CYP2C9: acenocoumarol 1863 to 1869

*2 = CYP2C9 gene variant with decreased activity, *3 = CYP2C9 gene variant with strongly decreased activity, CI = confidence interval, Cl_{or} = oral clearance, EM = extensive metaboliser (*1/*1) (normal CYP2C9 enzyme activity), HR = hazard ratio, IM = intermediate metaboliser, other genotype (decreased CYP2C9 enzyme activity due to a gene variant with decreased activity other than *2 or *3), INR = international normalised ratio, MR = metabolic ratio, NS = non-significant, OR = odds ratio, PM = poor metaboliser, other genotype (strongly decreased CYP2C9 enzyme activity involving one or two gene variants with decreased activity other than *2 or *3), RR = relative risk, S = significant, VKORC1 = vitamin K epoxide reductase complex subunit 1

Brief summary and justification of choices:
Acenocoumarol consists of a racemic mixture. The anticoagulant effect of the S-enantiomer is more potent than that of the R-enantiomer. However, the S-enantiomer is eliminated more rapidly, which makes the R-enantiomer predominantly responsible for the anticoagulant effect. The S-enantiomer is almost fully metabolised by CYP2C9 by hydroxylation. The R-enantiomer is metabolised by CYP1A2, CYP3A4, CYP2C9 and CYP2C19.
CYP2C9 gene variants leading to decreased metabolic capacity of the enzyme, cause increased S-acenocoumarol plasma concentrations and to a lesser extent increased R-acenocoumarol plasma concentrations. As confirmed in literature, these gene variants reduce the required acenocoumarol dose. However, as indicated below, there is insufficient evidence to recommend an adjustment of the initial dose, the frequency of INR monitoring or the choice of medicine. The risk of bleeding is not significantly increased in patients with an allele variant, possibly because INR is regularly monitored in all patients. The Dutch Pharmacogenetic Working Group therefore decides that no action is required (yes/no-interactions).
Initial dose
Verhoef 2013 did not find any significant differences in adverse events, thromboembolism and undercoagulation/ overcoagulation between treatment guided by a genotype-based algorithm and a non-genotype-based algorithm. Zhang 2017 also did not find any significant differences in the subgroup of patients with one CYP2C9 or VKORC1 variant and in the subgroup with two or more CYP2C9 or VKORC1 variants. This means that there is no proof that treatment for patients with a CYP2C9 or VKORC1 variant improves when genotype is considered when initiating therapy. Likewise, Cerezo-Manchado 2016 did not find any significant differences in bleeding events, thromboembolism and undercoagulation/overcoagulation between treatment guided by a genotype-based algorithm and physician management, despite an improvement in the percentage of patients reaching stable dose in the first 90 days of treatment.
Verhoef 2012 only found an elevated risk of undercoagulation/overcoagulation in patients with a *2 or *3 allele in the first 4 weeks of treatment. There were no further differences after the first 4 weeks of treatment. This suggests that genotype variants are mainly at risk on initiation of therapy. However, given the results of Verhoef 2013 and Zhang 2017, there is insufficient evidence to recommend adjusting the initial dose.
Choice of medicine
The article by Visser investigating the situation in the Netherlands found a relatively small difference for bleeding (HR for major bleeding: 1.83). Articles that related to other countries ranged from no increased risk of major bleeding to an increase by OR = 2.41.
The higher risk of bleeding for patients with CYP2C9 polymorphisms is not unacceptable and does not justify withholding anticoagulant therapy or switching to direct-acting oral anticoagulant therapy. Whereas all direct-acting oral anticoagulants (rivaroxaban, apixaban, dabigatran and edoxaban) are authorised for the treatment of venous thromboembolism, the prevention of recurrent venous thromboembolism and the prevention of venous thromboembolism in patients with atrial fibrillation, only rivaroxaban, apixaban and dabigatran are authorised for the prevention of thromboembolism in patients undergoing hip or knee replacement surgery. In addition, none of the direct-acting oral anticoagulants is authorised for use in patients with heart valve abnormalities.
Frequency of INR monitoring

Recommending a change in the frequency of INR monitoring by the National INR Monitoring Service (trombosedienst) is not meaningful: INR is always measured more frequently when the INR is not stable. Patients starting anticoagulant therapy at the hospital are often guided by residents or internists. There is also insufficient evidence that more frequent monitoring of patients with an allele variant is meaningful in this situation. One article found a longer time to achieving stable INR within target for some patients with an allele variant. Another article found no effect. Jiménez-Varo 2014 found an increased risk of INR > 6, but not of major bleeding for patients with a CYP2C9 *3 variant. However, INR values were determined twice a week until the first therapeutic INR in this study. Shorter intervals are considered not useful, because of the time required to reach a stable INR after a dose adjustment. Cerezo-Manchado 2014 found a shorter time to INR > 4 for patients with a CYP2C9 variant. However, the INR 72 hours after start of therapy was a good predictor of INR > 4 independently of genotype. This suggests that the INR-based dose adaption was suboptimal.
The results generated by Visser, 2005 and Beinema, 2007 suggest a (stronger) increase in INR by NSAIDs in patients with an allele variant than in wild-type patients. However, these results are not confirmed by research groups outside the Netherlands. INR is not monitored more frequently in patients using NSAIDs at this time, because this does not usually lead to increased INR. There is insufficient evidence to advise more frequent INR monitoring in patients with an allele variant using NSAIDs.
Overview of kinetic and clinical effects
You can find a detailed overview of the observed kinetic and clinical effects 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.

<table>
<tr><th>Source</th><th>Code</th><th>Effect</th><th>Comments</th></tr>
<tr>
<td>ref. 1
Varnai R et al. CYP2C9 and VKORC1 in therapeutic dosing and safety of acenocoumarol treatment: implication for clinical practice in Hungary.
Environ Toxicol Pharmacol 2017;56:282-289.
PubMed PMID: 29055218.</td>
<td>3

(*1/*2+ *1/*3+ *2/*2+ *2/*3+ *3/*3): A</td>
<td>For 117 patients on stable acenocoumarol maintenance dose, major and clinically relevant non-major bleeding events in the preceding 12 months were evaluated. The bleeding events were haematoma (n = 35), bleeding wounds (n= 23), bleeding nose (n =21), bleeding gums (n = 11), blood in stool (n = 11), and haematuria (n =9). The INR target was 2.0-3.0. The mean acenocoumarol treatment period was 5.9 years (range 0.2-27 years).
Relevant co-medication was not excluded.

Genotyping:
- 75x *1/*1
- 28x *1/*2
- 7x *1/*3
- 3x *2/*2
- 2x *2/*3
- 2x *3/*3

Results:
<table>
<tr><td colspan="7">Results compared to *1/*1:</td></tr>
<tr><td></td><td>*3/*3</td><td>*2/*3</td><td>*2/*2</td><td>*1/*3</td><td>*1/*2</td><td>value for *1/*1</td></tr>
<tr><td>bleeding events</td><td colspan="5">No association with the CYP2C9 genotype (NS).</td><td></td></tr>
<tr><td>overanticoagulation</td><td colspan="5">Carriers of CYP2C9 *2 and/or *3 without the VKORC1 variant that reduces dose requirement, had 6.33% over-anticoagulation (NS).</td><td></td></tr>
<tr><td rowspan="2">acenocoumarol dose</td><td>x 0.73 (NS)</td><td>x 0.41 (NS)</td><td>x 0.90 (NS)</td><td>x 0.97 (NS)</td><td>x 0.97 (NS)</td><td rowspan="2">2.41 mg/day</td></tr>
<tr><td colspan="5">Multivariate linear regression showed a significant effect of CYP2C9 genotype on acenocoumarol dose (S).</td></tr>
<tr><td colspan="7">Carriers of a combination of CYP2C9*2,*3 and the VKORC1 variant that reduces dose requirement, had 34% over-anticoagulation.</td></tr>
<tr><td colspan="7">VKORC1 genotype, CYP2C9 genotype and age together explained 30.4% of acenocoumarol dosing variability.</td></tr>
</table>
</td>
<td>Author’s conclusion:
”Most impact on dose reduction is accountable for CYP2C9*2/*3 (59%) and for VKORC1*2/*2 (45.5%), and on dose increase for newly evaluated VKORC1*3/*4 (22.5%) diplotypes. Being a carrier of combination of VKORC1*2 and CYP2C9*2,*3 polymorphisms, rather than of one of these SNPs, is associated with higher risk of over-anticoagulation (up to 34.3%) in long-term acenocoumarol treatment. Correlation between the studied diplotypes and bleeding events could not be revealed.”</td>
</tr>
</table>

<table>
<tr><td>ref. 1, continuation</td><td></td><td>Note: Genotyping was for *2 and *3. These are the most important gene variants in this Hungarian population.</td><td></td></tr>
<tr><td>ref. 2
Kalpana SR et al.
Influence of VKORC1 and CYP2C9 polymorphisms on daily acenocoumarol dose requirement in South Indian patients with mechanical heart valves.
Clin Appl Thromb Hemost
2017;23: 876-882.
PubMed PMID: 27335128.</td><td>3

(*1/*2+ *1/*3+ *2/*2+ *2/*3): A</td><td>205 patients on acenocoumarol therapy had a stable therapeutic INR between 2 and 3.5 for at least 3 months.
Antiepileptics, including phenytoin and carbamazepine and antituberculous treatment were excluded. Other relevant co-medication was not excluded (16% used digoxin, 5.8% furosemide and 1.5% amiodarone).

Genotyping:
- 161x *1/*1
- 13x *1/*2
- 29x *1/*3
- 1x *2/*2
- 1x *2/*3

Results:<table><tr><td colspan="2">Acenocoumarol dose compared to *1/*1 (2.71 mg/day):</td></tr><tr><td>*1/*2</td><td>x 0.79 (NS)</td></tr><tr><td>*1/*3</td><td>x 0.83 (NS)</td></tr><tr><td>*2/*2</td><td>x 0.85 (NS)</td></tr><tr><td>*2/*3</td><td>x 1.11 (NS)</td></tr><tr><td colspan="2">The acenocoumarol dose was lower for carriers of a CYP-2C9 variant (*1/*2 + *1/*3 + *2/*2 + *2/*3) compared to non-carriers (*1/*1) (S).</td></tr><tr><td colspan="2">Co-medication with furosemide and digoxin decreased the required acenocoumarol dose. These drugs are known to potentiate the effect of acenocoumarol by releasing it from plasma protein and increasing the concentration of the free active form in the plasma.</td></tr></table>
Note: Genotyping was for *2 and *3. These are the most important gene variants in this Indian population.</td><td>Author’s conclusion:
”Presence of a mutant allele of VKORC1 (-1639A & 1173T) and CYP2C9 genes increased the odds of requiring a lower mean dosage of acenocoumarol.”</td></tr>
<tr><td>ref. 3
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.</td><td>3</td><td>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 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.

Genotyping:
- 187x *1/*1
- 64x *1/*2
- 53x *1/*3
- 12x *2/*2
- 8x *2/*3
- 1x genotype unknown (clinical algorithm, ≥ 75 years)</td><td>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.”</td></tr>
</table>

ref. 3, continuation	genotype-guided versus not genotype-guided therapy: AA	Results: 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 similar results.		
			< 75 years, Dutch	NS	58.5%
			≥ 75 years, Dutch	NS	58.9%
			< 75 years, Greek	NS	65.3%
			≥ 75 years, Greek	NS	63.0%
		% of time with a suprathe-rapeutic 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%
			≥ 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%

<table>
<tr>
<td>ref. 3, continuation</td>
<td></td>
<td>
<table>
<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 rowspan="11">% 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>< 75 years, one CYP2C9 or VKORC1 variant</td><td>NS</td><td>18.6%</td></tr>
<tr><td>< 75 years, two or more CYP2C9 and/or VKORC1 variants</td><td>NS</td><td>16.6%</td></tr>
<tr><td>≥ 75 years, no CYP2C9 and VKORC1 variants</td><td>NS</td><td>35.1%</td></tr>
<tr><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>≥ 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>< 75 years</td><td>NS</td><td>19.9%</td></tr>
<tr><td>≥ 75 years</td><td>+ 9.9% (S)</td><td>22.4%</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>19.4%</td></tr>
<tr><td>≥ 75 years, Dutch</td><td>NS</td><td>20.4%</td></tr>
<tr><td>< 75 years, Greek</td><td>NS</td><td>20.6%</td></tr>
<tr><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. 4
Cerezo-Manchado JJ et al. Genotype-guided therapy improves initial acenocoumarol dosing. Results from a prospective randomised study. Thromb Haemost 2016;115:117-25. PubMed PMID: 26538428.</td>
<td>3</td>
<td>178 patients starting acenocoumarol were treated for 6 months. The first dose was administered to all patients according to the physician’s criteria (based on age, body surface area and co-medication). From 72 hours on, the dose was calculated based on INR in the physician management group (n = 92), whereas genetic data (CYP2C9, VKORC1 and CYP-4F2) were also considered in the genotype-guided dosing group (n = 86). For genotype-guided dosing, the algorithm in Cerezo-Manchado 2013, was adjusted to include the INR_{72h} and the subsequent INR values for the following doses. Then, the new acenocoumarol dose was calculated from the predicted dose calculated with the former algorithm and applying the following equation; NewDose = PrevDose + [C1*(INR*INR) + (C2*INR) + C3], with C1, C2 and C3 having different values for the 3rd, 4th and 5th dose. The INR target was 2.0-3.0. Only patients with atrial fibrillation and thus not receiving low-molecular-weight heparin as additional anticoagulant were included. One patient without CYP2C9 and VKORC1 variants, originally randomised to the genotype-guided group, was withdrawn from the study and not included in the data analysis and this summary. Due to a system failure, the algorithm did not modify the previous dose of 23 mg/week, despite the</td>
<td>Author’s conclusion: ”Genotype-guided dosing was associated with a higher percentage of patients with steady dose than routine practice when starting oral anticoagulation with acenocoumarol.”</td>
</tr>
</table>

ref. 4, continuation

patient having INR 1.2 on this dose on day 23. Patients included in the physician management group were genotyped when the study had finished.
Adverse events included major and minor bleeding events, thromboembolic complications and hospitalisations related to treatment.
Relevant co-medication was not excluded.
A power calculation, based on dose estimates within 20% of real dose for the algorithm and within 40% of real dose for physician management, showed a requirement of 88 patients per arm.

Genotyping:
- 105x *1/*1
- 47x *1/*2
- 20x *1/*3
- 2x *2/*2
- 3x *2/*3
- 1x *3/*3

Results:

Genotype-based algorithm versus physician management:			
			value for physician management
% of patients with stable dose	after 90 days	x 1.56 (S)	25%
	after 6 months	trend for an increase, p = 0.056 (NS)	72%
% of patients who achieved a stable anticoagulation period	in the first 90 days	increase (S)	
	in the first 6 months	increase (S)	
median time to stable dose	after 90 days	trend for a decrease, p = 0.097 (NS)	90 days
	after 6 months	NS	111 days
median time to first therapeutic INR		NS	11 days
% of time with therapeutic INR		x 1.11 (S)	45%
% of patients with an INR > 4	after 90 days	NS	26%
	after 6 months	NS	29%
median number of INR's determined	after 90 days	NS	8
	after 6 months	NS	13
% of adverse events	after 90 days	NS	12%
	after 6 months	NS	16%
% of major bleeding	after 90 days	NS	1%
	after 6 months	NS	1%
% of minor bleeding	after 90 days	NS	9%
	after 6 months	NS	11%
% of thromboembolic events	after 90 days	NS	1%
	after 6 months	NS	3%
% of hospitalisations related to treatment	after 90 days	NS	1%
	after 6 months	NS	1%

genotype-guided versus not genotype-guided therapy : AA[#]

<table>
<tr><td>ref. 4, continuation</td><td></td><td>Note: Genotyping was for *2 and *3. These are the most important gene variants in this Spanish population.</td><td></td></tr>
<tr><td>ref. 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.
PubMed PMID: 25519826.</td><td>4

*1/*2: A
*1/*3: A
*2/*3: AA</td><td>217 patients on acenocoumarol therapy had a stable therapeutic INR between 2 and 3.5 for at least 3 months.
Co-medication potentially interacting with acenocoumarol was excluded.

Genotyping:
- 176x *1/*1
- 12x *1/*2
- 28x *1/*3
- 1x *2/*3

Results:
<table>
<tr><td colspan="2">Acenocoumarol dose compared to *1/*1 (4.1 mg/day):</td></tr>
<tr><td>*1/*2</td><td>x 0.49 (S)</td></tr>
<tr><td>*1/*3</td><td>x 0.39 (S)</td></tr>
<tr><td>*2/*3</td><td>x 0.37</td></tr>
<tr><td colspan="2">CYP2C9 *3 explained 16.4% of the dose variation in this South-Indian population.</td></tr>
</table>
Note: Genotyping was for *2 and *3. These are the most important gene variants in this Indian population.</td><td>Author’s conclusion:
”The CYP2C9 *1*2, CYP2C9 *1*3, and CYP2C9 *2*3 variant genotypes significantly reduced the dose by 56.7% (2.0 mg), 67.6% (1.6 mg), and 70.3% (1.5 mg) than wild-type carriers 4.1 mg.”</td></tr>
<tr><td>ref. 6
Jiménez-Varo E et al.
Pharmacogenetics role in the safety of acenocoumarol therapy.
Thromb Haemost 2014;112:522-36.
PubMed PMID: 24919870.</td><td>3</td><td>128 patients were treated with acenocoumarol for 7 months. The first dose (usually 14-15 mg/week) was administered to all patients according to the physician’s criteria (based on age, weight and co-medication). Each patient received low-molecular-weight heparin until the first therapeutic INR. From day 3-4 on, the dose was titrated based on the INR values. INR values were determined twice a week until the first therapeutic INR, and once a week while within the therapeutic range (2.0-3.0). The frequency of INR measurements was increased when therapeutic INR was lost. The percentage of time within the therapeutic range was 33% during the first month and 61% in the 1-7 months period.
Major bleeding (a reduction in the haemoglobin level ≥ 20 g/l, transfusion ≥ 2 units of blood, or symptomatic bleeding in a critical area or organ) did not occur. There were 16 episodes of minor bleeding in 16 patients. One patient presented three episodes of transient ischaemic attack (TIA), the first in the first month and the last two in the fifth month of therapy.
Co-medication with CYP2C9 inhibitors was not excluded.
For the first month, only data were included of the 123 patients who had a minimum of four INR determinations in this period.
Odds ratios were calculated by multivariate analyses.

Genotyping:
- 76x *1/*1
- 33x *1/*2
- 14x *1/*3
- 3x *2/*2
- 1x *2/*3
- 1x *3/*3

Results:
<table>
<tr><td colspan="4">Odds ratios compared to the *1-allele:</td></tr>
<tr><td></td><td></td><td>*2-allele</td><td>*3-allele</td></tr>
<tr><td rowspan="3">bleeding events</td><td>0-1 months</td><td>NS</td><td>NS</td></tr>
<tr><td>0-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td>1-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td>INR > 6</td><td>0-1 months</td><td>NS</td><td>NS</td></tr>
</table></td><td>Author’s conclusion:
”VKORC1, CYP-2C9*3, APOE and ABCB1 genotypes should be considered in prevention of overanticoagulation and bleeding events in the initiation of acenocoumarol therapy.”</td></tr>
</table>

<table>
<tr><td>ref. 6, continuation</td><td>(*1/*3+ *2/*3+ *3/*3): D

(*1/*2+ *2/*2+ *2/*3): AA</td><td>
<table>
<tr><td></td><td>0-7 months</td><td>NS</td><td>OR = 5.5 (95% CI: 1.8-17)</td></tr>
<tr><td></td><td>1-7 months</td><td>NS</td><td>OR = 4.2 (95% CI: 1.2-14)</td></tr>
<tr><td></td><td colspan="3">In univariate analysis, the percentage of patients with INR > 6 during the whole period of 7 months and in the 1-7 months period was increased, and the time to INR > 6 was decreased for patients with the *3-allele compared to patients without the *3-allele (S).</td></tr>
<tr><td>INR > 4</td><td>0-1 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>0-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>1-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td colspan="3">In univariate analysis, the time to INR > 4 was decreased for patients with the *3-allele compared to patients without the *3-allele (S).</td></tr>
<tr><td>% of patients with stable dose</td><td>0-1 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>0-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>1-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td>% of time with therapeutic INR</td><td>0-1 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>0-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>1-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td colspan="3">There was no difference in the percentage of time with therapeutic INR for patients with one or more CYP2C9 variants compared to patients without a CYP2C9 variant, neither during the first month nor during the first 7 months of treatment (NS).</td></tr>
<tr><td>% of time with supratherapeutic INR (> 3.0)</td><td>0-1 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>0-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>1-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td colspan="3">In univariate analysis, the percentage of time with supratherapeutic INR was increased for patients with one or more CYP2C9 variants compared to patients without a CYP2C9 variant during the first month of treatment (S), but not during the whole period of 7 months (NS). The same was true for patients with the *2-allele compared to patients without the *2-allele.</td></tr>
<tr><td>% of time with subtherapeutic INR (< 2.0)</td><td>0-1 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>0-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>1-7 months</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td colspan="3">In univariate analysis, the percentage of time with subtherapeutic INR was decreased for patients with one or more CYP2C9 variants compared to patients without a CYP2C9 variant during the first month of treatment (S), but not during the whole period of 7 months (NS). The same was true for patients with the *2-allele compared to patients without the *2-allele.</td></tr>
</table>
Note: Genotyping was for *2 and *3. These are the most important gene variants in this Spanish population.
</td><td></td></tr>
<tr><td>ref. 7
Cerezo-Manchado JJ et al.
Effect of VKORC1, CYP2C9 and CYP4F2 genetic variants in early outcomes during acenocoumarol</td><td>3</td><td>941 patients were treated with acenocoumarol for 3 months. The loading doses were administered independently of genotypes, based on physician’s criteria and according to the INR target defined by clinical diagnosis. Subsequent doses were adjusted according to INR values. 29% of patients reached an INR > 4 in the 3 months treatment period. 19% of patients had an INR_{72h} > 2.5.
Some patients with missing values for at least one of the determinants or those who did not reach a stable phase within</td><td>Author’s conclusion:
”In addition to VKORC1 and CYP2C9, CYP4F2 gene has a slight but significant role in reaching INR ></td></tr>
</table>

<table>
<tr>
<td>treatment.
Pharmacogenomics 2014;15:987-96.
PubMed PMID: 24956252.

ref. 7, continuation</td>
<td>(*1/*3+ *2/*3): A

(*1/*2+ *2/*2): A</td>
<td>6 months were excluded from the multivariate analyses.

Genotyping:
- 569x *1/*1
- 241x *1/*2
- 99x *1/*3
- 19x *2/*2
- 13x *2/*3

Results:
<table>
<tr><td colspan="4">Hazard ratios compared to *1/*1:</td></tr>
<tr><td></td><td></td><td>*1/*2+*2/*2</td><td>*1/*3+*2/*3</td></tr>
<tr><td rowspan="4">time to INR > 4</td><td>multivariate analysis</td><td>-</td><td>HR = 1.19 (95% CI: 1.12-1.26)</td></tr>
<tr><td>univariate analysis</td><td>HR = 1.37 (95% CI: 1.04-1.80)</td><td>HR = 2.71 (95% CI: 2.05-3.75)</td></tr>
<tr><td colspan="3">24% of *1/*1 reached INR > 4 in the 3 months treatment period.</td></tr>
<tr><td colspan="3">The INR_{72h} was a good predictor of INR > 4, independently of genotype.</td></tr>
<tr><td>time to stable dose</td><td>multivariate analysis</td><td>-</td><td>NS</td></tr>
<tr><td>% of patients with INR_{72h} > 2.5</td><td>multivariate analysis</td><td>-</td><td>HR = 1.12 (95% CI: 1.03-1.24)</td></tr>
</table>

<table>
<tr><td colspan="2">Acenocoumarol stable dose compared to *1/*1 (13 mg/ week):</td></tr>
<tr><td>*1/*2</td><td>x 1.0</td></tr>
<tr><td>*1/*3</td><td>x 0.77</td></tr>
<tr><td>*2/*2</td><td>x 0.92</td></tr>
<tr><td>*2/*3</td><td>x 0.77</td></tr>
<tr><td colspan="2">S for (*1/*3+*2/*3) compared to (*1/*1+*1/*2+*2/*2)</td></tr>
</table>

Note: Genotyping was for *2 and *3. These are the most important gene variants in this Spanish population.</td>
<td>2.5 during the first weeks of acenocoumarol therapy.”</td>
</tr>
<tr>
<td>ref. 8
Verhoef TI et al.
A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon.
N Engl J Med 2013;369:2304-12.
PubMed PMID: 24251360.</td>
<td>3

geno-</td>
<td>Patients without prior exposure to coumarin therapy were treated with acenocoumarol for 12 weeks. During the first 5 to 7 days, patients were treated on the basis of an algorithm that incorporated CYP2C9 and VKORC1 genotypes (n=190) or on the basis of an algorithm incorporating clinical information only (n=187). The INR target was 2.0-3.0. Relevant co-medication was not excluded. Amiodarone usage was included in the dose algorithm. Patients with venous thromboembolism (17%) were often given low-molecular-weight heparin until reaching therapeutic INR.

Genotyping:
- 218x *1/*1
- 72x *1/*2
- 61x *1/*3
- 15x *2/*2
- 9x *2/*3
- 2x *3/*3

Genotype-based algorithm versus clinical algorithm:
- The time in therapeutic range throughout the treatment did</td>
<td>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.’</td>
</tr>
</table>

<table>
<tr>
<td>ref. 8, continuation

Baranova EV et al. Dosing algorithms for vitamin K antagonists across VKORC1 and CYP-2C9 genotypes. J Thromb Haemost 2017;15:465-472. PubMed PMID: 28063245.</td>
<td>type-guided versus not genotype-guided therapy : AA</td>
<td>not increase (NS)
- The time in therapeutic range in the first 4 weeks did not increase (NS)
- There was no difference in the incidence of adverse events and thromboembolism (NS)
- There was no difference in the percentage of patients with an INR ≥ 4, the percentage of time with INR ≥ 4 of < 2, the time to achieving INR in the therapeutic range and the time to reaching a stable dose (NS)

When the acenocoumarol and phenprocoumon data were pooled, the percentage of time in therapeutic range was higher in the first 4 weeks of treatment 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 CYP-2C9 and or VKORC1 variant:
<table>
<tr><td colspan="4">Genotype-based algorithm versus clinical algorithm:</td></tr>
<tr><td></td><td>genotype group</td><td>first 4 weeks</td><td>first 12 weeks</td></tr>
<tr><td>% of time in the therapeutic range</td><td>no CYP2C9 and VKORC1 variants</td><td>+ 14.68% (S, but only a trend after Bonferroni correction (significance for p < 0.001) (NS, p = 0.002))</td><td>trend for an increase, p = 0.087 (NS)</td></tr>
<tr><td></td><td>one or more CYP2C9 variants and no VKORC1 variant</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>no CYP2C9 variants and one VKORC1 variant</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>one or more CYP2C9 variants and one VKORC1 variant</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>no CYP2C9 variants and two VKORC1 variants</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>one or more CYP2C9 variants and two VKORC1 variants</td><td>NS</td><td>NS</td></tr>
<tr><td>% of time with a supra-therapeutic INR (> 3.0)</td><td>no CYP2C9 and VKORC1 variants</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>one or more CYP2C9 variants and no VKORC1 variant</td><td>NS</td><td>NS</td></tr>
</table>
</td>
<td>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–CYP-2C9 genetic subgroups.
EU-PACT genetic-guided dose initiation algorithms for acenocoumarol and phenprocoumon could have predicted the dose overcautiously in</td>
</tr>
</table>

<table>
<tr><td>ref. 8, continuation</td><td></td><td>
<table>
<tr><td></td><td>no CYP2C9 variants and one VKORC1 variant</td><td>NS</td><td>NS</td></tr>
<tr><td></td><td>one or more CYP2C9 variants and one VKORC1 variant</td><td>trend for a decrease, p = 0.098 (NS)</td><td>NS</td></tr>
<tr><td></td><td>no CYP2C9 variants and two VKORC1 variants</td><td>trend for a decrease, p = 0.087 (NS)</td><td>trend for a decrease, p = 0.057 (NS)</td></tr>
<tr><td></td><td>one or more CYP2C9 variants and two VKORC1 variants</td><td>- 20.50% (S, but NS after Bonferroni correction)</td><td>NS</td></tr>
<tr><td>% of time with a sub-therapeutic INR (< 2.0)</td><td>no CYP2C9 and VKORC1 variants</td><td>- 20.29% (S, before and after Bonferroni correction)</td><td>trend for a decrease, p = 0.083 (NS)</td></tr>
<tr><td></td><td>one or more CYP2C9 variants and no VKORC1 variant</td><td>NS</td><td>NS</td></tr>
<tr><td></td><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></td><td>one or more CYP2C9 variants and one VKORC1 variant</td><td>NS</td><td>NS</td></tr>
<tr><td></td><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></td><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>
</td><td>the VKORC1 AA–CYP2C9*1*1 subgroup. Adjustment of the genotype-guided algorithm could lead to a higher benefit of genotyping.’</td></tr>
<tr><td>ref. 9
Gschwind L et al. Impact of CYP2C9 polymorphisms on the vulnerability to pharmacokinetic drug-drug interactions during acenocoumarol treatment. Pharmacogenomics 2013:14;745-53. PMID: 23651023.</td><td>3</td><td>115 patients who started acenocoumarol therapy were followed for 35 days. The INR target was 2.0-3.0. 35 patients had a CYP2C9 inhibitor as co-medication.

Genotyping:
- 74x *1/*1
- 26x *1/*2
- 2x *2/*2
- 9x *1/*3
- 3x *2/*3
- 1x *3/*3

Results:</td><td>Authors’ conclusion:
‘These findings support the fact that CYP2C9 genotyping could be useful to identify patients requiring closer monitoring, especially when a drug-drug interaction is suspected.’</td></tr>
</table>

ref. 9, continuation	(*2+*3): B *3: A *2: AA	- Presence of *2 and/or *3 increased the risk of an INR ≥ 4 (HR=1.7; 95% CI: 1.19-2.44) (S) - CYP2C9 inhibitors increased the risk of an INR ≥ 4 to the same extent in *1/*1 patients as in (*2 and/or *3) patients (HR=2.7; 95% CI: 1.19-6.12 and HR=2.9; 95% CI: 1.29-6.54 respectively; difference in HRs was NS) - *3: the dose decreased by 35% versus *1/*1 (S) - *2 had no significant effect on the maintenance dose	
ref. 10 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.	3 (*1/*2+ *2/*2): A (*1/*3+ *3/*3+ *2/*3): D	The data from 1420 acenocoumarol users in three different studies were analysed. 12% of the patients were from the Schalekamp 2006 study, which is also included separately in this risk analysis. This was the only study that included data on the first 6 months of treatment. Data until 18 months of treatment were derived from the other two studies. The INR target for all patients was 2.0-3.5. Relevant co-medication was not excluded. There were no significant differences in the percentage of patients using amiodarone in the different genotype groups. Genotyping: - 938x *1/*1 - 312x (*1/*2 + *2/*2) - 170x (*1/*3 + *2/*3 + *3/*3) (*1/*2 + *2/*2) versus *1/*1: - No difference in the risk of INR < 2 throughout the treatment period (NS) - The risk of INR > 3.5 in the first month increased by 22% (from 41% to 50% of the patients) (S). There were no differences after the first month (NS). - The risk of INR > 6 in the first month increased non-significantly by 75% (from 4% to 7% of the patients) (NS). There were no differences after the first month (NS). (*1/*3 + *2/*3 + *3/*3) versus *1/*1: - The risk of INR < 2 in the first month decreased by 17% (from 65% to 54% of the patients) (S). There were no differences after the first month (NS). - The risk of INR > 3.5 in the first month increased by 24% (from 41% to 51%) (S). There were no differences after the first month (NS). - The risk of INR > 6 in the first month increased by 125% (from 4% to 9%) (S). There were no differences after the first month (NS).	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.'
ref. 11 Esmerian MO et al. Influence of CYP2C9 and VKORC1 polymorphisms on warfarin and acenocoumarol in a sample of Lebanese people. J Clin Pharmacol 2011;51:1418-28. PMID: 21148049.	3	133 patients received a maintenance dose of acenocoumarol. The INR target was 2.0-3.0 (n=100) or 2.5-3.5 (n=33). INR 1.7-4.0 was considered an INR within the therapeutic range. Relevant co-medication, such as anti-platelet therapy or CYP2C9 inhibitors, was not excluded. Genotyping: - 84x *1/*1 - 24x *1/*2 - 15x *1/*3 - 4x *2/*2 - 4x *2/*3 - 2x *3/*3 Results: - No association of *2 and *3 with the incidence of major or minor bleeding events since the start of therapy (NS).	Authors' conclusion: 'The reduction in weekly dose is driven by mainly VKORC1, followed by CYP2C9*3 variants.'

ref. 11, continuation	*1/*2: AA *3/*3: AA (*2/*3+ *2/*2+ *1/*3): A	Many patients who were hospitalised for major bleeding had INRs within the target range. - No differences in the frequency of CYP2C9 alleles between patients within or outside the therapeutic range (NS) - No differences in the time to achieving stable therapeutic INR between *1/*1 patients and patients with one or two allele variants (n=40) (NS) - *2 and *3 had no effect on the maintenance dose (NS) - No differences in maintenance dose between *3/*3 and (*1/*1 + *1/2) (NS) - The maintenance dose decreased by 34% (from 19 to 13 mg/week) for (*2/*3 + *2/*2 + *1/*3) versus (*1/*1 + *1/2) (S) NOTE: The authors stated that the sample size should have been 200 to demonstrate a 20% difference in acenocoumarol dose for CYP2C9*3. The sample size required was not calculated for bleeding and time to therapeutic INR.	
ref. 12 Cadamuro J et al. Genetic determinants of acenocoumarol and phenprocoumon maintenance dose requirements. Eur J Clin Pharmacol 2010;66:253-60. PMID: 20020283.	4 *1/*2: AA *2/*2: AA *1/*3 + *2/*3 + *3/*3: A	80 patients, 44x *1/*1, 21x *1/*2, 7x *1/*3, 3x *2/*2, 2x *2/*3, 3x *3/*3, acenocoumarol users, significance maintained after correction for relevant co-medication; Maintenance dose (corrected for age, sex and last INR) versus *1/*1: - *1/*2: 16% decrease from 19.74 to 16.64 mg/week (NS) - *1/*3: 36% decrease from 19.74 to 12.56 mg/week (S for *1/*3, *2/*3 and *3/*3 pooled) - *2/*2: 14% increase from 19.74 to 22.48 mg/week (NS) - *2/*3: 6% decrease from 19.74 to 18.64 mg/week (S for *1/*3, *2/*3 and *3/*3 pooled) - *3/*3: 69% decrease from 19.74 to 6.2 mg/week (S for *1/*3, *2/*3 and *3/*3 pooled) CYP2C9*3 is an independent variable for the maintenance dose (multivariable regression analysis). Age, sex, last INR and VKORC1 and CYP2C9 genotypes together account for 58% of the variation in the maintenance dose.	Authors' conclusion: 'These results reveal that interindividual variability in weekly acenocoumarol maintenance dose requirement is mainly dependent on the VKORC1 1173C>T and the CYP2C9*3 alleles. VKORC1 and CYP2C9 genotyping might provide helpful information to prevent serious bleeding events in subjects receiving acenocoumarol.'
ref. 13 Wijnen PA et al. Variant VKORC1 and CYP2C9 alleles in patients with diffuse alveolar hemorrhage caused by oral anti-coagulants. Mol Diagn Ther 2010;14:23-30. PMID: 20121287.	3 *1/*2 + *1/*3 + *2/*2 + *2/*3 + *3/*3: F	Case-control study including 63 cases (diffuse alveolar bleeding), on acenocoumarol (n=61) or phenprocoumon (n=2), loading dose 6-4-2-2 or 6-4-4-4 mg, co-medication affecting INR was taken by 60% of the cases; The causes of death in 59% of the cases were mainly complications related to heart failure in combination with diffuse alveolar bleeding. Case versus control group: - 1.3-fold increase in the percentage of patients with an allele variant (increase from 38.1% to 49.2%) (S) - 1.14-fold increase in the allele frequency of *2 (increase from 13.9% to 15.9%) (NS) - 1.98-fold increase in the allele frequency of *3 (increase from 6.4% to 12.7%) (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. 14 Teichert M et al. Genotypes associated with reduced activity of VKORC1 and CYP2C9 and their modification of acenocoumarol anti-	3	1525 patients, 1003x *1/*1, 321x *1/*2, 141x *1/*3, 30x *2/*2, 28x *2/*3, 2x *3/*3, loading dose 8-4-4 mg, relevant co-medication not excluded, but correction of the weekly dose after 6 weeks for co-medication affecting CYP2C9; The INR on day 4 was 2.7 among *1/*1 patients and the weekly dose after 6 weeks was 16.9 mg/week. *1/*2 versus *1/*1:	Authors' conclusion: 'Each CYP2C9 variant allele present reduced the required dosage by 1.8 mg/week. Our conclusion

coagulation during the initial treatment period. Clin Pharmacol Ther 2009;85:379-86. **ref. 14, continuation**	*1/*2: A *1/*3: A *2/*2: A *2/*3: A *3/*3: A *2/*2 + *2/*3 + *3/*3: B	- The INR on day 4 increased by 0.20 (S) - The risk of INR ≥ 6 on day 4 did not increase significantly - The weekly dose after 6 weeks decreased by 2.27 mg/ week (S) *1/*3 versus *1/*1: - The INR on day 4 increased by 0.16 (NS) - The risk of INR ≥ 6 on day 4 did not increase significantly - The weekly dose after 6 weeks decreased by 3.71 mg/ week (S) (*1/*2 + *1/*3) versus *1/*1: - The risk of INR ≥ 6 over six weeks did not increase significantly - The risk of bleeding over 6 weeks did not increase significantly *2/*2 versus *1/*1: - The INR on day 4 increased by 0.49 (S) - The risk of INR ≥ 6 on day 4 did not increase significantly - The weekly dose after 6 weeks decreased by 5.12 mg/week (S) *2/*3 versus *1/*1: - The INR on day 4 increased by 0.53 (S) - The risk of INR ≥ 6 on day 4 did not increase significantly - The weekly dose after 6 weeks decreased by 6.46 mg/week (S) *3/*3 versus *1/*1: - The INR on day 4 increased by 0.52 (NS) - The risk of INR ≥ 6 on day 4 did not increase significantly - The weekly dose after 6 weeks decreased by 9.44 mg/ week (S) (*2/*2 + *2/*3 + *3/*3) versus *1/*1: - Increased risk of INR ≥ 6 over six weeks (OR = 2.73; 95% CI = 1.28-5.86) - The risk of bleeding over 6 weeks did not increase significantly There was a significant multiplicative interaction between the effects of CYP2C9 and VKORC1 on the weekly dose. A greater proportion of the difference in dose requirement was explained by the VKORC1 genotype than by the CYP2C9 genotype (28% versus 5%).	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.'
ref. 15 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 *1/*2: F *1/*2 + *2/*2 + *2/*3: F *1/*3 + *2/*3 +	Case-control study including 89 cases (major gastrointestinal bleeding; 45x *1/*1, 25x *1/*2, 8x *1/*3, 4x *2/*2, 3x *2/*3, 4x *3/*3) and 177 controls (no bleeding), acenocoumarol usage, co-medication affecting INR was present; Three cases died as a result of bleeding. - Increased risk of major gastrointestinal bleeding for *1/*2 (OR = 2.41; 95% CI = 1.24-4.69). The risk did not increase significantly for the other genotypes. - Risk of bleeding versus (no *2) with dose ≤ 15 mg/ week: - (no *2) and > 15 mg: OR not significantly increased - *2 and > 15 mg: OR = 3.56 (95% CI 1.14-11.11) - Risk of bleeding versus (no *3) with dose ≤ 15 mg/ week: - *3 and > 15 mg: OR not significantly increased - The CYP2C9 inhibitor amiodarone potentiates the effect of	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 amio-

ref. 15, continuation	*3/*3: AA	polymorphisms on the risk of bleeding. Risk of bleeding versus (no VKORC1) homozygous variant, no *2 and no *3) without amiodarone: - (no VKORC1 homozygous variant, no *2 and no *3) with amiodarone: OR not significantly increased - (VKORC1 homozygous variant, *2 or *3) without amiodarone: OR = 1.89 (95% CI 1.08-6.26) - (VKORC1 homozygous variant, *2 or *3) 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 VKORC1 homozygous variant, no *2 and no *3) without acetylsalicylic acid: - (no VKORC1 homozygous variant, no *2 and no *3) with acetylsalicylic acid: OR not significantly increased - (VKORC1 homozygous variant, *2 or *3) without acetylsalicylic acid: OR = 1.89 (95% CI 1.08-3.31) - (VKORC1 homozygous variant, *2 or *3) with acetylsalicylic acid: OR = 8.97 (95% CI 1.66-48.34)	darone or aspirin. … Genotyping of these alterations may be advisable in those patients taking amiodarone or aspirin.'
ref. 16 Markatos CN et al. VKORC1 and CYP-2C9 allelic variants influence acenocoumarol dose requirements in Greek patients. Pharmacogenomics 2008;9:1631-8.	3 *1/*2: AA *1/*3: AA *2/*2: AA *2/*3: AA *1/*3 + *2/*3: A	98 patients, 57x *1/*1, 25x *1/*2, 12x *1/*3, 1x *2/*2, 3x *2/*3, acenocoumarol for ≥ 2 months and stable INR for ≥ 4 weeks (2.0-3.0), co-medication affecting INR not excluded, but there was no significant association between statins and triazole derivatives (CYP2C9 inhibitors) and acenocoumarol dose; Maintenance dose versus *1/*1: - *1/*2: 14% decrease from 2.91 to 2.51 mg/day (NS) - *1/*3: 41% decrease from 2.91 to 1.73 mg/day (NS) - *2/*2: ~12% increase from 2.91 to ~3.26 mg/day (NS) - *2/*3: 56% decrease from 2.91 to 1.28 mg/day (NS) - (*1/*2 + *2/*2 + *2/*3): 14% decrease from 2.91 to 2.51 mg/day (NS) - (*1/*3 + *2/*3): 44% decrease from 2.91 to 1.64 mg/day (S). Patients with wild-type VKORC1 only: 33% decrease from 3.67 to 2.45 mg/day (S). There was a significant association between CYP2C9 and maintenance dose. A greater proportion of the difference in dose requirement was explained by the VKORC1 genotype than by the CYP2C9 genotype (40% versus 12%). 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 patients.'
ref. 17 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 *3/*3: AA *1/*2 + *2/*2: AA	220 patients, 132x *1/*1, 48x *1/*2, 25x *1/*3, 6x *2/*2, 5x *2/*3, 4x *3/*3, loading dose 4-4-2 mg, co-medication affecting INR not excluded, but co-medication did not have a significant effect on INR on day 4 and was not associated with the dose requirement; The dose in week 7 was determined for patients with an INR target of 2.0-3.0 (n=187). *3/*3 versus *1/*1: - The INR on day 4 increased by 2.7 from 2.9 to 5.6 (NS) - The risk of INR ≥ 6 on day 4 increased by 558% (NS) (*1/*2 + *2/*2) versus *1/*1: - The INR on day 4 increased by 0.4 from 2.9 to 3.3 (NS) - The risk of INR ≥ 6 on day 4 increased by 239% (NS) - The dose in week 7 decreased by 17% from 19.0 to 15.8 mg/week (NS)	Authors' conclusion: 'Both the detection of the VKORC1*2, *3 and *4 haplotypes, as well as the CYP2C9*3 variant allele, might be useful to select not only the most sensitive patients, exposed to a higher risk of over-anticoagulation, but also the most resistant ones, exposed to the risk

ref. 17, continuation	*1/*3 + *2/*3 + *3/*3: A	(*1/*3 + *2/*3 + *3/*3) versus *1/*1: - The INR on day 4 increased by 0.8 from 2.9 to 3.7 (NS) - The risk of INR ≥ 6 on day 4 increased by 181% (NS) - The dose in week 7 decreased by 26% from 19.0 to 14.1 mg/week (S). CYP2C9 and VKORC1 independently influence the INR on day 4 and together with age explain 26% of the variation in this INR. A greater proportion of the difference in dose requirement was explained by the VKORC1 genotype than by the CYP-2C9 genotype (12% versus 5%).	of thrombosis recurrence.'
ref. 18 González-Conejero R et al. The genetic interaction between VKORC1 c1173t and calumenin a29809g modulates the anticoagulant response of acenocoumarol. J Thromb Haemost 2007;5:1701-6.	3 *1/*3 + *2/*3 + *3/*3: AA	100 patients with non-valvular atrial fibrillation, 63x *1/*1, 13x *1/*2, 13x *1/*3, 6x *2/*2, 6x (*2/*3 or *3/*3), loading dose 3-3-3 mg, INR target 2.0-3.0, co-medication affecting INR excluded; (*1/*3 + *2/*3 + *3/*3) versus (*1/*1 + *1/*2 + *2/*2): - The INR on day 3 increased by 0.09 from 1.88 to 1.97 (NS) - The maintenance dose decreased by 9.1% from 17.5 to 15.9 mg/week (NS)	Authors' conclusion: 'Using this approximation, we did not find a correlation between the response to acenocoumarol (INR and required dose) and the CYP2C*9 genotype.'
ref. 19 Beinema MJ et al. The influence of NSAIDs on coumarin sensitivity in patients with CYP-2C9 polymorphism after total hip replacement surgery. Mol Diagn Ther 2007;11:123-8.	3 *1/*2 + *1/*3: D *1/*2 + *1/*3 + *2/*2 + *2/*3: AA	100 patients who underwent total hip replacement, 65x *1/*1, 22x *1/*2, 8x *1/*3, 4x *2/*2, 1x *2/*3, low molecular weight heparins (5700 IU/day) for the first 5-13 days (until INR > 2.0, but for at least 5 days), acenocoumarol initiated on day 1, age-dependent loading dose ranging from 2-2 to 4-4 mg, INR target 1.8-3.5, co-medication with NSAIDs (n=52) and other co-medication affecting INR not excluded; (*1/*2 + *1/*3) versus *1/*1: - 3.8-fold increase in the percentage of patients with INR > 4.9 on one or more days during the first week (from 6% to 23%) (S) - (*1/*2 + *1/*3): percentage of patients with INR > 4.9 higher in the NSAID group than in the non-NSAID group (39% versus 0%) (S) *1/*1: no difference between both groups (2.9% versus 9.7%) (NS) - No difference in the mean daily INR for all patients and for non-NSAID users (NS) - Increased mean daily INR for NSAID users (S) (*1/*2 + *1/*3 + *2/*2 + *2/*3) versus *1/*1: - Non-significant increase in the percentage of patients with INR > 4.9 on one or more days during the first week (NS) - (*1/*2 + *1/*3 + *2/*2 + *2/*3): percentage of patients with INR > 4.9 higher in the NSAID group than in the non-NSAID group (32% versus 0%) (S) - *1/*1: no difference between both groups (2.9% versus 9.7%) (NS)	Authors' conclusion: 'In the group of patients with a CYP2C9 variant (*2 or *3 alleles), only concomitant use of a NSAID resulted in INRs > 4.9.'
ref. 20 Mark L et al. Cytochrome P450 2C9 polymorphism and acenocoumarol therapy. Kardiol Pol 2006;64:397-402.	3	421 patients, 276x *1/*1, 78x *1/*2, 55x *1/*3, 3x *2/*2, 9x *2/*3, acenocoumarol for ≥ 6 months, co-medication affecting INR not excluded, but no association between co-medication and bleeding events; *1/*2 versus *1/*1: - The maintenance dose decreased by 22% from 2.90 to	Authors' conclusion: 'In patients with CYP2C9*2 and *3 alleles the frequency of minor bleeding complications

ref. 20, continuation	*1/*2: A *1/*3: A *2/*2: AA *2/*3: A *1/*2 + *2/*2 + *2/*3: D *1/*2 + *1/*3 + *2/*2 + *2/*3: D	2.27 mg/day (S) - No difference in the percentage of patients with INR > 6 (both 29%) (NS) *1/*3 versus *1/*1: - The maintenance dose decreased by 31% from 2.90 to 2.01 mg/day (S) - 1.5-fold increase in the percentage of patients with INR > 6 (from 29% to 44%) (NS) *2/*2 versus *1/*1: - The maintenance dose decreased by 12% from 2.90 to 2.55 mg/day (NS) - The percentage of patients with INR > 6 decreased from 29% to 0% (NS) *2/*3 versus *1/*1: - The maintenance dose decreased by 55% from 2.90 to 1.31 mg/day (S) - 2.3-fold increase in the percentage of patients with INR > 6 (from 29% to 67%) (NS) (*1/*2 + *2/*2 + *2/*3) versus *1/*1: - 1.9-fold increase in the percentage of patients with minor bleeding (from 14% to 27%) (S) (*1/*2 + *1/*3 + *2/*2 + *2/*3) versus *1/*1: - 1.3-fold increase in the percentage of patients with INR > 6 (from 29% to 37%) (S) - Increased risk of minor bleeding: OR = 1.99 (95% CI 1.20-1.33) - Non-significant increase in the risk of major bleeding (NS)	and the occurrence of high INR values were significantly higher, but there was no difference in the rate of major bleedings.'
ref. 21 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 *1/*3 + *2/*3 + *3/*3: B *1/*2 + *2/*2: A	231 patients, 147x *1/*1, 34x *1/*2, 42x *1/*3, 4x *2/*2, 2x *2/*3, 2x *3/*3, loading dose 6-4-2 mg, no relevant co-medication; - The risk of INR ≥ 6 was increased in carriers of both CYP2C9 and VKORC1 polymorphisms versus no or one polymorphism (corr.HR = 3.85, S). The risk was non-significantly increased in carriers of one polymorphism (VKORC1 or CYP2C9). - The time to stable INR was increased in carriers ≥ 1x *3 allele versus *1/*1 (corr. HR = 0.59, S). There was no difference between *2 and *1/*1 (corr. HR = 1.16, NS) - The mean daily dose was 0.55 mg lower in carriers ≥ 1x *3 allele than in *1/*1 patients (S). It was 0.29 mg lower for *2 (S). NOTE: VKORC1 genotype is not associated with the time to reaching stable INR, but it was with a lower daily dose. A greater proportion of the difference in dose requirement was explained by the VKORC1 genotype than by the CYP2C9 genotype (21.4% versus 4.9%).	
ref. 22 Visser LE et al. Allelic variants of cytochrome P450 2C9 modify the interaction between nonsteroidal anti-inflammatory drugs	3	973 patients, 668x *1/*1, 205x *1/*2, 20x *2/*2, 63x *1/*3, 17x *2/*3 of whom 148 on phenprocoumon and 825 on acenocoumarol; - *1/*2: the maintenance dose decreased from 16.1 to 14.0 mg/wk versus *1/*1, RR INR ≥ 6 = 1.08 - *1/*3: the maintenance dose decreased from 16.1 to 12.5 mg/wk versus *1/*1, RR INR ≥ 6 = 1.46	

and coumarin anticoagulants. Clin Pharmacol Ther 2005;77:479-85. **ref. 22, continuation**	*1/*2: AA *1/*3: AA *2/*2: AA *2/*3: AA *1/*2 + *1/*3 + *2/*2 + *2/*3: D	- *2/*2: the maintenance dose decreased from 16.1 to 12.0 mg/wk versus *1/*1, RR INR ≥ 6 = 0.98 - *2/*3: the maintenance dose decreased from 16.1 to 10.8 mg/wk versus *1/*1, RR INR ≥ 6 = 1.46 The RR of an INR ≥ 6.0 was not significantly increased versus *1/*1 for any of the genotypes. The RR was lower for phenprocoumon than for acenocoumarol (0.60 versus 1.00). The INR was ≥ 6.0 in 415 patients. NSAIDs increased the risk of INR ≥ 6 more strongly in patients with an allele variant than in patients with the *1/*1 genotype (OR 3.78 (95% CI 2.02-7.09) and 1.69 (95% CI 1.05-2.69) respectively). This effect was greater for patients with a *3 allele than for patients with a *2 allele (OR 10.8 (95% CI 2.57-34.6) and 2.98 (95% CI 1.09-7.02) respectively).	
ref. 23 Visser LE et al. The risk of bleeding complications in patients with cytochrome P450 CYP-2C9*2 or CYP2C9*3 alleles on acenocoumarol or phenprocoumon. Thromb Haemost 2004;92:61-6.	4 *1/*2 + *1/*3 + *2/*2 + *2/*3: F	996 patients including 841 on acenocoumarol and 155 on phenprocoumon, 685x *1/*1, 311x variant genotype (210x *1/*2, 63x *1/*3, 23x *2/*2, 15x *2/*3), mean follow-up 481 days, co-medication not known; <u>Both coumarins pooled:</u> - Variant genotype: the risk of major and minor bleeding was not increased in the first 90 days, but there was a significantly increased risk of major bleeding after 460 days. - *1/*2 or *2/*2: HR for major + minor, minor, major bleeding 1.11 (NS), 1.02 (NS) and 1.60 (NS) respectively. - *1/*3 or *2/*3: HR for major + minor, minor, major bleeding 0.69 (NS), 0.49 (S) and 1.69 (NS) respectively. <u>For acenocoumarol:</u> - Variant genotype: HR major + minor bleeding was 1.05 (NS), HR minor bleeding was 0.89 (NS), HR major bleeding was 1.83 (S).	Authors' conclusion: 'In our study, CYP-2C9 genotype was not associated with a higher rate of bleeding events during the first 90 days of therapy. The higher risk in patients with variant alleles on acenocoumarol was only found for major and fatal bleeding events but not for minor events.'
ref. 24 Morin S et al. Pharmacogenetics of acenocoumarol pharmacodynamics. Clin Pharmacol Ther 2004;75:403-14.	3 *1/*3: A *1/*2: AA *2/*2: AA *2/*3: AA *3/*3: AA	263 healthy subjects, 170x *1/*1, 45x *1/*2, 32x *1/*3, 4x *2/*2, 1x *3/*3, 9x *2/*3, 2x *1/*5, single 4-mg dose of acenocoumarol, measurement after 24 hours, no co-medication; *Kinetic endpoint* - *2 and/or *3: S- and R-acenocoumarol below the detection limit in 229 and 36 subjects respectively, no significant difference in C_{min} versus *1/*1. *Clinical endpoints* - *1/*3: the INR increased from 1.24 to 1.42 versus *1/*1 (S), the factor VII ratio decreased from 60 to 39 (S). *3 allele explained 12% of the variation in pharmacodynamic response to acenocoumarol - Other genotypes: no significant difference in INR or factor VII ratio versus *1/*1.	
ref. 25 Schalekamp T et al. Acenocoumarol stabilization is delayed in CYP2C9*3 carriers. Clin Pharmacol Ther 2004;75:394-402.	4 *1/*2 + *2/*2: A	231 patients, 147x *1/*1, 38x *2 (*1/*2, *2/*2), 46x *3 (*1/*3, *2/*3, *3/*3), acenocoumarol loading regimen 6-4-2 mg, ≥ 3 months, no CYP2C9 inhibitors or inducers as co-medication; - *1/*2 or *2/*2: no difference in chance of achieving stability within 6 months versus *1/*1. The risk of INR > 6.0 was non-significantly increased, corrected HR was 1.38 for the total duration of therapy, 1.61 for the first 30 days. The INR on day 4 of therapy was 0.1 units lower versus *1/*1 (NS). There was no difference in mean dose. - *1/*3 or *2/*3 or *3/*3: lower chance of achieving stability	Authors' conclusion: 'Our study demonstrates that the CYP2C9*3 allele, but not the CYP-2C9*2 allele, is associated with the following: a decreased chance to achieve stability,

ref. 25, continuation	*1/*3 + *2/*3 + *3/*3: D	within 6 months versus *1/*1 (corrected HR 0.62), achieving stability took 15 days longer (S). The risk of INR > 6.0 was increased (S, corrected HR 3.80), especially during the first 30 days (corrected HR 5.59). The INR on day 4 of therapy increased from 2.7 to 3.2 versus *1/*1 (S). The dose decreased by 3.5 mg/week (S). There was an increased chance of INR within range versus *1/*1 or *1/*2 or *2/*2 (S, OR 3.1).	an increased risk for severe over-anticoagulation (INR >6.0), a higher initial fourth-day INR after a standard aceno-coumarol starting dose, and a lower acenocoumarol dose need.'
ref. 26 Visser LE et al. The risk of overantico-agulation in patients with cytochrome P450 CYP2C9*2 or CYP2C9*3 alleles on acenocoumarol or phenprocoumon. Pharmacogenetics 2004;1427-33.	3 *2/*2: D *1/*2: A *1/*3: A *2/*3: A	1124 patients, 771x *1/*1, 239x *1/*2, 73x *1/*3, 23x *2/*2, 18x *2/*3, 970 acenocoumarol users, mean follow-up 1.8 years, CYP2C9 inhibitors as co-medication; <u>With and without co-medication:</u> Higher INR after initial dose for all genotypes variant, significant for *1/*2 and *2/*2. No difference in INR versus *1/*1 after the second dose. Significantly higher INR in the first 6 weeks versus *1/*1 for *1/*2, *2/*2 and *2/*3, 98x INR ≥ 6.0, including 11% who experienced a bleeding event. Trend towards an increased risk of INR ≥ 6, significant for *2/*2 (RR 3.5). <u>Without co-medication</u> (754x): Significantly decreased dose versus *1/*1: - *1/*2: from 17.9 to 15.5 mg/wk - *1/*3: from 17.9 to 13.9 mg/wk - *2/*2: from 17.9 to 13.1 mg/wk - *2/*3: from 17.9 to 11.8 mg/wk	
ref. 27 Thijssen HH et al. Acenocoumarol pharmacokinetics in relation to cyto-chrome P450 2C9 genotype. Clin Pharmacol Ther 2003;7461-8.	3 *1/*2: AA *1/*3: A *2/*3: A *2/*2: AA	<u>1st study</u>: 26 healthy subjects, 9x *1/*1, 7x *1/*2, 6x *1/*3, 3x *2/*3, 1x *2/*2, single 8-mg acenocoumarol dose, measurements 4, 7, and 24 hours after administration, no co-medication; - *1/*2: no differences in concentration between both enantiomers versus *1/*1 after 4, 7 and 24 hours. - *1/*3: the S-acenocoumarol concentration after 7 hours increased from 5.4 to 14.6 ng/mL versus *1/*1 (S). Other time points and R-enantiomer: no significant differences versus *1/*1. - *2/*3: the S-acenocoumarol concentration after 7 hours increased from 5.4 to 16.6 ng/mL versus *1/*1 (S). Other time points and R-enantiomer: no significant differences versus *1/*1. - *2/*2: no differences in concentration between both enantiomers versus *1/*1 after 4, 7 and 24 hours. The S-acenocoumarol concentration after 24 hours was below the detection limit for all genotypes. <u>2nd study</u>: 6 healthy subjects, 3x *1/*1, 3x *1/*3, single 8-mg acenocoumarol dose, no co-medication; - *1/*3: The Cl_{or} of S-acenocoumarol decreased from 19.8 to 10.9 L/hr versus *1/*1 (S), the t½ increased from 1.0 to 2.0 hours (S). The AUC increased non-significantly from 205.9 to 388.9 h·µg/L. The AUC, Cl_{or} and t½ of R-acenocoumarol were non-significantly different versus *1/*1.	
ref. 28 Verstuyft C et al. Genetic and environmental risk	3	Case-control study including 75 cases (INR > 4.0) and 75 controls (INR ≤ 4.0), on acenocoumarol (41 cases and 41 controls) or warfarin or fluindione, co-medication affecting INR was present;	Authors' conclusion: 'In the present study, the CYP2C9

factors for oral anticoagulant overdose. Eur J Clin Pharmacol 2003;58:739-45. **ref. 28, continuation**	*1/*2 + *1/*3 + *2/*2 + *2/*3 + *3/*3: A	- The incidence of *2 and/or *3 was not significantly different between cases and controls for acenocoumarol and warfarin together. - For acenocoumarol: the mean daily dose did not differ significantly between *1/*1 and (*2 and/or *3) for the cases and the controls.	genetic polymorphism was not found to be a significant risk factor for oral anticoagulant overdose.' KNMP comment: A reason for not finding differences may be the limit of INR > 4.0.
ref. 29 Tassies D et al. Pharmacogenetics of acenocoumarol: cytochrome P450 CYP2C9 polymorphisms influence dose requirements and stability of anticoagulation. Haematologica 2002;87:1185-91.	3 *1/*2 + *2/*2: A *1/*3 + *2/*3: A	325 patients, target INR 2.5, constant acenocoumarol dose ≥ 3 controls, 169x *1/*1, 90x *1/*2, 48x *1/*3, 7x *2/*2, 11x *2/*3, co-medication not known; - *1/*2 or *2/*2: the maintenance dose decreased from 17.1 to 14.6 mg/wk versus *1/*1 (S). No differences in time within INR range, or in distribution of genotypes between dose groups. - *1/*3 or *2/*3: the maintenance dose decreased from 17.1 to 11.2 mg/wk versus *1/*1 (S). The time within INR range decreased from 75.1 to 64.7% versus *1/*1 (S). Of the 170 patients using ≤ 2 mg/day, 27.0% had a *3 allele, while this was 8.4% in the group who used > 2 mg/day (S, OR 4.77). Of the 45 patients using ≤ 1 mg/day, the OR was 3.12, which was a significant difference versus *1/*1. 43.9% had an INR > 4.5 and 17.1% an INR > 7.0 during the first 10 days, which was a significant increase versus non-*3 genotypes (11.6 and 0.01% respectively). The incidence of bleeding events was not increased. 84 patients known to have had bleeding events on acenocoumarol, target INR 2.5 linked to 84 controls without bleeding events; - No significant differences in dose and CYP2C9 genotype distribution between cases and controls. NOTE: alongside CYP2C9*3, age (> 70 years) was also a determinant for a lower acenocoumarol maintenance dose.	
ref. 30 Hermida J et al. Differential effects of 2C9*3 and 2C9*2 variants of cytochrome P-450 CYP-2C9 on sensitivity to acenocoumarol. Blood 2002;99:4237-9.	3 *1/*2 + *2/*2: A *1/*3 + *3/*3: A	108 patients, 93x *1/*1, 26x *1/*2, 3x *2/*2, 14x *1/*3, 1x *3/*3, target INR 2.0-3.2, constant acenocoumarol dose ≥ 3 months, co-medication not known; - *2: higher risk of lower acenocoumarol dose (corr. OR 2.70, 95% CI 1.11-1.17). - *3: higher risk of lower acenocoumarol dose (corr. OR 6.02, 95% CI 1.50-24.18).	
ref. 31 Verstuyft C et al. Early acenocoumarol overanticoagulation among cytochrome P450 2C9 poor metabolizers. Pharmacogenetics 2001;11:735-7.	2 *3/*3:D *3/*3:D	Patient 1, 18 years: INR= 9 without bleeding events after 3 days of 4 mg/day acenocoumarol. Dosing interrupted for 2 days then resumed at 0.5 mg/day gave INR 2-3. No co-medication. Genotype was *3/*3. Patient 2, 82 years: INR > 9 without bleeding events after 4 days of 4 mg/day acenocoumarol. Dosing interrupted for 3 days then resumed at 0.5 mg/day gave INR 2-3. The patient used the CYP2C9 inhibitor amiodarone (200 mg/day) + other co-medication. Genotype was *3/*3.	
ref. 32 Thijssen HH et al. Altered pharmacoki-	2 *3/*11:	Patient had an INR > 8 after a loading regimen of 4, 2 and 1 mg acenocoumarol. Stable INR of 2-3 after 5 weeks with dose regimen 1-1-0-1-1-0 mg/day.	Authors' conclusion: 'This case sug-

netics of R- and S-acenocoumarol in a subject heterozygous for CYP2C9*3. Clin Pharmacol Ther 2001;70:292-8. and Rettie AE et al. A case study of acenocoumarol sensitivity and genotype-phenotype discordancy explained by combinations of polymorphisms in VKORC1 and CYP-2C9. Br J Clin Pharmacol 2006;62:617-20.	D	Rettie et al.: the patient was *3/*11 and VKORC1 homozygous variant. Case-control study with this patient as the case, *3/*11, and 1 control, *1/*1. Single dose of 8 mg acenocoumarol, co-medication not known; - *3/*11: the S-acenocoumarol AUC increased from 140 to 2280 h·µg/L, the t½ from 1.8 to 8.1 h, and the Cl_{or} decreased from 28.5 to 1.8 L/h versus *1/*1. The R-acenocoumarol AUC increased from 2060 to 4090 h·µg/L, the t½ from 6.6 to 10.2 h, and the Cl_{or} decreased from 1.9 to 1 L/h.	gests that *CYP-2C9*11* should be included in routine test panels for genotyping of oral anticoagulant patients.'
ref. 33 Thijssen HH et al. The possession of the CYP2C9*3 allele is associated with low dose requirement of acenocoumarol. Pharmacogenetics 2000;10:757-60.	4 *1/*3 + *2/*3: A *1/*2: AA	35 patients, ≥ 3 months stable anticoagulant therapy on acenocoumarol, no relevant co-medication; - 13x dose ≤ 1 mg/day: 3x *1/*1, 2x *1/*2, 7x *1/*3, 1x *2/*3; the chance of *3 is significantly increased versus the 2-5 mg/day dose group (OR 24.3) and versus the ≥ 7 mg/day dose group (OR 17.0). The chance of *2 was NS different from the other two dose groups. The R-acenocoumarol C_{ss} decreased from 27.4 to 16.2 ng/mL versus the 2-5 mg/day dose group (NS). - 13x dose 2-5 mg/day: 9x *1/*1, 4x *1/*2; - 9x dose ≥ 7 mg/day: 8x *1/*1, 1x *1/*2; the R-acenocoumarol C_{ss} increased from 27.4 to 30.9 ng/mL versus the 2-5 mg/day dose group (NS).	

Risk group	Polymorphism for VKORC1, use of CYP2C9 inhibitors

Comments:

- After 2006, studies that only looked at an association with the maintenance dose, but in which the maintenance dose was not determined per genotype or genotype group (for example, genome-wide association or case-control studies) and cases that were identified based only on the INR were not included in the status report. The reason for this is that these articles supplied insufficient new data.
  The only articles included after 2010 are those that included more than 100 patients, as other articles supplied insufficient new data.
- **Dose algorithms:**
  Articles investigating dose algorithms were only included if the algorithm found was stated in the article.
  - 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:
    Log_{10} (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. PubMed PMID: 25519826.
An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 217 South-Indian patients. The algorithm was validated in the same cohort (by comparing the predicted doses with those predicted by a clinical algorithm in patients requiring either a low dose (≤ 10.5 mg/week), intermediate dose (≥ 10.5 mg/week and ≤ 35 mg/week) or high dose (≥ 35 mg/week)). The algorithm explained 61.5% of the variation in dose requirement. CYP2C9 *3 was responsible for 16.4% of the variation in dose requirement, while VKORC1 -1639G>A explained 28.6% of the variation in dose requirement.
The algorithm found was:
Log_{10} dose = 0.436 - 0.004*(age) + 0.018*(BMI) - 0.239*(VKORC1 -1639G>A) - 0.163*(CYP2C9*2) - 0.293*(CYP2C9*3) + 0.043*(CYP4F2) - 0.142*(GGCX) + 0.057*(VKORC1 rs7294)
- Cerezo-Manchado JJ et al. Creating a genotype-based dosing algorithm for acenocoumarol steady dose. Thromb Haemost 2013;109:146-153.
An algorithm for the acenocoumarol maintenance dose was developed on the basis of data from 973 patients. The validation cohort consisted of 2683 patients. The algorithm explained 48% of the variation in dose requirement. CYP2C9 was responsible for 5.7% of the variation in dose requirement, while VKORC1 explained 23% of the variation in dose requirement.
The algorithm found was:
√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 = √height in cm*(weight in kg)/3600
- Smires FZ et al. Influence of genetics and non-genetic factors on acenocoumarol maintenance dose requirement in Moroccan patients. J Clin Pharm Ther. 2012;37:594-8. PMID: 22486182.
See summary in the risk analysis. The authors developed the following algorithm:
Acenocoumarol dose (mg/week) = 28.32 /7.24 (if INR target between 3.0-4.0) or +14.48 (if INR target between 3.5-4.5) - 6.30*number of VKORC1 variant alleles – 7.57*number of CYP2C9 variant alleles.
This algorithm explained 36.2% of the dose variation.
- 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 a target INR of 2.0-3.5. The algorithm was validated in a cohort including 100 patients. The algorithm explained 41.4% of the variation in dose requirement. None of the CYP2C9 polymorphisms were significantly associated with acenocoumarol sensitivity or resistance. The minor influence of CYP2C9 in this algorithm may be explained by the low frequency of CYP2C9*2 and *3 in this population.
The algorithm found was:
Dose (mg/day) = 3.082 - 0.013*(smoking, 1 for smoker and 0 for non-smoker) - 0.433*(sex, 1 for male and 0 for female) - 0.004*(age in years) + indication (0.327 for mitral and aortic valve replacement and -0.092 for aortic valve replacement) + 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 *1/*2) - 0.456 (CYP2C9 *1/*3) + 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 a target INR of 2.0-3.5. The algorithm was validated in an independent dataset including 168 acenocoumarol users, of whom no height or weight parameters were known. As the acenocoumarol half-life is low, no separate loading dose is needed. The loading dose can therefore be calculated by multiplying the calculated maintenance dose per day by three and administering that quantity over the first 3 days of therapy. The algorithm explained 52.6% of the variation in dose requirement, and the CYP2C9 polymorphism explained 4.5% 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 CC) – 0.572 (if VKORC1 CT) – 1.267 (if VKORC1 TT) – 0.027 * age (years) + 0.271 (if female) + 0.009 * height (cm) + 0.010 * weight (kg) – 0.377 (if amiodarone user)
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: The median acenocoumarol doses predicted by the EU-PACT algorithm were significantly higher than the median stable doses for the 140 Greek patients who achieved stable acenocoumarol doses in the EU-PACT trial. The predicted doses were also significantly too high for the following subgroups: CYP2C9 *1/*1, CYP2C9 *1/*2, normal responders (patients having either no CYP2C9 and VKORC1 variant or one variant other than CYP2C9*3), sensitive responders (patients having either CYP2C9 *1/*3 or CYP2C9 *2/*2 in combination with no or one VKORC1 variants or CYP2C9 *2/*3 in combination with no VKORC1 variant or CYP2C9 *1/*2 in combination with one or two VKORC1 variants or CYP2C9 *1/*1 in combination with two VKORC1 variants), highly sensitive responders (patients having either CYP2C9 *3/*3 or having CYP2C9 *2/*3 in combination with one or two VKORC1 variants or CYP2C9 *1/*3 or CYP2C9 *2/*2 in combination with two VKORC1 variants).

- 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 high-dose or low-dose acenocoumarol was developed on the basis of data from 193 acenocoumarol users with a target INR 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)) obtained by adding up the number of wild-type alleles of five polymorphisms (CYP2C9*2, CYP2C9*3, VKORC1 -1639G>A, VKORC1 497T>G and VKORC1 1173C>T) and to express that number as a percentage of the maximum score. NOTE: as the authors did not consider that VKORC1 -1639G>A and VKORC1 1173C>T are linked, they inadvertently included the greater effect of this polymorphism in their algorithm.
  The mean AGS was significantly higher in the high-dose group (> 28 mg/week) than in the low-dose group (< 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 needing 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 a target INR 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 CC, 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)

- Cost-effectiveness
  Schalekamp et al., 2006 reports that there are various scenarios where the cost-effectiveness of CYP2C9-based acenocoumarol therapy could be plausible:
  "The marginal cost to avoid 1 major bleeding episode by CYP2C9 genotyping appears to be sensitive to a number of parameters. Some of these parameters are virtually unknown (reduction of major bleeding rate in carriers of a CYP2C9 polymorphism), vary between populations (major bleeding rate in wild-type subjects and prevalence of CYP2C9 polymorphisms), or change in time (cost of genotyping). These uncertainties, especially the ability to reduce the major bleeding rate by CYP2C9 genotyping, prevent us from concluding unequivocally that CYP2C9 genotyping is valuable in addition to INR monitoring in anticoagulation clinics. However, our base case example, our sensitivity analyses, and our threshold analysis all show that, even in a setting characterized by intensive INR monitoring, CYP2C9 genotyping could be a cost-effective strategy under certain circumstances and a potentially useful addition to INR monitoring."

Date of literature search: 26 January 2018.

	Genotype	Code	Gene-drug interaction	Action	Date
Dutch Pharmacogenetics Working Group decision	*1/*2	4 F	Yes	No	14 May 2018
	*1/*3	4 F	Yes	No	
	*2/*2	4 F	Yes	No	
	*2/*3	4 F	Yes	No	
	*3/*3	4 F	Yes	No	

	IM	4 F	Yes	No	
	PM	4 F	Yes	No	

Mechanism:
Acenocoumarol consists of a racemic mixture. The anticoagulant effect of the S-enantiomer is more potent than that of the R-enantiomer. However, the S-enantiomer is eliminated more rapidly, which makes the R-enantiomer predominantly responsible for the anticoagulant effect.
The S-enantiomer is almost fully metabolised by CYP2C9 by hydroxylation. The R-enantiomer is metabolised by CYP1A2, CYP3A4, CYP2C9 and CYP2C19.
A genetic polymorphism in CYP2C9 leads to decreased metabolic capacity of the enzyme, which may cause increased S-acenocoumarol plasma concentrations and to a lesser extent increased R-acenocoumarol plasma concentrations.