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► **To cite this version:**

D. Werner, U. Werner, A. Wuerfel, A. Grosch, H. G. Lestin, et al.. Pharmacogenetic characteristics of patients with complicated phenprocoumon dosing. *European Journal of Clinical Pharmacology*, 2009, 65 (8), pp.783-788. 10.1007/s00228-009-0639-2 . hal-00534951

HAL Id: hal-00534951

<https://hal.science/hal-00534951>

Submitted on 11 Nov 2010

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Pharmacogenetic characteristics of patients with complicated phenprocoumon dosing

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Received: 2 November 2008 / Accepted: 18 February 2009 / Published online: 25 March 2009
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Abstract

Purpose Anticoagulation therapy with coumarins necessitates a strict individualization of dosing. Whereas the impacts of the cytochrome P450 2C9 (*CYP2C9*) and vitamin K epoxide reductase (*VKORC1*) polymorphisms on warfarin dosing are clearly established, the role of these genetic variants on dosing and the safe use of phenprocoumon are less well investigated and, to a certain degree, controversial. **Methods** We studied the most frequent functional polymorphisms of *VKORC1*, *CYP2C9*, and *CYP3A5* in 60 consecutive patients demonstrating complicated phenprocoumon-mediated anticoagulation and in 120 controls. **Results** The frequencies of the less active *VKORC1* haplotype A-group alleles ($p < 0.0001$) and of *CYP2C9* genotypes with two variant alleles ($p = 0.035$) were higher in the patient cohort than in the control group, while the frequency of patients carrying only one variant *CYP2C9* allele was unchanged relative to the control subjects (RR 1.2; $p = 0.49$).

Conclusion The data suggest a fundamental role of *VKORC1* haplotypes and a minor role of *CYP2C9* variants in the anticoagulation property of phenprocoumon.

Keywords Anticoagulation · Coumarins · *CYP2C9* · *CYP3A5* · Pharmacogenetics · Phenprocoumon · *VKORC1*

Introduction

Anticoagulation by antagonists of vitamin K epoxide reductase (*VKORC1*) is a mainstay of clinical approaches to the prevention and treatment of thrombotic and embolic diseases. The international normalized ratio (INR) has to be kept within a small window to balance the therapeutic efficacy of the drug against the risk of bleeding. Coumarin dosing needs to be individualized due to effects exerted by the genetic makeup of the patient and by clinical cofactors on the pharmacodynamics and pharmacokinetics. Oral anticoagulants are the leading class of drugs causing drug-associated adverse effects that result in hospitalization [1, 2].

While warfarin is the preferred oral anticoagulant in many countries, such as Canada and the USA, phenprocoumon is the anticoagulant of choice in some continental European countries, including Germany. The seminal works of Aithal et al. [3] and Rieder et al. [4] demonstrated the marked effects of polymorphisms in the genes coding for cytochrome P450 (*CYP*) 2C9 and *VKORC1* on the pharmacokinetics and pharmacodynamics of warfarin, respectively. The *2 and *3 polymorphisms in *CYP2C9* cause amino acid exchanges underlying the metabolic impairment in carriers of the *2 and *3 alleles [5, 6]. The presence of one variant allele causes a clinically relevant reduction of clearance for some, but not all *CYP2C9* substrates [7]. The *VKORC1*-haplotype groups A and B differ in RNA expression and, consequently, *VKORC1* activity [4]. Patients carrying alleles of haplotype-group A exhibit reduced *VKORC1* expression; as a result, less warfarin is needed to inhibit the enzyme. Several studies

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have demonstrated that variant alleles have an impact on anticoagulant-dosing and the frequency of over-anticoagulation. Moreover, randomized studies designed to steer warfarin-dosing according to the *CYP2C9* or *VKORC1* genotype have demonstrated a trend towards a more correct dosing of warfarin in genotype-based strategies than in conventional dosing strategies [8, 9].

Whereas the impacts of polymorphisms in *CYP2C9* and *VKORC1* on dosing of warfarin are clearly established, the role of these genetic variants for dosing and safety of phenprocoumon are less well investigated and, to some degree, controversial. The results from pharmacokinetic studies suggest a minor impact of *CYP2C9* polymorphisms on the clearance of the more active S-enantiomer. The elimination of S-phenprocoumon appears to be moderately reduced only when two variant alleles are present. In contrast to these pharmacokinetic findings, the results of a recent Dutch clinical study suggest that a reduced phenprocoumon-dosing should be considered in patients carrying at least one variant *CYP2C9* allele [10]. As opposed to warfarin, the metabolism of phenprocoumon appears to depend on CYP3A enzymes [11, 12]. The activity of the CYP3A cytochromes exhibits marked interindividual variation, and although this variability is not well understood at the present time, it appears to be partly attributable to a polymorphism in intron 3 of *CYP3A5* that causes the generation of non-functional transcripts [13]. Most Caucasians are homozygous for this non-functional *3 allele and thus lack CYP3A5 expression.

The aim of this study was to identify the genetic factors associated with difficulties in phenprocoumon-induced anticoagulation. Sixty consecutive patients with either overanticoagulation, low dosing of phenprocoumon, or a slow reversal of anticoagulation after a pause in the drug treatment were genotyped for functional polymorphisms in *CYP2C9*, *CYP3A5*, and *VKORC1*. Genotype and allele frequencies were compared to those of 120 healthy Caucasian controls from northern Germany.

Methods

Patient characteristics

The patient cohort consisted of 60 consecutive patients (21 women; mean age 71.6 ± 11.1 years) recruited from a single center. All patients had clinically complicated anticoagulation or difficulties in dose titration and met one of the following criteria: (1) low phenprocoumon requirement ≤ 1.5 mg per day (46 patients, mean weekly dose requirement 8.3 ± 2.5 mg); (2) over-anticoagulation for at least 1 week after standard initial phenprocoumon dosing (seven patients, 18.2 ± 3.1 mg over the first 3 days); (3) prothrom-

bin time unchanged for at least 1 week after a pause in phenprocoumon administration for an operation or coronary intervention (seven patients). Indications for oral anticoagulation were atrial fibrillation (45 patients), implantation of artificial heart valves (12 patients), and venous thromboembolism (three patients). Exclusion criteria were concomitant hepatic disease, overt heart failure, systemic inflammation, or sepsis. The study was approved by the ethics committee of the federal state of Mecklenburg-West Pommern. Written informed consent was obtained from all study participants. The allele and genotype frequencies of polymorphisms in *VKORC1*, *CYP2C9*, and *CYP3A5* were compared to frequencies obtained in 120 local healthy control probands (69 women, mean age 35.0 ± 10.7 years) not receiving to anticoagulation medication.

Analytic and statistic methods

Genotypes of *CYP2C9* were detected by a commercial available PCR test (MutaREAL CYP2C9; Immundiagnostik AG, Bensheim, Germany). *VKORC1* and *CYP3A5* genotyping were performed with forced mutation-PCR-restriction fragment length polymorphisms. The rs8050894 in *VKORC1* was used to detect alleles of the haplogroup A, which is in almost complete linkage disequilibrium with the other polymorphism of the A-haplotypes [4]. The forward and reverse primers used were *AATAATGC CAGCTAGCTGCTCATCCC* and *CCTGGGGACCTAG GATGTCT*, respectively. The PCR product (133 bp) was digested with *MspI*. Only the 6853G-variant is split into two fragments (109+24 bp). The *CYP3A5* gene was genotyped for the signature-polymorphism of the *3 allele (rs776746). The primers used were *GAGGAGGAGC TCTTTTGTCTTTTCG* and *CCCTAGTTGTACGACACA CAGC*. The resulting PCR products were cut by *EcoRV* to yield two fragments in the *1 alleles (131+26 bp); the amplicons of the *3 alleles remain uncut. All PCRs were performed under standard conditions with Taq polymerase (Peqlab, Erlangen, Germany).

Statistical analyses were performed by Fisher's exact test, the Fisher–Freeman–Halton test, and the determination of exact confidence intervals (CI) of the respective genotype and allele frequencies. Statistical significance was assumed at *p* values of <0.05 .

Results

Genotype and allele frequencies in metabolic enzymes

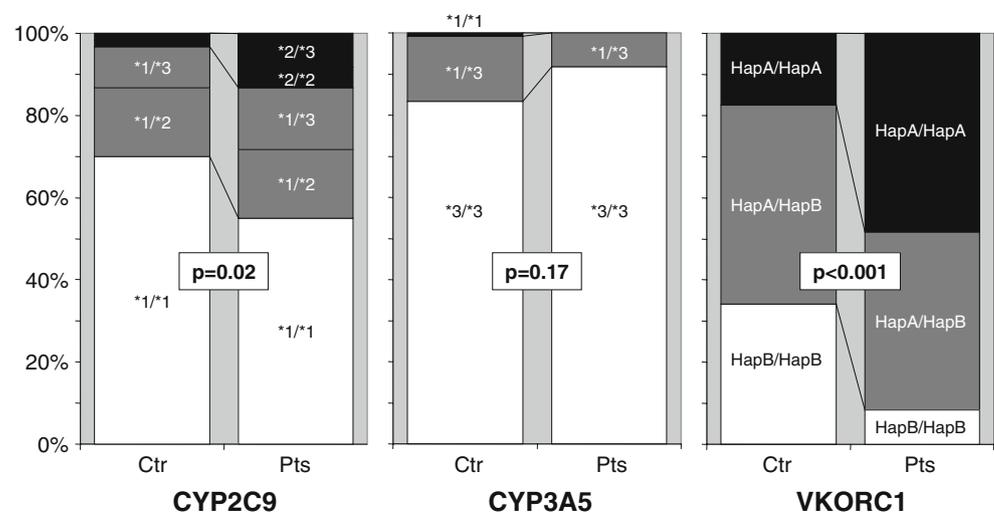
CYP2C9 allele and genotype frequencies in the patient cohort were statistically different from those of the control cohort (Table 1, Fig. 1). The relative frequencies of variant

Table 1 Genotype and allele frequencies of CYP2C9 and CYP3A5 with confidence intervals in the 60 patients and 120 healthy controls

CYP2C9					
Study group	Genotypes	n	Proportion (95% CI)	n	Proportion (95% CI)
Control	*1/*1	84	0.70 (0.62–0.77)	84	0.70 (0.61–0.78)
	*1/*2	20	0.17 (0.11–0.24)	32	0.27 (0.19–0.36)
	*1/*3	12	0.10 (0.06–0.17)		
	*2/*2	2	0.02 (0.01–0.06)	4	0.03 (0.01–0.08)
	*2/*3	2	0.02 (0.01–0.06)		
	*3/*3	0	0 (<0.01–0.03)		
Patients	*1/*1	33	0.55 (0.42–0.68)	33	0.55 (0.42–0.68)
	*1/*2	10	0.17 (0.08–0.29)	19	0.32 (0.20–0.45)
	*1/*3	9	0.15 (0.07–0.27)		
	*2/*2	3	0.05 (0.01–0.14)	8	0.13 (0.06–0.25)
	*2/*3	5	0.08 (0.03–0.18)		
	*3/*3	0	0 (<0.01–0.06)		
Control	Alleles	n	Proportion (95% CI)	n	Proportion (95% CI)
	*1	200	0.83 (0.78–0.88)	200	0.83 (0.78–0.88)
	*2	26	0.11 (0.07–0.15)	40	0.17 (0.12–0.22)
Patients	*1	85	0.71 (0.62–0.79)	85	0.71 (0.62–0.79)
	*2	21	0.18 (0.11–0.25)	35	0.29 (0.21–0.38)
	*3	14	0.12 (0.07–0.19)		
CYP3A5					
Control	Genotypes	n	Proportion (95% CI)		
	*3/*3	100	0.83 (0.75–0.90)		
	*1/*3	19	0.16 (0.1–0.24)		
Patients	*3/*3	55	0.92 (0.82–0.97)		
	*1/*3	5	0.08 (0.028–0.18)		
	*1/*1	0	0 (<0.001–0.06)		
Control	Alleles	n	Proportion (95% CI)		
	*3	219	0.91 (0.87–0.95)		
Patients	*3	115	0.96 (0.91–0.99)		
	*1	5	0.04 (0.01–0.09)		

CYP, Cytochrome P450; CI, confidence interval

Fig. 1 Distribution of the cytochrome P450 2C9 and 3A5 genotypes (*CYP2C9*, *CYP3A5*), and the vitamin K epoxide reductase (*VKORC1*) genotype (%) in the 60 patients (*Pts*) and 120 controls (*Ctr*)



CYP2C9 alleles (*2, *3) was 1.75-fold higher (95% CI 1.17–2.60) in the patients than in the Caucasian control subjects ($p=0.02$). This increase was driven by a significant increase in the number of patients carrying two variant alleles. Fourfold more patients than control subjects had two non-wildtype alleles per genome (*2/*2 or *2/*3) (95% CI 1.25–12.7; $p=0.022$). In contrast, there was no significant difference between the patients and controls in terms of the frequency of carrying one variant *CYP2C9* allele (*1/*2 or *1/*3; RR 1.19; 95% CI 0.7–1.9; $p=0.49$).

Due to frequent homozygosity for the *CYP3A5**3 allele, Caucasians only rarely express functional *CYP3A5*. In the control cohort, the allele frequency of the functional *1 allele was determined to be 0.09 (Table 1, Fig. 1). In contrast, in patients with difficult anticoagulation, the frequency of the functional allele was 0.04 (RR 0.42; 95% CI 0.18–1.23; $p=0.13$). In accordance with this result, the frequency of carriers of at least one functional *CYP3A5* (*1/*3 and *1/*1) allele was 0.17 in the control cohort and 0.08 in the patient cohort, respectively ($p=0.17$).

The frequency of the major haplotypic *VKORC1* polymorphism affecting the pharmacodynamics of oral anticoagulants is given in Table 2. We detected major differences in allele and genotype frequencies between patients with difficult anticoagulation and controls (both $p<0.0001$). There were clearly more patients than controls with the transcriptionally less active haplotype A-group alleles (Fig. 1). The risk of carrying two functionally less active haplotype A-group alleles was 2.7-fold (95% CI 1.7–4.4) higher in patients than in controls ($p=0.0001$). Conversely, the frequency of carriers of two haplotype B-group alleles was only 0.24 (95% CI 0.10 – 0.59) in the patient group compared to the controls ($p<0.0001$).

Table 2 Genotype and haplotype frequencies of *VKORC1* with confidence intervals in the 60 patients and 120 healthy controls

<i>VKORC1</i>			
Study group	Genotypes	<i>n</i>	Proportion (95% CI)
Controls	HapB/HapB	41	0.34 (0.26–0.43)
	HapA/HapB	58	0.48 (0.39–0.58)
	HapA/HapA	21	0.18 (0.11–0.25)
Patients	HapB/HapB	5	0.08 (0.03–0.18)
	HapA/HapB	26	0.43 (0.31–0.58)
	HapA/HapA	29	0.48 (0.35–0.62)
Haplotypes			
Controls	HapB	140	0.58 (0.52–0.65)
	HapA	100	0.42 (0.35–0.48)
Patients	HapB	36	0.30 (0.22–0.39)
	HapA	84	0.70 (0.61–0.78)

Discussion

We have compared the frequencies of functional polymorphisms implicated in the pharmacogenetics of phenprocoumon in consecutive patients with clinically complicated anticoagulation or a very low phenprocoumon requirement with those in controls from the same geographic area. A previous work by Schalekamp et al. showed that a low phenprocoumon requirement (weekly dose <10 mg) was associated with a seven- to nine-fold higher frequency of over-anticoagulation (INR>6.0) compared to patients with a weekly dose >22 mg phenprocoumon [14].

Our data suggest a major contribution of the *VKORC1* polymorphism and a minor contribution of *CYP2C9* polymorphisms to the occurrence of clinically complicated phenprocoumon-mediated anticoagulation.

The frequency of *CYP2C9* genotypes with two non-wildtype alleles was fourfold higher in the patient cohort than in the control group. In accordance with this result, the frequency of two reduced-function alleles in the patients was 5.7-fold higher than that recently published in a meta-analysis on *CYP2C9* variants in Caucasians [15]. Patients with only one reduced-function allele per genome were not over-represented (Fig. 1). Both findings are in good agreement with the moderate influence of the *CYP2C9* genotype on phenprocoumon clearance in probands [16]. In contrast to findings on the pharmacokinetics of warfarin or acenocoumarol, this latter study detected no effects on phenprocoumon kinetics in probands carrying one wildtype and one variant allele. In line with these findings, the results from two recent studies suggest that dosing of phenprocoumon does not differ in patients with a *1/*2 or *1/*3 genotype as compared to *1/*1 patients [17, 18]. These data are, however, at variance with findings by Dutch investigators who found lower dose requirements and an elevated risk of over-anticoagulation in patients carrying one variant *CYP2C9* allele [10]. Notably, the latter study found no gene-dose effect of variant alleles, which conflicts with the reports of a lower clearance of phenprocoumon in patients carrying two variant alleles [16]. The clearance of other coumarin-class anticoagulants is reduced in patients with a single variant allele per genome and more profoundly reduced in patients with two variant alleles [7, 12].

The clearance of some drugs (e.g., tacrolimus, sirolimus) has been shown to be reduced in patients homozygous for the inactive *CYP3A5**3 allele [19, 20]. The frequency of the *3 allele was determined to be 83% in a sample of the Dutch population [21] and also 83% in our control cohort. In accordance with a potential role of *CYP3A5* in the clearance of phenprocoumon, we detected fewer carriers of the functional *1 allele in our patient cohort (8 vs. 16.7% in controls), although the difference failed to reach statistical

significance. Thus, the role of the *CYP3A5* polymorphism in phenprocoumon therapy needs further investigation.

With respect to *VKORC1*, the frequency of patients homozygous for the less expressing group-A haplotypes was more than double that of the control subjects ($p < 0.001$). These data underscore the central role of *VKORC1* haplotypes in anticoagulation with all coumarin-class drugs. The underlying factors determining the different expressions levels of the *VKORC1* haplotypes have not yet been definitively established. Yuan et al. proposed an enhanced promoter activity of the -1639-G-variant (part of the group B-haplotype) of the *VKORC1* as compared to the A-variant of group A haplotypes [22].

Two prospective studies demonstrated an improved prediction of steady-state warfarin-dosing when the *CYP2C9* [9] or both the *CYP2C9* and the *VKORC1* genotype [8] were taken into account. This is a major and clinically relevant achievement and has led to the recent suggestion by the Federal Drug Administration to reduce warfarin dosing in patients with a specific genetic makeup. However, despite the marked impact of *CYP2C9* and *VKORC1* polymorphisms on warfarin dose, no prospective study has as yet demonstrated a reduction in the number of clinical events when pharmacogenetically guided dosing is practiced. Another recent prospective study demonstrated an increased propensity of patients with *VKORC1* group-A alleles, and to a lesser extent with variant *CYP2C9* genotypes, to develop overanticoagulation even after an INR within the therapeutic range had been achieved initially [23]. Thus, these patients appear to exhibit a sustained increased susceptibility for factors interfering with the pharmacodynamics and pharmacokinetics of warfarin.

Whether a prognostic benefit by coumarins is achieved or not is dependent on an extraordinarily delicate balance: an anticoagulation too low may cause bleeding, which is unopposed by a prognostic gain in terms of the prevention of embolism. On the other hand, even moderate elevations of INR values above the established therapeutic window are associated with an increased bleeding propensity, which again abates the beneficial effects of the treatment [24–26]. The risk–benefit ratio is further influenced by patient- and disease-based factors that modify the risk for thromboembolic complications.

With respect to phenprocoumon, *VKORC1* polymorphisms appear to be of major importance in determining the occurrence of clinical difficulties in anticoagulation, whereas the role of *CYP2C9* appears to be limited to those genotypes with two variant alleles. Genotyping information on these polymorphisms could assist the clinician in deciding whether or not to initiate a coumarin-based anticoagulation in patients with an intermediate or low risk for thromboembolism. However, the value of this genotyping has still to be determined in prospective studies.

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