VKORC1: acenocoumarol 1909/1910

AA = homozygous allele variant (= -1639 AA = 1173 TT) (strongly increased coumarin sensitivity), CI = confidence interval, Cl_{or} = oral clearance, GA = heterozygous (= -1639 GA = 1173 CT) (increased coumarin sensitivity), GG = homozygous wild type allele (= -1639 GG = 1173 CC) (normal coumarin sensitivity), HR = hazard ratio, INR = international normalised ratio, MR = metabolic ratio, NS = non-significant, OR = odds ratio, S = significant, SmPC = Summary of Product Characteristics, VKORC1 = vitamin K epoxide reductase complex 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:
Acenocoumarol reduces blood clotting by inhibition of VKORC1 enzyme activity. Mutations in the VKORC1 gene may lead to reduced production of the VKORC1 protein. Lower acenocoumarol doses are then needed to achieve the desired INR.
AA has an obvious effect on the maintenance dose. One study also found an increased risk of bleeding (significance for the trend GG-GA-AA). Two studies found an increased risk of bleeding for GA + AA. Two Dutch studies found that the risk of INR > 6 increased significantly. One study showed no significant difference in clinical effect for all genotypes combined when using a pharmacogenetic dose algorithm for the first 5-7 days. A later study showed this to be also true for patients with two or more VKORC1 and/or CYP2C9 variants. 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. Moreover, GA is the most common genotype among European Caucasians, 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. This is why a decision was made 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 clinical and kinetic effects per genotype in the background information text of the gene-drug interactions on 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: The studies by 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.
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, it was 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), a decision was made 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 is a decrease to 49% of the maintenance dose for GG (ranging from 37-60%, median 50%, in different studies). This was translated to a recommendation to reduce the initial dose to 50%, to be more achievable in clinical practice. A decision was also made to recommend additional monitoring at hospitals, as patients are initiated on anticoagulant therapy by residents or internists.

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. Four

studies found no increased risk of bleeding for GA or GA + AA.
The studies by Spreafico 2008, Teichert 2009 and Verhoef 2012 found no increased risk of INR > 6.

Recommendation concerning pre-emptive genotyping, including justification of choices:
The Dutch Pharmacogenetics Working Group considers genotyping before starting acenocoumarol to be to be beneficial for drug safety. It is advised to genotype the patient before (or directly after) drug therapy has been initiated to guide drug and 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). 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 and a Spanish study on gastro-intestinal bleeding. 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). 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 ≥ 3 (3 points for three or more publications with level of evidence score ≥ 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 ≥ 3 (only points for NNG ≤ 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
ref. 1 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.	3	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 < 75 years of age and 47 patients ≥ 75 years of age) and 165 received control treatment (103 patients < 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 < 75 years of age and 31 patients ≥ 75 years of age) and 126 in the control group (77 patients < 75 years of age and 49 patients ≥ 75 years of age). Of the patients < 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 treat-	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

<table>
<tr>
<td>ref. 1, continuation</td>
<td>genotype-guided versus not genotype-guided therapy : AA</td>
<td>ment.
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.

Genotyping:
- 116x GG
- 144x GA
- 64x AA
- 1x genotype unknown (clinical algorithm, ≥ 75 years)

Results:
<table>
<tr><td colspan="4">Genotype-based algorithm versus clinical algorithm:</td></tr>
<tr><td></td><td></td><td></td><td>value for the clinical algorithm</td></tr>
<tr><td rowspan="14">% of time in the therapeutic range</td><td>< 75 years, no CYP2C9 and VKORC1 variants</td><td>NS</td><td>58.9%</td></tr>
<tr><td>< 75 years, one CYP2C9 or VKORC1 variant</td><td>NS</td><td>65.2%</td></tr>
<tr><td>< 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>< 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="2">A per-protocol analysis showed similar results.</td><td></td></tr>
<tr><td>< 75 years, Dutch</td><td>NS</td><td>58.5%</td></tr>
<tr><td>≥ 75 years, Dutch</td><td>NS</td><td>58.9%</td></tr>
<tr><td>< 75 years, Greek</td><td>NS</td><td>65.3%</td></tr>
<tr><td>≥ 75 years, Greek</td><td>NS</td><td>63.0%</td></tr>
<tr><td rowspan="4">% of time with a suprathe-rapeutic INR (> 3.0)</td><td>< 75 years, no CYP2C9 and VKORC1 variants</td><td>NS</td><td>10.7%</td></tr>
<tr><td>< 75 years, one CYP2C9 or VKORC1 variant</td><td>NS</td><td>16.2%</td></tr>
<tr><td>< 75 years, two or more CYP2C9 and/or VKORC1 variants</td><td>NS</td><td>23.8%</td></tr>
<tr><td>≥ 75 years, no CYP2C9 and VKORC1 variants</td><td>NS</td><td>7.4%</td></tr>
</table>
</td>
<td>among older patients.”</td>
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<tr><td>ref. 1, continuation</td><td></td><td>
<table>
<tr><td></td><td>≥ 75 years, one CYP2C9 or VKORC1 variant</td><td>NS</td><td>21.2%</td></tr>
<tr><td></td><td>≥ 75 years, two or more CYP2C9 and/or VKORC1 variants</td><td>NS</td><td>16.2%</td></tr>
<tr><td></td><td>< 75 years</td><td>NS</td><td>18.8%</td></tr>
<tr><td></td><td>≥ 75 years</td><td>NS</td><td>15.9%</td></tr>
<tr><td></td><td colspan="2">A per-protocol analysis showed similar results.</td><td></td></tr>
<tr><td></td><td>< 75 years, Dutch</td><td>NS</td><td>22.0%</td></tr>
<tr><td></td><td>≥ 75 years, Dutch</td><td>NS</td><td>20.8%</td></tr>
<tr><td></td><td>< 75 years, Greek</td><td>trend for a decrease, p = 0.09 (NS)</td><td>14.1%</td></tr>
<tr><td></td><td>≥ 75 years, Greek</td><td>- 7.7% (S)</td><td>13.8%</td></tr>
<tr><td>% of time with a subthera-peutic INR (< 2.0)</td><td>< 75 years, no CYP2C9 and VKORC1 variants</td><td>NS</td><td>30.4%</td></tr>
<tr><td></td><td>< 75 years, one CYP2C9 or VKORC1 variant</td><td>NS</td><td>18.6%</td></tr>
<tr><td></td><td>< 75 years, two or more CYP2C9 and/or VKORC1 variants</td><td>NS</td><td>16.6%</td></tr>
<tr><td></td><td>≥ 75 years, no CYP2C9 and VKORC1 variants</td><td>NS</td><td>35.1%</td></tr>
<tr><td></td><td>≥ 75 years, one CYP2C9 or VKORC1 variant</td><td>trend for an increase, p = 0.06 (NS)</td><td>18.0%</td></tr>
<tr><td></td><td>≥ 75 years, two or more CYP2C9 and/or VKORC1 variants</td><td>trend for an increase, p = 0.08 (NS)</td><td>17.1%</td></tr>
<tr><td></td><td>< 75 years</td><td>NS</td><td>19.9%</td></tr>
<tr><td></td><td>≥ 75 years</td><td>+ 9.9% (S)</td><td>22.4%</td></tr>
<tr><td></td><td colspan="2">A per-protocol analysis showed similar results.</td><td></td></tr>
<tr><td></td><td>< 75 years, Dutch</td><td>NS</td><td>19.4%</td></tr>
<tr><td></td><td>≥ 75 years, Dutch</td><td>NS</td><td>20.4%</td></tr>
<tr><td></td><td>< 75 years, Greek</td><td>NS</td><td>20.6%</td></tr>
<tr><td></td><td>≥ 75 years, Greek</td><td>+ 11.5% (S)</td><td>23.3%</td></tr>
</table>

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.
</td><td></td></tr>
<tr><td>ref. 2
Cerezo-Manchado JJ et al.
Effect of VKORC1, CYP2C9 and CYP-4F2 genetic variants in early outcomes</td><td>3</td><td>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.

Genotyping:
- 320x GG</td><td>Authors' conclusion:
'Over-anticoagulation: international normalized ratio [INR] >2.5 at 72 h was the strongest</td></tr>
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during acenocou-
marol treatment.
Pharmacogenomics
2014;15:987-96.
PMID: 24956252.
 	
ref. 2, continuation
	
	GA: A
	AA: A
		- 465x GA
		- 156x AA
		
		Results versus GG:
		- maintenance dose:
		  - GA: decrease by 24% from 17 mg to 13 mg/week (S for the trend GG, GA, AA)
		  - AA: decrease by 47% from 17 mg to 9 mg/week (S for the trend GG, GA, AA)
		- percentage of patients with an INR > 4:
		  - GA: increase with HR 1.96 (95% CI: 1.44-2.67) (S)
		  - AA: increase with HR: 4.18 (95% CI: 3.08-5.98) (S)
		- time to stable dose:
		  - GA: median increase (HR: 1.104; 95% CI: 1.018-1.197) (S for the trend GG, GA, AA)
		  - 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)
		- INR > 2.5 after 72 hours of treatment:
		  - GA: increase (HR: 2.19; 95% CI: 1.811-2.654) (S for the trend GG, GA, AA)
		  - AA: increase (HR: 2.19; 95% CI: 1.811 - 2.654) (S for the trend GG, GA, AA)
			factor affecting INR >4, although VKORC1 and CYP2C9 genotypes also independently led to the same outcome.'
			
			Maintenance dose versus GG:
			GA: 76%
			AA: 53%

ref. 3 Verhoef TI et al. A randomized trial of genotype-guided dosing of acenocou-marol and phenpro-coumon. NEJM 2013;369:2304-12. PMID:24251360. Baranova EV et al. Dosing algorithms for vitamin K anta-	3 geno-type-guided versus not ge-notype-guided therapy : AA	Patients without prior exposure to coumarin therapy were treated with acenocoumarol for 12 weeks. The dose adminis-tered 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. Genotyping: - 125x GG - 177x GA - 75x AA Genotype-based algorithm versus clinical algorithm: - the time that the INR was in the therapeutic range through-out the treatment did not increase (NS) - 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 ≥ 4, the percentage of the time with an INR ≥ 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 differen-ces 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:	Authors' conclu-sion: 'Genotype-guided dosing of aceno-coumarol or phen-procoumon did not improve the per-centage of time in the therapeutic range during the 12 weeks after the initiation of thera-py.' Authors' conclu-sion: 'Four weeks after

gonists across VKORC1 and CYP-2C9 genotypes. J Thromb Haemost 2017;15:465-472. PubMed PMID: 28063245. **ref. 3, continuation**		Genotype-based algorithm versus clinical algorithm:			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–CYP-2C9 genetic subgroups. EU-PACT genetic-guided dose initiation algorithms for acenocoumarol and phenprocoumon could have predicted the dose overcautiously in the VKORC1 AA–CYP2C9*1*1 subgroup. Adjustment of the genotype-guided algorithm could lead to a higher benefit of genotyping.’
			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
			no CYP2C9 variants and one VKORC1 variant	NS	NS
			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	no CYP2C9	- 20.29% (S,	trend for a

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<table>
<tr><td rowspan="6">time with a sub-therapeutic INR (< 2.0)</td><td>and VKORC1 variants</td><td>before and after Bonferroni correction)</td><td>decrease, p = 0.083 (NS)</td></tr>
<tr><td>one or more CYP2C9 variants and no VKORC1 variant</td><td>NS</td><td>NS</td></tr>
<tr><td>no CYP2C9 variants and one VKORC1 variant</td><td>NS</td><td>trend for an increase, p = 0.081 (NS)</td></tr>
<tr><td>one or more CYP2C9 variants and one VKORC1 variant</td><td>NS</td><td>NS</td></tr>
<tr><td>no CYP2C9 variants and two VKORC1 variants</td><td>+ 19.89% (S, before and after Bonferroni correction)</td><td>+ 12.99% (S, but NS after Bonferroni correction)</td></tr>
<tr><td>one or more CYP2C9 variants and two VKORC1 variants</td><td>trend for an increase, p = 0.075 (NS)</td><td>NS</td></tr>
<tr><td colspan="4">Results were similar after sensitivity analysis for both coumarins separately and in the per-protocol dataset.</td></tr>
</table>
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<tr>
<td>ref. 4
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.</td>
<td>3

GA: C

AA: D</td>
<td>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
- 211x AA

GA versus GG:
- no difference in the percentage of patients with INR > 6 during the entire treatment time (NS)
- factor 0.85 decrease in the percentage of patients with INR < 2 during the first month (from 73% to 62%) (S)
- factor 0.82 decrease in the percentage of patients with INR < 2 during the second and third month (from 49% to 40%) (S)
- no difference in the percentage of patients with INR < 2 during the remainder of the treatment time (NS)
- factor 1.5 increase in the percentage of patients with INR > 3.5 during the first month (from 30% to 45%) (S)
- no difference in the risk INR > 3.5 during the remainder of the treatment time (NS)

AA versus GG:
- factor 4 increase in the percentage of patients with INR > 6 during the first month (from 3% to 12%) (S)</td>
<td>Authors’ conclusion:
“Patients with polymorphisms in CYP2C9 and VKORC1 had a higher risk of over-anticoagulation (up to 74%) and a lower risk of under-anticoagulation (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.”</td>
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ref. 4, continuation		- no difference in the risk of INR > 6 after the first month (NS) - factor 0.62 decrease in the percentage of patients with INR < 2 during the first month (from 73% to 45%) (S) - trend towards a factor 0.8 decrease in the percentage of patients with INR < 2 during the second and third month (from 49% to 39%) (NS; p = 0.05) - no difference in the percentage of patients with INR < 2 during the remainder of the treatment time (NS) - factor 2.5 increase in the percentage of patients with INR > 3.5 during the first month (from 30% to 74%) (S) - factor 1.6 increase in the percentage of patients with INR > 3.5 during the second and third month (from 40% to 62%) (S) - no difference in the percentage of patients with INR > 3.5 during the remainder of the treatment time (NS) NOTE: Genotyping was for the polymorphism 1173C>T.	
ref. 5 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.	3 GA: A AA: A	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. - maintenance dose versus GG: - GA: decrease by 35% from 26 mg to 17 mg/week (S) - AA: decrease by 50% from 26 mg to 13 mg/week (S) - 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. - time required to achieve first therapeutic INR after the start of the treatment (n=40): no difference between the genotypes (NS)	Authors' conclusion: "The reduction in weekly dose is driven by mainly VKORC1, followed by CYP2C9*3 variants." **Maintenance dose versus GG:** GA: 65% (S) AA: 50% (S)
ref. 6 Kovac MK et al. The c.-1639G>A polymorphism of the VKORC1 gene in Serbian population: retrospective study of the variability in response to oral anticoagulant therapy. Blood Coagul Fibrinolysis 2010;21:558-63.	3 GA: C AA: D	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 per week in week 2 and 1x per week upon achieving a therapeutic INR. Amiodarone was excluded, but other relevant co-medication was not. - maintenance dose versus GG: - GA: decrease by 27% from 26 mg to 19 mg/week (S for the trend) - AA: decrease by 62% from 26 mg to 10 mg/week (S) - % patients with INR > 4 during the first 3 months after starting treatment: - GG: 0% - GA: 7% (S for the trend) - AA: 47% (S for the trend) - on average, the INR was more elevated for AA than for GA (6.2 versus 4.9) (S) - % patients with bleeding episodes during the first 3 months after starting treatment: - GG: 0% - GA: 3% (S for the trend) - AA: 14% (S for the trend)	Authors' conclusion: "VKORC1 c.-1639 G>A polymorphism significantly influenced VKA dose and represented a good predictor of individuals predisposed to unstable anticoagulation. Pharmacogenetic testing could predict a high risk of overdose among 28.5% of our patients, carriers of AA genotype, before the initiation of anticoagulation." **Maintenance dose versus GG:** GA: 73% (S) AA: 38% (S)

ref. 6, continuation	GA: AA[#] AA: AA[#]	- The bleeding episodes were divided into 36.5% blood in the faeces, 36.5% bruises on the skin, 18% blood in the urine and 9% nosebleeds. Transfusion with fresh frozen plasma was required in 45% of the patients to stop the bleeding and reduce the INR to the therapeutic range. - % patients with INR > 4 after achieving the first stable anti-coagulation period: - GG: 8% - GA: 16% (NS) - AA: 54% (S) - % patients with INR < 2 after achieving the first stable anti-coagulation period: - GG: 40% - GA: 8% (S) - AA: 19% (S)	
ref. 7 Cadamuro J et al. Genetic determinants of acenocoumarol and phenprocoumon maintenance dose requirements. Eur J Clin Pharmacol 2010;66:253-60.	3 GA: A AA: A	80 patients (33x GG, 35x GA, 12x AA) on maintenance therapy with acenocoumarol. Relevant co-medication was taken by 24% of the patients. Maintenance dose (corrected for age, gender and last INR) versus GG: - GA: decrease by 25% from 21.56 mg to 16.08 mg/week (S for the trend) - AA: decrease by 51% from 21.56 mg to 10.36 mg/week (S for the trend) VKORC1 genotype is an independent variable for the maintenance dose (multivariable regression analysis). Age, gender, last INR and VKORC1 and CYP2C9 genotypes combined account for 58% of the variation in the maintenance dose. NOTE: Genotyping was for the polymorphism 1173C>T.	Authors' conclusion: "These results reveal that inter-individual variability in weekly acenocoumarol maintenance dose requirement is mainly dependent on the VKORC1 1173C>T and the CYP2C9*3 alleles." **Maintenance dose versus GG:** GA: 75% (S) AA: 48% (S)
ref. 8 Wijnen PA et al. Variant VKORC1 and CYP2C9 alleles in patients with diffuse alveolar hemorrhage caused by oral anticoagulants. Mol Diagn Ther 2010;14:23-30.	3 GA+AA: F	Case-control study involving 63 cases (diffuse alveolar bleeding) with acenocoumarol (n=61) or phenprocoumon (n=2) (loading dose 6-4-2-2 or 6-4-4-4 mg). The control group consisted of healthy volunteers. Co-medication that can affect coagulation was taken by 60% of the cases. A total of 59% of the cases died, primarily due to complications related to heart failure in combination with diffuse alveolar bleeding. Cases (patients) versus control group: - factor 1.15 increase in the percentage of patients with a variant allele (increase from 70.5% to 81.0%) (S) - factor 1.18 increase in the allele frequency of T (increase from 47.7% to 56.3%) (NS)	Authors' conclusion: "Genotyping of four SNPs for VKORC1 and CYP2C9 polymorphisms is useful in predicting a high probability of the occurrence of diffuse alveolar hemorrhage in patients receiving oral anticoagulants."
ref. 9 Stepien E et al. A vitamin K epoxide reductase-oxidase complex gene polymorphism (-1639 G>A) and interindividual variability in the dose-effect of vitamin K antagonists. J Appl Genet 2009;50:399-403.	4 GA: AA AA: A	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 coumarin 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 < 2.0. - maintenance dose versus GG: - GA: decrease by 26% from 6.8 mg to 5.0 mg/day (NS) - AA: decrease by 40% from 6.8 mg to 4.1 mg/day (S versus GG+GA)	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."

ref. 9, continuation		Multiple linear regression analysis demonstrated that the VKORC1 polymorphism is an independent variable for the acenocoumarol dose.	**Maintenance dose versus GG:** GA: 74% (S) AA: 60% (S)
ref. 10 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.	3 GA: A AA: D	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 CYP-2C9 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. GA versus GG: - increase in the INR on day 4 by 0.35 (S) - 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. - 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. - decrease in the weekly dose after 6 weeks by 5.09 mg/week (S) AA versus GG: - increase in the INR on day 4 by 0.66 (S) - 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. - increased risk of INR ≥ 6 during the first 6 weeks (OR = 2.46 (S)) - 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. - decrease in the weekly dose after 6 weeks by 10.2 mg per week (S) 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 explained by the VKORC1 genotype than by the CYP2C9 genotype (28% versus 5% respectively). NOTE: Genotyping was for the polymorphism 1173C>T.	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 over-anticoagulation in patients with variant alleles of the VKORC1 and CYP-2C9 genes." **Maintenance dose versus GG:** GA: 73% (S) AA: 49% (S)

ref. 11
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.
	3
	
	GA+AA: F
		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.
		
		- no increase in the risk of severe gastrointestinal bleeding for GA and AA (NS)
		- risk of bleeding versus GG with dose ≤ 15 mg/week:
		  - GG and > 15 mg: OR non-significantly increased
		  - GG+AA and ≤ 15 mg: OR non-significantly increased
		  - GA+AA and > 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.
			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. …

ref. 11, continuation	AA: F	- 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. - 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: - (no AA, no CYP2C9 polymorphism) with amiodarone: OR non-significantly increased - (AA and/or CYP2C9 polymorphism) without amiodarone: OR = 1.89 (95% CI: 1.08-6.26) - (AA and/or CYP2C9 polymorphism) with amiodarone: OR = 9.97 (95% CI: 1.75-56.89) - 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: - (no AA, no CYP2C9 polymorphism) with acetylsalicylic acid: OR non-significantly increased - (AA and/or CYP2C9 polymorphism) without acetylsalicylic acid: OR = 1.89 (95% CI: 1.08-3.31) - (AA and/or CYP2C9 polymorphism) with acetylsalicylic acid: OR = 8.97 (95% CI: 1.66-48.34)	Genotyping of these alterations may be advisable in those patients taking amiodarone or aspirin."
ref. 12 Markatos CN et al. VKORC1 and CYP2C9 allelic variants influence acenocoumarol dose requirements in Greek patients. Pharmacogenomics 2008;9:1631-8.	3 GA: A AA: A	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; Maintenance dose versus *1/*1: - GA: decrease by 19% from 3.54 mg to 2.85 mg/day (S for the trend) - AA: decrease by 63% from 3.54 mg to 1.3 mg/day (S for the trend) 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). NOTE: The authors' assumption that statins and triazole derivatives are CYP2C9 inhibitors is not entirely correct.	Authors' conclusion: "VKORC1-1639 G>A, CYP2C9*2 and CYP2C9*3 polymorphisms were found to predispose to acenocoumarol sensitivity in Greek." **Maintenance dose versus GG:** GA: 81% (S) AA: 37% (S)
ref. 13 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	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). GA versus GG (significance not determined): - increase in the INR on day 4 by 0.5 from 2.5 to 3.0 - increase in the incidence of INR ≥ 6 on day 4 by a factor 2.5 from 1.7% to 4.2% - decrease of the dose in week 7 by 13% from 20.7 to 18.0 mg/week AA versus GG:	Authors' conclusion: "Two copies of the VKORC1*2 haplotype were associated with a 45% dose reduction and an increased risk of over-anticoagulation."

ref. 13, continuation	AA: C	- increase in the INR on day 4 by 2.0 from 2.5 to 4.5 (S) - increase in the incidence of INR ≥ 6 on day 4 by a factor 13 from 1.7% to 22% (NS) - decrease of the dose in week 7 by 45% from 20.7 to 11.4 mg/week (S) CYP2C9 and VKORC1 independently affect the INR on day 4 and - together with age - account for 26% of the variability in this INR. A larger proportion of the difference in required dose was explained by the VKORC1 genotype than by the CYP2C9 genotype (12% versus 5% respectively). 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. NOTE: Genotyping was for the polymorphism 1173C>T.	
ref. 14 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	100 Caucasian men (< 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. GA+AA versus GG: - increased sensitivity to treatment during the first 3 days: median INR from 1.74 to 2.07 (S by 19%) - the required maintenance dose of acenocoumarol decreased from on average 19.5 to 15.8 mg/week (S by 19%) - increase in the percentage of patients with INR ≥ 3.5 after first 3 days from 2.7% to 12.7% (NS by 370%) NOTE: Genotyping was for the polymorphism 1173C>T.	Authors' conclusion: "Only VKORC1 genotype had significant impact on the efficacy of therapy."
ref. 15 Schalekamp T et al. VKORC1 and CYP2C9 genotypes and acenocoumarol anticoagulation status: interaction between both genotypes affects overanticoagulation. Clin Pharmacol Ther 2006;80:13-22.	4 GA: A AA: A	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. - risk of INR > 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) NOTE: Genotyping was for the polymorphism 1173C>T.	Authors' conclusion: "Being a carrier of a combination of polymorphisms of VKORC1 and CYP2C9, rather than of one of these polymorphisms, is associated with severe overanticoagulation. The time to achieve stability is mainly associated with the CYP2C9 genotype." **Maintenance dose versus GG:** GA: 80% (S) AA: 52% (S)
ref. 16 Montes R et al. The c.-1639G > A	3	The VKORC1-genotype was determined in 113 patients with stable anticoagulation on low dose (≤ 7 mg/week; n=42), medium dose (> 7 and < 28 mg/week; n=42) and high dose (≥	Authors' conclusion: "The A allele of the

polymorphism of the VKORC1 gene is a major determinant of the response to acenocoumarol in anticoagulated patients. Br J Haematol 2006;133:183-7. **ref. 16, continuation**	GA+AA : A AA: A	28 mg/week; n=21) acenocoumarol. There was no correction for co-medication. - 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.	c.-1639G > A polymorphism of VKORC1 is therefore associated with a low-dose requirement for acenocoumarol in patients receiving anticoagulant therapy."
ref. 17 Reitsma PH et al. A C1173T dimorphism in the VKORC1 gene determines coumarin sensitivity and bleeding risk. PLoS Med 2005;2:e312.	3 GA: A AA: A	Case-control study including 110 patients with a history of bleeding on coumarin 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. - 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%) NOTE: Genotyping was for the polymorphism 1173C>T.	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." **Maintenance dose versus GG:** GA: 72% (S) AA: 53% (S)
ref. 18 Bodin L et al. Cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKORC1) genotypes as determinants of acenocoumarol sensitivity. Blood 2005;106:135-140.	3 GA: A AA: A	222 healthy, white volunteers (72x GG, 110x GA, 40x AA) received a single dose of 4 mg acenocoumarol. INR was measured after 24 hours. The % change in INR following a single dose: - GG: 12% - GA: 21% (S by 75%) - AA: 42% (S by 250%)	

[#] In these cases, there was a significant difference between GG and GA or AA, but the clinical effect was more favourable for GA or AA than for GG. As the purpose of classification of the severity of the effect is to classify negative effects, code AA is used for a positive effect.

Risk group	use of CYP2C9 inhibitors, CYP2C9 polymorphisms.

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:
    - Maagdenberg H et al. The pediatric acenocoumarol dosing algorithm: The Children Anticoagulation and Pharmacogenetics Study. J Thromb Haemost 2018 Jun 23 [Epub ahead of print]. 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 anticoagulation 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^2) - 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.
    - 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 antibiotics 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 VKOR-C1 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 CYP-2C9*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*age(years)]+(0.009*BMI0-0.094 if CYP2C9*2 allele was present)-(0.099 if CYP2C9*3 allele was present)-(0.135 if VKORC1 GA genotype was present)-(0.285 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:
  Exp [1.79468 – 0.01373 age (years) + 0.00422 - weight (kg) + 0.00030589 - vitamin K (mcg/day) – 0.35744 if VKORC1 AG, – 0.66085 if VKORC1 AA, – 0.14129 if CYP2C9 non*1/*1, – 0.21131 if CrCl < 40 (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:
  $\sqrt{\text{weekly acenocoumarol dose}}=A+(-ay^2-by+c)*(dz^2+ez+f)+$[VKORC1 GG or GA or AA] + [CYP4F2 TT or CT or CC] + [CYP2C9 11 or 12 or 13 or 22 or 23 or 33]. y = age, z = $\sqrt{\text{height in cm}}$*(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:
Dose (mg/day) = 3.082-0.013(smoking status, 1 for smoker and 0 for non-smoker)-0.433(gender, 1 for male and 0 for female)–0.004(age in years) + indication (0.327 for DVR and -0.092 for AVR)+0.026(height in centimetres)+0.151 (weight in kilograms)-7.660(body surface area in cm^2)-0.862(VKORC1 GA)-2.257(VKORC1 AA)-0.049(CYP2C9*2 CT)-0.456(CYP2C9*3 AC)+0.449(CYP4F2 GA)+0.230(CYP4F2 AA)+0.245 (GGCX CG)+1.055 (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:
  √ (mean maintenance dose (mg/week)) = 4.117 – 0 (if CYP2C9*1/*1) – 0.093 (if CYP2C9*1/*2) – 0.519 (if CYP2C9*1/*3) – 0.435 (if CYP2C9*2/*2) – 0.466 (if CYP2C9*2/*3) – 1.375 (if CYP2C9*3/*3) – 0 (if VKORC1 GG) – 0.572 (if VKORC1 GA) – 1.267 (if VKORC1 AA) – 0.027 * age (years) + 0.271 (if female) + 0.009 * height (cm) + 0.010 * weight (kg) – 0.377 (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.
- 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:
  Log (dose (mg/day)) = 1.083 – 0.004 * age (years) – 0.188 * VKORC1 genotype (1 for GG, 2 for GA, 3 for AA) – 0.073 * 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: 12 July 2018.

	Genotype	Code	Gene-drug interaction	Action	Date
Dutch Pharmacogenetic Working Group decision	GA	4C	Yes	No	10 September 2018
	AA	4F	Yes	Yes	

Mechanism:

Coumarins exert their effect by inhibition of enzyme activity of the vitamin K 2,3-epoxide reductase complex subunit 1 (VKORC1). Mutations in the VKORC1 gene may lead to reduced production of the VKORC1 protein. This requires a lower coumarin dose 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. Inhibition of VKORC1 therefore results in reduced coagulation.

Clinical Implication Score:

Table 1: Definitions of the available Clinical Implication Scores

Potentially beneficial	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 +
Beneficial	PGx testing for this gene-drug pair is beneficial. It is advised to genotype the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection	3-5 +
Essential	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**
Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)		
• CTCAE Grade 3 or 4 (clinical effect score D or E)	+	
• CTCAE Grade 5 (clinical effect score F)	++	++
Level of evidence supporting the associated clinical effect grade ≥ 3		
• 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	+++	+++
Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3		
• 100 < NNG ≤ 1000	+	
• 10 < NNG ≤ 100	++	
• NNG ≤ 10	+++	
PGx information in the Summary of Product Characteristics (SmPC)		
• At least one genotype/phenotype mentioned	+	
OR		
• Recommendation to genotype	++	
OR		
• At least one genotype/phenotype mentioned as a contra-indication in the corresponding section	++	
Total Score:	10+	5+
Corresponding Clinical Implication Score:		Beneficial