapeutic INR range (standardised mean difference)</td><td>all (8 trials)</td><td>Not determined due to high heterogeneity</td><td></td></tr>
<tr><td></td><td>fixed initial dose (5 trials, n = 812)</td><td>+0.30 (95% CI: 0.08-0.53) (S)</td><td></td></tr>
<tr><td></td><td>not fixed initial dose (3 trials, n = 1,286)</td><td>NS</td><td></td></tr>
<tr><td></td><td>follow-up ≤ 28 days (3 trials, n = 1,218)</td><td>NS</td><td></td></tr>
<tr><td></td><td>follow-up > 28 days (5 trials, n = 880)</td><td>+0.25 (95% CI: -0.00-0.51) (NS, trend)</td><td></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>
</td><td>dies 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. 3
Franchini M et al. 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.</td><td>4</td><td>
Meta-analysis of 9 randomised controlled trials with 2,812 patients comparing genotype-guided dosing (n = 1,411) with non-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 non-genotype-guided therapy:</td></tr>
<tr><td></td><td></td><td></td><td>% in non-genotype-guided group</td></tr>
<tr><td>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></td><td colspan="3">2 trials with no major bleeds were excluded from the analysis (n = 481)</td></tr>
</table>
</td><td>Author’s conclusion:
”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>
</table>

<table>
<tr>
<td>ref. 3, continuation</td>
<td></td>
<td>
<table>
<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>standardised 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 hetero-geneity 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.
</td>
<td></td>
</tr>
<tr>
<td>ref. 4
Stergiopoulos K et al. Genotype-guided vs clinical dosing of warfarin and its analogues: meta-analysis of rando-mized clinical trials. JAMA Intern Med 2014;174:1330-8. PubMed PMID: 24935087.</td>
<td>4</td>
<td>Meta-analysis of 9 randomised controlled trials with 2,812 patients comparing genotype-guided dosing (n = 1,411) with non-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.

Results:
<table>
<tr><td colspan="4">Genotype-guided in comparison with non-genotype-guided therapy:</td></tr>
<tr><td></td><td></td><td></td><td>% in non-ge-notype-guided group</td></tr>
<tr><td>% of time within the therapeutic INR range (standardised 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>Thrombo-embolic events</td><td>7 trials, n = 2,586</td><td>NS</td><td>1.2%</td></tr>
<tr><td colspan="4">Analyses of sub-groups 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 with-in the therapeutic INR range. There was no heterogene-ity for the other outcomes.</td></tr>
<tr><td colspan="4">Indications for publication bias were not found.</td></tr>
</table>
</td>
<td>Author’s conclusion:
”In this meta-analy-sis 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 reduc-tion in major blee-ding or thrombo-embolic events compared with clinical dosing algorithms.”</td>
</tr>
<tr>
<td>ref. 5
Yang J et al. Influence of CYP2C9 and VKORC1 geno-</td>
<td>4</td>
<td>Meta-analysis of 22 studies with 6,272 patients. Patients were Caucasian in 15 studies, Caucasian or African-American in 3 studies, Asian in 3 studies and Brazilian in 1 study. The follow-up periods for bleeding or over-</td>
<td>Author’s conclu-sion:
”Both CYP2C9 and VKORC1</td>
</tr>
</table>

types 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.

ref. 5, continuation

*1/*3: D

*3/*3: C

(*1/*2 + *2/*2 + *2/*3): D

(*1/*3 + *2/*3 + *3/*3): D

anticoagulation varied from 3 weeks to 6 years.

Results:

Bleeding in comparison with *1/*1:

genotype	Total bleeding		Major bleeding	
	HR	95% CI	HR	95% CI
*1/*2	NS		Trend	0.95-5.40
*1/*3	2.05	1.36-3.10	2.43	1.17-5.06
*2/*2	Trend	0.80-19.66	-	
*2/*3	NS		-	
*3/*3	4.87	1.38-17.14	Trend	0.95-24.22

Number of studies and patients per genotype for both outcomes:
*1/*2: 3 studies, n = 49 and 2 studies, n = 45
*1/*3: 6 studies, n = 127 and 4 studies, n = 79
*2/*2: 2 studies, n = 6 and 0 studies
*2/*3: 2 studies, n = 7 and 0 studies
*3/*3: 4 studies, n = 10 and 2 studies, n = 7

The heterogeneity between the studies was high and significant for *1/*2 and total bleeding.

*2-carrier in comparison with *1/*1:

		HR	95% CI	Incidence for *1/*1
INR > 4 (5 studies, n = 1,672)		1.52	1.11-2.09	14%
	0-30 days	1.64	1.11-2.43	
	> 30 days	NS		
Total bleeding (4 studies, n = 561)		1.80	1.09-2.97	11%
	Caucasian	Trend	0.99-2.86	
	Asian	-		
Major bleeding (2 studies, n = 252)		2.56	1.15-5.69	10%

There was no significant heterogeneity between the studies.

There were no indications for publication bias.

Except for total bleeding, the HRs were not influenced by the omission of any individual study from the analyses.
For total bleeding, significance was lost after exclusion of either Margaglione 2000, Higashi 2002 or Lima 2008 from the analysis.

*3-carrier in comparison with *1/*1:

		HR	95% CI	Incidence for *1/*1
INR > 4 (6 studies, n = 1,803)		2.37	1.46-3.83	13%
	0-30 days	2.48	1.56-3.96	
	> 30 days	1.86	1.08-3.20	
Total bleeding (8 studies, n = 1,325)		1.95	1.38-2.78	23%
	Caucasian	1.90	1.18 -3.05	
	Asian	1.85	1.06-3.20	
Major bleeding (7 studies, n = 1,572)		2.55	1.29-5.02	6,5%

The heterogeneity between the studies was moderate but not significant for :
- INR > 4

There were no indications for publication bias.

genoty-pes are associated with an increased risk for warfarin over-anticoagulation. CYP2C9*3 is the main genetic risk factor for warfarin haemorrhagic complications."

ref. 5, continuation

The HRs were not influenced by the omission of any individual study from the analyses.

(*2- and/or *3-carrier) in comparison with *1:				
		HR	95% CI	Inci-den-ce for *1/*1
INR > 4 (11 studies, n = 4,717)		1.90	1.58-2.29	20%
	0-30 days	1.91	1.58-2.38	
	> 30 days	1.69	1.21-2.36	
Total bleeding (13 studies, n = 2,670)		1.68	1.34-2.11	22%
	Caucasian	1.42	1.05-1.92	
	Asian	1.85	1.06-3.20	
Major bleeding (7 studies, n = 1,572)		2.19	1.33-3.60	7.7%

The heterogeneity between the studies was moderate but not significant for:
- major bleeding

There were no indications for publication bias.

The HRs were not influenced by the omission of any individual study from the analyses.

ref. 6
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.

4

Meta-analysis of 34 studies. Most studies were retrospective cohorts.
The authors use the absence of 0 instead of 1 in the 95% confidence interval of odds ratios to distinguish significance from non-significance. They do not explain this in the article. They might be either looking at a logarithm of the odds ratio or at an odds difference.
Of the 34 studies in this meta-analysis, 9 were included in the meta-analysis of Yang 2013.

Results:

*1/*2: A

*1/*2 in comparison with *1/*1:			
			95% CI
Dose (mg/day) (difference in means) (16 studies)		-0.96	-1.73 - -0.19
	Caucasian	NS	
	Caucasian/Afro-American	-1.90	-3.51 - -0.29
	Indian/Chinese/Malay	NS	
Total bleeding (odds ratio (OR)) (4 studies)	Caucasian (all 4 studies)	NS	
	The result remained NS after exclusion of the only study that only counted serious or life-threatening bleeds.		

The heterogeneity between studies was significant for:
- dose, Caucasian

*1/*3: A

*1/*3 in comparison with *1/*1:			
			95% CI
Dose (mg/day) (difference in means) (25 studies)		-1.61	-2.79 - -0.43
	Caucasian	-1.79	-3.30 - -0.28
	Caucasian/Afro-American	NS	
	Chinese	-1.13	-1.52 - -0.74
	Japanese	-1.18	-1.78 - -0.58
	Indian/Chinese/Malay	-1.47	-2.16 - -0.78
Total bleeding (OR) (8 studies)		NS	
	Caucasian	NS	

Author's conclusion:
"Pooled effect estimates were significant in most ethnic groups for CYP2C9*3 and stable dose (mutant types requiring between 1.1 and 2.3 mg/day)."

<table>
<tr>
<td>ref. 6, continuation</td>
<td>*2/*2: A

*3/*3: C

(*2/*2 + *2/*3 + *3/*3): A</td>
<td>
<table>
<tr><td colspan="4">*2/*2 in comparison with *1/*1:</td></tr>
<tr><td colspan="3"></td><td>95% CI</td></tr>
<tr><td rowspan="4">Dose (mg/day) (difference in means) (9 studies)</td><td></td><td>-1.46</td><td>-2.00 - -0.92</td></tr>
<tr><td>Caucasian</td><td>NS</td><td></td></tr>
<tr><td>Caucasian/A-fro-American</td><td>NS</td><td></td></tr>
<tr><td colspan="3"></td></tr>
<tr><td rowspan="2">Total bleeding (OR) (4 studies)</td><td>Caucasian (all 4 studies)</td><td>NS</td><td></td></tr>
<tr><td colspan="3">The result remained NS after exclusion of the only study that only counted serious or life-threatening bleeds.</td></tr>
<tr><td colspan="4">The heterogeneity between studies was significant for:
- dose, Caucasian
- total bleeding</td></tr>
</table>
<table>
<tr><td colspan="4">*3/*3 in comparison with *1/*1:</td></tr>
<tr><td colspan="3"></td><td>95% CI</td></tr>
<tr><td rowspan="2">Dose (mg/day) (difference in means) (4 studies)</td><td></td><td>-1.64</td><td>-3.31 - -1.97</td></tr>
<tr><td>Caucasian</td><td>-2.29</td><td>-2.98 - -1.60</td></tr>
<tr><td rowspan="2">Total blee-ding (OR) (5 studies)</td><td></td><td>1.18</td><td>0.04-2.31 (S)</td></tr>
<tr><td>Caucasian</td><td>NS</td><td></td></tr>
</table>
<table>
<tr><td colspan="4">(*1/*2 + *1/*3) in comparison with *1/*1:</td></tr>
<tr><td colspan="3"></td><td>95% CI</td></tr>
<tr><td rowspan="3">Dose (mg/day) (difference in means) (6 studies)</td><td></td><td>-1.31</td><td>-2.20 - -0.42</td></tr>
<tr><td>Caucasian</td><td>-1.55</td><td>-2.38 - -0.72</td></tr>
<tr><td>Israeli-Jewish</td><td>-1.20</td><td>-1.41 - -0.99</td></tr>
<tr><td>Total blee-ding (OR) (2 studies)</td><td>Caucasian (both studies)</td><td>NS</td><td></td></tr>
</table>
<table>
<tr><td colspan="4">(*2/*2 + *2/*3 + *3/*3) in comparison with *1/*1:</td></tr>
<tr><td colspan="3"></td><td>95% CI</td></tr>
<tr><td rowspan="3">Dose (difference in means) (6 studies)</td><td></td><td>-3.56</td><td>-4.29 - -2.84</td></tr>
<tr><td>Caucasian</td><td>-3.35</td><td>-4.29 - -2.41</td></tr>
<tr><td>Israeli-Jewish</td><td>-3.60</td><td>-3.99 - -3.21</td></tr>
</table>
<table>
<tr><td colspan="4">(*2- and/or *3-carrier) in comparison with *1/*1:</td></tr>
<tr><td colspan="3"></td><td>95% CI</td></tr>
<tr><td rowspan="2">Longer time to stable dose (HR) (4 studies)</td><td>Caucasian</td><td>NS</td><td></td></tr>
<tr><td>Afro-American</td><td>NS</td><td></td></tr>
<tr><td>Longer time to therapeu-tic INR (2 studies)</td><td>Caucasian (both studies)</td><td>NS</td><td></td></tr>
</table>
There were not enough data for a meta-analysis of the influence of genotype on INR > 4 during the first week and on time in the therapeutic INR range.
</td>
<td></td>
</tr>
<tr>
<td>ref. 7
Lindh JD et al.
Influence of CYP2C9 genotype on warfarin dose requirements--a</td>
<td>4</td>
<td>Meta-analysis of 39 studies with 7,907 patients. 14 studies were performed in Europe, 13 in America and 12 in Asia. Studies were clinical cohorts or cross-sectional studies. Studies differed in target INR and in whether interacting drugs were excluded or not.</td>
<td>Author’s conclusion: ”Compared to the CYP2C9*1/*1 genotype, the</td>
</tr>
</table>

<table>
<tr>
<td>systematic review and meta-analysis. Eur J Clin Pharmacol 2009;65:365-75. PubMed PMID: 19031075.

ref. 7, continuation</td>
<td>*1/*2: A

*1/*3: A

*2/*2: A

*2/*3: A

*3/*3: A</td>
<td>In the meta-analyses, alleles other than *2 and *3 were considered wild type (*1).
Of the 39 studies in this meta-analysis, 7 were included in the meta-analysis of Yang 2013 and 18 in Jorgensen 2012.

Results:
<table>
<tr><td colspan="4">Dose in comparison with *1/*1 ($n_{*1/*1}$ = 5,749):</td></tr>
<tr><td>genotype</td><td></td><td></td><td>95% CI</td></tr>
<tr><td rowspan="4">*1/*2 (30 studies) ($n_{*1/*2}$ = 1,216)</td><td></td><td>-19.6%</td><td>-17.4 - -21.9</td></tr>
<tr><td>Caucasian</td><td>-19.9%</td><td>-17.4 - -22.4</td></tr>
<tr><td>Caucasian, no interacting drugs</td><td>-23.1%</td><td>-11.0 - -35.1</td></tr>
<tr><td>Caucasian, interacting drugs</td><td>-19.0%</td><td>-16.1 - -21.9</td></tr>
<tr><td rowspan="4">*1/*3 (37 studies) ($n_{*1/*3}$ = 759)</td><td></td><td>-33.7%</td><td>-29.4 - -38.1</td></tr>
<tr><td>Caucasian</td><td>-35.1%</td><td>-29.6 - -40.7</td></tr>
<tr><td>Caucasian, no interacting drugs</td><td>-40.9%</td><td>-32.2 - -49.5</td></tr>
<tr><td>Caucasian, interacting drugs</td><td>-33.4%</td><td>-25.1 - -41.7</td></tr>
<tr><td rowspan="4">*2/*2 (14 studies) ($n_{*2/*2}$ = 78)</td><td></td><td>-36.0%</td><td>-29.9 - -42.0</td></tr>
<tr><td>Caucasian</td><td>-36.8%</td><td>-28.5 - -45.1</td></tr>
<tr><td>Caucasian, no interacting drugs</td><td>-48.7%</td><td>-36.3 - -61.2</td></tr>
<tr><td>Caucasian, interacting drugs</td><td>-33.7%</td><td>-21.6 - -45.8</td></tr>
<tr><td rowspan="4">*2/*3 (17 studies) ($n_{*2/*3}$ = 81)</td><td></td><td>-56.7%</td><td>-49.1 - -64.3</td></tr>
<tr><td>Caucasian</td><td>-58.0%</td><td>-50.0 - -66.0</td></tr>
<tr><td>Caucasian, no interacting drugs</td><td>-42.0%</td><td>-23.9 - -60.0</td></tr>
<tr><td>Caucasian, interacting drugs</td><td>-58.8%</td><td>-47.5- -70.1</td></tr>
<tr><td rowspan="4">*3/*3 (7 studies) ($n_{*3/*3}$ = 24)</td><td></td><td>-78.1%</td><td>-72.0 - -84.3</td></tr>
<tr><td>Caucasian</td><td>-78.1%</td><td>-72.0 - -84.3</td></tr>
<tr><td>Caucasian, no interacting drugs</td><td>-</td><td></td></tr>
<tr><td>Caucasian, interacting drugs</td><td>-80.6%</td><td>-73.8 - -87.3</td></tr>
<tr><td colspan="4">The heterogeneity between the studies was significant for *1/*3, *2/*3 and *3/*3.</td></tr>
<tr><td colspan="4">There were no indications for publication bias.</td></tr>
<tr><td colspan="4">The variables age, African ethnicity, Asian ethnicity, exclusion of interacting drugs, and target INR had no significant influence on the dose reduction per genotype.
*1/*2, *1/*3 and *2/*2 showed a trend for a smaller dose reduction in studies with interacting drugs (p = 0.11-0.15).
*2/*2 showed a trend for an increase in dose reduction with age (p = 0.07).
*1/*2 showed a trend for an increase in dose reduction with the target INR (p = 0.13).</td></tr>
</table>
</td>
<td>CYP2C9*1/*2, CYP2C9*1/*3, CYP2C9*2/*2, CYP2C9*2/*3, and CYP2C9*3/*3 required warfarin doses that were 19.6, 33.7, 36.0, 56.7, and 78.1% lower, respectively. The impact of CYP2C9 genotype tended to be larger in patients without interacting drugs.”</td>
</tr>
<tr>
<td>ref. 8
Sanderson S et al. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. Genet Med 2005;7:97-104. PubMed PMID:</td>
<td>4</td>
<td>Meta-analysis of 9 studies with 2,775 patients. 8 of the 9 studies were performed in Caucasian patients. In the 9th study, 78% of patients was Caucasian and 22% Afro-Carib-bean. Studies were clinical cohorts, cross-sectional or case-control studies. Studies differed in target INR.
Of the 9 studies in this meta-analysis, 4 were included in the meta-analysis of Yang 2013, 7 in Jorgensen 2012 and 6 in Lindh 2009.

Results:
<table>
<tr><td colspan="4">*2-carrier in comparison with no *2:</td></tr>
<tr><td></td><td></td><td>95% CI</td><td>Value</td></tr>
</table>
</td>
<td>Author’s conclusion: ”Patients with CYP2C9*2 and CYP2C9*3 alleles have lower mean daily warfarin doses and a greater risk of bleeding.”</td>
</tr>
</table>

15714076.

ref. 8, continuation

(*1/*2 + *2/*2 + *2/*3): C

			for no *2:
Dose (mg/day and %) (8 studies)	-0.85 = -17%	-1.11- -0.60 = -22- -12%	5.0 mg/ day
Total bleeding (RR) (2 studies)	1.91	1.16-3.17	
There was no significant heterogeneity between the studies.			
Results were similar after exclusion of Highashi 2002 from the meta-analyses. In this study, the genotype frequencies were not in Hardy Weinberg equilibrium.			

(*1/*3 + *2/*3 + *3/*3): C

*3-carrier in comparison with no *3:			
		95% CI	Value for no *3:
Dose (mg/day and %) (8 studies)	-1.92 = -37%	-2.47- -1.37 = -48- -26%	5.2 mg/ day
Total bleeding (RR) (3 studies)	1.77	1.07-2.91	
The heterogeneity between the studies was high for the dose.			
Results were similar after exclusion of Highashi 2002 from the meta-analyses. In this study, the genotype frequencies were not in Hardy Weinberg equilibrium.			

(*1/*2 + *1/*3 + *2/*2 + *2/*3 + *3/*3): C

(*2- and/or *3-carrier) in comparison with (no *2 and *3):		
		95% CI
Dose (mg/day and %) (7 studies)	-1.71 = -27%	-2.20- -1.22
Total bleeding (RR) (2 studies)	2.26	1.36-3.75
The heterogeneity between the studies was high for the dose		
Results were similar after exclusion of Highashi 2002 from the meta-analyses. In this study, the genotype frequencies were not in Hardy Weinberg equilibrium.		

ref. 9
SmPC Coumadin (warfarin), USA, 28-10-15.

0

*1/*2: AA
*1/*3: A
*2/*2: A
*2/*3: A
*3/*3: A

Dosing:
The initial dose is influenced by:
• Clinical factors including age, ethnicity, body weight, gender, concomitant medications, and co-morbidities
• Genetic factors (CYP2C9 and VKORC1 genotypes)
Dosing Recommendations with Consideration of Genotype
Table 1 displays three ranges of expected maintenance Coumadin doses observed in sub-groups 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. Patients with CYP2C9 *1/*3, *2/*2, *2/*3, and *3/*3 may require more prolonged time (>2 to 4 weeks) to achieve maximum INR effect for a given dosage regimen than patients without these CYP variants.

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 deter-

ref. 9, continuation	IM: A PM: A	minants of warfarin dose. Clinical Pharmacology: *Pharmacokinetics* CYP2C9, a polymorphic enzyme, is likely to be the principal form of human liver CYP450 that modulates the in vivo anticoagulant activity of warfarin. Patients with one or more variant CYP2C9 alleles have decreased S-warfarin clearance. The S-enantiomer exhibits 2 to 5 times more anticoagulant activity than the R-enantiomer in humans, but generally has a more rapid clearance. *Pharmacogenomics* The S-enantiomer of warfarin is mainly metabolized to 7-hydroxywarfarin by CYP2C9, a polymorphic enzyme. The variant alleles, CYP2C9*2 and CYP2C9*3, result in decreased in vitro CYP2C9 enzymatic 7-hydroxylation of S-warfarin. The frequencies of these alleles in Caucasians are approximately 11% and 7% for CYP2C9*2 and CYP2C9*3, respectively. Other CYP2C9 alleles associated with reduced enzymatic activity occur at lower frequencies, including *5, *6, and *11 alleles in populations of African ancestry and *5, *9, and *11 alleles in Caucasians. 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	VKORC1 polymorphism, use of CYP2C9 inhibitors

Comments:

- We only included meta-analyses of more than five studies in the risk analysis. Smaller meta-analyses and separate studies did not contribute sufficiently to the evidence to be included.
- Dosing algorithms:
  Ethnicity predicts CYP2C9 enzyme activity also independent of CYP2C9 genotype (Hatta FH et al. Differences in CYP2C9 genotype and enzyme activity between Swedes and Koreans of relevance for personalized medicine: role of ethnicity, genotype, smoking, age, and sex. OMICS 2015;19:346-53. PubMed PMID: 25977991). For this reason, only algorithms based on a predominantly Caucasian population are included. For patients with an African or (East-)Asian heritage, other algorithms might give better results. For patients with an African heritage, also other CYP2C9 alleles, such as *6, *8 and *11, should be taken into account. Examples of algorithms for patients with an African heritage 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 heritage 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, Pub-Med 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 1173 CT – 1.6974 × VKORC1 1173 TT – 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 1173T – 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 1,022 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 * ethnicity (AA=0, EA=1) - 0.025161445 * age (in years) + 0.058138499 * gender (F=0, M=1) + 1.1848957 * BSA (kg/m^2) + 0.068020571 * smoking status (non-smoker=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 1,342 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 three days to minimise 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 bisoprolol.
- 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 4,043 patients. The algorithm was validated in a separate set of 1,009 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 1,523 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, gender was 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 gender) - 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 1,015 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 vein 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>:
  QALY = quality adjusted life year
  - 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 of 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. Patients who survived a major thrombo-embolic event while receiving a direct oral anticoagulant would resume the initial anticoagulation therapy. Those who experienced a major thrombo-embolic 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 non-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 was 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 non-genotype-guided warfarin therapy were derived from literature. Event rates after three 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 have been switched to aspirin. Other discontinuing patients were switched to warfarin.
- 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 normalised ratio values by more than 5 to 9 percentage points (costs less than 100,000 and 50,000 USD per QALY gained respectively). The midpoint of the result of two published randomised 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 three 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 standard 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 six 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), thrombo-embolism and deaths. For CYP2C9 variant patients, the reduction in bleeds was offset by the increased number of thrombo-embolic 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 standard 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 was 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 one 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 classifies the recommendations for warfarin dosing based on genotype in this guideline as strong. However, CPIC indicates that there are limited prospective data from randomised 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 non-genetic clinical algorithms and fixed-dose approaches in dose prediction (Gage 2008 and International Warfarin Pharmacogenetics Consortium 2009). They also predict the warfarin dose more accurately 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 Pharmacogenetics 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: 31 May 2016.

	Genotype	Code	Gene-drug interaction	Action	Date
Decision by the Dutch Pharmacogenetic Working Group	*1/*2	4 A	Yes	no	24 August 2016
	*1/*3	4 D	Yes	yes	
	*2/*2	4 A	Yes	yes	
	*2/*3	4 A	Yes	yes	
	*3/*3	4 C	Yes	yes	
	IM	0 A	Yes	yes	
	PM	0 A	Yes	yes	

Mechanism:
Warfarin consists of a racemic mixture. S-warfarin is a more potent anticoagulant than R-warfarin. S-warfarin is predominantly metabolised by CYP2C9, whereas R-warfarin is primarily metabolised by other enzymes.