Journal of Thrombosis and Haemostasis, **15**: 454–464 DOI: 10.1111/jth.13601

ORIGINAL ARTICLE

Age-stratified outcome of a genotype-guided dosing algorithm for acenocoumarol and phenprocoumon

Y. ZHANG,* A. DE BOER,* T. I. VERHOEF,† F. J. M. VAN DER MEER,‡ S. LE CESSIE,§¶ V. G. MANOLOPOULOS**†† and A. H. MAITLAND-VAN DER ZEE,*FOR THE EU-PACT GROUP[1]

**Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands; †Department of Applied Health Research, University College London, London, UK; ‡Department of Thrombosis and Hemostasis, Leiden University Medical Center; §Department of Medical Statistics and Bioinformatics, Leiden University Medical Center; ¶Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands; **Clinical Pharmacology Unit, Academic General Hospital of Evros; and ††Laboratory of Pharmacology, Medical School, Democritus University of Thrace, Alexandroupolis, Greece*

To cite this article: Zhang Y, de Boer A, Verhoef TI , van der Meer FJM, le Cessie S, Manolopoulos VG, Maitland-van der Zee AH, for the EU-PACT Group. Age-stratified outcome of a genotype-guided dosing algorithm for acenocoumarol and phenprocoumon. *J Thromb Haemost* 2017; **15**: 454–64.

Essentials

- The EU-PACT trial was used to investigate age on the interaction between coumarins and genotype.
- The results support the use of genotype-guided dosing for phenprocoumon in patients < 75 years.
- For patients ≥ 75 years the phenprocoumon algorithm should be revised and further tested.
- No influence of comorbidities and co-current drug use was found that could explain the differences.

Summary. *Background:* Age seemed to affect the interaction between coumarins and genotype in the acenocoumarol and phenprocoumon arm of the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial. *Objectives:* To investigate the effect of genotype-guided dosing stratified by age and the potential factors causing a difference. *Patients/Methods:* Data from the acenocoumarol/phenprocoumon arm of the EU-PACT trial were used. The percentages of time below the therapeutic range, time above the therapeutic range and time in the therapeutic range (TTR) during the initial 12 weeks of therapy were compared between the genotype-guided group and the control group among younger (< 75 years) and older (≥ 75 years) patients by the use of independent *t*-tests, and adjusted for sex, height, weight and co-medications by the use of linear regression. *Results:* Among younger phenprocoumon users, TTR during the first 12 weeks in the genotype-guided group ($n = 55$) was 9.5% (95% confidence interval [CI] 1.3 to 17.8) higher than in the control group ($n = 63$), with a remarkably lower percentage of time above this range (difference: − 9.6%, 95% CI − 19.0 to − 0.2) and a similar time below this range. Older patients dosed by the genotype-guided algorithm ($n = 24$) spent more time above the range (difference: 27.5%, 95% CI 12.9 to 42.0). 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. *Conclusions:* The genotype-guided algorithm for phenprocoumon in the EU-PACT trial benefitted younger patients more, but for older patients the algorithm needs to be revised and tested in further research.

Keywords: age groups; algorithms; coumarins; cytochrome P450 2C9; pharmacogenetics; vitamin K epoxide reductases.

Correspondence: Anke-Hilse Maitland-van der Zee, Division of Pharmacoepidemiology & Clinical Pharmacology, Faculty of Science, Utrecht University, PO Box 80 082, 3508 TB Utrecht, the Netherlands.
Tel.: +31 622 736 715; fax: +31 30 253 9166.
E-mail: a.h.maitland@uu.nl

[1]See Appendix for the full list of contributors

Received 28 May 2016
Manuscript handled by: M. Carrier
Final decision: M. Carrier, 2 December 2016

Introduction

Aging is one of the common causes of interindividual variation in the stable dose of coumarin derivatives [1,2]. With increasing age, the pharmacokinetics and pharmacodynamics of coumarins change [3]. This results in the fact that elderly patients, on average, require a lower dose than younger patients to maintain the same

anticoagulation effect [4]. In addition, elderly patients are more likely to have comorbidities, and they therefore receive a higher number of co-medications [5]. Both comorbidities [6] and co-medications [7] can influence the anticoagulation effect of coumarins, owing to drug–disease interactions or drug–drug interactions. Furthermore, elderly patients usually have a high risk of bleeding even without taking coumarins [8]. Therefore, it is important to take into account the patient's age when assessing the effect of coumarin therapy.

Previously, three dosing algorithms to optimize coumarin dosing including genetic and clinical factors were investigated in the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial and the Clarification of Optimal Anticoagulation through Genetics (COAG) trial [9–11]. Although these dosing algorithms include age as a parameter, none of them stratified patients by age in the primary outcomes report. The mean age of patients in the EU-PACT trial was ~ 68 years in both the acenocoumarol/phenprocoumon arm [9] and the warfarin arm [10]. In a reply to a commentary, it was shown that, among patients aged < 75 years, the group that used the genotype-guided algorithm had a higher percentage of time in the therapeutic International Normalized Ratio (INR) range (TTR) than the group dosed according to the non-genotype-guided algorithm during the 12 weeks after the initiation of therapy. In contrast, patients who were aged ≥ 75 years did not spend more time in range in the genotyped arm [12]. Therefore, age seemed to affect the interaction between coumarins and genotype.

After this intriguing finding, we wanted to present here further analyses of the acenocoumarol/phenprocoumon arm of the EU-PACT trial to assess the effect of genotype-guided dosing stratified by age. We also assessed the influence of potential factors such as comorbidities and concurrent drug use that may cause the differences in different age categories.

Methods

Patient selection and study design

Data from patients with at least 10 weeks of follow-up in the acenocoumarol/phenprocoumon arm of the EU-PACT trial [9] were used for the present study. In brief, the EU-PACT trial was a single-blind, randomized trial comparing a genotype-guided dosing algorithm [13] that included clinical variables and genotyping for *CYP2C9* and *VKORC1* with a dosing algorithm that included only clinical variables, for the initiation of acenocoumarol or phenprocoumon treatment in patients with atrial fibrillation or venous thromboembolism [9]. Details of the study design, outcome definitions, patients, and data collection, and the main results of this trial, are described elsewhere [9,13,14].

Outcome measures

The primary outcome in the present study was the percentage of time in the TTR (2.0–3.0) during the first 12 weeks of acenocoumarol or phenprocoumon treatment in different age groups. Rosendaal's method was used to calculate the TTR [15]. Other outcomes that we assessed were the percentage of time above and below the TTR, and the maintenance dose per day in the first stable period after initiation of anticoagulation therapy as defined in the EU-PACT trial [9].

Definition of patient groups

To determine the impact of age on the primary outcome of genotype-guided dosing, the interaction between age and treatment was examined beforehand (Figs S1 and S2). There was a trend towards an age interaction for phenprocoumon. Patients were then categorized into two age groups: younger (< 75 years) and older (≥ 75 years). In each age group, the outcomes were compared between the genotype-guided group and the control group.

We also determined the outcome in three genotype strata (no variant, one variant in either *CYP2C9* or *VKORC1*, and more than one variant). To evaluate the impact of the first maintenance dose, the differences in the maintenance dose calculated with the genotype-guided algorithm and the clinical algorithm were compared.

Potential confounding factors

The baseline patient characteristics of sex, height, weight, *CYP2C9* genotype, *VKORC1* genotype, comorbidity and concomitant medication were compared between younger and older patients. The comorbidities that we tested were hypertension, heart failure, myocardial infarction, hyperlipidemia, and diabetes mellitus, which were the most common and may have an impact on the anticoagulation effect [7,16]. The suspected concomitant drugs were defined as coumarin-potentiating drugs, including statins, proton-pump inhibitors, antidepressants [17–20], antibiotics [21], non-steroidal anti-inflammatory drugs, lactulose [22], aspirin, and enzyme inducers, which can reduce the effect of anticoagulation. Detailed information on the concomitant drugs used is shown in Table S1.

Statistical analysis

Only patients with at least 10 weeks of follow-up were included in the analyses; however, per-protocol analyses were also performed. The independent *t*-test and Pearson's chi-square test were used to compare the baseline characteristics. The primary and secondary outcomes were compared by calculating mean differences with 95% confidence intervals (CIs), by the use of independent-samples *t*-tests, and adjusted in a linear regression model for

height, weight, sex, and the concomitant drugs used (only enzyme inhibitors or inducers). The interaction of age and treatment was assessed with ANCOVA. Genotype proportions were tested for deviations from Hardy–Weinberg equilibrium with a chi-square test. For all calculations, a *P*-value of < 0.05 was considered to be statistically significant. All analyses were performed with IBM SPSS STATISTICS version 20.0 (IBM Corp., Armonk, NY, USA).

Results

Patients

The present study included a total of 484 patients (325 patients treated with acenocoumarol, and 159 patients treated with phenprocoumon) from the acenocoumarol/phenprocoumon arm of the EU-PACT trial [9], which excluded 64 patients who did not have at least 10 weeks of treatment. Of these, 160 acenocoumarol users were assigned to the genotype-guided group and 165 to the control group; 79 phenprocoumon users were included in the genotype-guided group and 80 in the control group. An additional number of patients were excluded from the per-protocol analysis for the reasons outlined in Table S2. Of these, 111 acenocoumarol users were assigned to the genotype-guided group and 126 to the control group; 49 phenprocoumon users were included in the genotype-guided group and 58 in the control group.

Most of the baseline characteristics of patients were similar between the genotype-guided group and the control group in both age groups and for both acenocoumarol users and phenprocoumon users (Table 1). Only among younger phenprocoumon-treated patients was a statistically significant difference shown for weight, which was 92 kg in the genotype-guided group and 85 kg in the control group. The characteristics of acenocoumarol-treated patients stratified by country of residence (the Netherlands and Greece) are shown in Table S3, and were similar between the genotype-guided group and the control group in both age groups.

Comorbidities and concomitant medication

There were no statistically significant differences in common comorbidities between the genotype-guided group and the control group per age group in phenprocoumon-treated patients (Table 2).

The concomitant medications suspected to interact with acenocoumarol or phenprocoumon are summarized in Table 3. No statistically significant difference was shown in the distribution of concomitant drug use between the genotype-guided group and the control group in the young or old age categories of acenocoumarol-treated and phenprocoumon-treated patients. During the initial therapy with phenprocoumon, for the younger age group, 33 patients (60.0%) in the genotype-guided group and 31 patients (49.2%) in the control group were taking at least one potentiating drug during the anticoagulant treatment, and for the older age group, these numbers were 10 (41.7%) and 10 (58.8%), respectively, in the two groups. Among younger patients treated with acenocoumarol, there were 70 patients (61.9%) concurrently using potentiating drugs in the genotype-guided group, and 63 (61.2%) in the control group. Only one patient used enzyme inducers, which might decrease the INR during therapy with acenocoumarol.

We also compared the comorbidities and concomitant drug use between the young and older patients (shown in Tables S4 and S5). For phenprocoumon users, no statistically significant difference was found. For acenocoumarol users, there were no statistically significant differences for the comorbidities except for hypertension and heart failure, which were more prevalent in the elderly group than in the younger group. The concomitant use of potentiating drugs, enzyme inhibitors or aspirin with acenocoumarol was also more prevalent in the older group than in the younger group.

TTR during the initial 12 weeks

In all phenprocoumon-treated patients, the difference in the TTR between the genotype-guided group and the control group was 2.5% [9]. However, the effect of genotype-guided dosing for patients aged < 75 years and for patients aged ≥ 75 years was different, as shown in Table 4. Among patients aged < 75 years, the TTR during the first 12 weeks was 64.1% in the genotype-guided group, and 55.7% in the control group, with an adjusted difference of 9.5% (95% CI 1.3 to 17.8). Younger patients treated with phenprocoumon also spent 9.6% less time (17.6% versus 27.1%, 95% CI -19.0 to -0.2) with an INR above 3. There was no difference in the percentage of time with an INR below 2. In contrast, among patients aged ≥ 75 years, genotype-guided dosing resulted in a lower TTR (adjusted difference of -17.9%, 95% CI -31.8 to -3.9) and a higher percentage of time with an INR above 3 (adjusted difference of 27.5%, 95% CI 12.9 to 42.0) than in the control group. The older patients with genotype-guided dosing also spent 9.7% less time with an INR below 2 than those in the control group; however, this difference was not statistically significant. A per-protocol analysis yielded similar results (shown in Table S6), although the difference in the TTR between the genotype-guided group and the control group was not statistically significant.

For acenocoumarol, among younger patients, the genotype-guided group got a TTR of 64.5%, which was a little higher (adjusted difference of 3.6%, 95% CI -2.9 to 10.1) than that in the control group (61.3%), whereas the opposite was found among older patients (adjusted difference of -4.2%, 95% CI -13.3 to 4.9), as shown in Table 4. However, none of these differences was statistically significant. The older patients in the genotype-

Table 1 Characteristics of patients dosed according to the genotype-guided algorithm and the controls stratified by age

	Acenocoumarol						Phenprocoumon					
	< 75 years			≥ 75 years			< 75 years			≥ 75 years		
Characteristics	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value
Patient number	113	103	–	47	62	–	55	63	–	24	17	–
Age (years), mean ± SD	62 ± 12	62 ± 10	1	81 ± 4	80 ± 4	0.42	62 ± 12	63 ± 9	0.76	79 ± 3	81 ± 4	0.24
Male sex, *n* (%)	82 (73)	62 (60)	0.05	23 (49)	32 (52)	0.78	36 (66)	39 (62)	0.69	13 (54)	11 (52)	0.23
Height (cm), mean ± SD	175 ± 10	174 ± 10	0.85	166 ± 10	165 ± 10	0.51	175 ± 9	176 ± 9.5	0.81	171 ± 9	165 ± 9	0.06
Weight (kg), mean ± SD	86 ± 16	86 ± 20	0.89	80 ± 11	76 ± 14	0.12	92 ± 17	85 ± 15	0.03	76 ± 13	73 ± 15	0.52
Race (white), *n* (%)	108 (95.6)	103 (100)		47 (100)	62 (100)		51 (92.7)	61 (96.8)		24 (100)	16 (94.1)	
CYP2C9 genotype, *n* (%)			0.62		1 missing	0.28		1 missing	0.86			0.50
*1/*1	68 (60.2)	55 (53.4)		26 (55.3)	38 (61.3)		37 (67.3)	41 (65.1)		15 (62.5)	14 (82.4)	
*1/*2	20 (17.7)	21 (20.4)		14 (29.8)	9 (14.8)		9 (16.4)	12 (6)		4 (16.7)	2 (11.8)	
*1/*3	19 (16.8)	17 (16.5)		6 (12.8)	11 (17.7)		7 (12.7)	6 (9.5)		4 (16.7)	1 (5.9)	
*2/*2	3 (2.7)	7 (6.8)		0	2 (3.2)		2 (3.6)	2 (3.2)		0	0	
*2/*3	3 (2.7)	3 (2.9)		1 (2.1)	1 (1.6)		0	1 (1.6)		1 (4.2)	0	
*3/*3	0 (0)	0		0	0		0	0 (0)		0	0	
HWE for *CYP2C9* genotype, *P* value	0.52	0.09		0.54	0.38			0.52		0.54	0.38	
VKORC1 genotype, *n* (%)			0.58		1 missing	0.74		1 missing	0.54			0.46
GG	45 (39.8)	34 (33.0)		18 (38.3)	19 (30.6)		16 (29.1)	24 (38.1)		7 (29.2)	8 (47.1)	
GA	45 (39.8)	45 (43.7)		22 (46.8)	32 (51.6)		25 (45.5)	25 (39.7)		14 (58.3)	8 (47.1)	
AA	23 (20.4)	24 (23.3)		7 (14.9)	10 (16.1)		14 (25.5)	13 (20.6)		3 (12.5)	1 (5.9)	
HWE for *VKORC1* genotype, *P* value	0.07	0.23		0.95	0.57		0.07	0.23		0.95	0.57	
Atrial fibrillation, *n* %	88 (78)	82 (80)	0.76	44 (93.6)	58 (94)	0.99	45 (82)	50 (79)	0.74	21 (88)	16 (94.1)	0.48

HWE, Hardy–Weinberg equilibrium; SD, standard deviation.

Table 2 Common comorbidities of patients with dosing according to the genotype-guided algorithm and the controls stratified by age

	Acenocoumarol						Phenprocoumon					
	< 75 years			≥ 75 years			< 75 years			≥ 75 years		
	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value
Patient number	113	103		47	62		55	63		24	17	
Common comorbidities												
Hypertension, *n* (%)	55 (48.7)	53 (51.5)	0.68	36 (76.6)	48 (77.4)	0.92	24 (43.6)	29 (46)	0.79	13 (54.2)	12 (70.6)	0.29
Heart failure, *n* (%)	14 (12.4)	9 (8.7)	0.39	9 (19.1)	18 (29.0)	0.24	2 (3.6)	0	0.13	0	0	–
Myocardial infarction, *n* (%)	6 (5.3)	4 (3.9)	0.62	3 (6.4)	4 (6.5)	0.99	4 (7.3)	1 (1.6)	0.13	0	1 (5.9)	0.23
Hyperlipidemia, *n* (%)	36 (31.9)	25 (24.3)	0.22	11 (23.4)	17 (27.4)	0.64	7 (12.7)	15 (23.8)	0.12	6 (25.0)	4 (23.5)	0.91
Diabetes, *n* (%)	21 (18.6)	19 (18.4)	0.98	16 (34.0)	12 (19.4)	0.08	4 (7.3)	4 (6.3)	0.84	1 (4.2)	1 (5.9)	0.80

Table 3 Concomitant drug use stratified by age and coumarins for the genotype-guided group and the control group separately

	Acenocoumarol						Phenprocoumon					
	< 75 years			≥ 75 years			< 75 years			≥ 75 years		
	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value	Genotype-guided group	Control group	*P*-value
Patient number	113	103		47	62		55	63		24	17	
Co-medication, *n* (%)	113 (100)	99 (96.1)		46 (97.9)	61 (98.4)		55 (100)	61 (96.8)		23 (95.8)	16 (94.1)	
Potentiating drugs, *n* (%)	70 (61.9)	63 (61.2)	0.91	38 (80.9)	47 (75.8)	0.53	33 (60.0)	31 (49.2)	0.24	10 (41.7)	10 (58.8)	0.28
Enzyme inibitors, *n* (%)	50 (44.2)	43 (41.7)	0.71	27 (57.4)	35 (56.5)	0.92	27 (49.1)	22 (34.9)	0.12	9 (37.5)	8 (47.1)	0.54
Amiodarone, *n* (%)	7 (6.2)	11 (10.7)	0.23	8 (17.0)	7 (11.3)	0.39	0	0	–	0	0	–
PPIs, *n* (%)	18 (16.0)	11 (10.7)	0.26	10 (21.3)	14 (22.6)	0.87	16 (29.1)	12 (19.0)	0.20	5 (20.8)	5 (29.4)	0.53
Statins, *n* (%)	34 (30.1)	29 (28.2)	0.76	13 (27.7)	16 (25.8)	0.83	10 (18.2)	14 (22.2)	0.59	5 (20.8)	4 (23.5)	0.84
Antidepressants, *n* (%)	4 (3.5)	2 (1.9)	0.48	0	5 (8.1)	–	5 (9.1)	2 (3.2)	0.18	0	0	–
Antibacterial drugs, *n* (%)	11 (9.7)	8 (7.8)	0.61	5 (10.6)	10 (16.1)	0.41	6 (10.9)	6 (9.5)	0.80	1 (4.2)	0	–
Other NSAIDs, *n* (%)	9 (8.0)	5 (4.8)	0.35	1 (2.1)	2 (3.2)	–	2 (3.6)	3 (4.8)	0.76	0	0	–
Lactulose, *n* (%)	2 (1.8)	0	–	1 (2.1)	1 (1.6)	–	0	0	–	0	0	–
Aspirin, *n* (%)	19 (16.8)	19 (18.4)	0.75	10 (21.3)	21 (33.9)	0.15	1 (1.8)	2 (3.2)	0.64	0	2 (11.8)	–
Digoxin, *n* (%)	12 (10.6)	11 (10.7)	0.34	8 (17.0)	9 (14.5)	0.72	6 (10.9)	4 (6.3)	0.38	0	3 (17.6)	–
Enzyme inducers, *n* (%)	0	1		1			0	0	–	0	0	–

NSAID, non-steroidal anti-inflammatory drug; PPI, proton-pump inhibitor.

Table 4 Percentage of time in, below and above the therapeutic range during 12 weeks after the initiation of treatment after stratification by age

	< 75 years					≥ 75 years				
	Genotype-guided group	Control group	Percentage difference (95% CI)	Adjusted difference (95% CI)	*P*-value*	Genotype-guided group	Control group	Percentage difference (95% CI)	Adjusted difference (95% CI)	*P*-value*
Phenprocoumon										
Number	55	63				24	17			
INR of 2–3 (%), mean ± SD	64.1 ± 19.8	55.7 ± 24.0	8.4 (0.3 to 16.5)	9.5 (1.3 to 17.8)	0.02	50.9 ± 21.5	63.3 ± 21.2	− 12.4 (− 26.1 to 1.3)	− 17.9 (− 31.8 to − 3.9)	0.01
INR of < 2 (%), mean ± SD	18.3 ± 16.0	17.2 ± 19.3	1.1 (− 5.4 to 7.6)	0.1 (− 6.6 to 6.8)	0.98	17.0 ± 18.3	26.9 ± 20.9	− 9.6 (− 22.3 to 2.6)	− 9.7 (− 22.5 to 3.3)	0.14
INR of > 3 (%), mean ± SD	17.6 ± 2.19	27.1 ± 26.5	− 9.5 (− 18.3 to − 0.6)	− 9.6 (− 19.0 to − 0.2)	0.05	32.1 ± 27.2	9.9 ± 16.5	22.3 (8.4 to 36.1)	27.5 (12.9 to 42.0)	< 0.01
Acenocoumarol										
Number	113	103				47	62			
INR of 2–3 (%), mean ± SD	64.5 ± 23.7	61.3 ± 24.4	3.2 (− 3.2 to 9.7)	3.6 (− 2.9 to 10.1)	0.28	57.0 ± 25.4	61.7 ± 21.2	− 4.7 (− 13.6 to 4.2)	− 4.2 (− 13.3 to 4.9)	0.37
INR of < 2 (%), mean ± SD	21.0 ± 21.8	19.9 ± 20.3	1.1 (− 4.5 to 6.8)	0.1 (− 5.6 to 5.6)	0.98	32.1 ± 27.0	22.4 ± 19.7	9.7 (0.5 to 19.0)	9.9 (1.0 to 18.8)	0.03
INR of > 3 (%), mean ± SD	14.5 ± 18.6	18.8 ± 21.5	− 4.3 (− 9.8 to 1.1)	− 3.7 (− 9.2 to 1.9)	0.19	10.9 ± 15.5	15.9 ± 16.9	− 5.1 (− 11.3 to 1.2)	− 5.7 (− 11.9 to 0.4)	0.17
Greece										
Number	48	42				32	43			
INR of 2–3 (%), mean ± SD	66.1 ± 27.0	65.3 ± 26.6	0.8 (− 10.5 to 12.1)	1.5 (− 9.8 to 12.9)	0.79	57.3 ± 25.7	63.0 ± 20.8	− 5.6 (− 16.4 to 5.1)	− 3.8 (− 15.2 to 7.6)	0.51
INR of < 2 (%), mean ± SD	25.6 ± 25.0	20.6 ± 21.5	5.0 (− 4.8 to 14.9)	4.4 (− 5.5 to 14.3)	0.38	36.4 ± 27.2	23.3 ± 19.5	13.1 (2.4 to 23.9)	11.5 (0.1 to 2.8)	0.05
INR of > 3 (%), mean ± SD	8.3 ± 13.9	14.1 ± 18.3	− 5.8 (−12.7 to 1.1)	− 5.9 (− 12.9 to 1.0)	0.09	6.3 ± 9.5	13.8 ± 15.6	− 7.5 (− 13.7 to − 1.7)	− 7.7 (− 14.2 to − 1.3)	0.02
The Netherlands										
Number	65	61				15	19			
INR of 2–3 (%), mean ± SD	63.3 ± 21.0	58.5 ± 22.5	4.8 (− 2.9 to 12.5)	5.5 (− 2.3 to 13.2)	0.17	56.4 ± 25.7	58.9 ± 22.4	− 2.5 (− 19.3 to 14.3)	− 4.5 (− 23.9 to 14.8)	0.63
INR of < 2 (%), mean ± SD	17.6 ± 18.5	19.4 ± 19.6	− 1.8 (− 8.5 to 4.9)	− 2.5 (− 9.3 to 4.4)	0.47	23.0 ± 25.0	20.4 ± 20.5	2.6 (− 13.3 to 18.5)	5.4 (− 11.6 to 22.3)	0.53
INR of > 3 (%), mean ± SD	19.0 ± 20.9	22.0 ± 23.1	− 3.0 (− 10.8 to 4.7)	− 3.0 (− 11.0 to 5.0)	0.46	20.6 ± 20.9	20.8 ± 19.0	− 0.1 (− 14.1 to 13.9)	− 0.8 (− 15.3 to 13.7)	0.91

CI, confidence interval; INR, International Normalized Ratio; SD, standard deviation.
**P*-value for the difference adjusted for height, weight, sex, enzyme inhibitors, and enzyme inducers.

guided group had a higher percentage of time with an INR below 2 than those in the control group (adjusted difference of 9.9%, 95% CI 1.0 to 18.8). There was no statistically significant difference in the percentage of time with an INR below 2 among younger patients treated with acenocoumarol. Regarding the percentage of time with an INR above 3, the genotype-guided group and the control group did not differ significantly for either the younger or the older patients. A per-protocol analysis showed similar results (Table S6).

Effect of algorithms stratified by genotype variants

As shown in Table 5, among younger patients treated with phenprocoumon, the effect of genotype-guided dosing as compared with the control group was most remarkable (14.0% difference in TTR, $P = 0.04$) if there were two or more variants of *CPY2C9* or *VKORC1*. There were no statistically significant differences between the two groups in patients with only one variant of either *CPY2C9* or *VKORC1*. In patients without variation in either *CPY2C9* or *VKORC1*, genotype-guided dosing gave a 9.4% improvement in the TTR as compared with the control group; however, the difference was not statistically significant. Older patients without variation in *CPY2C9* and *VKORC1* dosed according to the genotype-guided algorithm achieved a similar TTR as the patients dosed according to the clinical algorithm used in the control group. However, the percentage of time above the range in the genotype-guided algorithm group was higher than in the control group. None of the differences was statistically significant. For the older patients with only one variant of either *CYP2C9* or *VKORC1*, genotype-guided dosing led to a lower TTR and less time below an INR of 2, whereas 21.3% spent more time above the TTR ($P = 0.04$).

For acenocoumarol, both in younger and in older patients without variation or with one variant of either *CPY2C9* or *VKORC1*, genotype-guided dosing led to a higher TTR; however, the difference was not statistically significant. In contrast, among patients with two or more variants of either *CPY2C9* or *VKORC1*, genotype-guided dosing resulted in a higher TTR in younger patients but a lower TTR in older patients, also without a statistically significant difference. These data are shown in Table S7.

The initial predicted maintenance dose stratified by genotype variants is shown in Table 6. The dose calculated according to the genotype-guided algorithm was compared with the dose calculated according to the clinical algorithm. Generally, with use of the genotype-guided algorithm, both younger and older patients would be prescribed a higher dose if they had no variants, a similar dose if they had one variant allele, and a lower dose if they had more than one variant allele, as compared with the dose calculated according to the clinical algorithm, either for phenprocoumon users or for acenocoumarol users.

Table 5 Effect of genetic variants on anticoagulation control in genotyped and control patients among younger and elderly phenprocoumon users

	< 75 years						≥ 75 years					
	Genotype-guided group		Control group		Percentage difference (95% CI)	*P*-value	Genotype-guided group		Control group		Percentage difference (95% CI)	*P*-value
	%, mean ± SD	*N*	%, mean ± SD	*N*			%, mean ± SD	*N*	%, mean ± SD	*N*		
Percentage of time in therapeutic INR range												
No variation	63.3 ± 19.2	12	53.9 ± 24.5	18	9.4 (− 7.8 to 26.6)	0.27	58.1 ± 9.1	5	56.0 ± 28.5	5	2.0 (− 32.8 to 36.9)	0.89
One variant	62.4 ± 19.4	21	63.0 ± 20.2	18	− 0.6 (− 13.5 to 12.3)	0.93	56.7 ± 24.6	12	67.2 ± 18.5	11	− 10.5 (− 29.5 to 8.5)	0.27
Two or more variants	66.1 ± 21.2	22	52.1 ± 25.9	27	14.0 (0.2 to 27.8)	0.05	35.6 ± 14.8	7	55.6	1		–
Percentage of time with INR of < 2.0												
No variation	22.4 ± 18.2	12	30.0 ± 22.8	18	− 7.5 (− 23.6 to 8.6)	0.42	15.6 ± 17.2	5	30.8 ± 24.8	5	− 15.1 (− 46.2 to 15.9)	0.29
One variant	18.7 ± 17.9	21	18.3 ± 16.3	18	0.4 (− 10.8 to 11.6)	0.94	16.4 ± 20.9	12	27.3 ± 19.8	11	− 10.8 (− 28.5 to 6.9)	0.42
Two or more variants	15.6 ± 12.6	22	8.0 ± 13.3	27	7.7 (0.2 to 15.2)	0.05	19.0 ± 16.8	7	3.5	1		–
Percentage of time with INR of > 3.0												
No variation	14.2 ± 19.5	12	16.1 ± 21.6	18	− 1.9 (− 17.8 to 14.0)	0.81	26.3 ± 19.3	5	13.2 ± 21.4	5	13.1 (− 16.7 to 42.9)	0.34
One variant	18.9 ± 23.7	21	18.8 ± 21.8	18	0.2 (− 14.7 to 15.0)	0.98	26.8 ± 30.7	12	5.5 ± 11.3	11	21.3 (0.9 to 41.7)	0.04
Two or more variants	18.3 ± 22.2	22	40.0 ± 27.6	27	− 21.7 (− 36.0 to − 7.4)	0.00	45.3 ± 23.7	7	40.8	1		–

CI, confidence interval; INR, International Normalized Ratio; SD, standard deviation.

Table 6 The mean difference between the doses calculated for phenprocoumon users with the genotype-guided algorithm and the clinical algorithm

	Genotype-guided group					Control group				
Stratified by genotype	*N*	Dose calculated with the genotype-guided algorithm, mean ± SD	Dose calculated with the clinical algorithm, mean ± SD	Difference	*P*-value	*N*	Dose calculated with the clinical algorithm, mean ± SD	Dose calculated with the genotype-guided algorithm, mean ± SD	Difference	*P*-value
Age < 75 years										
Pooled	55	2.2 ± 0.6	2.3 ± 0.3	− 0.20	0.05	62	2.2 ± 0.3	2.2 ± 0.6	0.0	0.79
No variation	12	3.0 ± 0.3	2.4 ± 0.4	0.60	0.00	18	2.2 ± 0.2	2.9 ± 0.3	− 0.7	0.00
One variant	21	2.2 ± 0.2	2.2 ± 0.2	0.00	0.40	18	2.2 ± 0.3	2.3 ± 0.2	− 0.1	0.11
Two or more variants	22	1.6 ± 0.4	2.3 ± 0.3	− 0.70	0.00	26	2.2 ± 0.4	1.6 ± 0.4	0.6	0.00
Age ≥ 75 years										
Pooled	24	1.8 ± 0.5	1.8 ± 0.2	0.00	0.96	17	1.7 ± 0.2	1.9 ± 0.4	− 0.2	0.10
No variation	5	2.4 ± 0.2	1.8 ± 0.2	0.60	0.00	5	1.7 ± 0.3	2.2 ± 0.3	− 0.6	0.00
One variant	12	1.9 ± 0.3	1.9 ± 0.3	0.00	0.65	11	1.8 ± 0.2	1.8 ± 0.2	0	0.35
Two or more variants	7	1.3 ± 0.4	1.7 ± 0.1	− 0.40	0.02					

SD, standard deviation.

Discussion

The present study shows that there is an interaction between age and genotype-guided dosing for phenprocoumon during the initial period of use. An age cut-off point of 75 years was chosen to stratify patients into a younger age group and an older age group. For younger patients, genotype-guided dosing increased the TTR by 9.3% and reduced the time above the TTR by 9.5%. However, for patients who were aged ≥ 75 years, genotype-guided dosing did not show an improvement as compared with patients who were treated according to a clinical algorithm (including the same factors as the genetic algorithm, except for the genetic variants). For acenocoumarol users, the point estimates of the effect were in the same direction. However, there were no statistically significant differences between the age groups.

Previously, the EU-PACT trial [9] reported that genotype-guided dosing of acenocoumarol or phenprocoumon did not statistically significantly improve the TTR during the 12 weeks after the initiation of therapy. However, this outcome was the mean value based on subjects of all ages. When patients were stratified by age groups, in the younger age group genotype-guided dosing resulted in a higher TTR (difference of 5.1%, $P = 0.05$) than dosing according to the clinical algorithm [12]. However, that was a combined result for acenocoumarol and phenprocoumon. In the present study, by stratifying the patients by age, we provided evidence that, among patients aged < 75 years, genotype-guided dosing for phenprocoumon could lead to a statistically significant improvement in the TTR. However, patients aged ≥ 75 years did not benefit from the current pharmacogenetic algorithm for phenprocoumon [23]. The pharmacogenetic algorithm may be considered to perform worse than the clinical algorithm in this age group, because the percentage of time spent above the target range was higher than in the clinical algorithm group, which may cause harm to these patients. However, we should also consider the limitation of the dosing algorithm. Age might not be correctly captured. Previously, it has been shown that the clinical algorithm for phenprocoumon has a tendency to result in underdosing relative to the genotype-guided algorithm [13]. Our present study also shows that, in patients without variant alleles of *CYP2C9* and *VKORC1*, the dose predicted by the clinical algorithm would be significantly lower than that predicted by the genotype-guided algorithm in both the younger group and the older group. Elderly patients in the ≥ 75-year group are likely to require an age-related lower dose of anticoagulant, so the lower percentage of time above the TTR in the control group of phenprocoumon users might be partly explained by the lower dose predictions of the clinical algorithm than of the genotype-guided algorithm. Therefore, the increased time above the TTR might not represent an interaction with genotype, but an insufficient age-related dose correction in the genotype-guided algorithm.

Our data were obtained from a randomized control trial, so the baseline characteristics were similar between the genotype algorithm-guided and the clinical algorithm-guided groups. After stratification by age, most of the baseline characteristics of the trial population were still balanced between the genotype-guided group and the control group, except for the mean weight among

younger phenprocoumon-treated patients. However, it is unlikely that the younger patients dosed according to the genotype-guided algorithm got a higher TTR was because of their higher mean weight.

We tested whether there was a difference in existing comorbidities that might influence the dose response for coumarins [7]. However, they were equally distributed between the genotype-guided group and the control group. Furthermore, our outcomes were adjusted for the co-medications; however, these did not differ between age groups, and so also cannot not explain our findings.

One suggested explanation for our findings is related to the different physical conditions and drug metabolism between young and old populations [3]. Although coumarin doses were inversely related to age [1,2], the rate of decline of dose requirement was not necessarily similar between the young and old groups. For instance, among younger patients, the dose requirement decreased strongly with increase in age, whereas, among elderly patients, the decrease in dose requirement with age was less pronounced [1]. Previously, another study reported a pharmacogenetic-based dosing algorithm that failed to identify older patients who needed a lower daily dose (with two variants of *VKORC1*) of warfarin [23]. In the present study, we compared the first prescribed maintenance dose and the TTR in three genetically defined strata. The outcome in patients aged < 75 years was in accordance with the predicted first prescribed dose in patients without, with one and with two or more *CYP2C9* and *VKORC1* variants. However, the dose response was not necessarily as expected among patients aged ≥ 75 years. For instance, doses predicted by the genotype-guided algorithm and the clinical dosing algorithm were similar for the older patients with only one variant of either *CYP2C9* or *VKORC1*; however, patients in the genotype-guided group had a higher percentage of time above the TTR. Without stratification by age, the genotype-guided algorithm was not able to accurately predict dosage for either younger or older patients.

Another possible limitation of the dosing algorithm could be that we only included *CYP2C9* and *VKORC1* genotypes that are common in Caucasian populations. Besides the genetic variants and clinical factors used in the present algorithm, there might be some undetected variants that accounted for the differences in the effect of pharmacogenetic dosing in older patients. For instance, if patients have variants other than *CYP2C9*2* and *CYP2C9*3* that can reduce enzyme activity, they will be misclassified as having the *CYP2C9*1/*1* genotype. For those patients, the dose predicted by the algorithm will be inaccurate and higher than the actual required dose. This could partly explain why, in the present study, the doses in the patients with no variants are not accurately predicted by the genotype-guided algorithm. However, it is important to note that these variants are only expected to be present in a small percentage of the patients. It would be important to consider the inclusion of rare genetic variants as well as making a better age adjustment for older patients when applying the dosing algorithm.

In the present study, unlike in patients treated with phenprocoumon, the TTRs of the patients treated with the genotype-guided dose for acenocoumarol were not statistically significant different from those of the patients who received the clinical algorithm dose, among either the younger patients or the older patients. A possible explanation for this finding is that the half-life of acenocoumarol [24] is considerably shorter than the half-life of phenprocoumon [25]. Therefore, we used a different dose adjustment strategy after the loading period in the EU-PACT trial [9]. This might account for the different stratified outcomes between acenocoumarol and phenprocoumon.

Several limitations of our study should be considered. First, this study is a subgroup analysis of a prospective randomized trial, leading to small sample sizes in different strata, especially in the older subset of the phenprocoumon patients. This reduced the power and caused a large CI. Second, the stratified analysis was not part of the original study design of the EU-PACT trial, so this *post hoc* analysis with multiple testing might cause chance findings. It is important to conduct further studies to test a separate dose algorithm for older patients. Third, our study used the TTR and the percentage of time spent below and above the TTR as outcomes, which are surrogate outcomes for evaluating the quality of anticoagulation, whereas clinical events are more important in clinical practice.

In conclusion, we found, in the EU-PACT trial, that *VKORC1* and *CYP2C9* genotype together with clinical factors could improve the accuracy in predicting the initial dose of phenprocoumon in patients aged < 75 years during the initial 12 weeks of treatment. For patients aged ≥ 75 years, the algorithm should be revised and tested in further research.

Addendum

Y. Zhang was involved in conception and design of the study, acquisition of data, analysis and interpretation of data, drafting of the manuscript, and statistical analysis. A. de Boer was involved in conception and design of the study, analysis and interpretation of data, critical revision of the manuscript, and supervision. T. I. Verhoef was involved in conception and design of the study, acquisition of data, and critical revision of the manuscript. F. J. M. van der Meer was involved in conception and design of the study, acquisition of data, and critical revision of the manuscript. S. le Cessie was involved in analysis and interpretation of data, and critical revision of the manuscript. V. G. Manolopoulos was

involved in conception and design of the study, critical revision of the manuscript, and supervision. A. H. Maitland-van der Zee was involved in conception and design of the study, analysis and interpretation of data, critical revision of the manuscript, obtaining funding, and supervision.

Acknowledgements

The authors would like to acknowledge all of the patients who participated in the EU-PACT trial and all of the staff at the anticoagulant clinics and hospitals where patient recruitment was conducted. This work was supported by the European Commission Seventh Framework Program (HEALTH-F2-2009-223062) and partially supported by the China Scholarship Council.

Disclosure of Conflict of Interests

A. H. Maitland-van der Zee reports receiving an unrestricted research grant from GlaxoSmithKline, outside the submitted work. The other authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. There is a trend towards an age interaction on the primary outcome of genotype-guided dosing for phenprocoumon users (A) but not for acenocoumarol users (B).
Fig. S2. There is a trend towards an age interaction on the primary outcome of genotype-guided dosing for phenprocoumon users (A) but not for acenocoumarol users (B).
Table S1. Concomitant drug use stratified by age and coumarins for the genotype-guided group and the control group separately.
Table S2. Protocol violations and numbers of patients excluded from the per-protocol analyses.
Table S3. Characteristics of patients dosing by the genotype-guided algorithm and the control stratified by age (acenoucoumarol users in the Netherlands and Greece).
Table S4. Characteristics of patients stratified by age.
Table S5. The comparison of concomitant drug use between younger and older patients, respectively, in the genotype-guided group and the control group.
Table S6. Percentage of time in, below and above the therapeutic range during 12 weeks after the initiation of treatment after stratification by age (per-protocol analysis).
Table S7. Effect of genetic variants on anticoagulation control in genotyped and control patients among younger and elderly acenocoumarol users.

Appendix

EU-PACT group members

A. de Boer, Utrecht, the Netherlands; A. Daly, Newcastle upon Tyne, UK; F. Kamali, Newcastle upon Tyne, UK; A.-H. Maitland-van der Zee, Utrecht, the Netherlands; K. Redekop, Rotterdam, the Netherlands; V. G. Manolopoulos, Alexandroupolis, Greece; M. Pirmohamed, Liverpool, UK; F. R. Rosendaal, Leiden, the Netherlands; and M. Wadelius, Uppsala, Sweden.

References

1 Garcia D, Regan S, Crowther M, Hughes RA, Hylek EM. Warfarin maintenance dosing patterns in clinical practice: implications for safer anticoagulation in the elderly population. *Chest* 2005; **127**: 2049–56.
2 Cesar JM, Garcia-Avello A, Navarro JL, Herraez MV. Aging and oral anticoagulant therapy using acenocoumarol. *Blood Coagul Fibrinolysis* 2004; **15**: 673–6.
3 Mangoni AA, Jackson SH. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. *Br J Clin Pharmacol* 2004; **57**: 6–14.
4 Redwood M, Taylor C, Bain BJ, Matthews JH. The association of age with dosage requirement for warfarin. *Age Ageing* 1991; **20**: 217–20.
5 Fleg JL, Aronow WS, Frishman WH. Cardiovascular drug therapy in the elderly: benefits and challenges. *Nat Rev Cardiol* 2011; **8**: 13–28.
6 Holbrook AM, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M, Wells PS. Systematic overview of warfarin and its drug and food interactions. *Arch Intern Med* 2005; **165**: 1095–106.
7 Penning-van Beest FJ, van Meegen E, Rosendaal FR, Stricker BH. Characteristics of anticoagulant therapy and comorbidity related to overanticoagulation. *Thromb Haemost* 2001; **86**: 569–74.
8 Fang MC, Go AS, Hylek EM, Chang Y, Henault LE, Jensvold NG, Singer DE. Age and the risk of warfarin-associated hemorrhage: the anticoagulation and risk factors in atrial fibrillation study. *J Am Geriatr Soc* 2006; **54**: 1231–6.
9 Verhoef TI, Ragia G, de Boer A, Barallon R, Kolovou G, Kolovou V, Konstantinides S, Le Cessie S, Maltezos E, van der Meer FJ, Redekop WK, Remkes M, Rosendaal FR, van Schie RM, Tavridou A, Tziakas D, Wadelius M, Manolopoulos VG, Maitland-van der Zee AH; EU-PACT Group. A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon. *N Engl J Med* 2013; **369**: 2304–12.
10 Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, Kesteven P, Christersson C, Wahlstrom B, Stafberg C, Zhang JE, Leathart JB, Kohnke H, Maitland-van der Zee AH, Williamson PR, Daly AK, Avery P, Kamali F, Wadelius M; EU-PACT Group. A randomized trial of genotype-guided dosing of warfarin. *N Engl J Med* 2013; **369**: 2294–303.
11 Kimmel SE, French B, Kasner SE, Johnson JA, Anderson JL, Gage BF, Rosenberg YD, Eby CS, Madigan RA, McBane RB, Abdel-Rahman SZ, Stevens SM, Yale S, Mohler ER 3rd, Fang MC, Shah V, Horenstein RB, Limdi NA, Muldowney JA 3rd, Gujral J, *et al*. A pharmacogenetic versus a clinical algorithm for warfarin dosing. *N Engl J Med* 2013; **369**: 2283–93.
12 Maitland-van der Zee AH, de Boer A, Manolopoulos VG; EU-PACT Group. Genotype-guided dosing of vitamin K antagonists. *N Engl J Med* 2014; **370**: 1765–6.

13 van Schie RM, Wessels JA, le Cessie S, de Boer A, Schalekamp T, van der Meer FJ, Verhoef TI, van Meegen E, Rosendaal FR, Maitland-van der Zee AH; EU-PACT Study Group. Loading and maintenance dose algorithms for phenprocoumon and acenocoumarol using patient characteristics and pharmacogenetic data. *Eur Heart J* 2011; **32**: 1909–17.
14 van Schie RM, Wadelius MI, Kamali F, Daly AK, Manolopoulos VG, de Boer A, Barallon R, Verhoef TI, Kirchheiner J, Haschke-Becher E, Briz M, Rosendaal FR, Redekop WK, Pirmohamed M, Maitland van der Zee AH. Genotype-guided dosing of coumarin derivatives: the European pharmacogenetics of anticoagulant therapy (EU-PACT) trial design. *Pharmacogenomics* 2009; **10**: 1687–95.
15 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.
16 Nelson WW, Choi JC, Vanderpoel J, Damaraju CV, Wildgoose P, Fields LE, Schein JR. Impact of co-morbidities and patient characteristics on international normalized ratio control over time in patients with nonvalvular atrial fibrillation. *Am J Cardiol* 2013; **112**: 509–12.
17 Teichert M, van Noord C, Uitterlinden AG, Hofman A, Buhre PN, De Smet PA, Straus S, Stricker BH, Visser LE. Proton pump inhibitors and the risk of overanticoagulation during acenocoumarol maintenance treatment. *Br J Haematol* 2011; **153**: 379–85.
18 van Schie RM, Verhoef TI, Boejharat SB, Schalekamp T, Wessels JA, le Cessie S, Rosendaal FR, van der Meer FJ, de Boer A, Maitland-van der Zee AH. Evaluation of the effect of statin use on the acenocoumarol and phenprocoumon maintenance dose. *Drug Metabol Drug Interact* 2012; **27**: 229–34.
19 Wedemeyer RS, Blume H. Pharmacokinetic drug interaction profiles of proton pump inhibitors: an update. *Drug Saf* 2014; **37**: 201–11.
20 Sansone RA, Sansone LA. Warfarin and antidepressants: happiness without hemorrhaging. *Psychiatry (Edgmont)* 2009; **6**: 24–9.
21 Visser LE, Penning-van Bees FJ, Kasbergen AA, De Smet PA, Vulto AG, Hofman A, Stricker BH. Overanticoagulation associated with combined use of antibacterial drugs and acenocoumarol or phenprocoumon anticoagulants. *Thromb Haemost* 2002; **88**: 705–10.
22 Visser LE, Penning-van Beest FJ, Wilson JH, Vulto AG, Kasbergen AA, De Smet PA, Hofman A, Stricker BH. Overanticoagulation associated with combined use of lactulose and acenocoumarol or phenprocoumon. *Br J Clin Pharmacol* 2004; **57**: 522–4.
23 Schwartz JB, Kane L, Moore K, Wu AH. Failure of pharmacogenetic-based dosing algorithms to identify older patients requiring low daily doses of warfarin. *J Am Med Dir Assoc* 2011; **12**: 633–8.
24 Thijssen HH, Drittij MJ, Vervoort LM, 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.
25 Ufer M. Comparative pharmacokinetics of vitamin K antagonists: warfarin, phenprocoumon and acenocoumarol. *Clin Pharmacokinet* 2005; **44**: 1227–46.