

Eva Hummers-Pradier · Stephan Hess  
Ibrahim M. Adham · Thomas Papke  
Burkert Pieske · Michael M. Kochen

## Determination of bleeding risk using genetic markers in patients taking phenprocoumon

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**Abstract Background:** In patients on oral anticoagulation with warfarin, genetic variations of the cytochrome P 450–CYP2C9 have recently been associated with very low warfarin requirements. Patients needing low doses had an increased risk for bleeding complications. In Germany, phenprocoumon (having a similar metabolic pathway) is the most commonly employed vitamin K antagonist. Treatment is usually monitored by general practitioners (GPs).

**Objectives:** To determine whether CYP2C9 variant alleles can serve as risk markers in general-practice patients anticoagulated with phenprocoumon.

**Methods:** All adult anticoagulated patients in 12 teaching general practices and one university outpatient clinic were to be recruited. Blood samples were taken from 185 patients during routine anticoagulation controls and tested for CYP2C9 mutations. Subjects answered a questionnaire concerning bleeding complications, drug intolerance, and personal and family medical history. Phenprocoumon dosages required for stable anticoagulation were recorded. Odds ratios (OR) with 95% confidence intervals (CI) were calculated based on 2-way cross-tabulations and multivariate logistic regression models, *t*-tests used where appropriate.

**Results:** Bleeding was reported by 19% of the patients, 2.2% of whom had suffered life-threatening bleeding. CYP2C9 variants were carried by 26.3% of 179 patients tested (17.9% \*1/\*2, 7.8% \*1/\*3, 0.6% \*2/\*3). While presence of a \*2 allele was not associated with an increased risk (OR 0.35, CI 0.10–1.24), carriers of the rare \*3 alleles had a higher risk of bleeding (OR 3.10, CI 1.02–9.40). With regard to bleeding, carrying CYP2C9\*3 was highly specific (94%), though sensitivity was low at 17%; post-test probability of bleeding was 40%.

**Conclusions:** CYP2C9\*3 variants are associated with an increased bleeding risk in patients anticoagulated with phenprocoumon. Screening can identify patients with a high risk of bleeding. Appropriate clinical consequences (restricted indication for anticoagulation, careful induction, adjustment of target INR, closer monitoring or self-testing of INR) as well as the cost-effectiveness of screening for variant CYP2C9 with regard to patient outcomes should be subject of further research.

**Keywords** Anticoagulants · Cytochrome P-450 CYP2C9 polymorphism · General practice · Genetic markers · Phenprocoumon

E. Hummers-Pradier (✉) · S. Hess · M. M. Kochen  
Department of General Practice,  
University of Göttingen Medical School,  
Humboldtallee 38, 37073 Göttingen, Germany  
E-mail: ehummer@gwdg.de  
Tel.: +49-551-398360  
Fax: +49-551-399530

I. M. Adham · T. Papke  
Department of Human Genetics,  
University of Göttingen Medical School,  
Heinrich-Düker-Weg 12,  
37073 Göttingen, Germany

B. Pieske  
Department of Cardiology and Pulmonology,  
University of Göttingen Medical School,  
Robert-Koch-Strasse 40,  
37075 Göttingen, Germany

### Introduction

Oral anticoagulation is a high-risk therapy: Incidence of bleeding complications has been reported as 10–17 per 100 patient-years (any severity) and 2–5 per 100 patient-years (major bleeding) [1]. In Germany, some 172.5 million defined daily doses (DDD) of phenprocoumon were prescribed in 2000 [2], corresponding to approximately 473,000 anticoagulated patients and some 10,000–80,000 patients with bleeding complications per year.

Phenprocoumon, the drug almost exclusively used for oral anticoagulation in Germany [2, 3], is a vitamin K antagonist similarly structured to warfarin (with a much longer half-life: 6.5 vs 1.5 days) [4], but considered to be less prone to drug interactions. [5] Despite the differences in molecular structure and pharmacokinetics, the

metabolism of both warfarin and phenprocoumon depends (to some extent) on the CYP2C9 isoenzyme of the cytochrome P450.[6] The exact pathway of the phenprocoumon metabolism, however, remains to be clarified. Interindividual differences in drug response caused by variable metabolizing enzyme activity due to genetic variants of CYP2C9 have been described. Next to a wild-type allele *CYP2C9\*1*, two variant alleles with decreased enzyme activity have been described: people with a *\*1/\*2* polymorphism have a somewhat reduced enzyme activity, carriers of *\*1/\*3* or *\*2/\*2* genotypes have a significantly reduced metabolism, and *\*2/\*3* and *\*3/\*3* carriers are classified as slow metabolizers [7].

In 1999, Aithal et al. reported that selected anticoagulated patients presenting genetic variants of *CYP2C9* often needed very low doses of warfarin, and that low-dose patients had more than a threefold increased risk of major bleeding complications (a direct association of bleeding risk and presence of a variant allele was, however, not demonstrated) [8]. Margaglione et al. [9] and Higashi et al. [10] have shown a two- to threefold increased bleeding risk in warfarin-anticoagulated patients with any variant *CYP2C9* allele who were followed up in specialized clinics. In contrast to the UK, where patients are usually followed up in hospital-based anticoagulation clinics, in Germany most patients with oral anticoagulation are managed in general practice (an approach which has shown to be feasible in the UK also) [11, 12].

The aim of our study was to determine whether *CYP2C9* polymorphisms are relevant for patients taking phenprocoumon, and to evaluate their feasibility and validity as risk markers in a (unselected) cross-sectional sample of anticoagulated general practice patients [9, 13, 14, 15, 16].

## Methods

The study was approved by the Göttingen University ethics committee.

The prevalence of *\*3* polymorphisms in general populations is reported to be 7–10% [17, 18]. On this basis a sample size of 170–500 would be necessary to demonstrate a sixfold increased bleeding risk with a 5% significance level and a power of 80%.

All 25 teaching general practices in the city of Göttingen, as well as the university outpatient clinic for cardiovascular diseases, were invited to participate. Five of the twelve participating practices (response rate 48%) were run by one GP, 6 had two GPs, and 1 was a group practice with four partners. Respondents were asked to recruit all their patients taking phenprocoumon. Eligible patients were determined using the practice computers. After obtaining written informed consent, an extra 9 ml of venous blood (EDTA-filled vacutainer) was drawn during a routine INR control, sent to the local institute of human genetics, and frozen until genotyping of *CYP2C9*. Samples were identified by a patient code number to insure blind analysis. Additionally, patients and GPs (or practice staff) were asked to complete a short questionnaire on the indication, duration frequency of INR controls, past bleeding, complications (site and severity), concurrent chronic disorders and treatments, as well as a history of drug intolerance. Patients and GPs were also asked to provide information on dosage schedules and past INR values (copy of the patients' anticoagulation pass or

relevant chart documentation). The questionnaires were returned by mail or collected by one of the researchers. Questionnaires and blood samples were matched by means of the patient code number.

Phenprocoumon dose requirements were determined as follows[12]: The six most recent INR values within the therapeutic range (target INR as recommended for each indication [19]) were determined in each patient's documentation.

Blood samples were analyzed using PCR-based procedures as described by Stubbins [20]. DNA fragments were examined for restriction fragment length polymorphisms (RFLPs); incomplete digestion was ruled out by application of controls in every batch and double-checking suspect samples. Genotyping was blind with regard to patient identity and questionnaire responses; results were matched with the questionnaires by means of the patient code number in the department of GP. Descriptive statistics, *t*-tests, odds ratios (OR), and 95% confidence intervals (CI) based on 2×2 contingency tables were calculated, relating occurrence of bleeding with presence of variant alleles, phenprocoumon requirement, target INR, and comedication. To assess the influence of variant alleles on bleeding compared with other possible factors, multivariate (logistic regression) models were determined using SAS Version 8.1 [21]. Accuracy of genotyping as a diagnostic test was calculated according to Sackett. [22].

## Results

From August 1999 to February 2000, 185 patients (137 general practice and 48 hospital outpatients) were included into the study. Fifty-eight general practice patients could not be included because they did not contact their practice within the research period, refused consent, or were not recruited by their GP for logistic reasons. Information on these patients could not be gathered due to the particularities of the German healthcare system (no gatekeeping, no practice lists). Five practices had included all their eligible patients, 5 practices between 50 and 100%, two practices had recruited less than half of their patients. A mean of 14 patients (range 2–31) were recruited per practice. Patient characteristics and indications for anticoagulation are summarized in Table 1.

Phenprocoumon dosages could be retrieved for 155 patients (83.8%), documentation of INR and/or dosages was incomplete in the others. A vast majority of patients (88.1%) took other long-term drugs in addition to phenprocoumon (mean number of drugs 3.6, 95% CI 3.3–3.9). Comorbidity was documented in 159 patients (86%, average number of codiagnoses 2.6, 95% CI 2.3–2.9) 92.5% of whom were treated (the most frequent comorbidities and comedications are shown in Table 2).

## Genotypes

Genotyping of *CYP2C9* alleles was performed for 179 (of the 185) patients (blood samples did not arrive at the institute of human genetics in six cases: four hospital outpatients and two general practice patients). Wild-type (*\*1/\*1*) alleles were most common ( $n = 132$ , 73.7%); 26.3% had *CYP2C9* polymorphisms: *\*1/\*2* genotype in 32 patients (17.9%), *\*1/\*3* genotype in 14 patients

**Table 1** Patient characteristics ( $n = 185$ )

Age (years)		Median	64
		25–75% Range	55–73
Women (%)			43
Indications for anticoagulation (multiple answers possible)	Cardiac arrhythmia (%)		31.4
	Valve replacement (%)		22.7
	Venous thromboembolism (%)		19.5
	Congestive heart failure (%)		9.2
	Coronary heart disease (%)		8.6
	Arteriosclerosis (%)		5.9
	Other valvular heart disease (%)		5.9
	Other, none of the above (%)		3.2
Treatment duration (years)		Mean	5.6
		95% CI	4.8–6.6
INR self-controls (%)			7.7
Control interval (weeks)		Mean	2.8
		95% CI	2.6–3.0
Target INR	1.5–2.5 (%)		10.6
	2.0–3.0 (%)		65.0
	3.0–4.5 (%)		24.4
Bleeding	Minor (%)		9.7
	Serious (%)		2.2
	Life-threatening: gastroin- testinal, cerebral, hip joint (%)		2.2
	Severity not specified (%)		4.9
Reported drug intolerance	Total	<i>n</i>	27
		%	14.6
	Penicillin/ antibiotics	<i>n</i>	6
		%	3.2
	Aspirin	<i>n</i>	5
		%	2.7
	Diclofenac	<i>n</i>	2
		%	1.1
	Losartan	<i>n</i>	1
		%	0.5
Other/not specified	<i>n</i>	13	
	%	7.0	

**Table 2** Comorbidity and comedication, frequent disorders and drugs ( $n = 185$ , multiple answers possible)

		Occurrence (%)
Concomitant chronic disorders	Total	86.0
	Hypertension	39.5
	Coronary heart disease	25.4
	Diabetes	23.8
	Dyslipidemia	23.8
	Chronic heart failure	17.8
	Others, none of the above	18.4
Concomitant long-term drug therapy	Total	88.1
	Diuretics	49.7
	Digitalis	43.8
	ACE inhibitors	43.8
	Beta-blockers	27.6
	Cardiac vasodilators	25.4
	Others, none of the above	10.3

(7.8%), and  $*2/*3$  genotype in 1 (0.6%) patient. Mean treatment duration did not differ significantly between wild-type or variant allele carriers, nor did target INR or age. Significantly less  $*2$  carriers were on comedication compared with wild-type carriers (76 versus 92%,  $P = 0.01$ ), the difference between wild-type and  $*3$  carriers was not significant (80% vs 92%). There was no significant difference in the mean phenprocoumon doses

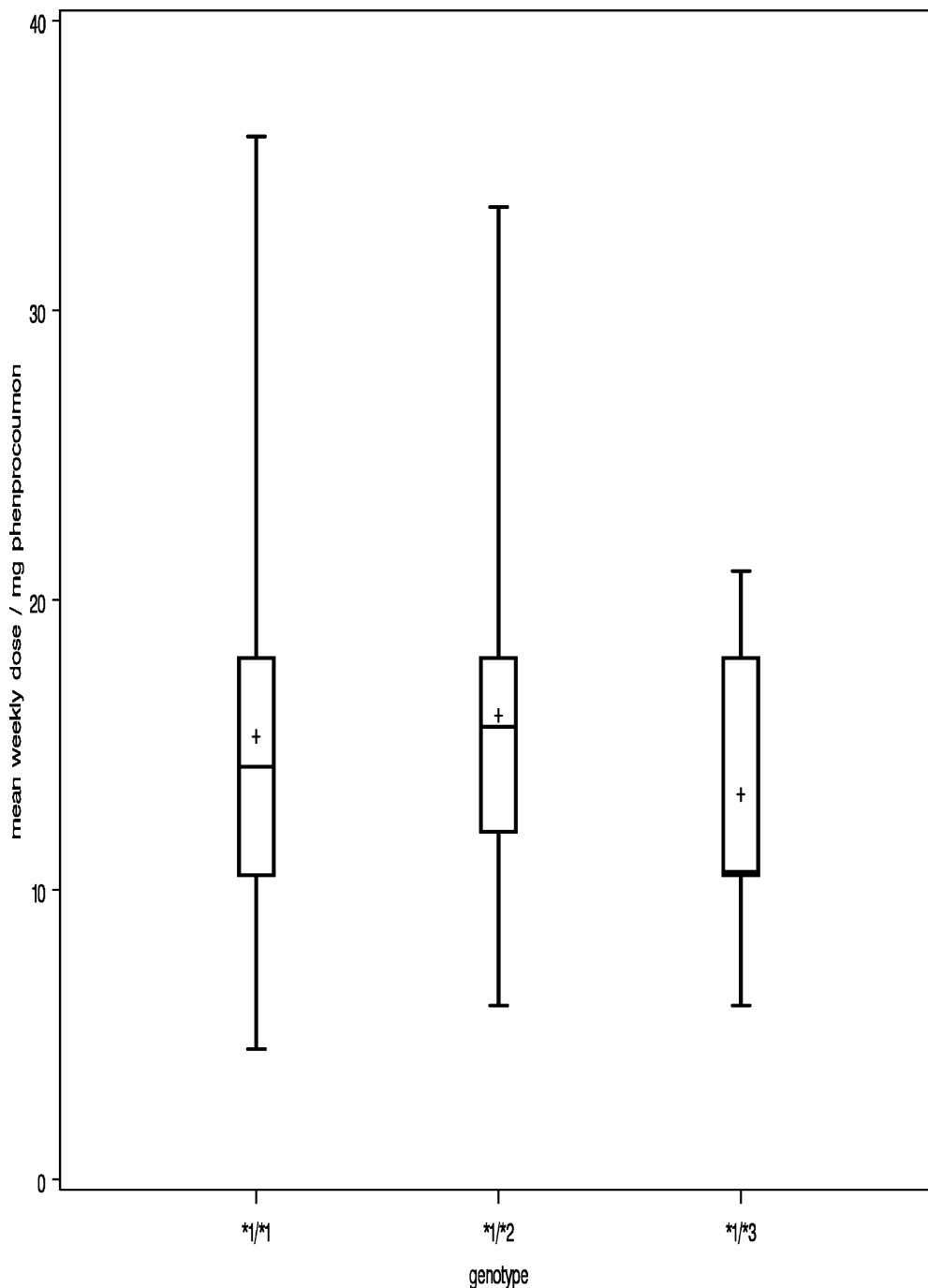
required by patients with wild-type, (15.29 mg/week, 95% CI 14.13–16.45),  $*1/*2$  (16.02 mg/week, 95% CI 13.53–18.51), and  $*1/*3$  (13.29 mg/week, 95% CI 10.45–16.13) genotype (no dosage information was available for the only  $*2/*3$  patient); details on dose distributions are shown in Fig. 1.

However, patients who had bled required significantly lower weekly doses than patients without hemorrhagic complications: 12.58 mg (95% CI 10.92–14.23) vs 15.90 mg (95% CI 14.80–17.00). Comorbidity and comedication were not associated with increased bleeding risk, nor were individual codiagnoses or drug classes (details not shown).

Carriers of a  $CYP2C9*2$  polymorphism did not have an increased bleeding risk (OR 0.35, CI 0.10–1.24). However, a threefold increased bleeding risk (OR 3.10, CI 1.02–9.40) was detected in the 8.4% of our patients carrying  $*3$  polymorphisms ( $*1/*3$  and  $*2/*3$ ), resulting in a positive predictive value of 40%, i.e., four of ten patients with  $*3$  polymorphisms will suffer bleeding complications; details are shown in Tables 3 and 4.

Risk identification by  $CYP2C9*3$  genotype persisted in a multivariate (logistic regression) analysis with sex, age (less than 60 years or 60 years and older), target INR, presence of drug intolerance, comedication, and

**Fig. 1** Weekly doses of phenprocoumon according to the *CYP2C9* genotype. Means (cross), medians (horizontal bar), quartiles (box) and 10–90 percentiles (whiskers)



**Table 3** \*3 *CYP2C9* polymorphism as a diagnostic indicator for bleeding

		Bleeding complications		
		Present	Absent	Total
Test positive	*3 Polymorphism	6	9	15
Test negative	Wild-type or *1/*2	29	135	164
	Total	35	144	179

presence of a \*3 polymorphism as predictive variables model (OR 3.64, 95% CI 1.08–12.26). Reporting drug intolerance was also associated with an increased

bleeding risk (OR 3.57, 95% CI 1.34–9.51), as well as age over 60 years (OR 3.37, CI 1.12–10.20). The other predicting variables had no significant influence.

**Table 4** Test characteristics of \*3 *CYP2C9* polymorphism as a diagnostic indicator for bleeding

Sensitivity (%)	17
Specificity (%)	94
Positive predictive value (%)	40
Negative predictive value (%)	82
Prevalence of bleeding (%)	20
Pre-test odds	0.25
Likelihood ratio for positive test	2.74
Post-test odds	0.68
Post-test probability (%)	40
Number needed to treat/test	5

## Discussion

Identifying carriers of variant *CYP2C9* alleles proved to be feasible in general practice, as genotyping could be performed on a blood sample taken during a routine INR control, and is relatively inexpensive. The prevalence of variant alleles was lower in our cross-sectional sample (27%) than in Aithal's control groups (48% in anticoagulated patients, 40% in community controls) [8], and frequencies reported in European volunteer populations (33–35%) [17, 18].

In a relatively small sample of unselected general practice patients, screening for variant *CYP2C9* alleles could identify patients with a high bleeding risk: carriers of rare genotypes \*1/\*3 and \*2/\*3 proved to have a 3 times higher risk of bleeding complications than patients with wild-type or the more common \*1/\*2 variant alleles. This is in line with previous reports of *CYP2C9*\*2 carriers having only a moderately reduced enzyme activity (which is in some contrast to in vitro studies [23]), while *CYP2C9*\*3 carriers have significantly reduced function [7]. Phenprocoumon, like warfarin, is a substrate for *CYP2C9* [6], and genetic variance in the metabolism appears to have significant influence on patients' bleeding risk. Consequently, a revision of the recent proposal to use phenprocoumon instead of warfarin in patients presenting variant *CYP2C9* alleles should be considered [24]. The third vitamin K antagonist used for anticoagulation, acenocoumarol, does not seem to be a safe alternative either [25]. More studies may be necessary to determine and compare the metabolic pathways and pharmacogenetic implications for all three drugs.

Bleeding risk may be affected by several factors including vitamin K intake, which is difficult to measure reliably as questionnaires on dietary habits are of low validity [26]. Patient compliance as well as the quality of care (e.g., anticoagulation control, dose adjustment) are likely to influence the individual bleeding risk. However, assessing the quality of primary care and its influence on patient outcomes was not the focus of this study. To be useful in general practice patients, risk markers have to be prominent in patients with variable dietary habits, comorbidity, and compliance levels. Despite the sensitivity of "genetic testing" for bleeding being only 17%, our results indicate that, due to the high specificity

(94%), *CYP2C9* screening for \*3 polymorphisms can identify 8.4% of patients—a subgroup with a particularly high risk of 40%.

However, as all anticoagulated patients have (or should have) regular INR controls, the additional value of genetic screening and the impact on the management of high-risk patients remains to be determined. Closer monitoring of drug interactions and INR values could be a consequence, with self-control of INR being a management option [27, 28]. To maximize the benefit, genotyping should probably be performed before the induction of anticoagulation. A slower, low-dose induction under close supervision of INR would then be chosen for patients at risk. Indications for anticoagulation or drugs interacting with anticoagulants (particularly those metabolized via *CYP2C9*) may be set more restrictively for patients with variant alleles, and target INR values may have to be adjusted according to individual risks for bleeding and thrombotic events. Risk adjustment for genotype could also be included in computer systems for dosage advice [29], though these devices are only occasionally used in Germany. Effectiveness of these measures with regard to bleeding and thromboembolic risk would have to be subject of further studies. Larger population-based trials are necessary to determine the number of bleeding episodes that could be prevented by genetic screening of patients requiring anticoagulation.

Some methodological problems may influence the validity of our study (some of them typical for studies in a general practice setting). For confidentiality and feasibility, patient recruitment was not centrally organized, but was done by individual GPs. Though GPs were instructed to include all their patients on phenprocoumon into our trial, the inclusion rate was variable and incomplete in many practices, as can be commonly observed in studies where patients are recruited by their GP, particularly in countries without a list system [30, 31]. For most patients, detailed information on the induction period of phenprocoumon could not be provided, nor could difficulties in dose adjustment be reliably recalled. In Germany, induction of anticoagulation often takes place when patients are still in hospital and is then not documented in the patient's pass or the GPs' charts.

As frequently seen in general practice-based surveys, documentation quality was variable between and within practices. However, recall or documentation bias is unlikely to affect the study outcome, as genotyping was performed blind with regard to patients' identity or questionnaire responses. As there was not contact between the researchers and patients, we had to rely on patients' answers on the questionnaires, which had been kept short and concise in order to ensure a high acceptance. For example, instead of asking patients how often and when they had experienced bleeding in several years (which seemed likely to elicit recall bias rather than reliable data), patients were asked whether there had been bleeding episodes at all. This approach did not

allow for expression as bleeding episodes per patient year (and thus for controlling bleeding risk for duration of therapy). However, in our population, bleeding risk was of similar magnitude to that reported in other surveys [32], and there was no significant difference in duration of treatment between patients with or without bleeding complications.

In our cross-sectional study, patients having suffered adverse events of phenprocoumon may be underrepresented due to death or switch to other forms of treatment. If these (early) adverse events were related to rare *CYP2C9* variants, it seems possible that the overall importance of the *CYP2C9* genotype for phenprocoumon-induced bleeding was underestimated (and carriers of rare variant alleles underrepresented). The mean length of treatment being 5 years, patients in our study can be expected to be stabilized on phenprocoumon, but still the *CYP2C9* genotype stands out as a risk factor for bleeding.

The high rate of comorbidity, typical for (mostly) elderly patients in general practice, explains the high comedication of anticoagulated patients [33, 34] and highlights the need for monitoring with regard to drug interactions and susceptibility to complications. Previous studies did not report details on comedication [35], they excluded patients “with drug interactions as apparent cause for low-dose requirements” [8] or adjusted for any [9] or *CYP2C9*-dependent [10] comedication. Judicious use of genetic testing may be justified in patients starting oral anticoagulation or presenting additional risk factors, including consumption of drugs that may increase hypocoagulation or have a *CYP2C9*-dependent metabolism. Intolerance of losartan as well as diclofenac (and other NSAIDs) reported by a few patients merits further studies, because metabolism of losartan and diclofenac depends on *CYP2C9*. [36, 37] We cannot exclude, however, that the association of bleeding risk and reported drug intolerance was confounded by patients reporting GPs’ recommendations to avoid certain drugs (such as aspirin) as “drug intolerance.”

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