

## Pharmacogenetics of acenocoumarol: cytochrome P450 CYP2C9 polymorphisms influence dose requirements and stability of anticoagulation

DOLORS TÀSSIES, CAROLINA FREIRE, JOSEFINA PIJOAN, SANTIAGO MARAGALL, JOAN MONTEAGUDO, ANTONI ORDINAS, JOAN CARLES REVERTER

Hemotherapy and Hemostasis Department. Hospital Clinic and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

**Background and Objectives.** Cytochrome P450 CYP2C9 (CYP2C9) is the main enzyme implicated in coumarinic metabolism. Variant alleles, CYP2C9\*2 and CYP2C9\*3, have been related to decreased enzymatic activity, but their clinical relevance in acenocoumarol metabolism has not been established. We investigated CYP2C9 polymorphisms in relation to acenocoumarol dose requirement, stability of anticoagulation and bleeding.

**Design and Methods.** CYP2C9 genotyping was performed in 325 acenocoumarol-treated patients (INR target between 2.0 and 3.0) and in an additional group of 84 patients with repeated bleeding.

**Results.** Patients with the wild-type CYP2C9\*1/\*1 genotype (n=169) required a higher maintenance dose of acenocoumarol (17.1±8.7 mg/week) than did patients with the CYP2C9\*2 (14.6±6.4 mg/week,  $p<0.05$ , N=97) or the CYP2C9\*3 allele (11.2±6.2 mg/week,  $p<0.001$ , n=59). Out of 170 patients requiring a low-dose of acenocoumarol ( $\leq 2$  mg/day), 27.1% carried the CYP2C9\*3 allele, while among the patients requiring higher doses, 8.4% had CYP2C9\*3 (OR=4.77, 95% CI = 2.40-9.48,  $p<0.001$  vs. CYP2C9\*1/\*1 patients). In the multivariate analysis, independent predictive variables for low-dose acenocoumarol requirements were age >70 years (OR=3.73, 95%CI=2.29-6.08,  $p<0.001$ ), and the CYP2C9\*3 allele (OR=4.75, 95%CI=2.36-9.55,  $p<0.001$ ). Carriers of CYP2C9\*3 spent less time within the therapeutic range (64.7±23.1%) than did patients with the CYP2C9\*1/\*1 genotype (75.1±22.0%,  $p<0.01$ ), and more frequently had an INR >4.5 at the initiation of treatment (43.9% vs. 11.6%,  $p<0.001$ ), but did not show repeated bleeding more frequently (19.0% vs. 15.5%,  $p=NS$ ).

**Interpretation and Conclusions.** CYP2C9\*3 is related to lower acenocoumarol dose requirements, a higher frequency of over-anticoagulation at the initiation of therapy and an unstable anticoagulant response.

©2002, Ferrata Storti Foundation

Key words: oral anticoagulants, acenocoumarol, CYP2C9, polymorphism, pharmacogenetics.

## Thrombosis

research paper

**haematologica** 2002; 87:1185-1191

[http://www.haematologica.org/2002\\_11/1185.htm](http://www.haematologica.org/2002_11/1185.htm)



Correspondence: Dr. Dolors Tàssies, MD, Hemotherapy and Hemostasis Department, Hospital Clinic, Villarroel 170, 08036 Barcelona, Spain. Phone: international +34.9.32275439. Fax: international +34.9.32279369. E-mail: dtassies@clinic.ub.es

Treatment with oral anticoagulants has improved the prognosis of patients with thromboembolic disease. However, optimal oral anticoagulation control is usually hampered by significant interindividual variability in dose requirements for a given target level of anticoagulation. Extreme sensitivity to coumarinics may make it difficult to achieve the appropriate dosage and anticoagulation stability, and, consequently, predispose patients to bleeding complications.<sup>1</sup> With the development of pharmacogenetics, there is increasing evidence that interindividual sensitivity to coumarinics may be largely genetically determined.<sup>2</sup> Polymorphisms of the cytochrome P450 2C9 enzyme family (CYP2C9) have recently been associated with impaired elimination of warfarin and over-anticoagulation in certain patients.<sup>3</sup> Warfarin is available as a racemic mixture of two enantiomers (R)- and (S)-. The clinical effect of warfarin relies mainly on (S)-warfarin, which is pharmacologically more active than (R)-warfarin. (S)-warfarin is mainly metabolized by CYP2C9.<sup>4</sup> Allelic variants of CYP2C9, CYP2C9\*2 (Arg144Cys) and CYP2C9\*3 (Ile359Leu), have less catalytic activity than the wild type CYP2C9\*1 (Arg144/Ile359).<sup>5,6</sup> Both alleles, CYP2C9\*2 and CYP2C9\*3, have been associated with decreased warfarin dose requirements.<sup>3,7-10</sup> A relationship between this polymorphism and increased risk of bleeding in patients receiving warfarin has been reported in some studies but not confirmed by others.<sup>3,7,9</sup> While warfarin is more frequently prescribed in the United States and the United Kingdom, acenocoumarol is the oral anticoagulant widely used in continental Europe. Racemic acenocoumarol has several pharmacologically significant differences in regard to warfarin. It has a half-life about four times shorter than that of warfarin. Furthermore, the very rapid metabolism of (S)-acenocoumarol (half-life less than 2 hours) makes the therapeutic effect almost exclusively due to (R)-acenocoumarol.<sup>11</sup> (S)-acenocoumarol, like (S)-warfarin, is metabolized almost exclusively by CYP2C9,

while (R)-acenocoumarol is metabolized mainly by CYP2C9 but CYP2C19 and CYP1A2 also take part.<sup>12</sup> The extent to which CYP2C9 contributes to the overall clearance of warfarin and acenocoumarol is also different. CYP2C9 is responsible for about 85% of the clearance of (S)-warfarin, while it accounts for only about 40% of the clearance of the clinically significant (R)-enantiomer of acenocoumarol.<sup>13</sup> Consequently, warfarin and acenocoumarol may differ in their propensity to interact with other CYP2C9 substrates or inhibitors, and genetic CYP2C9 variants may, in a different manner, affect the metabolism of these drugs.

At present, the significance of the CYP2C9 polymorphism on acenocoumarol dose requirements is less known than for warfarin. Moreover, the contribution of CYP2C9 polymorphism to the risk of bleeding or dosage stability in patients receiving acenocoumarol has not been established. The aim of the present study was to investigate the effect of the CYP2C9 polymorphism on dose requirements, anticoagulation stability and bleeding complications in a cohort of acenocoumarol-treated patients.

### Design and Methods

The Oral Anticoagulation Unit in the Hospital Clínic of Barcelona is attended by more than 3,500 patients on acenocoumarol therapy. In this unit, patients are monitored by their international normalized ratio (INR), the dose of acenocoumarol is prescribed and the date for the next control is fixed. Data from patients treated with oral anticoagulants have been recorded in a computer program since 1993. Patients are examined by medical staff at the start of oral anticoagulation, are instructed about acenocoumarol management, and followed-up in the case of any relevant event such as concomitant illness, bleeding complications, surgical, invasive or odontological procedures or changes in medication. Data on every INR determination and clinical event are registered by computer.

### Patients

After providing informed consent, patients with a target INR of 2.5 (therapeutic range from 2.0 to 3.0), and a stable acenocoumarol dose requirement for at least the three last controls, not taking any medication known to interfere with acenocoumarol, and without significant liver or cardiac dysfunction were consecutively selected for CYP2C9 genotyping from among the patients seen in the Anticoagulation Unit during a one-week period. Data on the INR determinations, acenocoumarol dose and bleeding episodes were obtained from computer files. In order to evaluate the risk of over-anticoagulation at the

initiation of acenocoumarol therapy in relation to the CYP2C9 polymorphisms, genotyped patients followed in our institution from the initiation of their therapy were selected. INR values and clinical events at the initiation of acenocoumarol therapy were obtained from computer files. These patients received acenocoumarol in a homogeneous protocol (9 mg in the first 72 hours).

In order to establish the relationship between bleeding complications and the CYP2C9 polymorphisms we selected a group of 84 patients treated with acenocoumarol with a target INR of 2.5 known to have had repeated bleeding, from the whole group of anticoagulated patients attending our institution, and an additional control group of 84 acenocoumarol-treated patients without bleeding matched for age, sex, and reason for anticoagulation.

### Samples

Samples were obtained at the patients' regular appointments for INR testing. No additional blood was extracted for the study. Venous blood samples were drawn from a clean antecubital venipuncture and collected in tubes containing 3.8% trisodium citrate (1:9, vol:vol) (Becton Dickinson, Rutherford, NJ, USA). For genotype studies, 100  $\mu$ L of whole blood were transferred into tubes containing lysis buffer (5 mol/L guanidine thiocyanate, 1.3% (w/v) Triton X-100, and 50 mmol/L Tris/HCl, pH 6.4), and frozen at  $-80^{\circ}\text{C}$  until tested. For INR determinations, platelet-free plasma was obtained by centrifugation at 2,000 g for 10 min at  $22^{\circ}\text{C}$ .

Prothrombin time was measured on an automated CA 6000 coagulometer (Dade Behring, Marburg, Germany) using human placental thromboplastin (Thromborel, Dade Behring). The INR was calculated by the formula  $\text{INR} = (\text{patient time}/\text{control time})^{\text{ISI}}$ , where ISI is the international sensitivity index.

### DNA studies

Genomic DNA was extracted from 100  $\mu$ L of whole blood by a silica gel column method (QIAamp DNA blood mini kit, Qiagen GmbH, Hilden, Germany). For detection of the CYP2C9\*2 and CYP2C9\*3 variants a protocol based on a polymerase chain reaction (PCR) technique and endonuclease digestion was used with minor modifications.<sup>14</sup> For CYP2C9\*2 (T<sub>416</sub>→C) the following primers were used: 5'-GGA TAT GAA GCA GTG AAG GAA-3', forward primer, position 221-241, and 5'-GGC CTT GGT TTT TCT CAA CTC-3', reverse primer, position 469-449. PCR was carried out on 25  $\mu$ L volume samples, in a Techne Progene thermal cycler (Techne, Cambridge, UK) with 35 cycles at  $95^{\circ}\text{C}$  for 50 sec,  $61^{\circ}\text{C}$  for 1

min, and 72°C for 3 min, and a final extension cycle of 7 min at 72°C. After amplification, 5 µL of the PCR product were digested overnight at 37°C with 10U of the A<sub>va</sub>I restriction enzyme (Roche Diagnostics, Basel, Switzerland). The digested product was analyzed in a 2.8% agarose gel electrophoresis (Metaphor, FMC Bioproducts, Rockland, ME, USA) and visualized under UV light after staining with ethidium bromide. Samples with T<sub>416</sub> had a single 429 bp band, while samples with C<sub>416</sub> gave 363 and 57 bp bands.

For CYP2C9\*3 allele amplification a mismatched PCR primer was used that creates a Nsi I restriction site when an A in 1061 is present: 5'-TGC ACG AGG TCC AGA GAT GC-3', forward primer position 1041-1060. Reverse primer: 5'-AAA CAT GGA GTT GCA GTG TAG-3' position (+36)-(+16) downstream from the exon 7/intron 7 junction. PCR was carried out on 25 µL volume samples, with 35 cycles at 95°C for 50 sec, 59°C for 1 min, and 72°C for 2 min, and a final extension cycle of 7 min at 72°C. After amplification, 5 µL of the PCR product were digested overnight at 37°C with 10U of the Nsi I restriction enzyme (Roche Diagnostics). The digested product was analyzed in a 4% agarose gel electrophoresis. Samples with A<sub>1061</sub> showed 110 bp and 21 bp bands.

#### Statistical analysis

Results are shown as mean±SD and/or as median and ranges. Odd ratios (OR) were calculated and the 95% confidence intervals (95% CI) were determined by Woolf's method.<sup>15</sup> Comparisons were made by the  $\chi^2$  test or by the analysis of variance followed by Bonferroni's test. Multivariate analysis was performed by the logistic regression method using the BMDP statistical software package for PC. The percentage of time spent within the therapeutic range was determined by the step method proposed by Rosendaal *et al.*<sup>16</sup>

## Results

### Study group

A total of 325 consecutive patients who fulfilled the selection criteria were genotyped for the CYP2C9 polymorphisms. There were 180 men and 145 women, with a mean age of 70.2±12.3 years. Patients were receiving acenocoumarol for treatment of deep venous thrombosis (DVT) and pulmonary embolism (PE) (15.7%), primary prophylaxis of cardioembolic disease (56.6%), secondary prophylaxis of thromboembolism (24.9%), or for other reasons (2.8%). The mean duration of anticoagulant therapy was 40.1±29.8 months. The distribution of the CYP2C9 genotype was as follows:

CYP2C9\*1/\*1: 169 patients (52.0%); CYP2C9\*1/\*2: 90 patients (27.7%); CYP2C9\*1/\*3: 48 patients (14.8%); CYP2C9\*2/\*2: 7 patients (2.2%); and CYP2C9\*2/\*3: 11 patients (3.4%); no patient was homozygous for allele CYP2C9\*3. The allele frequencies were 0.73 for CYP2C9\*1, 0.18 for CYP2C9\*2, and 0.09 for CYP2C9\*3.

### CYP2C9 polymorphisms and maintenance dose of acenocoumarol

The acenocoumarol dose required to achieve a therapeutic INR was very variable. In the whole series, the median dose was 13 mg/week (range 2.3 to 61 mg/week), and the mean dose was 15.3±8.0 mg/week. The doses of acenocoumarol in relation to the CYP2C9 genotype are depicted in Table 1 and Figure 1. Patients with the CYP2C9\*1/\*1 wild-type genotype (n=169) required a significantly higher maintenance dose of acenocoumarol (17.1±8.7 mg/week) than patients with the CYP2C9\*2 allele (2C9\*1/\*2 plus 2C9\*2/\*2 genotypes, n=97) (14.6±6.4 mg/week,  $p<0.05$ ) or patients carrying the CYP2C9\*3 allele (2C9\*1/\*3 and 2C9\*2/\*3 genotypes, n= 59) (11.2±6.2 mg/week,  $p<0.001$ ).

When we considered patients requiring a weekly acenocoumarol dose of 14 mg (2 mg/day) or less as a *low-dose* group, and those requiring a weekly acenocoumarol dose of 7 mg (1 mg/day) or less as a *very low-dose* group, in the whole series, 170 patients were in the *low-dose* group, and 45 patients were in the *very low-dose* group. In the former group, 46 patients (27.1%) were carriers of the CYP2C9\*3 allele, while only 13 (8.4%) of the patients requiring higher doses had this allele ( $p<0.001$  in comparison to the CYP2C9\*1/\*1 patients) (Table 2). However, no significant differences were seen for the CYP2C9\*2 allele carriers (Table 2). Similarly, in the *very low-dose* group, 16 patients (35.6%) were carriers of the CYP2C9\*3 allele, in contrast to 43 (15.4%) in the group requiring higher doses ( $p<0.005$  in comparison to the CYP2C9\*1/\*1 patients) (Table 2). No significant differences were seen for the CYP2C9\*2 allele carriers. In the multivariate analysis, independent predictive variables for requiring a *low dose* of acenocoumarol were older age (> 70 years) (OR=3.73, 95%CI: 2.29-6.08,  $p<0.001$ ), and the presence of the CYP2C9\*3 allele (OR= 4.75, 95%CI: 2.36-9.55,  $p<0.001$ ). For *very low-dose* requirements of acenocoumarol, the predictive variables were again older age (> 70 years) (OR= 2.98, 95%CI: 1.50-7.23,  $p<0.001$ ), and the CYP2C9\*3 allele (OR= 2.33, 95%CI: 1.64-6.89,  $p<0.001$ ). There were no significant differences in CYP2C9\*3 allele prevalence between the patients

**Table 1. CYP2C9 genotype and maintenance dose of acenocoumarol.**

CYP2C9	N	Mean dose (mg/week)	SD	Median dose (mg/week)	Range
*1/*1	169	17.1	8.7	15.8	2.3- 61
*1/*2	90	14.4	6.3	13.5	3.5- 37.3
*1/*3	48	11.0	5.1	10.5	2.3- 22
*2/*2	7	17.0	8.1	13.0	8.2- 27
*2/*3	11	12.4	9.9	11.6	2.6- 32.6
total	325	15.27	8.0	13	2.3- 61

aged 70 years or less (18.7%) and those aged more than 70 years (17.8%).

#### CYP2C9 polymorphisms and stability of anticoagulation

Considering the time spent within or outside the therapeutic range, carriers of the CYP2C9\*3 allele had an INR within the therapeutic range for a lower percentage of time ( $64.7 \pm 23.1\%$ ) than did patients with the CYP2C9\*1/\*1 genotype ( $75.1 \pm 22.0\%$ ,  $p < 0.01$ ). However, in the patients with the CYP2C9\*2 allele (2C9\*1/\*2 plus 2C9\*2/\*2 genotypes) there were no significant differences in stability of anticoagulation ( $71.0 \pm 20.5\%$  of time with INR within the therapeutic range) in comparison to that of the CYP2C9\*1/\*1 genotype patients.

A total of 222 of the genotyped patients have been followed up in our institution since the initiation of their anticoagulant therapy. Among the carriers of the CYP2C9\*3 allele ( $n = 41$ ), 18 (43.9%) had at least one INR value over 4.5 during the first 10 days following initiation of their therapy, and 7 (17.1%) had at least one INR value over 7.0. In contrast, among the 181 non-carriers of the CYP2C9\*3 allele, 21 (11.6%) had at least one INR value over 4.5 during the first days of therapy ( $p < 0.001$ ), and only one (0.01%) had at least one INR value over 7.0 ( $p < 0.001$ ). However, this greater frequency of over-anticoagulation at the initiation of therapy in carriers of CYP2C9\*3 was not accompanied by a greater number of bleeding episodes.

#### CYP2C9 polymorphisms and bleeding complications

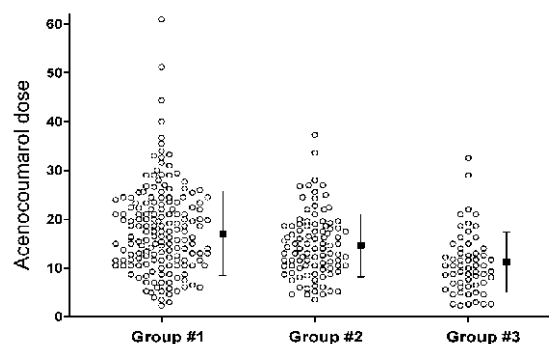
Of the selected 84 patients known to have had repeated bleeding complications, 39 were men and 45 were women: their mean age was  $71.3 \pm 11.1$  years (median = 74 years, range: 24-88). Bleeding episodes were muco-cutaneous (60%), genito-urinary (18%), digestive (15%), muscular (5%), articular (1%), and mediastinal (1%). INR at the time of bleeding was available for 91% of events. Of them

**Table 2. CYP2C9 genotype and low-dose acenocoumarol requirements.**

CYP2C9	mg/week		OR (95% CI)	mg/week		OR (95% CI)
	$\leq 14$ N= 170	$> 14$ N= 155		$\leq 7$ N= 45	$> 7$ N= 280	
*1/*1	72	97	—	18	151	—
*1/*2 + *2/*2	52	45	1.56 (0.94-2.57)	11	86	1.07 (0.48-2.38)
*1/*3 + *2/*3	46	13	4.77 (2.40-9.48)	16	43	3.12 (1.47-6.63)

46.2% had an INR above the therapeutic range, while 53.8% had an INR within the therapeutic range. The mean INR at the time of bleeding was  $3.41 \pm 2.38$ . Patients were receiving acenocoumarol for treatment of DVT-PE (16.7%), primary prophylaxis for cardioembolic events (53.5%) or secondary prophylaxis (29.8%). The selected control group was constituted by 84 patients on acenocoumarol therapy without bleeding complications and was formed of 39 men and 45 women with a mean age of  $71.3 \pm 11.1$  years (median = 74 years, range: 24-88).

Controls were receiving acenocoumarol for the treatment of DVT-PE (16.7%), primary prophylaxis for cardioembolic events (53.5%), and secondary prophylaxis (29.8%). The mean time on acenocoumarol treatment was  $43.5 \pm 30.9$  months in the group of repeated bleeding patients and  $45.8 \pm 31.6$  months in the control group. In repeated bleeding patients, the mean dose of acenocoumarol was  $13.3 \pm 6.1$  mg/week (median = 12.3 mg/week, range: 2.6-37.3), whereas it was  $14.2 \pm 7.9$  mg/week (median = 12.3 mg/week range: 2.6-40.0) ( $p = ns$ ) in control patients. CYP2C9 genotype distribution was similar



**Figure 1. Mean dose of acenocoumarol (mg/week) in relation to CYP2C9 alleles. Group #1: CYP2C9\*1/\*1. Group #2: CYP2C9\*1/\*2 and CYP2C9\*2/\*2. Group #3: CYP2C9\*1/\*3 and CYP2C9\*2/\*3. Squares are means and error bars are standard deviations.**

in both groups of patients. In the group with repeated hemorrhage the genotype was CYP2C9\*1/\*1 in 39 patients (46.4%), CYP2C9\*1/\*2 in 26 (31.0%), CYP2C9\*1/\*3 in 14 (16.7%), CYP2C9\*2/\*2 in 3 (3.6%), and CYP2C9\*2/\*3 in 2 (2.4%). In the control group it was CYP2C9\*1/\*1 in 44 patients (52.4%), CYP2C9\*1/\*2 in 25 (29.8%), CYP2C9\*1/\*3 in 9 (10.7%), CYP2C9\*2/\*2 in 2 (2.4%), and CYP2C9\*2/\*3 in 4 (4.8%) ( $p=ns$ ).

## Discussion

Optimal monitoring of acenocoumarol treatment is hampered by broad interindividual variability in maintenance dose requirements, ranging by more than 20 fold in our series. This fact, as well as the narrow therapeutic index of acenocoumarol puts patients at risk of overdosage during the period of establishing the individualized dose of anticoagulant. Identification of factors influencing this variability is of great importance to provide accurate dosing and to prevent adverse effects.

CYP2C9 is the major enzyme involved in the metabolism of the oral anticoagulants warfarin, phenprocoumon, and acenocoumarol.<sup>13,17</sup> Allelic variants, CYP2C9\*2 and CYP2C9\*3, have been reported to have decreased enzymatic activity in the metabolism of various substrates, including warfarin.<sup>5,6,18,19</sup> Likewise, CYP2C9\*3 allele has been related to a lower oral clearance and to an increased half-life of elimination of (S)-acenocoumarol.<sup>20</sup> From a clinical point of view, allelic variants of CYP2C9 have been associated with increased sensitivity to warfarin. Significantly lower warfarin dose requirements have been reported for patients carrying either the CYP2C9\*2 or the CYP2C9\*3 polymorphism.<sup>3,7-10,21,22</sup> Moreover, isolated cases of extreme sensitivity to warfarin have been reported in homozygous CYP2C9\*3 patients<sup>23,24</sup> and one CYP2C9\*2/\*3 double heterozygous patient,<sup>25</sup> although in some of these patients additional factors such as advanced age may have been involved in the hypersensitivity.

The influence of the CYP2C9 polymorphisms on sensitivity to acenocoumarol therapy is less known. Thijssen *et al.*<sup>26</sup> recently reported a higher prevalence of CYP2C9\*3 in a group of 13 patients requiring a low dose of acenocoumarol compared to in 22 patients requiring higher doses. In addition, two patients homozygous for CYP2C9\*3 who were over-anticoagulated early after the initiation of acenocoumarol therapy have been reported.<sup>27</sup> In our series we found that heterozygous CYP2C9\*3 patients required a significantly lower dose of acenocoumarol to achieve the target INR than did patients

with the wild type CYP2C9\*1/\*1 genotype, while the effect of CYP2C9\*2 allele did not seem to be clinically relevant.

Our results are in keeping with those recently published by Hermida *et al.*,<sup>28</sup> who found a minor effect of CYP2C9\*2 allele on the dose of acenocoumarol necessary to provide anticoagulation. The weak effect on acenocoumarol sensitivity exerted by the CYP2C9\*2 allele contrasts with the situation for warfarin, given that low dose requirements of this latter drug have also been associated with the CYP2C9\*2 allele,<sup>3,8,9</sup> and suggests that the function of this enzyme variant with respect to acenocoumarol metabolism is not significantly impaired.

The sensitivity to acenocoumarol in CYP2C9\*3 carriers may be attributed to a diminished metabolism of (S)-acenocoumarol.<sup>26</sup> Interestingly, (S)-acenocoumarol is clinically inactive because of its extremely short half-life. Consequently, polymorphisms diminishing the rate of metabolism of (S)-acenocoumarol may produce an accumulation of this potent enantiomer. As deduced from our study, it is sufficient to have only one CYP2C9\*3 allele in order to have a clinically increased sensitivity to acenocoumarol. In keeping with this observation, Thijssen *et al.* recently demonstrated a very reduced metabolism of (S)-acenocoumarol in one patient heterozygous for CYP2C9\*3 who was hypersensitive to acenocoumarol.<sup>20</sup>

In addition to the CYP2C9\*3 allele, older age was also an independent variable related to a lower acenocoumarol requirement in our series. It is well-known that elderly patients are particularly sensitive to oral anticoagulants, requiring an estimated reduction in the maintenance dose of warfarin of 0.5 mg/day per decade.<sup>29</sup> The mechanism of this increased sensitivity is poorly understood but several pharmacokinetic and pharmacodynamic changes may be implicated.<sup>29</sup> The presence of the CYP2C9\*3 polymorphism may make older patients especially prone to acenocoumarol overdose. Since, in recent years, there has been a marked increase in the indications for oral anticoagulants among the elderly, CYP2C9 genotyping would help to identify a risk group of patients hypersensitive to acenocoumarol.

There is scarce information on whether the presence of CYP2C9 polymorphisms influences the stability of anticoagulant response. Initial studies reported a greater difficulty in establishing the warfarin dose at the initiation of therapy in patients carrying CYP2C9 polymorphisms,<sup>3</sup> and a recent study seems to confirm the effect of any allele variant on the risk of above-range INRs.<sup>30</sup> We found that establishing an adequate dose of aceno-

coumarol at the initiation of anticoagulation was more difficult in patients who are carriers of the CYP2C9\*3 allele, who presented above-range INR values more frequently. In contrast, in a large study performed by Taube *et al.*<sup>8</sup> in patients on maintenance treatment with warfarin, having a variant allele did not increase the likelihood of severe over-anticoagulation or stability of the anticoagulation. In our study, carriers of the CYP2C9\*3 allele spent a significantly less time with an INR within the therapeutic range of anticoagulation than did patients with the wild-type genotype. Reduced clearance of acenocoumarol in patients with the CYP2C9\*3 allele may make them more susceptible to even slight environmental changes that would affect the remaining genotypes to a lesser extent.

Bleeding complications have been related to CYP2C9 polymorphisms in several studies in patients receiving warfarin. Aithal *et al.*<sup>3</sup> found that patients stabilized on low doses of warfarin who had one or more CYP2C9 allele variant were four times more likely to develop major bleeding complications.

Similarly, in an study including 180 patients, Margaglione *et al.*<sup>9</sup> found that patients with both local sites of potential bleeding and CYP2C9 allele variants had a higher risk of bleeding, and recently Higashi *et al.*<sup>30</sup> reported an increased risk of bleeding in carriers of one or more CYP2C9 allele variant.

These results were not, however, confirmed by a large study (including 233 patients) in which heterozygous CYP2C9\*3 patients did not have more bleeding complications.<sup>7</sup>

To our knowledge, the influence of CYP2C9 polymorphisms on bleeding complications in patients on acenocoumarol therapy has not yet been reported. We found no association between repeated bleeding episodes and CYP2C9 polymorphisms. Owing to the controversial results reported concerning bleeding complications, it has been argued that the risk of bleeding may be especially high at the initiation of therapy in carriers of CYP2C9 polymorphisms due to unexpected over-dosing.<sup>3</sup> Indeed, we found that carriers of the CYP2C9\*3 allele were more prone to suffer over-anticoagulation at the initiation of therapy, although this did not result in a higher rate of bleeding. The lack of agreement among studies on the risk of bleeding and CYP2C9 polymorphisms may have several explanations, including differences in selected populations and the presence of additional predisposing bleeding factors: furthermore, in our study a different coumarinic was evaluated. As bleeding in carriers of CYP2C9 variants may be attributed to the more frequent and longer periods during which these

patients have high INR values, management of over-anticoagulation in patients treated with acenocoumarol may be easier because of the shorter half-life of this compound and partially explain why CYP2C9\*3 was not related to bleeding in our series.

Finally, some confounding factors on the pharmacogenetics of oral anticoagulants remain to be analyzed. Among the group of patients requiring the lower doses of acenocoumarol, more than 40% had the wild-type genotype (CYP2C9\*1/\*1). In some, lower dose requirements may be attributed to an age greater than 70 years, but it is possible that additional unidentified CYP2C9 variants may be present in the population. In fact, CYP2C9\*4,<sup>31</sup> and CYP2C9\*5<sup>32</sup> allele variants, related to reduced catalytic enzyme efficiency, have recently been described, although these alleles are not found in Caucasians. In addition, the CYP2C9\*3 genotype induces an accumulation of (S)-acenocoumarol, but there is still a dependent anticoagulant effect of (R)-acenocoumarol, which is also metabolised by CYP2C19 and CYP1A2. Thus, polymorphisms in other cytochromes may also affect acenocoumarol metabolism. In conclusion, genotyping for the CYP2C9\*3 polymorphism may be useful for predicting sensitivity to acenocoumarol treatment. This may be important in selected groups of patients, such as elderly people or individuals at a high risk of bleeding, who can take advantage of optimized monitoring in order to reduce the risk of over-anticoagulation and thus, increase the safety and efficacy of acenocoumarol therapy.

#### Contributions and Acknowledgments

DT and JCR designed the study, interpreted the results and wrote the paper. CF, JP and DT performed the laboratory studies. DT, SM, JM and JCR recruited the patients, were the clinicians responsible for the management of the patients and acquired the data. AO is the head of the department and gave the final approval for the manuscript's submission. All the authors critically revised the manuscript.

#### Disclosures

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

#### References

1. Hirsh J, Dalen JE, Anderson DR, Poller L, Bussey H, Ansell J, et al. Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. *Chest* 2001; 119:8S-21S.
2. Meyer UA. Pharmacogenetics and adverse drug reactions. *Lancet* 2000; 1:1667-71.
3. Aithal GP, Day CP, Kesteven P, Daly AK. Association of

- polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999; 353:717-9.
4. Rettie AE, Korzekwa KR, Kunze KL, Lawrence RF, Eddy AC, Aoyama T, et al. Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in etiology of (S)-warfarin-drug interactions. *Chem Res Toxicol* 1992; 5:54-9.
  5. Rettie AE, Wienkers LC, Gonzalez FJ, Trager WF, Korzekwa KR. Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. *Pharmacogenetics* 1994; 4:39-42.
  6. Yamazaki H, Inoue K, Chiba K, Ozawa N, Kawai T, Suzuki Y et al. Comparative studies on the catalytic roles of cytochrome P450 2C9 and its Cys- and Leu-variants in the oxidation of warfarin, flurbiprofen and diclofenac by human liver microsomes. *Biochem Pharmacol* 1998; 56: 243-51.
  7. Ogg M, Brennan P, Meade T, Humphries SE. CYP2C9\*3 allelic variant and bleeding complications. *Lancet* 1999; 354:1124.
  8. Taube J, Halsall, Baglin T. Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. *Blood* 2000; 96:1816-9.
  9. Margaglione M, Colaizzo D, D'Andrea G, Brancaccio V, Ciampa A, Grandone E, et. al. Genetic modulation of oral anticoagulation with warfarin. *Thromb Haemost* 2000; 84:775-8.
  10. Freeman BD, Zehnbauer BA, McGrath S, Borecki I, Buchman TG. Cytochrome P450 polymorphisms are associated with reduced warfarin dose. *Surgery* 2000; 128:281-5.
  11. Godbillon J, Richard J, Gerardin A, Meinertz T, Kasper W, Jahnchen E. Pharmacokinetics of the enantiomers of acenocoumarol in man. *Br J Clin Pharmacol* 1981; 12:621-9.
  12. Thijssen HH, Flinois JP, Beaune PH. Cytochrome P4502C9 is the principal catalyst of racemic acenocoumarol hydroxylation reactions in human liver microsomes. *Drug Metab Dispos* 2000; 28:1284-90.
  13. Hermans JJ, Thijssen HH. Human liver microsomal metabolism of the enantiomers of warfarin and acenocoumarol: P450 isozyme diversity determines the differences in their pharmacokinetics. *Br J Pharmacol* 1993; 110:482-90.
  14. Wang SL, Huang JD, Lai MD, Tsai JJ. Detection of CYP2C9 polymorphisms based on the polymerase chain reaction in Chinese. *Pharmacogenetics* 1995; 5:37-42.
  15. Woolf B. On estimating the relation between blood group and disease. *Ann Human Genet* 1955; 19:251-3.
  16. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briet E. A method to determine the optimal intensity of oral anticoagulant therapy. *Thromb Haemost* 1993; 69:236-9.
  17. He M, Korzekwa KR, Jones JP, Rettie AE, Trager WF. Structural forms of phenprocoumon and warfarin that are metabolized at the active site of CYP2C9. *Arch Biochem Biophys* 1999; 372:16-28.
  18. Crespi CL, Miller VP. The R144C change in the CYP2C9\*2 allele alters interaction of the cytochrome P450 with NADPH:cytochrome P450 oxidoreductase. *Pharmacogenetics* 1997; 7:203-10.
  19. Takanashi K, Tainaka H, Kobayashi K, Yasumori T, Hosakawa M, Chiba K. CYP2C9 Ile359 and Leu359 variants: enzyme kinetic study with seven substrates. *Pharmacogenetics* 2000; 10:95-104.
  20. Thijssen HHW, Drittij MJ, Vervoort LMT, de Vries-Hanje JC. Altered pharmacokinetics of R- and S- acenocoumarol in a subject heterozygous for CYP2C9\*3. *Clin Pharmacol Ther* 2001; 70:292-8.
  21. Furuya H, Fernandez-Salguero P, Gregory W, Taber H, Steward A, Gonzalez FJ, et al. Genetic polymorphism of CYP2C9 and its effect on warfarin maintenance dose requirement in patients undergoing anticoagulation therapy. *Pharmacogenetics* 1995; 5:389-92.
  22. Takahashi H, Kashima T, Nomizo Y, Muramoto N, Shimizu T, Nasu K, et al. Metabolism of warfarin enantiomers in Japanese patients with heart disease having different CYP2C9 and CYP2C19 genotypes. *Clin Pharmacol Ther* 1998; 63:519-28.
  23. Steward DJ, Haining RL, Henne KR, Davis G, Rushmore TH, Trager WF, et al. Genetic association between sensitivity to warfarin and expression of CYP2C9\*3. *Pharmacogenetics* 1997; 7:361-7.
  24. Tabrizi AR, McGrath SD, Blinder MA, Buchman TG, Zehnbauer BA, Freeman BD. Extreme warfarin sensitivity in siblings associated with multiple cytochrome P450 polymorphisms. *Am J Hematol* 2001; 67:144-6.
  25. Bloch A, Ben-Chetrit E, Muszkat M, Caraco Y. Major bleeding caused by warfarin in a genetically susceptible patient. *Pharmacotherapy* 2002; 22:97-101.
  26. Thijssen HHW, Verkooyen IWC, Frank LL. The possession of the CYP2C9\*3 allele is associated with low dose requirement of acenocoumarol. *Pharmacogenetics* 2000; 10:757-60.
  27. Verstuyft C, Morin S, Robert A, Loriot MA, Beaune P, Jailion P, et al. Early acenocoumarol overanticoagulation among cytochrome P450 2C9 poor metabolizers. *Pharmacogenetics* 2001; 11:735-7.
  28. Hermida J, Zarza J, Alberca I, Montes R, Lopez ML, Molina E, et al. Differential effects of 2C9\*3 and 2C9\*2 variants of cytochrome P-450 CYP2C9 on sensitivity to acenocoumarol. *Blood* 2002; 99:4237-9.
  29. Hylek EM. Oral anticoagulants. Pharmacologic issues for use in the elderly. *Clin Geriatr Med* 2001; 17:1-13.
  30. Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, et al. Association between CYP2C9 genetic variants and anticoagulant-related outcomes during warfarin therapy. *JAMA* 2002; 287:1690-8.
  31. Imai J, Ieiri I, Mamiya K, Miyahara S, Furuumi H, Nanba E, et al. Polymorphisms of the cytochrome P450 (CYP) 2C9 gene in Japanese epileptic patients: genetic analysis of the CYP2C9 locus. *Pharmacogenetics* 2000; 10:85-9.
  32. Dickmann LJ, Rettie AE, Kneller MB, Kim RB, Wood AJJ, Stein CM, et al. Identification and functional characterization of a new CYP2C9 variant (CYP2C9\*5) expressed among African Americans. *Mol Pharmacol* 2001; 60:382-7.

## PEER REVIEW OUTCOMES

### Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. José A. Páramo, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Páramo and the Editors. Manuscript received May 22, 2002; accepted September 27, 2002.

### What is already known on this topic

Few studies have addressed the important issue of individual variability to anticoagulant treatment on the basis of the genetic polymorphisms of P450CYP2C9. Only one previous report has presented similar findings. That means the results are original.

### What this study adds

A new approach for the control of oral anticoagulant therapy on an individual basis.

### Potential implications for clinical practice

Oral anticoagulants are widely used all over the world, so that the study has relevant clinical applications.

José A. Páramo, Associate Editor (Pamplona, Spain)