

## VKORC1: acenocoumarol #

2562/2563‡

AA = homozygous for the variant allele (= -1639 AA = 1173 TT) (strongly increased VKA sensitivity), CI = confidence interval,  $Cl_{or}$  = oral clearance, GA = heterozygous (= -1639 GA = 1173 CT) (increased VKA sensitivity), GG = homozygous wild type allele (= -1639 GG = 1173 CC) (normal VKA sensitivity), HR = hazard ratio, INR = international normalised ratio, MR = metabolic ratio, NS = non-significant, OR = odds ratio, S = significant, SmPC = Summary of Product Characteristics, VKA = vitamin K antagonist, VKORC1 = vitamin K epoxide reductase complex subunit 1

**Disclaimer:** The KNMP Pharmacogenetics Working Group 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.

### **Brief summary and justification of choices:**

Acenocoumarol exerts its anticoagulant effect by inhibiting the activity of the VKORC1 enzyme. Variations in the VKORC1 gene can result in reduced production of the enzyme. In patients with these gene variations, the acenocoumarol dose required to attain the therapeutic INR is lower.

The homozygous variant genotype AA has an obvious effect on the maintenance dose (Cerezo-Manchado 2014, Esmerian 2011, Kovac 2010, Cadamuro 2010, Stepien 2009, Teichert 2009, Markatos 2008, Spreafico 2008, Montes 2006, and Reitsma 2005). A study also found an increased risk of bleeding (significant for the trend GG-GA-AA) (Kovac 2010). Two studies found an increased risk of bleeding for GA + AA (Wijnen 2010 and Montes 2008). Two Dutch studies found that the risk of INR > 6 increased significantly (Teichert 2009 and Verhoef 2012). A study showed no significant difference in clinical effect for all genotypes combined when using a pharmacogenetic dose algorithm for the first 5-7 days (Verhoef 2013). A later study showed this to be also true for patients with two or more VKORC1 and/or CYP2C9 variants (Zhang 2017). However, this might be due to the algorithm being suboptimal. Based on the observed clinical effects for AA, the KNMP Pharmacogenetics Working Group decided that a recommendation to reduce the initial dose is required for this gene-drug interaction (yes/yes-interaction).

GA has a less obvious effect on the maintenance dose (Cerezo-Manchado 2014, Esmerian 2011, Kovac 2010, Cadamuro 2010, Stepien 2009, Teichert 2009, Markatos 2008, Spreafico 2008, Montes 2006, and Reitsma 2005). Moreover, GA is the most common genotype among European Whites, and the standard dose will therefore be largely based on this genotype. There is only limited evidence to support an increased risk of bleeding or an increased risk of INR > 6.0 (Kovac 2010, Wijnen 2010, Montes 2008, and Spreafico 2008). This is why the KNMP Pharmacogenetics Working Group decided that this concerns a gene-drug interaction, but that no action is required (yes/no-interaction). You can find a detailed overview of the observed effects per genotype in the background information text of the corresponding gene-drug interaction in the KNMP Kennisbank. You might also have access to this background information text via your pharmacy or physician electronic decision support system.

More detailed substantiation of the choice per genotype is given below.

**AA:** Kovac 2010 and Wijnen 2010 found an increased risk of bleeding for GA + AA. However, Teichert 2009 did not reach the same conclusion for GA and AA separately and Montes 2008 reached this conclusion only for high doses or other risk factors. Esmerian 2011 and Reitsma 2005 found no difference in risk. The Dutch studies by Teichert 2009 and Verhoef 2012 found an increased risk of INR > 6. In Teichert 2009, measurements were performed on the day after an initial dose of 8-4-4 mg acenocoumarol (OR = 3.54). Verhoef 2012 found an increased risk during the first month of use. Another study involving a small group of patients found no significant difference after an initial dose of 4-2-2 mg acenocoumarol (Spreafico 2008). As the regular checks by the Thrombosis Service do not offer protection against an INR > 6 on the day after the initial dose, the KNMP Pharmacogenetics Working Group decided to recommend adjustment of the initial dose. As the initial dose used by the Thrombosis Service differs for patients < 70 years (6-4-2 mg) and for patients ≥ 70 years or with relative contra-indication(s) (either 4-2-1 or 3-2-1 mg), the KNMP Pharmacogenetics Working Group decided to recommend a percentage decrease in the initial dose equivalent to the decrease in the maintenance dose for AA. The weighted mean of the calculated decrease in maintenance dose for AA compared to GG is a decrease to 49% of the maintenance dose (range 37-60%, median 50%)

(based on a total of 602 AA from 9 studies) (Cerezo Manchado 2014, Esmerian 2011, Kovac 2010, Cadamuro 2010, Stepien 2009, Teichert 2009, Markatos 2008, Schalekamp 2006, and Reitsma 2005). This was translated to a decrease to 50% of the normal dose, to be more achievable in clinical practice. The weighted mean of the calculated decrease in maintenance dose for AA compared to GG+GA is a decrease to 58% of the maintenance dose (range 42-70%, median 59%) This was translated to a decrease to 60% of the normal dose, to be more achievable in clinical practice. Because dosing in Whites will be mainly based on the groups with genotypes GA and GG, the KNMP Pharmacogenetics Working Group decided to recommend adjustment of the therapy for AA by starting with 60% of the normal (i.e. not genotype guided) dose. The KNMP Pharmacogenetics Working Group also decided to recommend additional monitoring in hospitals, where patients are initiated on anticoagulant therapy by residents or internists.

If a VKORC1 AA patient also has a CYP2C9 variant necessitating reduction of the start dose, both dose recommendations should be applied, i.e. if the CYP2C9-based recommendation is to use 75% of the normal start dose, 60% of 75% (= 45%) of the normal start dose should be used. The reason that both dose reductions should be applied, is that gene variants of VKORC1 and CYP2C9 influence the response to acenocoumarol in separate and independent ways (i.e. by reducing the amount of VKORC1 protein (and thus the amount of acenocoumarol needed for inhibition) and by reducing the acenocoumarol clearance, respectively).

GA: There is no direct evidence for an increased risk of bleeding for GA. Kovac 2010 found an increased risk of bleeding for the trend GG, GA, AA. Wijnen 2010 found an increased risk of bleeding for GA + AA. Montes 2008 found an increased risk of bleeding for GA + AA, but only for high doses or other risk factors. Three studies found no increased risk of bleeding for GA or GA + AA (Esmerian 2011, Teichert 2009, and Reitsma 2005). Spreafico 2008, Teichert 2009 and Verhoef 2012 found no increased risk of INR > 6.

#### **Recommendation concerning pre-emptive genotyping, including justification of choices:**

The KNMP Pharmacogenetics Working Group considers genotyping before starting acenocoumarol to be to be beneficial for drug safety. It is advised to consider genotyping the patient before (or directly after) drug therapy has been initiated to guide dose selection.

The clinical implication of the gene-drug interaction scores 5 out of the maximum of 10 points (with pre-emptive genotyping considered to be beneficial for scores ranging from 3 to 5 points) (see also the clinical implication score tables at the end of this risk analysis):

Despite very careful dose titration by the Dutch Thrombosis Service, the percentage of patients developing INR > 6 (severity code D corresponding to CTCAE grade 3) was enhanced for patients homozygous for the variant VKORC1 allele (VKORC1 -1639 AA) compared to patients homozygous for the wild type allele (VKORC1 -1639 GG) (Verhoef 2012 and Teichert 2009). In addition, two studies reporting also fatal bleeding found an increased bleeding risk for AA or GA+AA compared to GG (severity code F corresponding to CTCAE grade 5). It concerned a Dutch case-control study on diffuse alveolar bleeding (Wijnen 2010) and a Spanish study on gastro-intestinal bleeding (Montes 2008). The maximum severity of CTCAE grade 5 results in the maximum of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (2 points for CTCAE grade 5).

Five studies confirmed VKORC1 -1639 AA to result in a severe clinical effect (score of D or F corresponding to CTCAE grade 3 or 5) (Verhoef 2012, Kovac 2010, Wijnen 2010, Teichert 2009 and Montes 2008). This results in the maximum of 3 points for the second criterion of the clinical implication score, the level of evidence supporting an associated clinical effect grade  $\geq 3$  (3 points for three or more publications with level of evidence score  $\geq 3$ ).

The number needed to genotype was deduced from the increase in the percentage of patients with bleeding for VKORC1 -1639 AA. INR > 6 only has a severity code D (CTCAE grade 3), because an increase in INR > 6 corresponds to an increase in bleeding. However, the incidence of bleeding is much lower than the incidence of INR > 6 and patients do not notice INR > 6 if it does not result in bleeding. For this reason, INR > 6 is not suitable for calculation of the number needed to genotype to prevent a serious adverse event. Reitsma 2005 investigated major bleeding, but only mentioned odds ratios, not the incidence of major bleeding in VKORC1 -1639 GG or in the general population. For this reason, the number needed to genotype cannot be calculated from this publication. Like Reitsma 2005, Teichert 2009 does not report a significant difference in bleeding between VKORC1 -1639 AA and VKORC1 -1639 GG, but does report a numerical difference. In this study, the percentage of patients with at least one bleeding event was 4.0% for VKORC1 -1639 AA and 3.6% for VKORC1 -1639 GG, while VKORC1 -1639 AA was present in 15% of the patients. Thus, genotype-guided therapy could maximally avoid bleeding in 0.4% (1 in 250) of VKORC1 -1639 AA and 6.67 patients should be genotyped to find one VKORC1 -1639 AA. This amounts to a number needed to genotype of 1667 to avoid bleeding in 1 patient. To avoid serious bleeding in 1 patient this number would be even higher. A calculated number needed to genotype higher than 1667 results in 0 out of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) to prevent one clinical effect grade  $\geq 3$  (only points for NNG  $\leq 1000$ ).

The Summary of Product Characteristics (SmPC) of acenocoumarol does not mention any VKORC1 phenotype or genotype. This results in 0 out of the maximum of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (only points for at least one genotype/phenotype mentioned in the SmPC).

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																																					
<p><b>ref. 1</b> Zhang Y et al. Age-stratified outcome of a genotype-guided dosing algorithm for acenocoumarol and phenprocoumon. J Thromb Haemost 2017;15:454-464. PubMed PMID: 27992949.</p>	3	<p>Data from the 325 patients in Verhoef 2013 who had at least 10 weeks follow-up were reanalysed. Of these patients, 160 received genotype-guided treatment (113 patients &lt; 75 years of age and 47 patients ≥ 75 years of age) and 165 received control treatment (103 patients &lt; 75 years of age and 62 patients ≥ 75 years of age). After exclusion of patients due to protocol violations, 111 patients remained in the genotype-guided group (80 patients &lt; 75 years of age and 31 patients ≥ 75 years of age) and 126 in the control group (77 patients &lt; 75 years of age and 49 patients ≥ 75 years of age). Of the patients &lt; 75 years of age, 58% was Dutch and the remaining 42% was Greek. Of the patients ≥ 75 years of age, 31% was Dutch and the remaining 69% was Greek. All INRs were measured during the first 12 weeks of treatment. The majority of patients used relevant co-medication. Amiodarone usage was included in the dose algorithm. Differences in percentages of time in or outside the therapeutic range were adjusted for height, weight, sex, enzyme inhibitors, and enzyme inducers.</p> <p>Genotyping: - 116x GG - 144x GA - 64x AA - 1x genotype unknown (clinical algorithm, ≥ 75 years)</p> <p>Results:</p> <table border="1"> <thead> <tr> <th colspan="4">Genotype-based algorithm versus clinical algorithm:</th> </tr> <tr> <th></th> <th></th> <th></th> <th>value for the clinical algorithm</th> </tr> </thead> <tbody> <tr> <td rowspan="7">% of time in the therapeutic range</td> <td>&lt; 75 years, no CYP2C9 and VKORC1 variants</td> <td>NS</td> <td>58.9%</td> </tr> <tr> <td>&lt; 75 years, one CYP2C9 or VKORC1 variant</td> <td>NS</td> <td>65.2%</td> </tr> <tr> <td>&lt; 75 years, two or more CYP2C9 and/or VKORC1 variants</td> <td>NS</td> <td>59.6%</td> </tr> <tr> <td>≥ 75 years, no CYP2C9 and VKORC1 variants</td> <td>NS</td> <td>53.4%</td> </tr> <tr> <td>≥ 75 years, one CYP2C9 or VKORC1 variant</td> <td>NS</td> <td>60.9%</td> </tr> <tr> <td>≥ 75 years, two or more CYP2C9 and/or VKORC1 variants</td> <td>NS</td> <td>66.7%</td> </tr> <tr> <td>&lt; 75 years</td> <td>NS</td> <td>61.3%</td> </tr> <tr> <td>≥ 75 years</td> <td>NS</td> <td>61.7%</td> </tr> <tr> <td colspan="4">A per-protocol analysis showed</td> </tr> </tbody> </table>	Genotype-based algorithm versus clinical algorithm:							value for the clinical algorithm	% of time in the therapeutic range	< 75 years, no CYP2C9 and VKORC1 variants	NS	58.9%	< 75 years, one CYP2C9 or VKORC1 variant	NS	65.2%	< 75 years, two or more CYP2C9 and/or VKORC1 variants	NS	59.6%	≥ 75 years, no CYP2C9 and VKORC1 variants	NS	53.4%	≥ 75 years, one CYP2C9 or VKORC1 variant	NS	60.9%	≥ 75 years, two or more CYP2C9 and/or VKORC1 variants	NS	66.7%	< 75 years	NS	61.3%	≥ 75 years	NS	61.7%	A per-protocol analysis showed				<p>Author's conclusion: "For acenocoumarol users, there were no significant differences between the genotype-guided and control groups for most outcomes, except for a lower percentage of time below the range among older patients."</p>
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ref. 1, continuation

geno-  
type-  
guided  
versus  
not geno-  
type-  
guided  
therapy  
: AA

	similar results.		
	< 75 years, Dutch	NS	58.5%
	≥ 75 years, Dutch	NS	58.9%
	similar results.		
	< 75 years, Greek	NS	65.3%
	≥ 75 years, Greek	NS	63.0%
% of time with a supratherapeutic INR (> 3.0)	< 75 years, no CYP2C9 and VKORC1 variants	NS	10.7%
	< 75 years, one CYP2C9 or VKORC1 variant	NS	16.2%
	< 75 years, two or more CYP2C9 and/or VKORC1 variants	NS	23.8%
	similar results.		
	≥ 75 years, no CYP2C9 and VKORC1 variants	NS	7.4%
	≥ 75 years, one CYP2C9 or VKORC1 variant	NS	21.2%
	≥ 75 years, two or more CYP2C9 and/or VKORC1 variants	NS	16.2%
	< 75 years	NS	18.8%
	≥ 75 years	NS	15.9%
	A per-protocol analysis showed similar results.		
	< 75 years, Dutch	NS	22.0%
	≥ 75 years, Dutch	NS	20.8%
	< 75 years, Greek	trend for a decrease, p = 0.09 (NS)	14.1%
	≥ 75 years, Greek	- 7.7% (S)	13.8%
	% of time with a subtherapeutic INR (< 2.0)	< 75 years, no CYP2C9 and VKORC1 variants	NS
< 75 years, one CYP2C9 or VKORC1 variant		NS	18.6%
< 75 years, two or more CYP2C9 and/or VKORC1 variants		NS	16.6%
similar results.			
≥ 75 years, no CYP2C9 and VKORC1 variants		NS	35.1%
≥ 75 years, one CYP2C9 or VKORC1 variant		trend for an increase, p = 0.06 (NS)	18.0%
≥ 75 years, two or more CYP2C9 and/or VKORC1 variants		trend for an increase, p = 0.08 (NS)	17.1%
< 75 years		NS	19.9%
≥ 75 years		+ 9.9% (S)	22.4%
A per-protocol analysis showed similar results.			

ref. 1, continuation			< 75 years, Dutch	NS	19.4%	
			≥ 75 years, Dutch	NS	20.4%	
			< 75 years, Greek	NS	20.6%	
			≥ 75 years, Greek	+ 11.5% (S)	23.3%	
			Note: The authors indicate that the lack of a significant difference between the genotype-guided and clinical algorithms for acenocoumarol, could be due to the dose adjustment strategy after the loading period. Because of the shorter half-life of acenocoumarol compared to phenprocoumon, this dose adjustment strategy differed between the two anticoagulants.			
ref. 2 Cerezo-Manchado JJ et al. Effect of VKORC1, CYP2C9 and CYP4F2 genetic variants in early outcomes during acenocoumarol treatment. Pharmacogenomics 2014;15:987-96. PMID: 24956252.	3		This study monitored 941 patients who started with acenocoumarol for a period of 90 days. The INR target value differed between patients. Relevant co-medication was not excluded.			Authors' conclusion: 'Over-anticoagulation: international normalized ratio [INR] >2.5 at 72 h was the strongest factor affecting INR >4, although VKORC1 and CYP2C9 genotypes also independently led to the same outcome.'  <b>Maintenance dose versus GG:</b> GA: 76% AA: 53%
	GA: A AA: A	<p>Genotyping:</p> <ul style="list-style-type: none"> <li>- 320x GG</li> <li>- 465x GA</li> <li>- 156x AA</li> </ul> <p>Results versus GG:</p> <ul style="list-style-type: none"> <li>- maintenance dose: <ul style="list-style-type: none"> <li>- GA: decrease by 24% from 17 mg to 13 mg/week (S for the trend GG, GA, AA)</li> <li>- AA: decrease by 47% from 17 mg to 9 mg/week (S for the trend GG, GA, AA)</li> </ul> </li> <li>- percentage of patients with an INR &gt; 4: <ul style="list-style-type: none"> <li>- GA: increase with HR 1.96 (95% CI: 1.44-2.67) (S)</li> <li>- AA: increase with HR: 4.18 (95% CI: 3.08-5.98) (S)</li> </ul> </li> <li>- time to stable dose: <ul style="list-style-type: none"> <li>- GA: median increase (HR: 1.104; 95% CI: 1.018-1.197) (S for the trend GG, GA, AA)</li> <li>- AA: median increase by 33% from 60 to 80 days (HR: 1.104; 95% CI: 1.018-1.197) (S for the trend GG, GA, AA)</li> </ul> </li> <li>- INR &gt; 2.5 after 72 hours of treatment: <ul style="list-style-type: none"> <li>- GA: increase (HR: 2.19; 95% CI: 1.811-2.654) (S for the trend GG, GA, AA)</li> <li>- AA: increase (HR: 2.19; 95% CI: 1.811 - 2.654) (S for the trend GG, GA, AA)</li> </ul> </li> </ul>				
ref. 3 Verhoef TI et al. A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon. NEJM 2013;369:2304-12. PMID:24251360.	3		Patients without prior exposure to vitamin K antagonist therapy were treated with acenocoumarol for 12 weeks. The dose administered during the first 5-7 days was guided by an algorithm that included CYP2C9 and VKORC1 genotypes (n=190) or guided by an algorithm based on clinical information only (n=187). The INR target was 2.0-3.0. Relevant co-medication was not excluded. Use of amiodarone was incorporated in the dose algorithm. Patients with venous thromboembolism (17%) were commonly given low-molecular-weight heparin until achieving therapeutic INR.			Authors' conclusion: 'Genotype-guided dosing of acenocoumarol or phenprocoumon did not improve the percentage of time in the therapeutic range during the 12 weeks after the initiation of therapy.'
	genotype-	<p>Genotyping:</p> <ul style="list-style-type: none"> <li>- 125x GG</li> <li>- 177x GA</li> <li>- 75x AA</li> </ul> <p>Genotype-based algorithm versus clinical algorithm:</p> <ul style="list-style-type: none"> <li>- the time that the INR was in the therapeutic range throughout the treatment did not increase (NS)</li> </ul>				

**ref. 3, continuation**

guided versus not genotype-guided therapy : AA

- the time that the INR was in the therapeutic range during the first 4 weeks did not increase (NS)
- no difference in the incidence of adverse events and thromboembolism (NS)
- no difference in the percentage of the patients with an INR  $\geq 4$ , the percentage of the time with an INR  $\geq 4$  or  $< 2$ , the time until achieving an INR within the therapeutic range and the time until achieving a stable dose (NS)

When the acenocoumarol and phenprocoumon data were pooled, the time that the INR was in the therapeutic range in the first 4 weeks of treatment was higher for the genotype-based algorithm than for the clinical algorithm (52.8% and 47.5% of the time respectively) (S). There were no differences in weeks 5-8 and weeks 9-12. However, the results of Baranova 2017 suggested the higher percentage of time in therapeutic range in the first 4 weeks to be due to the patients without a CYP2C9 and or VKORC1 variant:

Genotype-based algorithm versus clinical algorithm:			
	genotype group	first 4 weeks	first 12 weeks
% of time in the therapeutic range	no CYP2C9 and VKORC1 variants	+ 14.68% (S, but only a trend after Bonferroni correction (significance for $p < 0.001$ ) (NS, $p = 0.002$ ))	trend for an increase, $p = 0.087$ (NS)
	one or more CYP2C9 variants and no VKORC1 variant	NS	NS
	no CYP2C9 variants and one VKORC1 variant	NS	NS
	one or more CYP2C9 variants and one VKORC1 variant	NS	NS
	no CYP2C9 variants and two VKORC1 variants	NS	NS
	one or more CYP2C9 variants and two VKORC1 variants	NS	NS
% of time with a supra-therapeutic INR ( $> 3.0$ )	no CYP2C9 and VKORC1 variants	NS	NS
	one or more CYP2C9 variants and no VKORC1 variant	NS	NS

Authors' conclusion:  
 'Four weeks after therapy initiation, genotype-guided dosing increased the mean percentage of time in the therapeutic INR range in the VKORC1 GG-CYP2C9\*1\*1 subgroup as compared with the non-genetic dosing (difference of 14.68%). For the VKORC1 AA-CYP2C9\*1\*1 subgroup, there was a higher risk of under-anticoagulation with the genotype-guided algorithm (difference of 19.9%). Twelve weeks after therapy initiation, no statistically significant differences in anticoagulation control between trial arms were noted across the VKORC1-CYP2C9 genetic subgroups. EU-PACT genetic-guided dose initiation algorithms for acenocoumarol and phenprocoumon could have predicted the dose over-cautiously in the

Baranova EV et al. Dosing algorithms for vitamin K antagonists across VKORC1 and CYP2C9 genotypes. J Thromb Haemost 2017;15:465-472. PubMed PMID: 28063245.

ref. 3, continuation		no CYP2C9 variants and one VKORC1 variant	NS	NS	VKORC1 AA-CYP2C9*1*1 subgroup. Adjustment of the genotype-guided algorithm could lead to a higher benefit of genotyping.'	
		one or more CYP2C9 variants and one VKORC1 variant	trend for a decrease, p = 0.098 (NS)	NS		
		no CYP2C9 variants and two VKORC1 variants	trend for a decrease, p = 0.087 (NS)	trend for a decrease, p = 0.057 (NS)		
		one or more CYP2C9 variants and two VKORC1 variants	- 20.50% (S, but NS after Bonferroni correction)	NS		
		% of time with a sub-therapeutic INR (< 2.0)	no CYP2C9 and VKORC1 variants	- 20.29% (S, before and after Bonferroni correction)		trend for a decrease, p = 0.083 (NS)
			one or more CYP2C9 variants and no VKORC1 variant	NS		NS
			no CYP2C9 variants and one VKORC1 variant	NS		trend for an increase, p = 0.081 (NS)
			one or more CYP2C9 variants and one VKORC1 variant	NS		NS
			no CYP2C9 variants and two VKORC1 variants	+ 19.89% (S, before and after Bonferroni correction)		+ 12.99% (S, but NS after Bonferroni correction)
			one or more CYP2C9 variants and two VKORC1 variants	trend for an increase, p = 0.075 (NS)		NS
			Results were similar after sensitivity analysis for both vitamin K antagonists separately and in the per-protocol dataset.			
		ref. 4	3	Data from 1,420 acenocoumarol users from 3 different studies were analysed. 12% of the patients participated in the Schalekamp 2006 study, which is also included separately in this risk analysis. This was the only study that generated data on the first 6 months of treatment. Data up to 18 months were derived from the other two studies. The INR target was 2.0-3.5 for all patients. Relevant co-medication was not excluded.		Genotyping: - 499x GG - 696x GA
Verhoef TI et al. Long-term anticoagulant effects of the CYP2C9 and VKORC1 genotypes in acenocoumarol users. J Thromb Haemost 2012;10:606-14. PMID: 22252093.						

<p><b>ref. 4, continuation</b></p>	<p>GA: C  AA: D</p>	<p>- 211x AA</p> <p>GA versus GG:</p> <ul style="list-style-type: none"> <li>- no difference in the percentage of patients with INR &gt; 6 during the entire treatment time (NS)</li> <li>- factor 0.85 decrease in the percentage of patients with INR &lt; 2 during the first month (from 73% to 62%) (S)</li> <li>- factor 0.82 decrease in the percentage of patients with INR &lt; 2 during the second and third month (from 49% to 40%) (S)</li> <li>- no difference in the percentage of patients with INR &lt; 2 during the remainder of the treatment time (NS)</li> <li>- factor 1.5 increase in the percentage of patients with INR &gt; 3.5 during the first month (from 30% to 45%) (S)</li> <li>- no difference in the risk INR &gt; 3.5 during the remainder of the treatment time (NS)</li> </ul> <p>AA versus GG:</p> <ul style="list-style-type: none"> <li>- factor 4 increase in the percentage of patients with INR &gt; 6 during the first month (from 3% to 12%) (S)</li> <li>- no difference in the risk of INR &gt; 6 after the first month (NS)</li> <li>- factor 0.62 decrease in the percentage of patients with INR &lt; 2 during the first month (from 73% to 45%) (S)</li> <li>- trend towards a factor 0.8 decrease in the percentage of patients with INR &lt; 2 during the second and third month (from 49% to 39%) (NS; p = 0.05)</li> <li>- no difference in the percentage of patients with INR &lt; 2 during the remainder of the treatment time (NS)</li> <li>- factor 2.5 increase in the percentage of patients with INR &gt; 3.5 during the first month (from 30% to 74%) (S)</li> <li>- factor 1.6 increase in the percentage of patients with INR &gt; 3.5 during the second and third month (from 40% to 62%) (S)</li> <li>- no difference in the percentage of patients with INR &gt; 3.5 during the remainder of the treatment time (NS)</li> </ul> <p>NOTE: Genotyping was for the polymorphism 1173C&gt;T.</p>	<p>(down to 45%) in the first month of treatment with acenocoumarol, but this effect diminished after 1-6 months. Knowledge of the patient's genotype therefore might assist physicians to adjust doses in the first month(s) of therapy."</p>
<p><b>ref. 5</b> Esmerian MO et al. Influence of CYP-2C9 and VKORC1 polymorphisms on warfarin and acenocoumarol in a sample of Lebanese people. J Clin Pharmacol 2011;51:1418-28.</p>	<p>3  GA: A AA: A</p>	<p>A total of 133 patients (33x GG, 57x GA, 43x AA) on maintenance therapy with acenocoumarol. The INR target was 2.0-3.0 (n=100) or 2.5-3.5 (n=33). An INR of 1.7-4.0 is considered an INR within the therapeutic range. Relevant co-medication was not excluded.</p> <p>- maintenance dose versus GG:</p> <ul style="list-style-type: none"> <li>- GA: decrease by 35% from 26 mg to 17 mg/week (S)</li> <li>- AA: decrease by 50% from 26 mg to 13 mg/week (S)</li> </ul> <p>- number of bleeding events since the start of the treatment: no difference between the genotypes (NS) Many of the patients who were admitted to the hospital with major bleeding had an INR within the target range.</p> <p>- time required to achieve first therapeutic INR after the start of the treatment (n=40): no difference between the genotypes (NS)</p>	<p>Authors' conclusion: "The reduction in weekly dose is driven by mainly VKORC1, followed by CYP2C9*3 variants."  <b>Maintenance dose versus GG:</b> GA: 65% (S) AA: 50% (S)</p>
<p><b>ref. 6</b> Kovac MK et al. The c.-1639G&gt;A polymorphism of the VKORC1 gene in Serbian population: retro-</p>	<p>3</p>	<p>A total of 200 patients (53x GG, 90x GA, 57x AA) on stable anticoagulation with acenocoumarol for at least 3 months. Therapeutic INR is defined as 2.0-3.0, independent of the individual target. No specific dose algorithm was used to calculate the dose. The INR was measured every 4 weeks after achieving a stable anticoagulant dose and when starting treatment the INR was measured 3x per week in week 1, 2x</p>	<p>Authors' conclusion: "VKORC1 c.-1639 G&gt;A polymorphism significantly influenced VKA dose and represented a good</p>



<p>oral anticoagulants. Mol Diagn Ther 2010;14:23-30.</p> <p><b>ref. 8, continuation</b></p>	<p>GA+AA : F</p>	<p>lar bleeding.</p> <p>Cases (patients) versus control group:</p> <ul style="list-style-type: none"> <li>- factor 1.15 increase in the percentage of patients with a variant allele (increase from 70.5% to 81.0%) (S)</li> <li>- factor 1.18 increase in the allele frequency of T (increase from 47.7% to 56.3%) (NS)</li> </ul>	<p>high probability of the occurrence of diffuse alveolar hemorrhage in patients receiving oral anticoagulants.”</p>
<p><b>ref. 9</b> Stepien E et al. A vitamin K epoxide reductase-oxidase complex gene polymorphism (-1639 G&gt;A) and interindividual variability in the dose-effect of vitamin K antagonists. J Appl Genet 2009;50:399-403.</p>	<p>4  GA: AA AA: A</p>	<p>57 patients (24x GG, 25x GA, 8x AA) on maintenance therapy with acenocoumarol (n=50) and warfarin (n=7). The INR target was 2.0-2.5. The warfarin dose was divided by 1.85 to convert it to an acenocoumarol dose. Co-medication that could influence the vitamin K antagonist metabolism was excluded and intake of products with a high vitamin K content was discouraged. A total of 5 patients (all GG) had an INR &lt; 2.0.</p> <ul style="list-style-type: none"> <li>- maintenance dose versus GG:</li> <li>- GA: decrease by 26% from 6.8 mg to 5.0 mg/day (NS)</li> <li>- AA: decrease by 40% from 6.8 mg to 4.1 mg/day (S versus GG+GA)</li> </ul> <p>Multiple linear regression analysis demonstrated that the VKORC1 polymorphism is an independent variable for the acenocoumarol dose.</p>	<p>Authors' conclusion: "Altogether, our study supports the hypothesis that identification of a SNP of the VKORC1 gene may help to achieve stable anticoagulation with the better VKA dose adjustment."</p> <p><b>Maintenance dose versus GG:</b> GA: 74% (S) AA: 60% (S)</p>
<p><b>ref. 10</b> Teichert M et al. Genotypes associated with reduced activity of VKORC1 and CYP2C9 and their modification of acenocoumarol anticoagulation during the initial treatment period. Clin Pharmacol Ther 2009;85:379-86.</p>	<p>3  GA: A  AA: D</p>	<p>1,525 patients (554x GG, 743x GA, 228x AA) on acenocoumarol for various indications. Loading dose 8-4-4 mg. Relevant co-medication was not excluded. The weekly dose after 6 weeks was corrected for co-medication affecting CYP2C9 and the INR target. The percentage of patients developing INR ≥ 6 was 2.0% after 4 days and 7.3% in the first 6 weeks. For GG, the INR was 2.5 on day 4 and the weekly dose after 6 weeks was 20.1 mg/week.</p> <p>GA versus GG:</p> <ul style="list-style-type: none"> <li>- increase in the INR on day 4 by 0.35 (S)</li> <li>- no significantly increased risk of INR ≥ 6 during the first 6 weeks. The incidence of INR ≥ 6 was 2.2% for GA and 0.9% for GG.</li> <li>- no increased risk of bleeding during the first 6 weeks (NS). The percentage of patients with at least 1 bleeding was 3.9% for GA and 3.6% for GG.</li> <li>- decrease in the weekly dose after 6 weeks by 5.09 mg/week (S)</li> </ul> <p>AA versus GG:</p> <ul style="list-style-type: none"> <li>- increase in the INR on day 4 by 0.66 (S)</li> <li>- increased risk of INR ≥ 6 on day 4 (OR = 3.54 (S)). The incidence of INR ≥ 6 was 3.1% for AA and 0.9% for GG.</li> <li>- increased risk of INR ≥ 6 during the first 6 weeks (OR = 2.46 (S))</li> <li>- no increased risk of bleeding during the first 6 weeks (NS). The percentage of patients with at least 1 bleeding was 4.0% for AA and 3.6% for GG.</li> <li>- decrease in the weekly dose after 6 weeks by 10.2 mg per week (S)</li> </ul> <p>There was a significant, multiplicative interaction between the effects of CYP2C9 and VKORC1 on the weekly dose. A larger proportion of the difference in required dose was</p>	<p>Authors' conclusion: "Each CYP2C9 variant allele present reduced the required dosage by 1.8 mg/week. Our conclusion was that an initial standard dosing regimen with acenocoumarol increases the risk of severe overanticoagulation in patients with variant alleles of the VKORC1 and CYP2C9 genes."</p> <p><b>Maintenance dose versus GG:</b> GA: 73% (S) AA: 49% (S)</p>

<p><b>ref. 10, continuation</b></p>		<p>explained by the VKORC1 genotype than by the CYP2C9 genotype (28% versus 5% respectively).</p> <p>NOTE: Genotyping was for the polymorphism 1173C&gt;T.</p>	
<p><b>ref. 11</b> Montes R et al. The influence of polymorphisms of VKORC1 and CYP2C9 on major gastrointestinal bleeding risk in anticoagulated patients. Br J Haematol 2008;143:727-33.</p>	<p>3</p> <p>GA+AA : F</p> <p>AA: F</p>	<p>Case control study involving 86 cases (severe gastrointestinal bleeding; 25x GG, 41x GA, 20x AA) and 175 controls (no bleeding), acenocoumarol use, relevant co-medication is present; 3 cases died as a result of the bleeding. The mean acenocoumarol dose was similar for cases and controls.</p> <ul style="list-style-type: none"> <li>- no increase in the risk of severe gastrointestinal bleeding for GA and AA (NS)</li> <li>- risk of bleeding versus GG with dose ≤ 15 mg/week: <ul style="list-style-type: none"> <li>- GG and &gt; 15 mg: OR non-significantly increased</li> <li>- GA+AA and ≤ 15 mg: OR non-significantly increased</li> <li>- GA+AA and &gt; 15 mg: OR = 10.10 (95% CI: 1.00-102.40). Significance was only achieved after correction for age, gender and duration of acenocoumarol treatment.</li> </ul> </li> <li>- there was a significant interaction for AA and the high dose (S). The result was the same after checking for factors such as use of amiodarone, acetylsalicylic acid or statins.</li> <li>- the CYP2C9 inhibitor amiodarone amplifies the effect of the polymorphisms on the risk of bleeding. Risk of bleeding versus (no AA, no CYP2C9 polymorphism) without amiodarone: <ul style="list-style-type: none"> <li>- (no AA, no CYP2C9 polymorphism) with amiodarone: OR non-significantly increased</li> <li>- (AA and/or CYP2C9 polymorphism) without amiodarone: OR = 1.89 (95% CI: 1.08-6.26)</li> <li>- (AA and/or CYP2C9 polymorphism) with amiodarone: OR = 9.97 (95% CI: 1.75-56.89)</li> </ul> </li> <li>- acetylsalicylic acid potentiates the effect of the polymorphisms on the risk of bleeding. Risk of bleeding versus (no AA, no CYP2C9 polymorphism) without acetylsalicylic acid: <ul style="list-style-type: none"> <li>- (no AA, no CYP2C9 polymorphism) with acetylsalicylic acid: OR non-significantly increased</li> <li>- (AA and/or CYP2C9 polymorphism) without acetylsalicylic acid: OR = 1.89 (95% CI: 1.08-3.31)</li> <li>- (AA and/or CYP2C9 polymorphism) with acetylsalicylic acid: OR = 8.97 (95% CI: 1.66-48.34)</li> </ul> </li> </ul>	<p>Authors' conclusion: "The risk of gastrointestinal bleeding during acenocoumarol therapy in carriers of any of the studied polymorphisms is severely increased with exposure to weekly doses of acenocoumarol higher than 15 mg or the use of amiodarone or aspirin. ... Genotyping of these alterations may be advisable in those patients taking amiodarone or aspirin."</p>
<p><b>ref. 12</b> Markatos CN et al. VKORC1 and CYP2C9 allelic variants influence acenocoumarol dose requirements in Greek patients. Pharmacogenomics 2008;9:1631-8.</p>	<p>3</p> <p>GA: A</p> <p>AA: A</p>	<p>98 patients (26x GG, 49x GA, 23x AA) on maintenance therapy with acenocoumarol (INR target 2.0-3.0). Relevant co-medication was not excluded, but statins and triazole derivatives (CYP2C9 inhibitors) had no significant association with the acenocoumarol dose;</p> <p>Maintenance dose versus *1/*1:</p> <ul style="list-style-type: none"> <li>- GA: decrease by 19% from 3.54 mg to 2.85 mg/day (S for the trend)</li> <li>- AA: decrease by 63% from 3.54 mg to 1.3 mg/day (S for the trend)</li> </ul> <p>There was a significant association between the VKORC1 genotype and the maintenance dose. A larger proportion of the difference in required dose was explained by the VKORC1 genotype than by the CYP2C9 genotype (40% versus 12% respectively).</p>	<p>Authors' conclusion: "VKORC1-1639 G&gt;A, CYP2C9*2 and CYP2C9*3 polymorphisms were found to predispose to acenocoumarol sensitivity in Greek."</p> <p><b>Maintenance dose versus GG:</b> GA: 81% (S) AA: 37% (S)</p>

<b>ref. 12, continuation</b>		NOTE: The authors' assumption that statins and triazole derivatives are CYP2C9 inhibitors is not entirely correct.	
<b>ref. 13</b> Spreafico M et al. Effects of CYP2C9 and VKORC1 on INR variations and dose requirements during initial phase of anticoagulant therapy. Pharmacogenomics 2008;9:1237-50.	3  GA: AA  AA: C	<p>220 patients (79x GG (0x *1/*1, 8x *1/*3, 4x *1/*4, 29x *3/*3, 32x *3/*4, 6x *4/*4), 93x GA (3x *1/*2, 60x *2/*3, 30x *2/*4), 48x AA (*2/*2)) on acenocoumarol (loading dose 4-4-2 mg). Relevant co-medication was not excluded, but co-medication did not have a significant effect on the INR on day 4 and was not associated with the required dose; The dose in week 7 was determined for patients with an INR target of 2.0-3.0 (n=187).</p> <p>GA versus GG (significance not determined):</p> <ul style="list-style-type: none"> <li>- increase in the INR on day 4 by 0.5 from 2.5 to 3.0</li> <li>- increase in the incidence of INR ≥ 6 on day 4 by a factor 2.5 from 1.7% to 4.2%</li> <li>- decrease of the dose in week 7 by 13% from 20.7 to 18.0 mg/week</li> </ul> <p>AA versus GG:</p> <ul style="list-style-type: none"> <li>- increase in the INR on day 4 by 2.0 from 2.5 to 4.5 (S)</li> <li>- increase in the incidence of INR ≥ 6 on day 4 by a factor 13 from 1.7% to 22% (NS)</li> <li>- decrease of the dose in week 7 by 45% from 20.7 to 11.4 mg/week (S)</li> </ul> <p>CYP2C9 and VKORC1 independently affect the INR on day 4 and - together with age - account for 26% of the variability in this INR.</p> <p>A larger proportion of the difference in required dose was explained by the VKORC1 genotype than by the CYP2C9 genotype (12% versus 5% respectively).</p> <p>NOTE: The authors divided the G allele into three different alleles (*1, *3 and *4). As no difference was found between the effect of *3 and *4 on acenocoumarol treatment and *1 occurs at very low frequencies, this distinction is not useful. Therefore, *1, *3 and *4 were combined for analysis of the results.</p> <p>NOTE: Genotyping was for the polymorphism 1173C&gt;T.</p>	Authors' conclusion: "Two copies of the VKORC1*2 haplotype were associated with a 45% dose reduction and an increased risk of over-anticoagulation."
<b>ref. 14</b> Gonzalez-Conejero R et al. The genetic interaction of VKORC1 c1173t/calumenin a29809g modulates the anticoagulant response of acenocoumarol. J Thromb Haemost 2007;5:1701-6.	4  GA+AA : A	<p>100 White men (&lt; 75 years; atrial fibrillation without involvement of the heart valves; 37x GG, 56x GA, 7x AA) received acenocoumarol (loading dose 3 mg/per day for 3 days, followed by individualisation based on INR). No relevant co-medication.</p> <p>GA+AA versus GG:</p> <ul style="list-style-type: none"> <li>- increased sensitivity to treatment during the first 3 days: median INR from 1.74 to 2.07 (S by 19%)</li> <li>- the required maintenance dose of acenocoumarol decreased from on average 19.5 to 15.8 mg/week (S by 19%)</li> <li>- increase in the percentage of patients with INR ≥ 3.5 after first 3 days from 2.7% to 12.7% (NS by 370%)</li> </ul> <p>NOTE: Genotyping was for the polymorphism 1173C&gt;T.</p>	Authors' conclusion: "Only VKORC1 genotype had significant impact on the efficacy of therapy."
<b>ref. 15</b> Schalekamp T et al. VKORC1 and CYP2C9 genotypes	4	<p>231 patients (81x GG, 111x GA, 39x AA) received acenocoumarol (loading dose 6-4-2 mg, followed by titration based on INR). No relevant co-medication; correction for use of NSAIDs and antibiotics.</p>	Authors' conclusion: "Being a carrier of a combination of

<p>and acenocoumarol anticoagulation status: interaction between both genotypes affects over-anticoagulation. Clin Pharmacol Ther 2006;80:13-22.</p> <p><b>ref. 15, continuation</b></p>	<p>GA: A AA: A</p>	<p>- risk of INR &gt; 6 versus GG: - with CYP2C9*1/*1 genotype, GA or AA: HR = 0.37 (NS) - with CYP2C9*2 or *3 genotype, GA or AA: HR = 4.16 (NS) - maintenance dose (mg/day): - GG: 2.80 (mean of all CYP2C9s) - GA: 2.80-0.56= 2.24 (S by 20%) - AA: 2.80-1.34= 1.46 (S by 48%) - time to stability (days) (mean all CYP2C9s): - GG: 36 (n=71) - GA: 35 (n=93) - AA: 26 (n=30)</p> <p>NOTE: Genotyping was for the polymorphism 1173C&gt;T.</p>	<p>polymorphisms of VKORC1 and CYP-2C9, rather than of one of these polymorphisms, is associated with severe overanticoagulation. The time to achieve stability is mainly associated with the CYP-2C9 genotype.”</p> <p><b>Maintenance dose versus GG:</b> GA: 80% (S) AA: 52% (S)</p>
<p><b>ref. 16</b> Montes R et al. The c.-1639G &gt; A polymorphism of the VKORC1 gene is a major determinant of the response to acenocoumarol in anticoagulated patients. Br J Haematol 2006;133:183-7.</p>	<p>3  GA+AA : A  AA: A</p>	<p>The VKORC1-genotype was determined in 113 patients with stable anticoagulation on low dose (<math>\leq 7</math> mg/week; n=42), medium dose (<math>&gt; 7</math> and <math>&lt; 28</math> mg/week; n=42) and high dose (<math>\geq 28</math> mg/week; n=21) acenocoumarol. There was no correction for co-medication.</p> <p>- The VKORC1-1639A allele occurs in 90.5% of the low dose group, 76.2% of the medium dose group and 28.6% of the high dose group (S) - The presence of the A allele increases the chances of needing a low dose: OR = 9.38 (S). This effect is primarily high for AA: OR = 44.2 (S) - The presence of the A allele reduces the chances of needing a high dose: OR = 0.04 (S) - CYP2C9 polymorphisms potentiate the effect of the VKORC1 polymorphism on the required dose.</p>	<p>Authors' conclusion: “The A allele of the c.-1639G &gt; A polymorphism of VKORC1 is therefore associated with a low-dose requirement for acenocoumarol in patients receiving anticoagulant therapy.”</p>
<p><b>ref. 17</b> Reitsma PH et al. A C1173T dimorphism in the VKORC1 gene determines coumarin sensitivity and bleeding risk. PLoS Med 2005;2:e312.</p>	<p>3  GA: A AA: A</p>	<p>Case-control study including 110 patients with a history of bleeding on vitamin K antagonist therapy and 220 patients with no history of bleeding. 61 cases (22x GG, 26x GA, 13x AA) and 135 controls (55x GG, 57x GA, 23x AA) using acenocoumarol. Co-medication was not known.</p> <p>- risk of bleeding (major bleeding) versus GG: - GA: OR = 1.1 (NS) - AA: OR = 1.4 (NS) - GA+AA: OR = 1.2 (NS) - GA+AA (calculation including all 121 GA+AA controls): OR = 1.4 (NS) - mean dose required to achieve a certain INR: - GG: 3.2 mg/day - GA: 2.3 mg/day (S by 28%) - AA: 1.7 mg/day (S by 47%)</p> <p>NOTE: Genotyping was for the polymorphism 1173C&gt;T.</p>	<p>Authors' conclusion: “These findings encourage taking further steps towards the evaluation of the use of VKORC1 genetic testing for bleeding prevention in individuals who receive VKA therapy.”</p> <p><b>Maintenance dose versus GG:</b> GA: 72% (S) AA: 53% (S)</p>
<p><b>ref. 18</b> Bodin L et al. Cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKORC1) genotypes as determinants of acenocoumarol</p>	<p>3  GA: A AA: A</p>	<p>222 healthy, white volunteers (72x GG, 110x GA, 40x AA) received a single dose of 4 mg acenocoumarol. INR was measured after 24 hours.</p> <p>The % change in INR following a single dose: - GG: 12% - GA: 21% (S by 75%) - AA: 42% (S by 250%)</p>	

sensitivity. Blood 2005;106:135-140.			
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Risk group	use of CYP2C9 inhibitors, CYP2C9 polymorphisms.
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### Comments:

- Articles relating to VKORC1 gene variations that led to acenocoumarol resistance were not included, because the prevalence of these VKORC1 gene variations is very low.  
From 2007, articles were only included if they showed a clinical effect or an effect of separate VKORC1 phenotypes on dose or kinetics, because articles that only showed that VKORC1 has an effect on kinetics or dose did not supply new information.  
From 2011, articles investigating the effect on dose or kinetics were only included if they involved at least 500 patients. Clinical studies were only included if they involved more than 200 patients and bleeding and/or INR > 4 of if they provided new information on such studies. The other articles supplied insufficient new information.
- Dose algorithms:
  - o Roco A et al. A pharmacogenetically guided acenocoumarol dosing algorithm for Chilean patients: a discovery cohort study. *Front Pharmacol* 2020 6;11:325. PMID: 32327994.  
An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 304 Chilean patients with an INR target range of 2.0-3.0. Of the patients, 90.2% was White and 9.8% Amerindian. The allele frequencies of VKORC1 -1639G>A, CYP2C9\*2 and CYP2C9 \*3 were 0.467, 0.081, and 0.041, respectively. CYP2C9 \*3 was not in Hardy-Weinberg equilibrium. The algorithm explained 50% of the variation in dose requirement, while an algorithm without pharmacogenetic parameters explained 19%.  
The algorithm was not validated in an independent cohort.  
The algorithm found was:  
Log weekly dose (mg) = 3.081 + 0.167 (if male) - 0.0081\*age (in years) - 0.055\*(initial INR) + 0.013\*BMI - 0.107 (if CYP2C9\*1/\*2) - 0.323 (if CYP2C9\*1/\*3) - 0.746 (if CYP2C9\*3/\*3) - 0.270 (if VKORC1 G/A) - 0.701 (if VKORC1 A/A).  
BMI = body mass index.
  - o Maagdenberg H et al. The pediatric acenocoumarol dosing algorithm: The Children Anticoagulation and Pharmacogenetics Study. *J Thromb Haemost* 2018;16:1732-42. PubMed PMID: 29935043.  
An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 80 Dutch children with a median age of 9.7 years. The algorithm explained 61.8% of the variation in dose requirement, while an algorithm without pharmacogenetic parameters explained 45.0%. VKORC1 was responsible for 19.2% of the variation in dose requirement, while CYP2C19 explained 4.4% and CYP2C9 3.9% of the variation. For VKORC1AA, the dose calculated with the algorithm was in between the dose calculated with the current guideline of the Dutch Federation of anti-coagulation clinics and the stable dose achieved during acenocoumarol treatment, with the latter two differing significantly from each other. In the current guideline dosing is only based on age group and weight. The algorithm overestimated the dose for obese patients with a BMI of more than 30.  
The algorithm was not validated in an independent cohort.  
The algorithm found was:  
Log daily dose (mg) = 0.105 + 0.316\*BSA (m<sup>2</sup>) - 0.102\*(Fontan circulation, yes=1; no=0) - 0.120\*(number of VKORC1 variant alleles) - 0.084\*(number of CYP2C18 variant alleles) - 0.090\*(number of CYP2C9 \*2 and \*3 variant alleles).  
BSA = body surface area.
  - o Elkhazraji A et al. Effect of CYP2C9, VKORC1, CYP4F2, and GGCX gene variants and patient characteristics on acenocoumarol maintenance dose: Proposal for a dosing algorithm for Moroccan patients. *Drug Discov Ther* 2018;12:68-76. PubMed PMID: 29760340.  
An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 217 Moroccan patients. The algorithm explained 35.9% of the variation in dose requirement, while an algorithm with only the pharmacogenetic parameters explained 33.7% of the variation. The linkage disequilibrium between the -1639 G>A and the 1173 C>T polymorphism was less than 100% in this Moroccan population.  
The algorithm was not validated in an independent cohort.  
The algorithm found was:

Log weekly dose = 1.925 - 0.108\*(VKORC1 1639 G>A) - 0.073\*(VKORC1 1173 C>T) - 0.093\*(CYP2C9 haplotype) - 0.003\*age (in years)

VKORC1 -1639 G>A: value 1 for GG; 2 for GA and 3 for AA.

VKORC1 1173C>T: value 1 for CC; 2 for CT and 3 for TT.

CYP2C9 haplotype: value 1 for \*1/\*1; 2 for \*1/\*2 or \*1/\*3 and 3 for \*2/\*2 or \*2/\*3.

- Ajmi M et al. Influence of genetic and non-genetic factors on acenocoumarol maintenance dose requirement in a Tunisian population. *Eur J Clin Pharmacol* 2018;74:711-722. PubMed PMID: 29479633.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 197 Tunisian patients. The validation cohort consisted of 49 patients. The algorithm explained 48.1% of the variation in dose requirement in the generation cohort. VKORC1 was responsible for 17.2% of the variation in dose requirement, while CYP2C9 explained 5% of the variation in dose requirement. The mean initial dose chosen by the clinician differed significantly from the mean maintenance dose, whereas the mean dose calculated with the algorithm did not.

The algorithm found was:

Mean maintenance dose (mg/day) = 3.680 - 0.036\*age (years) + 0.014\* weight (kg) + 0.633 (if anti-biotics used) - 0.428\*(number of CYP2C9\*3 variant allele(s)) + 0.437\*(number of VKORC1\*3 variant allele(s)) + 0.507\*(number of VKORC1\*4 variant allele(s)) - 0.711\*(number of VKORC1 -1639G>A variant allele(s)) + 0.634\*(number of CALU variant allele(s)) + (0.582 × number of CYP4F2 variant allele(s)).

NOTE: The polymorphism 1173C>T was determined in this study.

NOTE: VKORC1 \*3 and \*4 are both -1639 G alleles. Spreafico 2008 found \*3 and \*4 to be the most important -1639 G alleles (with \*1 having a low frequency) and found no difference between these alleles in the effect on acenocoumarol treatment.

- Ragia G et al. A novel acenocoumarol pharmacogenomic dosing algorithm for the Greek population of EU-PACT trial. *Pharmacogenomics* 2017;18:23-34. PubMed PMID: 27967328.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 140 Greek patients, who reached acenocoumarol stable dose in the EU-PACT trial (Verhoef 2013). The algorithm was computationally validated in the same cohort (by testing it on randomly selected groups of 70 patients from this cohort). The algorithm explained 53% of the variation in dose requirement. CYP2C9 was responsible for 3.8% of the variation in dose requirement, while VKORC1 explained 31.3% of the variation in dose requirement.

The algorithm found was:

Log10 (Dose) = 0.555 - 0.034\*CYP2C9 - 0.160\*VKORC1 - 0.004\*age [years] + 0.004\*weight [kg], CYP2C9 genotype is 1 for CYP2C9\*1/\*1, 2 for CYP2C9\*1/\*2, 3 for CYP2C9\*1/\*3, 4 for CYP2C9\*2/\*2 and 5 for CYP2C9\*2/\*3. VKORC1 genotype is 1 for GG, 2 for GA and 3 for AA.

- Tong HY et al. A new pharmacogenetic algorithm to predict the most appropriate dosage of acenocoumarol for stable anticoagulation in a mixed Spanish population. *PLoS One* 2016; 11:e0150456. PubMed PMID: 26977927.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 554 Spanish patients. The validation cohort consisted of 128 patients. The algorithm explained 52.8% of the variation in dose requirement in the generation cohort and 64% in the validation cohort. CYP2C9 was responsible for 14.3% of the variation in dose requirement, while VKORC1 explained 22.9% of the variation in dose requirement.

The algorithm found was:

Ln (mean weekly acenocoumarol dose) = 3.181 - 0.010\*age (years) + 0.005\*weight (kg) + 0.070 (if enzyme inducer is used) - 0.337 (if amiodarone is used) - 0.111 (if CYP2C9\*1/\*2) - 0.323 (if CYP2C9\*1/\*3) - 0.691 (if CYP2C9 \*2/\*2 or \*2/\*3 or \*3/\*3) - 0.302 (if VKORC1 GA) - 0.727 (if VKORC1 AA) + 0.214 (if CYP4F2 MM) + 0.086 (if INR target is 2.5-3.5).

- Krishna-Kumar D et al. An acenocoumarol dosing algorithm exploiting clinical and genetic factors in South Indian (Dravidian) population. *Eur J Clin Pharmacol* 2015;71:173-81.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 217 patients. The algorithm was not validated in an independent dataset. The algorithm explained 61.8% of the variation in dose requirement, with 29.2% being explained by VKORC1. The greatest proportion (28.6%) was explained by the VKORC1 polymorphism 1639G>A.

The algorithm found was:

Log10 dose = 0.436-0.004(age)+0.018(BMI)-0.239(VKORC1 -1639A)-0.163(CYP2C9\*2)-0.293(CYP2C9\*3)+0.043(CYP4F2)-0.142(GGCX)+0.057(VKORC1 rs7294)

- Pop TR et al. An acenocoumarol dose algorithm based on a South-Eastern European population. *Eur J Clin Pharmacol* 2013;69:1901-7.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 200 patients. The validation cohort consisted of 101 patients. The algorithm explained 41.1% of the variation in dose requirement in the group of 200 patients and explained 32.8% of the variation in

the validation cohort. VKORC1 was responsible for 17.6% of the variation in the group of 200 patients.

The algorithm found was:

$D = 1.402 - [0.005 * \text{age}(\text{years})] + (0.009 * \text{BMI} - 0.094 \text{ if CYP2C9}^*2 \text{ allele was present}) - (0.099 \text{ if CYP2C9}^*3 \text{ allele was present}) - (0.135 \text{ if VKORC1 GA genotype was present}) - (0.285 \text{ if VKORC1 AA genotype was present})$ .

- Wolkanin-Bartnik J et al. Impact of genetic and clinical factors on dose requirements and quality of anticoagulation therapy in Polish patients receiving acenocoumarol: dosing calculation algorithm. *Pharmacogenet Genomics* 2013; 23: 611-8.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 226 patients. The validation cohort consisted of 50 patients. The algorithm explained 49% of the variation in the dose requirement. In the validation cohort, the algorithm correctly predicted the dose for 70% of the patients. VKORC1 was responsible for 20.5% of the variation.

The algorithm found was:

$\text{Exp} [1.79468 - 0.01373 \text{ age (years)} + 0.00422 - \text{weight (kg)} + 0.00030589 - \text{vitamin K (mcg/day)} - 0.35744 \text{ if VKORC1 AG,} - 0.66085 \text{ if VKORC1 AA,} - 0.14129 \text{ if CYP2C9 non}^*1/^*1, - 0.21131 \text{ if CrCl} < 40 \text{ (mL/min)}]$ .

- Cerezo-Manchado JJ et al. Creating a genotype-based dosing algorithm for acenocoumarol steady dose. *Thrombosis and haemostasis* 2013; 109: 146-53.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 973 patients. The validation cohort consisted of 2,683 patients. The algorithm explained 48% of the variation in dose requirement, with 23% being explained by VKORC1.

The algorithm found was:

$\sqrt{\text{weekly acenocoumarol dose}} = A + (-ay^2 - by + c) * (dz^2 + ez + f) + [\text{VKORC1 GG or GA or AA}] + [\text{CYP4F2 TT or CT or CC}] + [\text{CYP2C9 11 or 12 or 13 or 22 or 23 or 33}]$ . y = age, z =  $\sqrt{\text{height in cm} * (\text{weight in kg}) / 3600}$

- Rathore SS et al. Therapeutic dosing of acenocoumarol: proposal of a population specific pharmacogenetic dosing algorithm and its validation in North Indians. *PloS ONE* 2012; 7:e37844. An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 125 North Indian patients with an INR target of 2.0-3.5. The algorithm was validated in a cohort of 100 patients. The algorithm explained 41.4% of the variation in dose requirement, with 21% being explained by VKORC1.

The algorithm found was:

$\text{Dose (mg/day)} = 3.082 - 0.013(\text{smoking status, 1 for smoker and 0 for non-smoker}) - 0.433(\text{gender, 1 for male and 0 for female}) - 0.004(\text{age in years}) + \text{indication (0.327 for DVR and -0.092 for AVR)} + 0.026(\text{height in centimetres}) + 0.151 (\text{weight in kilograms}) - 7.660(\text{body surface area in cm}^2) - 0.862(\text{VKORC1 GA}) - 2.257(\text{VKORC1 AA}) - 0.049(\text{CYP2C9}^*2 \text{ CT}) - 0.456(\text{CYP2C9}^*3 \text{ AC}) + 0.449(\text{CYP4F2 GA}) + 0.230(\text{CYP4F2 AA}) + 0.245 (\text{GGCX CG}) + 1.055 (\text{GGCX GG})$

- van Schie RM et al. Loading and maintenance dose algorithms for phenprocoumon and acenocoumarol using patient characteristics and pharmacogenetic data. *Eur Heart J* 2011; 32:1909–1917.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 375 acenocoumarol users with an INR target of 2.0-3.5. The algorithm was validated in an independent dataset including 168 acenocoumarol users, whose height and weight parameters were not known. As the half-life for acenocoumarol is short, a separate loading dose was not required. The loading dose can therefore be calculated by dividing 3 times the calculated maintenance dose per day over the first 3 days of treatment. The algorithm explained 52.6% of the variation in dose requirement, with the VKORC1 polymorphism explaining 27.2% of the variation. The mean absolute error in the calculated maintenance dose was 0.52 mg/day. These numbers were 49.0% and 0.57 mg/day respectively for the validation set. A randomised controlled trial is needed to test whether the use of this algorithm leads to improvement of control and safety of acenocoumarol therapy.

The algorithm found was:

$\sqrt{\text{(mean maintenance dose (mg/week))}} = 4.117 - 0 \text{ (if CYP2C9}^*1/^*1) - 0.093 \text{ (if CYP2C9}^*1/^*2) - 0.519 \text{ (if CYP2C9}^*1/^*3) - 0.435 \text{ (if CYP2C9}^*2/^*2) - 0.466 \text{ (if CYP2C9}^*2/^*3) - 1.375 \text{ (if CYP2C9}^*3/^*3) - 0 \text{ (if VKORC1 GG)} - 0.572 \text{ (if VKORC1 GA)} - 1.267 \text{ (if VKORC1 AA)} - 0.027 * \text{age (years)} + 0.271 \text{ (if female)} + 0.009 * \text{height (cm)} + 0.010 * \text{weight (kg)} - 0.377 \text{ (if amiodarone user)}$

NOTE: The polymorphism 1173C>T was determined in this study.

- Verde Z et al. A novel, single algorithm approach to predict acenocoumarol dose based on CYP2C9 and VKORC1 allele variants. *PLoS One* 2010; 5:e11210.

A single algorithm to predict which patients would require a high or low dose of acenocoumarol was developed on the basis of data from 193 acenocoumarol users with an INR target of 3.0-4.0 or 2.0-

3.0 The algorithm was not validated in an independent dataset. The algorithm consists of a single number (the acenocoumarol dose genotype score (AGS)), which is obtained by adding up the number of wild-type alleles of 5 polymorphisms (CYP2C9\*2, CYP2C9\*3, VKORC1 -1639G>A, VKORC1 497T>G and VKORC1 1173C>T) and expressing this number as a percentage of the maximum score. NOTE: as the authors did not take into consideration that VKORC1 -1639G>A and VKORC1 1173C>T are linked, they unknowingly included the greater effect of this polymorphism in their algorithm.

The mean AGS was significantly higher in the group with a high dose (> 28 mg/week) than in the group with a low dose (< 7 mg/week). Patients with an AGS > 70 had an increased chance of requiring a high dose (OR = 3.347; 95% CI = 1.112-10.075). Patients with an AGS ≤ 60 had an increased chance of requiring a low dose (OR = 2.356; 95% CI = 1.094-5.073). The results were the same after correction for relevant co-medication.

- o Markatos CN et al. VKORC1 and CYP2C9 allelic variants influence acenocoumarol dose requirements in Greek patients. Pharmacogenomics 2008;9:1631-8.

An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 98 acenocoumarol users with an INR target of 2.0-3.0. The algorithm was not validated.

The algorithm found was:

$$\text{Log (dose (mg/day))} = 1.083 - 0.004 * \text{age (years)} - 0.188 * \text{VKORC1 genotype (1 for GG, 2 for GA, 3 for AA)} - 0.073 * \text{CYP2C9 genotype (1 for *1/*1, 2 for *1/*2, 3 for *1/*3, 4 for *2/*2, 5 for *2/*3)}$$

Date of literature search: 4 June 2025.

	Genotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	GA	4C	Yes	No	29 September 2025
	AA	4F	Yes	Yes	

#### Mechanism:

Vitamin K antagonists (VKAs) 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 production of the VKORC1 protein. As a consequence, lower doses of VKAs are required for inhibition of this protein. VKORC1 regenerates reduced vitamin K (vitamin K 2,3-epoxide) to the active oxidised form (vitamin K hydroquinone). Vitamin K is an essential co-factor for carboxylation of glutamic acid residues on coagulation factors II, VII, IX and X and the anticoagulation proteins C, S and Z. For this reason, inhibition of VKORC1 diminishes coagulation.

#### Clinical Implication Score:

Table 1: Definitions of the available Clinical Implication Scores

<b>Potentially beneficial</b>	PGx testing for this gene-drug pair is potentially beneficial. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the DPWG recommends adhering to the gene-drug guideline	0-2 +
<b>Beneficial</b>	PGx testing for this gene-drug pair is beneficial. It is advised to consider genotyping the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection	3-5 +
<b>Essential</b>	PGx testing for this gene-drug pair is essential for drug safety or efficacy. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection	6-10 +

Table 2: Criteria on which the attribution of Clinical Implication Score is based

Clinical Implication Score Criteria	Possible Score	Given Score
<b>Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)</b>		
• CTCAE Grade 3 or 4 (clinical effect score D or E)	+	
• CTCAE Grade 5 (clinical effect score F)	++	++
<b>Level of evidence supporting the associated clinical effect grade ≥ 3</b>		
• One study with level of evidence score ≥ 3	+	
• Two studies with level of evidence score ≥ 3	++	
• Three or more studies with level of evidence score ≥ 3	+++	+++
<b>Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3</b>		

<ul style="list-style-type: none"> <li>• 100 &lt; NNG ≤ 1000</li> <li>• 10 &lt; NNG ≤ 100</li> <li>• NNG ≤ 10</li> </ul>	<ul style="list-style-type: none"> <li>+</li> <li>++</li> <li>+++</li> </ul>	
<b>PGx information in the Summary of Product Characteristics (SmPC)</b> <ul style="list-style-type: none"> <li>• At least one genotype/phenotype mentioned</li> </ul> OR <ul style="list-style-type: none"> <li>• Recommendation to genotype</li> </ul> OR <ul style="list-style-type: none"> <li>• At least one genotype/phenotype mentioned as a contra-indication in the corresponding section</li> </ul>	<ul style="list-style-type: none"> <li>+</li> <li>++</li> <li>++</li> </ul>	
<b>Total Score:</b>	10+	5+
<b>Corresponding Clinical Implication Score:</b>		Beneficial