Journal of Thrombosis and Haemostasis, **15**: 465–472 DOI: 10.1111/jth.13615

BRIEF REPORT

Dosing algorithms for vitamin K antagonists across *VKORC1* and *CYP2C9* genotypes

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To cite this article: Baranova EV, Verhoef TI, Ragia G, le Cessie S, Asselbergs FW, de Boer A, Manolopoulos VG, Maitland-van der Zee AH, for the EU-PACT Group. Dosing algorithms for vitamin K antagonists across *VKORC1* and *CYP2C9* genotypes. *J Thromb Haemost* 2017; **15**: 465–72.

Essentials

- Prospective studies of pharmacogenetic-guided (PG) coumarin dosing produced varying results.
- EU-PACT acenocoumarol and phenprocoumon trials compared PG and non-PG dosing algorithms.
- Sub-analysis of EU-PACT identified differences between trial arms across *VKORC1-CYP2C9* groups.
- Adjustment of the PG algorithm might lead to a higher benefit of genotyping.

Summary. *Background:* The multicenter, single-blind, randomized EU-PACT trial compared the safety and efficacy of genotype-guided and non-genetic dosing algorithms for acenocoumarol and phenprocoumon in patients with atrial fibrillation or deep vein thrombosis. The trial showed no differences in the primary outcome between the two dosing strategies. *Objectives:* To explore possible reasons for the lack of differences between trial arms by performing a secondary analysis of EU-PACT data in order to evaluate the performance of both dosing algorithms across *VKORC1–CYP2C9* genetic subgroups. *Patients/Methods:* Anticoagulation control measured according to an International Normalized Ratio (INR) below (INR of < 2), within (INR of 2–3) and above (INR of > 3) the therapeutic range was compared across *VKORC1–CYP2C9* subgroups. Owing to a low number of patients in each subgroup, trials for acenocoumarol and phenprocoumon were combined for analysis. *Results:* Four weeks after therapy initiation, genotype-guided dosing increased the mean percentage of time in the therapeutic INR range (PTIR) in the *VKORC1* GG–*CYP2C9**1*1 subgroup as compared with the non-genetic dosing (difference of 14.68%, 95% confidence interval [CI] 5.38–23.98). 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%; 95% CI 11.6–28.2). Twelve weeks after therapy initiation, no statistically significant differences in anticoagulation control between trial arms were noted across the *VKORC1–CYP2C9* genetic subgroups. *Conclusions:* EU-PACT genetic-guided dose initiation algorithms for acenocoumarol and phenprocoumon could have predicted the dose overcautiously in the *VKORC1* AA–*CYP2C9**1*1 subgroup. Adjustment of the genotype-guided algorithm could lead to a higher benefit of genotyping.

Keywords: acenocoumarol; drug dosing biomarkers; pharmacogenetics; phenprocoumon; randomized controlled trial.

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Clinical Trial Registration: NCT01119274; NCT01119261

Received 18 May 2016
Manuscript handled by: J.-B. Hansen
Final decision: J.-B. Hansen, 15 December 2016

Introduction

The coumarin anticoagulants acenocoumarol and phenprocoumon are commonly used in many countries for the

prevention of thromboembolic complications of atrial fibrillation (AF) and for the treatment of deep vein thrombosis. Owing to a narrow therapeutic window and large interpersonal and intrapersonal variability in coumarin dose requirements, the dose-finding process during therapy initiation remains a challenge, leading to an increased number of bleeding episodes and hospitalizations [1]. Among many factors that influence coumarin dose variability, including patients' anthropomorphic characteristics, (non)-compliance, diet, comorbidities, and comedications, genetic variants in the vitamin K epoxide reductase (*VKORC1*) and hepatic drug-metabolizing enzyme cytochrome P450 2C9 (*CYP2C9*) genes are responsible for a large proportion of variation in the dose required [1]. Taken together, *VKORC1* -1639 G>A (rs9923231), *CYP2C9**2 (rs1799853) and *CYP2C9**3 (rs1057910) polymorphisms explain up to 30–40% of dose variability, and have been associated with anticoagulation effects of coumarins in populations of European descent [1–3]. The utility of pharmacogenetic-guided (PG) coumarin prescribing during therapy initiation has recently been investigated in prospective randomized trials [4–7]. Two warfarin trials (the European Pharmacogenetics of Anticoagulant Therapy [EU-PACT] warfarin arm, and the Clarification of Optimal Anticoagulation through Genetics [COAG] trial) produced divergent results [5,6]. The EU-PACT warfarin trial showed a 7% improvement in the mean percentage of time in the therapeutic International Normalized Ratio (INR) range (PTIR) (INR of 2.0–3.0) with PG dosing as compared with the UK standard clinical practice [5]. Conducted in the USA, the COAG trial, in contrast, demonstrated no difference in PTIR between PG dosing algorithms, including genetic and clinical information, and non-PG dosing algorithms, including only clinical information [6]. Similarly, the combined EU-PACT acenocoumarol and phenprocoumon arms showed no statistically significant difference in PTIR over a period of 12 weeks between the PG and the non-PG control arm [4].

To explore the potential reasons for these findings, we performed subanalyses of EU-PACT acenocoumarol and phenprocoumon data stratified by the *VKORC1* and *CYP2C9* genotypes. We aimed to investigate whether the effect of PG and non-PG dosing on anticoagulation control in certain genetic subgroups differed from the overall effect in the whole trial population, and whether any differences across subgroups were present 4 weeks and 12 weeks after the start of treatment.

Materials and methods

Trial design and participants

We used the combined data of two multicenter, single-blind, randomized controlled trials, comparing a PG with a non-PG dosing algorithm for the initiation of acenocoumarol or phenprocoumon treatment in patients with AF and in patients with venous thromboembolism (VTE) (www.clinicaltrials.gov NCT01119274 and NCT01119261) [4]. A detailed description of EU-PACT trial design, procedures and results can be found elsewhere [4,8]. In brief, patients aged ≥ 18 years diagnosed with AF or VTE, initiating acenocoumarol or phenprocoumon therapy for at least 12 weeks, having a target INR in the low-intensity range, and being able to attend scheduled visits, were recruited and randomized to either of the dosing groups [4]. During the first 5–7 days of the trial, doses of acenocoumarol and phenprocoumon were determined by use of a PG algorithm in the intervention group and by use of a non-PG algorithm in the control group. The EU-PACT loading and maintenance dosing algorithms were developed and validated by van Schie *et al*., and are described in detail elsewhere [9,10]. Non-PG algorithms predicted dose on the basis of age, height, weight, gender, and amiodarone use, whereas PG algorithms also used *VKORC1* -1639 G>A, *CYP2C9**2 and *CYP2C9**3 genotypes. After the initial 5–7 days, dose adjustments were performed by the use of INR values, in accordance with the local clinical practice of participating trial centers. Coumarin doses, INR and the occurrence of possible adverse events were monitored during the 12-week follow-up.

Patients taking acenocoumarol were recruited at the Department of Cardiology and the Department of Internal Medicine of the Democritus University of Thrace in Alexandroupolis, Greece, and at the Cardiology Department of the Onassis Cardiac Surgery Center in Athens, Greece. Phenprocoumon and acenocoumarol patients were recruited at four anticoagulant clinics in the Netherlands from November 2010 to March 2013. The trial protocol was approved by the Leiden Medical Ethics Committee in the Netherlands, and by the Scientific Council and Ethics Committee of the Academic General Hospital of Alexandroupolis and the institutional review board of the Onassis Cardiac Surgery Center in Athens, Greece. All patients provided written informed consent upon inclusion into the trial.

Outcome measures

The primary outcome of the EU-PACT trial was PTIR during 12 weeks following the start of therapy with acenocoumarol or phenprocoumon, calculated with the linear interpolation method of Rosendaal *et al*. [11]. The secondary outcomes included, among others, the percentage of time with an INR of > 4 and an INR of < 2, the time needed to reach a therapeutic INR, the time needed to achieve a stable dose, and the percentage of patients with a stable dose within 12 weeks [4]. In the present analysis, trial participants were stratified by *VKORC1* and *CYP2C9* genotypes, and differences in the INR response 4 weeks and 12 weeks after therapy initiation

were assessed across the subgroups. The time intervals were chosen on the basis of the follow-up duration and consideration of earlier reports indicating the importance of PG dosing during the first few weeks of therapy. Owing to low rates of thromboembolic events, minor bleeds and major bleeds in the trial, these outcomes were not evaluated.

Statistical analysis

In EU-PACT, a sample size of 200 patients per group was required to detect a 7% improvement in PTIR over a period of 12 weeks. The sample size calculation was based on a standard deviation of 23%, a two-sided significance level of 5%, and an 80% power. As a consequence of low enrollment, both acenocoumarol and phenprocoumon trials were concluded before the enrollment goal had been reached, and were combined for analysis. Patients with a follow-up of at least 27 days were included in the analyses 4 weeks after therapy initiation. For analyses 12 weeks after therapy initiation, data of patients with a minimum of 69 days of follow-up were used.

The combined effect of *VKORC1* and *CYP2C9* variants on anticoagulation control was investigated by creating six subgroups, in which each of the three *VKORC1* genotypes was combined with either the wild-type (WT) or the variant *CYP2C9* alleles, as previously described [2,9,12–14]. Owingt to a low number of patients with *CYP2C9* variant alleles, homozygous and heterozygous *CYP2C9**2 and *CYP2C9**3 carriers were placed into the same subgroup, as follows:

VKORC1 GG–*CYP2C9**1/*1 (WT–WT);
VKORC1 GG–*CYP2C9**1/*2, *2/*2, *1/*3, *2/*3, and *3/*3 (WT–any variant);
VKORC1 GA–*CYP2C9**1/*1 (GA–WT);
VKORC1 GA–*CYP2C9**1/*2, *2/*2, *1/*3, *2/*3 and *3/*3 (GA–any variant);
VKORC1 AA–*CYP2C9**1/*1 (AA–WT);
VKORC1 AA–*CYP2C9**1/*2, *2/*2, *1/*3, *2/*3 and *3/*3 (AA–any variant).

Between-group differences in baseline characteristics were assessed with a two-sample *t*-test and a chi-squared test as appropriate. Ninety-five per cent confidence intervals (CIs) were constructed for the differences in mean PTIR, and mean time above and below the therapeutic INR range. Means and 95% CIs were also calculated for the INR measurements in week 1 of the trial. A two-sample *t*-test was performed to assess differences in the INR response between trial arms across genetic subgroups. A two-sided *P*-value of < 0.05 was considered to be nominally statistically significant. After the Bonferroni correction for multiple testing, a *P*-value threshold of < 0.001 was considered to be statistically significant. All analyses were carried out with SPSS STATISTICS for Windows, version 23.0 (IBM Corp., Armonk, NY, USA).

Results and discussion

Data were available for 548 trial participants, of whom seven patients of the non-PG arm were excluded because of missing genotypes, leaving 273 patients in the PG group and 268 patients in the control group (Fig. 1). The baseline clinical characteristics of the trial population are shown in Table 1. The demographic characteristics were comparable between the intervention group and the control group. The most frequent indication for coumarin therapy was AF. The results of analyses across *VKORC1–CYP2C9* subgroups over the first 4 weeks and 12 weeks of treatment are shown in Tables 2 and 3. Four weeks after therapy initiation in the PG *VKORC1* GG–*CYP2C9**1*1 subgroup, a nominally statistically significant 14.7% increase in PTIR was observed (54.9% ± 23.9% versus 40.2% ± 27.0%, difference of 14.68%, 95% CI 5.38–23.98; $P = 0.002$, Table 2). In the *VKORC1* AA–*CYP2C9**1*1 subgroup, there was a higher risk of under-anticoagulation when patients were dosed with the PG strategy than when they were dosed with the non-PG strategy (29.1% ± 23.3% versus 9.3% ± 6.1%,

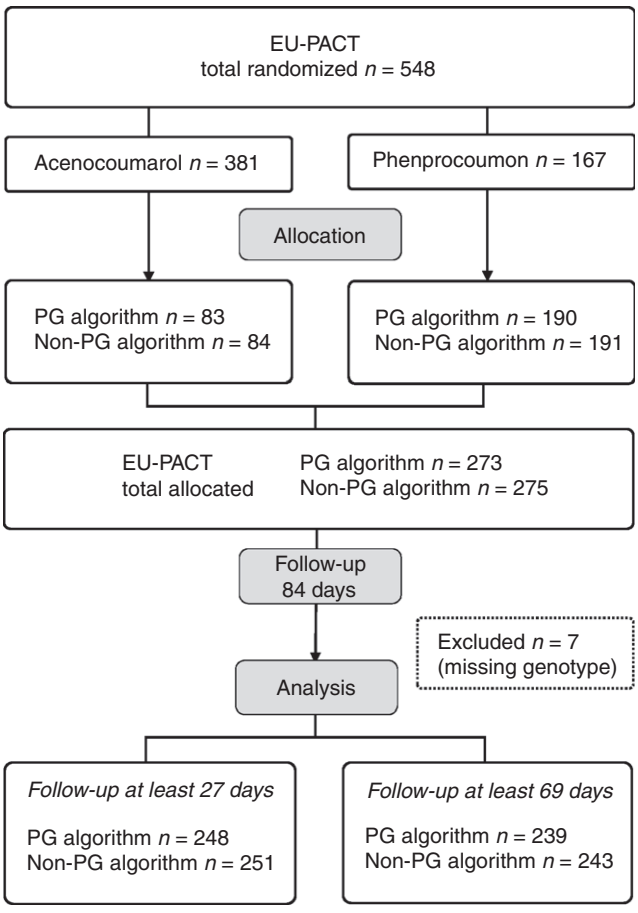

Figure 1. Flowchart of the patients included in analyses. PG, pharmacogenetic-guided. Non-PG, non-pharmacogenetic-guided (using clinical information only).

Table 1 Baseline clinical characteristics of the trial population

	Acenocoumarol		Phenprocoumon		Combined	
	PG $n = 190$	Non-PG $n = 191$	PG $n = 83$	Non-PG $n = 84$	PG $n = 273$	Non-PG $n = 275$
Age (years), mean ± SD	68 ± 14	68 ± 13	67 ± 11	67 ± 11	68 ± 13	68 ± 13
Male sex, *n* (%)	121 (64)	107 (56)	51 (61)	47 (56)	172 (63)	154 (56)
Caucasian, *n* (%)*	184 (97)	189 (99)	79 (95)	81 (96)	263 (96)	270 (98)
Atrial fibrillation, *n* (%)	158 (83)	158 (83)	68 (82)	70 (83)	226 (83)	228 (84)
Height (cm), mean ± SD	172 ± 11	171 ± 11	174 ± 9	173 ± 10	172 ± 10	171 ± 11
Weight (kg), mean ± SD	84 ± 15	82 ± 18	87 ± 17	83 ± 16	85 ± 16	82 ± 17
CYP2C9, *n* (%)						
*1*1	111 (58)	107 (57)	55 (66)	57 (70)	166 (61)	164 (60)
*1*2	39 (21)	33 (18)	14 (17)	14 (17)	53 (19)	47 (17)
*1*3	29 (15)	32 (17)	11 (13)	7 (9)	40 (15)	39 (14)
*2*2	4 (2)	11 (6)	2 (2)	2 (3)	6 (2)	13 (5)
*2*3	5 (3)	4 (2)	1 (1)	1 (1)	6 (2)	5 (2)
*3*3	2 (1)	0 (0)	0 (0)	0 (0)	2 (1)	0 (0)
Missing, *n* (%)	0 (0)	4 (2)	0 (0)	3 (4)	0 (0)	7 (3)
P-value of HWE for *CYP2C9*	0.37	0.002	0.66	0.77	0.89	0.002
VKORC1, *n* (%)						
GG	70 (37)	55 (29)	24 (29)	33 (41)	94 (34)	88 (32)
GA	84 (44)	93 (50)	40 (48)	33 (41)	124 (45)	126 (46)
AA	36 (19)	39 (20)	19 (23)	15 (19)	55 (20)	54 (20)
Missing, *n* (%)	0 (0)	4 (2)	0 (0)	3 (4)	0 (0)	7 (3)
P-value of HWE for *VKORC1*	0.23	0.97	0.77	0.20	0.23	0.47
Amiodarone use, *n* (%)	22 (12)	23 (12)	0 (0)	0 (0)	22 (8)	23 (8)

HWE, Hardy–Weinberg equilibrium; PG, pharmacogenetic-guided; SD, standard deviation. *Ethnicity was self-reported.

difference of 19.89%, 95% CI 11.60–28.18; $P < 0.001$, Table 2). Twelve weeks after therapy initiation, the difference in PTIR between trial arms was no longer statistically significant in the *VKORC1* GG–*CYP2C9**1*1 subgroup (62.9% ± 21.4% versus 55.7% ± 23.5%, difference of 7.23%, 95% CI − 1.06 to 15.52; $P = 0.087$; Table 3). For the PG-dosed *VKORC1* AA–*CYP2C9**1*1 subgroup, the percentage of time below the therapeutic range remained increased, but the difference was not statistically significant after the correction for multiple testing (21.9% ± 22.3% versus 8.9% ± 13.0%, difference of 12.99%, 95% CI 3.90–22.07; $P = 0.006$; Table 3). No statistically significant differences were observed for INR above the therapeutic range. Sensitivity analyses were also performed for coumarin separately and in the per-protocol dataset, and the results for subgroups were similar (data not shown).

This study addressed the issue of robustness of the EU-PACT dose prediction algorithms for acenocoumarol and phenprocoumon, and explored explanations for the lack of benefit of PG over non-PG dosing on anticoagulation control in this trial. The analysis of the EU-PACT data across the *VKORC1–CYP2C9* subgroups showed that the PG dosing strategy improved PTIR 4 weeks from therapy initiation only in the *VKORC1* GG–*CYP2C9**1*1 subgroup, whereas the *VKORC1* AA–*CYP2C9**1*1 subgroup was dosed overcautiously, and had an increased mean time below the therapeutic INR range. The use of a PG strategy could allow treatment to be started with higher doses in *VKORC1* GG–*CYP2C9**1*1 carriers, and thereby reduce under-anticoagulation and the risk of thrombosis. More time below the therapeutic INR range in the *VKORC1* GG–*CYP2C9**1*1 subgroup dosed with the EU-PACT clinical algorithm is in accordance with previous reports of an increased risk of subtherapeutic INRs in these patients when an insufficiently high dose is prescribed with a standardized dosing algorithm [12,13].

We suggest that one of the many reasons for the controversy regarding existing trial results could be the possible limitations of a particular dosing algorithm used. The prediction of coumarin dose variability is not entirely similar between existing algorithms. It depends on the characteristics of the derivation cohort and on the variables included in the algorithm, and could perform differently in another population [3,15]. The EU-PACT algorithms were developed in large populations of acenocoumarol and phenprocoumon users, but the derivation cohort did not contain a sufficient number of rare *CYP2C9* variant allele carriers to account for dose variability in these patients [9,10]. A recent randomized controlled trial of genotype-guided dosing of acenocoumarol used a PG dosing algorithm developed in a Spanish population that included the *VKORC1*, *CYP2C9* and *CYP4F2* genotypes [15]. In this trial, the PG approach was superior to standard care in terms of the number of patients with a stable dose and the mean percentage of therapeutic INRs after 90 days of follow-up [16]. Although there are certainly differences in study design

Table 2 Anticoagulation control 4 weeks after therapy initiation across *VKORC1–CYP2C9* subgroups

	INR 2–3 (%)				INR < 2 (%)				INR > 3 (%)			
VKORC1–CYP2C9 genotype	PG algorithm	Non-PG algorithm	Percentage difference (95% CI)	*P*	PG algorithm	Non-PG algorithm	Percentage difference (95% CI)	*P*	PG algorithm	Non-PG algorithm	Perentage difference (95% CI)	*P*
GG-*1*1	*n* = 60 54.88 ± 23.92	*n* = 58 40.20 ± 27.03	14.68 (5.38–23.98)	**0.002**†	*n* = 60 30.62 ± 22.75	*n* = 58 50.91 ± 30.86	− 20.29 (− 30.16 to − 0.43)	**< 0.001**‡	*n* = 60 14.50 ± 22.99	*n* = 58 8.89 ± 17.11	5.61 (− 1.79 to 13.02)	0.136
GG-variant	*n* = 28 49.73 ± 30.57	*n* = 28 40.96 ± 26.87	8.77 (− 6.65 to 24.19)	0.259	*n* = 28 27.56 ± 29.68	*n* = 28 38.41 ± 31.64	− 10.85 (− 27.28 to 5.59)	0.191	*n* = 28 22.71 ± 25.64	*n* = 28 20.63 ± 24.62	2.08 (− 11.38 to 15.55)	0.758
GA-*1*1	*n* = 62 56.18 ± 25.28	*n* = 60 53.17 ± 27.75	3.01 (− 6.50 to 12.52)	0.532	*n* = 62 28.10 ± 26.41	*n* = 60 25.01 ± 22.07	3.09 (− 5.65 to 11.83)	0.485	*n* = 62 15.72 ± 21.47	*n* = 60 21.82 ± 27.56	− 6.10 (− 14.94 to 2.74)	0.174
GA-variant	*n* = 50 53.15 ± 22.38	*n* = 56 50.79 ± 25.74	2.37 (− 6.98 to 11.71)	0.617	*n* = 50 26.99 ± 23.34	*n* = 56 21.46 ± 20.58	5.53 (− 2.93 to 13.99)	0.198	*n* = 50 19.85 ± 21.76	*n* = 56 27.75 ± 26.37	− 7.90 (− 12.27 to 1.48)	0.098
AA-*1/*1	*n* = 29 41.93 ± 24.62	*n* = 34 49.99 ± 27.54	− 8.07 (− 21.33 to 5.20)	0.229	*n* = 29 29.14 ± 23.29	*n* = 34 9.25 ± 6.12	19.89 (11.60 to 28.18)	**< 0.001**§	*n* = 29 28.93 ± 26.19	*n* = 34 40.76 ± 27.43	− 11.82 (25.41–1.76)	0.087
AA-variant	*n* = 19 54.89 ± 24.40	*n* = 15 45.45 ± 27.81	9.44 (− 8.81 to 27.70)	0.300	*n* = 19 26.22 ± 19.34	*n* = 15 15.16 ± 14.55	11.06 (− 1.18 to 23.31)	0.075	*n* = 19 18.89 ± 23.16	*n* = 15 39.39 ± 31.87	− 20.50 (− 39.72 to − 1.28)	0.037

CI, confidence interval; INR, International Normalized Ratio; PG, pharmacogenetic-guided. The frequencies of *VKORC1* and *CYP2C9* genotypes per subgroup in the PG arm were as follows. GG-*1*1: *VKORC1* GG, *n* = 60; *CYP2C9*1*1, *n* = 60. GG-variant: *VKORC1*, GG, *n* = 28; *CYP2C9*1*2, *n* = 15; *CYP2C9*1*3, *n* = 10; *CYP2C9*2*2, *n* = 1; *CYP2C9*2*3, *n* = 2; *CYP2C9*3*3, *n* = 0. GA-*1*1: *VKORC1* GA, *n* = 62; *CYP2C9*1*1, *n* = 62. GA-variant: *VKORC1* GA, *n* = 50; *CYP2C9*1*2, *n* = 26; *CYP2C9*1*3, *n* = 19; *CYP2C9*2*2, *n* = 4; *CYP2C9*2*3, *n* = 1; *CYP2C9*3*3, *n* = 0. AA-*1*1: *VKORC1* AA, *n* = 29; *CYP2C9*1*1, *n* = 29. AA-variant: *VKORC1* AA, *n* = 19; *CYP2C9*1*2, *n* = 8; *CYP2C9*1*3, *n* = 8; *CYP2C9*2*2, *n* = 0; *CYP2C9*2*3, *n* = 3; *CYP2C9*3*3, *n* = 0. The frequencies of *VKORC1* and *CYP2C9* genotypes per subgroup in the non-PG arm were as follows. GG-*1*1: *VKORC1* GG, *n* = 58; *CYP2C9*1*1, *n* = 58. GG-variant: *VKORC1* GG, *n* = 28; *CYP2C9*1*2, *n* = 16; *CYP2C9*1*3, *n* = 10; *CYP2C9*2*2, *n* = 1; *CYP2C9*2*3, *n* = 1; *CYP2C9*3*3, *n* = 0. GA-*1*1: *VKORC1* GA, *n* = 60; *CYP2C9*1*1, *n* = 60. GA-variant: *VKORC1* GA, *n* = 56; *CYP2C9*1*2, *n* = 22; *CYP2C9*1*3, *n* = 21; *CYP2C9*2*2, *n* = 9; *CYP2C9*2*3, *n* = 4; *CYP2C9*3*3, *n* = 0. AA-*1*1: *VKORC1* AA, *n* = 34; *CYP2C9*1*1, *n* = 34. AA-variant: *VKORC1* AA, *n* = 15; *CYP2C9*1*2, *n* = 8; *CYP2C9*1*3, *n* = 5; *CYP2C9*2*2, *n* = 2; *CYP2C9*2*3, *n* = 0; *CYP2C9*3*3, *n* = 0. The ± values are means with standard deviations. The carriers with the following *CYP2C9* genotypes were combined into the *CYP2C9* 'variant' category: *1*2, *1*3, *2*2, *2*3, and *3*3. After the Bonferroni correction for multiple testing, the threshold for statistical significance was $P < 0.001$. †$P = 2.2 \times 10-3$. ‡$P = 8.4 \times 10-5$. §$P = 1.1 \times 10-5$. Bold values indicate statistically significant differences for comparisons.

Table 3 Anticoagulation control 12 weeks after therapy initiation across *VKORC1–CYP2C9* subgroups.

	INR 2–3 (%)				INR < 2 (%)				INR > 3 (%)			
VKORC1–CYP2C9 genotype	PG algorithm	Non-PG algorithm	Percentage difference (95% CI)	*P*	PG algorithm	Non-PG algorithm	Percentage difference (95% CI)	*P*	PG algorithm	Non-PG algorithm	Percentage difference (95% CI)	*P*
GG-*1*1	*n* = 58 62.95 ± 21.38	*n* = 57 55.71 ± 23.45	7.23 (− 1.06 to 15.52)	0.087	*n* = 58 24.85 ± 23.50	*n* = 57 32.46 ± 23.14	− 7.61 (− 16.23 to 1.00)	0.083	*n* = 58 12.20 ± 16.19	*n* = 57 11.82 ± 17.26	0.38 (− 5.79 to 6.57)	0.902
GG-variant	*n* = 28 57.11 ± 25.92	*n* = 28 54.10 ± 22.50	3.01 (− 9.99 to 16.01)	0.644	*n* = 28 22.92 ± 23.36	*n* = 28 28.34 ± 24.01	− 5.42 (− 18.12 to 7.27)	0.395	*n* = 28 19.97 ± 21.84	*n* = 28 17.56 ± 21.49	2.41 (− 9.19 to 14.02)	0.678
GA-*1*1	*n* = 60 62.49 ± 22.68	*n* = 57 67.70 ± 19.41	− 5.51 (− 12.96 to 2.54)	0.186	*n* = 60 22.67 ± 21.87	*n* = 57 16.57 ± 14.76	6.10 (− 0.77 to 12.97)	0.081	*n* = 60 14.84 ± 22.18	*n* = 57 15.73 ± 17.63	− 0.89 (− 8.26 to 6.47)	0.811
GA-variant	*n* = 46 62.53 ± 25.36	*n* = 53 59.35 ± 24.88	3.18 (− 6.87 to 13.22)	0.532	*n* = 46 19.38 ± 20.46	*n* = 53 15.74 ± 16.52	3.64 (− 3.74 to 11.02)	0.330	*n* = 46 18.09 ± 23.84	*n* = 53 24.91 ± 24.88	− 6.82 (− 16.58 to 2.94)	0.169
AA-*1/*1	*n* = 28 59.69 ± 21.96	*n* = 34 62.06 ± 24.78	− 2.38 (− 14.40 to 9.65)	0.694	*n* = 28 21.89 ± 22.29	*n* = 34 8.90 ± 13.01	12.99 (3.90–22.07)	**0.006†**	*n* = 28 18.43 ± 17.29	*n* = 34 29.04 ± 24.35	− 10.61 (− 21.57 to 0.35)	0.057
AA-variant	*n* = 19 61.31 ± 25.86	*n* = 14 54.80 ± 25.47	6.51 (− 11.95 to 24.96)	0.478	*n* = 19 18.64 ± 19.11	*n* = 14 15.21 ± 15.44	3.43 (− 9.26 to 16.12)	0.585	*n* = 19 20.05 ± 22.98	*n* = 14 29.99 ± 26.83	− 9.94 (− 27.66 to 7.78)	0.261

CI, confidence interval; INR, International Normalized Ratio; PG, pharmacogenetic-guided. The frequencies of *VKORC1* and *CYP2C9* genotypes per subgroup in the PG arm were as follows. GG-*1*1: *VKORC1* GG, *n* = 58; *CYP2C9**1*1, *n* = 58. GG-variant: *VKORC1* GG, *n* = 28; *CYP2C9**1*2, *n* = 15; *CYP2C9**1*3, *n* = 10; *CYP2C9**2*2, *n* = 1; *CYP2C9**2*3, *n* = 2; *CYP2C9**3*3, *n* = 0. GA-*1*1: *VKORC1* GA, *n* = 60; *CYP2C9**1*1, *n* = 60. GA-variant: *VKORC1* GA, *n* = 46; *CYP2C9**1*2, *n* = 24; *CYP2C9**1*3, *n* = 18; *CYP2C9**2*2, *n* = 4; *CYP2C9**2*3, *n* = 0; *CYP2C9**3*3, *n* = 0. AA-*1*1: *VKORC1* AA, *n* = 28; *CYP2C9**1*1, *n* = 28. AA-variant: *VKORC1* AA, *n* = 19; *CYP2C9**1*2, *n* = 8; *CYP2C9**1*3, *n* = 8; *CYP2C9**2*2, *n* = 3; *CYP2C9**2*3, *n* = 0; *CYP2C9**3*3, *n* = 0. The frequencies of *VKORC1* and *CYP2C9* genotypes per subgroup in the non-PG arm were as follows. GG-*1*1: *VKORC1* GG, *n* = 57; *CYP2C9**1*1, *n* = 57. GG-variant: *VKORC1* GG, *n* = 28; *CYP2C9**1*2, *n* = 16; *CYP2C9**1*3, *n* = 10; *CYP2C9**2*2, *n* = 1; *CYP2C9**2*3, *n* = 1; *CYP2C9**3*3, *n* = 0. GA-*1*1: *VKORC1* GA, *n* = 57; *CYP2C9**1*1, *n* = 57. GA-variant: *VKORC1* GA, *n* = 53; *CYP2C9**1*2, *n* = 20; *CYP2C9**1*3, *n* = 20; *CYP2C9**2*2, *n* = 9; *CYP2C9**2*3, *n* = 4; *CYP2C9**3*3, *n* = 0. AA-*1*1: *VKORC1* AA, *n* = 34; *CYP2C9**1*1, *n* = 34. AA-variant: *VKORC1* AA, *n* = 14; *CYP2C9**1*2, *n* = 8; *CYP2C9**1*3, *n* = 5; *CYP2C9**2*2, *n* = 1; *CYP2C9**2*3, *n* = 0; *CYP2C9**3*3, *n* = 0. The ± values are means with standard deviations. The carriers with the following *CYP2C9* genotypes were combined into the *CYP2C9* 'variant' category: *1*2, *1*3, *2*2, *2*3, and *3*3. After the Bonferroni correction for multiple testing, the threshold for statistical significance was $P < 0.001$. †$P = 5.8 \times 10-3$. Bold values indicate statistically significant differences for comparisons.

between this trial and EU-PACT, it is possible that including extra genetic variants might affect the precision of dose prediction and impact on the outcome.

One of the study limitations is a small sample size and combination of the acenocoumarol and phenprocoumon data, which mens that pharmacologic differences between the two drugs and between their dosing algorithms were not accounted for. However, the results of our sensitivity analyses by drug did not show substantial differences. The *CYP2C9**2 and *CYP2C9**3 genotypes were combined in *VKORC1–CYP2C9* subgroups, but the low frequencies of *CYP2C9**2/*2, *CYP2C9**3/*3 and *CYP2C9**2/*3 genotypes in our data probably had minor effects on the results.

In conclusion, the use of PG algorithms for therapy initiation with acenocoumarol and phenprocoumon could be advantageous in certain patient subgroups, particularly during the first month of coumarin therapy. Adjustment and refinement of the EU-PACT PG algorithms could increase the benefit of genotyping for *VKORC1* and *CYP2C9* variant allele carriers. This study also highlights the need to consider potential limitations of dose prediction algorithms when interpreting the results of clinical trials of coumarin PG dosing.

Addendum

E. V. Baranova performed analysis and wrote the manuscript. T. I. Verhoef designed research and contributed to the data analysis. S. le Cessie contributed to the data analysis. T. I. Verhoef, G. Ragia, F. W. Asselbergs, S. le Cessie, A. de Boer, V. G. Manolopoulos, and A. H. Maitland-van der Zee critically revised the manuscript. Members of the EU-PACT group contributed to the study design, performed research, and critically revised the manuscript.

Acknowledgements

This work was supported by a grant from the European Commission Seventh Framework Programme (HEALTH F2 2009 223062) and by funding from GlaxoSmithKline (to A.-H. Maitland-van der Zee) and the Swedish Research Council (Medicine), the Swedish Heart–Lung Foundation, and Clinical Research Support at Uppsala University (to M. Wadelius). We thank all of the patients who participated in the EU-PACT trial, and all of the staff at the anticoagulant clinics and hospitals where the recruiting was conducted.

Disclosure of Conflict of Interests

E. V. Baranova reports receiving European Union FP7 Grant no. 602108, during the conduct of the study. A.-H. Maitland-van der Zee reports receiving EU FP7 Collaborative grant EU-PACT and EU FP7 Collaborative Grant PREDICTION-ADR, during the conduct of the study. The other authors state that they have no conflict of interest.

Appendix

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