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PHARMACOGENETICS

*CYP2C19*17* affects R-warfarin plasma clearance and warfarin INR/dose ratio in patients on stable warfarin maintenance therapy

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Abstract
Purpose We aimed to assess the influence of *CYP2C19*17* on R-warfarin clearance as well as the effect of *CYP2C19*, *CYP2C8*, *CYP2C9*, and *VKORC1* polymorphisms together with non-genetic factors on warfarin international normalized ratio (INR)/daily dose.
Methods One hundred fifty Caucasian Italian outpatients with data on steady-state plasma concentrations of S- and R-warfarin were genotyped for *CYP2C19* (**2*, **3*, **4*, **17*), *CYP2C9* (**2*, **3*), *CYP2C8*3*, and *VKORC1*2*. The statistical analysis was performed on the effect of genotypes/haplotypes, age, sex, and body weight on the clearance of warfarin enantiomers and dose-normalized INR.
Results R-warfarin clearance was 32 % higher in carriers of *CYP2C19*17* than in carriers of *CYP2C19*2* (mean 2.5 mL/min, 95 % confidence interval (CI) 2.3–2.8 vs. 1.9 mL/min, 95 % CI 1.7–2.2; $P_{\text{post hoc}}$=0.01). Patients with *CYP2C19*1/*1* genotype had an intermediate clearance (mean 2.1 mL/min, 95 % CI 1.8–2.4). The genotypes of *VKORC1*, *CYP2C9*, and *CYP2C19*, together with non-genetic factors (age, sex, and body weight) explained 52 % of the variability in warfarin INR/daily dose, of which *CYP2C19* genotypes accounted for 7 %.
Conclusions This is the first study to include the gain-of-function *CYP2C19*17* allele when assessing the impact of *CYP2C19* polymorphisms on the clearance of warfarin enantiomers. *CYP2C19* genotypes influenced the clearance of R-warfarin and contributed significantly to the variability in INR/daily dose, indirectly indicating a clinical relevance of R-warfarin.

Keywords Warfarin · Enantiomers · *CYP2C19* · *CYP2C9* · *VKORC1* · Gene polymorphisms · Pharmacokinetics

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Introduction

The oral anticoagulant warfarin, commonly used for the treatment and prevention of thromboembolic disorders, exerts its effect by inhibition of the vitamin K epoxide reductase (VKOR) [1]. Due to its narrow therapeutic index, large inter-individual variability in kinetics and dose requirement, and multiple drug interactions, dose titration, and follow-up of warfarin is guided by repeated measurements of the prothrombin time, expressed as the international normalized ratio (INR or PT-INR), with a target therapeutic range of 2.0–3.0 [2].

Warfarin is administered as a racemic (1:1) mixture of its S- and R-enantiomers. The two enantiomers differ in plasma clearance, elimination half-life, and the degree of protein-

binding. S-warfarin is considered to be 3–5 times more potent than R-warfarin as an inhibitor of VKOR [3], and is almost exclusively metabolized by the polymorphic cytochrome P450 CYP2C9 [4]. The metabolism of R-warfarin is, on the other hand, catalyzed by multiple CYP enzymes including CYP2C19, CYP3A4, CYP1A2, and possibly CYP2C8 [4, 5]. There is, by now, a large body of evidence confirming the impact of functional *CYP2C9* polymorphisms on S- (but not R-) warfarin clearance as well as on warfarin dose requirement [6, 7]. Polymorphisms in *CYP2C9* and the gene coding for vitamin K epoxide reductase complex subunit 1 (*VKORC1*) together with sex, age, and body weight explain about 50 % of the variability in warfarin dose requirement [8, 9]. Several dosing algorithms with *VKORC1* and *CYP2C9* genotypes included have been developed [10, 11] and compared to clinical algorithms in prospective randomized trials [12, 13].

Compared to S-warfarin, the pharmacokinetics (PK) and pharmacodynamics (PD) of R-warfarin are much less studied, possibly due to the widely held view that R-warfarin is unlikely to be of relevance for the clinical effects of the drug [3]. This has, however, been challenged recently by Maddison et al. [14] with a single-dose study in healthy subjects, where S- and R-warfarin were administered separately and in combination. Their data, together with the longer half-life, lower clearance, and higher steady-state concentration, indicated the contribution of R-warfarin to the hypoprothrombinemic effects of racemic warfarin. Thus, factors influencing the activity of enzymes involved in the metabolism of R-warfarin could potentially affect the dose–effect relationship of the racemic drug. In a single-dose study in Japanese healthy volunteers, CYP2C19 poor metabolizers had higher area under the plasma concentration-time curve (AUC) and longer elimination half-life of R-warfarin as compared to extensive metabolizers [15]. However, no significant effect of *CYP2C19*2* (the most common loss-of-function allele of *CYP2C19* in Caucasians) on R-warfarin clearance was observed by us in an Italian patient cohort [6].

*CYP2C19*17* is a gain-of-function allele associated with increased CYP2C19 activity as compared to *CYP2C19*1*, contributing to the variability in the metabolic capacity among extensive metabolizers of CYP2C19 [16]. It is prevalent among Caucasians with population allele frequencies of 18–32 % [16]. *CYP2C19*17* is also reported to co-appear almost exclusively with wild-type alleles (**1*) of *CYP2C9* and *CYP2C8* in Northern European populations [17]. This pattern of linkage disequilibrium (LD) has not yet been assessed in Italian population. Moreover, to the best of our knowledge, no study has so far addressed the potential impact of *CYP2C19*17* on the clearance of warfarin enantiomers or warfarin dose requirement. Similarly, the effect of *CYP2C8* genotype on the PK of warfarin enantiomers is unknown. Overall, the influence of genetic factors on the clearance of R-warfarin and the pharmacodynamic role of the R-enantiomer are not well understood.

The primary aims of this study were to assess the potential influence of *CYP2C19*17* and extended CYP2C haplotypes on R-warfarin clearance. The combined effect of *CYP2C19*, *CYP2C8*, *CYP2C9*, and *VKORC1* polymorphisms together with non-genetic factors on INR response in relation to dose was also studied.

Materials and methods

Patients and study design

This study was based on 150 Italian warfarin-treated patients included in two previously published studies, study I (93 patients) by Scordo et al. 2002 [6] and study II (57 patients) by Takahashi et al. 2006 [18]. All patients were of Caucasian origin and none was included in both studies I and II. Parts of the data have also been used in the pharmacometric NONMEM modeling described by Hamberg et al. [7]. All patients were on stable maintenance dose of warfarin, aimed to reach a target INR value between 2.0 and 3.0. The main demographic data of the two study cohorts and the total material of 150 patients are given in the Supplementary table. Patients who were taking drugs known to interfere with warfarin metabolism (such as nonsteroidal anti-inflammatory drugs, sulfonamides, antiepileptics, rifampin [INN, rifampicin], and amiodarone) were not included in the studies. Plasma samples were taken at steady state, 12–14 h after the last drug administration, for analysis of S- and R-warfarin concentrations and INR. Full details of study design and patient characteristics have been described elsewhere [6, 18]. Total plasma concentrations of S- and R-warfarin were analyzed by HPLC as described in [6]. Both clinical studies were approved by the Ethics Committee at the Azienda Ospedaliera di Padova, Padova, Italy, and all patients gave informed consent to participate. The genetic analysis linked to anonymous phenotype and genotype characteristics from previous studies was approved by the Regional Ethical Review Board in Stockholm 2014.

Warfarin plasma clearance and dose-normalized INR

Assuming complete oral bioavailability of warfarin, the oral plasma clearance (CL) of each enantiomer was calculated based on the equation: $CL=[(D/\tau)]/Css$ where D is the average daily dose of each enantiomer (half the weekly racemic dose divided by 7), τ is the dose interval (i.e., 24 h), and Css the total concentration of S- or R-warfarin, respectively, at steady state.

As a measure of the dose–effect relationship, the INR values were normalized by the average daily dose of the racemic drug (warfarin INR/daily dose).

Genotyping

DNA was extracted from peripheral leukocytes using the Cell Culture DNA kit (QIAGEN, Hilden, Germany). Five single-nucleotide polymorphisms (SNPs) were genotyped using validated TaqMan genotyping assays from Applied Biosystems according to the manufacturer's guidelines (for rs12248560C>T, *CYP2C19*17*, assay ID C__469857_10; for rs4244285G>A, *CYP2C19*2*, assay ID C_25986767_70; for rs4986893G>A, *CYP2C19*3*, assay ID C__27861809_10; for rs28399504A>G, *CYP2C19*4*, assay ID C__30634136_10; for rs10509681A>G, *CYP2C8*3*, assay ID C__25625782_20; for rs992323G>A, *VKORC1*2*, assay ID C__30403261_20). The analyses were carried out using an ABI Prism 7500 real-time PCR system or an Applied Biosystems StepOnePlus Real-Time PCR system. *CYP2C19*17* and **4* were genotyped successfully in all 150 patients and *CYP2C8*3* in 148 patients (DNA depleted in two patients). *CYP2C19*2* and**3* were analyzed in the 57 patients from study II only as these genotypes were already available from study I [6]. Similarly, *VKORC1*2* genotypes for patients from study II were already available [18]. Of the 93 patients in study I, 92 were successfully genotyped for *VKORC1*2* (DNA depleted in one patient). The genotypes rs1799853 (*CYP2C9*2*) and rs1057910 (*CYP2C9*3*) of all 150 patients were available since before as described [6, 18].

Statistics

Statistical analysis was carried out using STATISTICA 10 (StatSoft Inc., Tulsa, Oklahoma, USA). A two-tailed *P* value of up to 0.05 was considered to be statistically significant. The two subpopulations were compared regarding patient characteristics (age, sex, and body weight), warfarin dose, S/R warfarin concentration ratio, INR at steady state and genotype frequencies using Mann–Whitney test (for continuous variables) or Pearson's chi-square test (for categorical variables).

The probability of deviation from Hardy–Weinberg equilibrium (HWE) was calculated for each SNP. Pair-wise LD was characterized by *D* values and illustrated by the LD plots in Haploview (4.2). Haplotypes were inferred using Haploview (4.2) and UNPHASED (v3.1.6). The association of the *CYP2C* haplotypes with the clearance of the enantiomers was assessed using UNPHASED (v3.1.6), implementing maximum-likelihood inference. To reduce non-normality, the clearance values and the warfarin INR/daily dose were log-transformed before statistical assessment. Shapiro–Wilk test was used for assessing normality before vs. after log-transformation ($P<0.0001$ vs. $P=0.43$ for R-warfarin CL; $P<0.0001$ vs. $P=0.07$ for S-warfarin CL; $P<0.0001$ vs. $P=0.03$ for warfarin INR/daily dose). The results of statistical analysis are presented as antilog values. Log-transformation of the S/R concentration ratio did not result in reduction of non-normality (Shapiro–Wilk test before vs. after: $P<0.0001$ vs. $P<0.0001$). Variability in the S/R concentration ratio in relation to *VKORC1*2* was assessed using the non-parametric Kruskal–Wallis test.

Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to assess the effect of genetic polymorphisms on log(R-warfarin CL) and log(S-warfarin CL). The combined effect of genetic and demographic covariates on log(warfarin INR/daily dose) was assessed in linear regression models applying backwards stepwise regression. The adjusted value of R^2 obtained from the final model is considered a measure of the explained variability. Bonferroni test was used as post hoc analysis to test the statistical significance of specific comparisons.

To avoid small sample size effect in analysis (≤5 patients/group), the *CYP2C19* genotypes were grouped as follows: (1) subjects homozygous for *CYP2C19*1* (*CYP2C19 *1/*1*), (2) heterozygous and homozygous carriers of the rs4244285 A-allele only (*CYP2C19*2* carriers), (3) heterozygous and homozygous carriers of the rs12248560 T-allele only (*CYP2C19*17* carriers), and (4) carriers of the variant alleles of both rs4244285 and rs12248560 (*CYP2C19*2/*17*). No carriers of either *CYP2C19*3* or **4* were found among the 150 patients. The *CYP2C9* genotypes were grouped as follows: (1) *CYP2C9*1/*1*, (2) heterozygous carriers of the rs1799853 T-allele only (*CYP2C9*1/*2*), (3) heterozygous carriers of the rs1057910 C-allele only (*CYP2C9*1/*3*), and (4) subjects with two variant alleles (*CYP2C9*2/*2*, **3/*3* or **2/*3*).

Results

Characteristics of the 150 patients are summarized in Supplementary Table 1. The study population was between 22 and 87 years of age and all were on stable maintenance doses of warfarin (6.3–78.8 mg/week). All patients were treated for cardiovascular/ thromboembolic diseases with a target INR value between 2 and 3. The patients from study I had lower median body weight than patients from study II (75 vs. 82 kg, $P=0.004$), but showed no difference regarding age, warfarin dose, S/R warfarin concentration ratio, and INR at steady-state or genotype frequencies ($P>0.1$ in all cases). No deviation from HWE was shown for any of the SNPs analyzed. The frequency of the *CYP2C19*17* allele was 17 % in this Italian patient population. Significant pair-wise LD was observed only between rs1799853 (*CYP2C9*2*) and rs10509681 (*CYP2C8*3*), at a moderately high level $D=0.78$ (Supplementary Fig. 1).

CYP2C haplotype inference and association with warfarin clearance

Six *CYP2C* haplotypes with frequencies ≥2 % were inferred (Table 1). The more rare haplotypes together accounted for 5 % of the haplotypes. The haplotypes inferred by UNPHASED were the same as those inferred by Haploview regarding both structure composition and frequency distribution. Haplotype 1 consists of wild-type variants at all SNP loci (Table 1). Haplotypes 2, 3, 5, and 6 each included one single variant allele. The variant G-allele of rs10509681 (*CYP2C8*3*) co-appeared always with the variant T-allele of rs1799853 (*CYP2C9*2*) and formed haplotype 4. None of the six haplotypes was significantly associated with log(R-warfarin CL). Regarding S-warfarin, haplotypes 4, 5, and 6 containing variant alleles of *CYP2C9* SNPs showed significant reductions in log(S-warfarin CL) compared to the reference haplotype 1 ($P<0.0001$, data not shown).

The influence of CYP2C genotypes on R- and S-warfarin clearance

Because of the known effect of CYP2C9 on S-warfarin CL, the effect of *CYP2C19* genotypes was assessed together with the *CYP2C9* genotypes using two-way ANOVA. *CYP2C8*3* was not included as an independent variable in ANOVA since it was in strong LD with *CYP2C9*2*. Variability in log(R-warfarin CL) was significantly associated with *CYP2C19* genotype ($F_{3,143}=3.1$, $P=0.03$) but not with *CYP2C9* genotype ($F_{3,143}=1.9$, $P=0.1$).

*CYP2C19*17* carriers displayed a 1.3-fold higher ($P_{post\ hoc}=0.01$) mean R-warfarin clearance (antilog values: mean 2.5 mL/min, 95 % confidence interval (CI) 2.3–2.8) than *CYP2C19*2* carriers (1.9 mL/min, 95 % CI 1.7–2.2) (Fig. 1). These comparisons do not include heterozygous with *CYP2C19*2/*17* genotype. Patients with *CYP2C19*1/*1* genotype had their mean R-warfarin CL in-between the **17* and **2* carrier groups (2.1 mL/min, 95 % CI 1.8–2.4). Those who carried both *CYP2C19*2* and**17* alleles (**2/*17* genotype) showed a mean CL similar to that of the **17* carriers

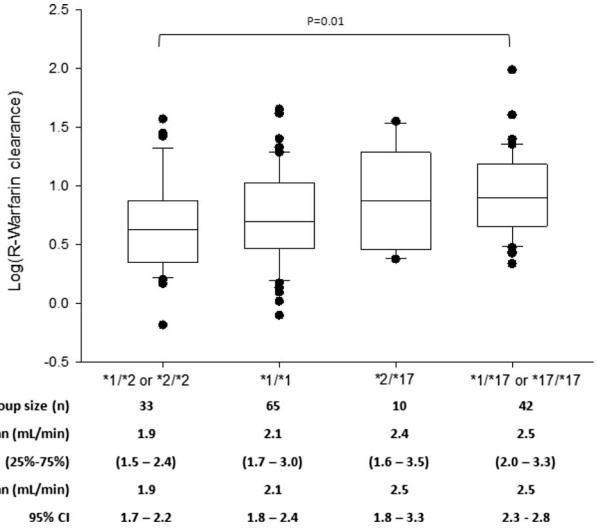

Fig. 1 Total R-warfarin clearance categorized by *CYP2C19* genotypes. Log-transformed R-warfarin clearance with median and inter quartile range are shown graphically and antilog values are presented numerically. The mean values and 95 % confidence interval (CI) were used in Bonferroni post hoc test

(2.5 mL/min, 95 % CI 1.8–3.3). However, no statistically significant differences in R-warfarin CL compared to the other groups were observed for the **1/*1* or the **2/*17* groups. The influence of *CYP2C19* genotype on R-warfarin CL was further assessed with adjustment for age and body weight using ANCOVA. The *CYP2C19* genotype remained a significant factor ($F_{3,118}=5.4$, $P=0.002$) with a consistent subgroup difference between carriers of *CYP2C19*17* and those of **2* ($P_{post\ hoc}=0.002$). Increase in age was associated with a decrease in log(R-warfarin CL) (Beta=−0.26, $F_{1,118}=9.8$, $P=0.002$) whereas body weight showed no significant influence.

On the other hand, no statistically significant association was observed between *CYP2C19* genotypes and the clearance of S-warfarin ($F_{3,143}=0.5$, $P=0.6$). *CYP2C9* genotype was a strong predictor of log(S-warfarin CL) ($F_{3,143}=38.5$, $P<0.001$). Mean S-warfarin CLs in the *CYP2C9*1/*2* (antilog values: 3.4 mL/min, 95 % CI 2.9–4.0) and **1/*3* genotype groups (3.0 mL/min, 95 % CI 2.6–3.6) were significantly lower than that in the *CYP2C9*1/*1* group (5.4 mL/min, 95 % CI 4.9–6.0; $P_{post\ hoc}=<0.0001$ in both cases), but higher

Table 1 The six main *CYP2C* haplotypes inferred in the study cohort of 150 patients

	Haplotype						
No.	CYP2C19*17	CYP2C19*2	CYP2C9*2	CYP2C9*3	CYP2C8*3	Count	Frequency
1	C	G	C	A	A	128	0.43
2	T	G	C	A	A	50	0.17
3	C	A	C	A	A	41	0.14
4	C	G	T	A	G	31	0.10
5	C	G	C	C	A	28	0.09
6	C	G	T	A	A	5	0.02
rare						13	0.05

than in carriers of two defect alleles (1.3 mL/min, 95 % CI 1.0–1.8; $P_{post\ hoc}$=<0.0001 in both cases). No significant difference in S-warfarin CL between the *CYP2C9*1/*2* and *CYP2C9*1/*3* groups was observed.

The influence of CYP2C genotypes on warfarin INR/daily dose

Approximately one third of the patients (47/150) had an INR value outside the target range (2.0–3.0), the minimum and maximum INR being 1.36 and 4.37, respectively. Therefore, warfarin INR/daily dose was used as a measure of the dose–effect relationship. When assessing variability in warfarin INR/daily dose, *CYP2C19* genotype was identified as a significant factor ($F_{3,112}$=2.9, P=0.04) in addition to the *CYP2C9* ($F_{3,112}$=13.7, P<0.0001) and *VKORC1* genotypes ($F_{2,112}$=31.7, P<0.0001, Table 2).

In subsequent subgroup comparison, warfarin INR/dose ratios were decreased by 37 and 42 % in carriers of *CYP2C19*17* (mean 0.46, 95 % CI 0.39–0.54) and subjects with the *CYP2C19*2/*17* genotype (0.43, 95 % CI 0.31–0.59), respectively, as compared to *CYP2C19*2* carriers (0.73, 95 % CI 0.56–0.96; $P_{post\ hoc}$=<0.01 in both cases). The *CYP2C19*1/*1* group had warfarin INR/dose values in-between the extremes (0.61, 95 % CI 0.52–0.73), with 16 % reduction compared to the *CYP2C19*2* carriers.

Compared to patients with *CYP2C9*1/*1* genotype (mean 0.46, 95 % CI 0.42–0.52), heterozygous, and homozygous carriers of *CYP2C9*2* or **3* displayed 1.4 to 2.4-fold higher warfarin INR/daily dose ratios (the **1/*2* group: 0.64, 95 % CI 0.52–0.78; the **1/*3* group: 0.77, 95 % CI 0.63–0.94; and the **2/*2*, **3/*3* or **2/*3* group: 1.12, 95 % CI 0.82–1.56, $P_{post\ hoc}$=<0.01 in all cases). Similarly, 1.5- and 2.3-fold higher mean warfarin INR/daily dose ratios were observed in heterozygous and homozygous carriers of *VKORC1*2* (0.61, 95 % CI 0.53–0.70 and 0.93, 95 % CI 0.79–1.09, respectively) than in the *VKORC1*1/*1* group (0.40, 95 % CI 0.35–0.46; $P_{post\ hoc}$<0.001 in both cases). Of the non-genetic factors included (age, body weight, and sex), only age was observed to be a significant covariate ($F_{1,112}$=8.6, P=0.004, Table 2). Increase in age was associated with an increase in warfarin INR/dose (Beta=0.19). The three genetic factors and age together explained 52 % of the variability in log (warfarin INR/daily dose) (adjusted R^2=0.52) with the *CYP2C19* genotypes accounting for 7 % of the variability (Table 2).

Discussion

This is, to our knowledge, the first study to include *CYP2C19*17* in the analysis of the effect of *CYP2C19* genotypes on the enantioselective PK and INR response of warfarin. A significant effect of *CYP2C19* genotype on R-warfarin clearance was observed, with carriers of *CYP2C19*17* showing on average 32 % higher clearance than carriers of *CYP2C19*2*. Patients with two functional *CYP2C19*1* alleles had clearance values in-between these two groups. On the other hand, no influence of *CYP2C19* genotypes on S-warfarin clearance was observed. Our data are in line with published in vitro studies supporting a major role for CYP2C19 in the metabolism of R-warfarin [5].

In our earlier study including part (93 patients) of the current study population, no significant effect of *CYP2C19* genotypes on the clearance of unbound R-warfarin was found [6]. At the time of that study, the *CYP2C19*17* allele had not yet been described. Carriers of the **17* allele were thus classified as *CYP2C19*1* carriers in that study. Analysis of the**17* allele in the current study allowed identification of a subgroup of patients with a higher predicted average CYP2C19 activity as compared to those carrying two *CYP2C19*1* alleles. The improved prediction of the phenotype together with a larger patient population is a possible explanation to the observed significant effect of the genotype on R-warfarin clearance. Similar to this study, a significant effect of *CYP2C19*17* on the PK of other CYP2C19 substrates such as omeprazole [19] and escitalopram [20] has been shown. It is to be noted that the

Table 2 Multivariate linear regression analysis of variables influencing log(warfarin INR/daily dose)

Factors	Degree of freedom	*F* statistic	*P* value	Partial eta-squared[(a)]	Observed power (alpha=0.05)
Age (years)	1	8.6	0.004	0.07	0.83
Sex			NS		
Body weight (kg)			NS		
CYP2C9 genotype	3	13.7	<0.0001	0.27	1.00
VKORC1 genotype	2	31.7	<0.0001	0.36	1.00
CYP2C19 genotype	3	2.9	0.04	0.07	0.67

CYP2C9 genotype: **2* and **3* analyzed; *VKORC1* genotype: **2* analyzed; *CYP2C19* genotype: **2*, **3*, **4* and **17* analyzed

Adjusted R^2 =0.52

NS not significant

[a] Displaying the proportion of variability in log(warfarin INR/daily dose) that can be explained by each factor

free fraction of R-warfarin is higher than that of S-warfarin (in average 1.18 and 1.08 %, respectively, for R- and S-warfarin in study I [6]). Calculating total instead of the free unbound clearance of the enantiomers (as in the present study due to the lack of data on the free fraction in study II) would thus be expected to underestimate rather than overestimate the contribution of the *CYP2C19* genotype on the clearance and, consequently, plasma exposure of free R-warfarin. This is of interest when discussing the possible contribution of the R-enantiomer for the pharmacological effects of warfarin.

In the present study, R-warfarin clearance was on average 10 % (though not statistically significantly) higher in the *CYP2C19*1/*1* genotype group as compared to carriers of *CYP2C19*2*. In a study in Asian healthy volunteers [15] a 30 % higher clearance of this enantiomer was found in homozygous extensive metabolizers (**1/*1* genotype) of CYP2C19 than in poor metabolizers (i.e., carriers of two loss-of-function alleles). No heterozygous carriers of *CYP2C19*2* or **3* were included in that study. As expected, due to the low allele frequency of *CYP2C19*2* (and lack of **3*) in Caucasians, only four patients in our cohort were homozygous for this allele, and heterozygous and homozygous carriers of **2* were thus grouped together for statistical analysis. A larger patient population with more poor metabolizers would be required for proper analysis of the gene–dose effect of *CYP2C19*2* in Caucasians. Another difference between the current study and that by Uno et al. [15] is that the Asian study addressed warfarin kinetics after a fixed single 10-mg dose while our data are from steady-state conditions using individually titrated warfarin doses.

The *CYP2C* haplotypes with frequencies higher than 10% in our Italian population were the same as those previously reported in Nordic populations [17]. Consistently, *CYP2C19*17* was observed with an allele frequency of 17 % and in strong LD with *CYP2C9*1* and *CYP2C8*1*. This result supports the observed correlation between *CYP2C19*17* and R-warfarin clearance being independent from the other SNPs assessed in this study. Our analysis also confirmed the moderately strong LD between *CYP2C8*3* and *CYP2C9*2*.

In addition to the effect of *CYP2C19* genotypes on R-warfarin clearance, an association with warfarin response was observed, using warfarin INR/daily dose as a marker. *CYP2C19* genotypes accounted for 7 % of the variance in warfarin INR/daily dose. Genetic (*VKORC1*, *CYP2C9*, and *CYP2C19*) and non-genetic (age, sex, and body weight) covariates together explained 52 % of the variability. Although the impact of *CYP2C19* genotypes was much smaller than that of *VKORC1* and *CYP2C9*, it was nevertheless a significant factor. This is in line with the results of a recent PK/PD study [14] suggesting that the R-enantiomer does indeed contribute to the anticoagulant effect of warfarin, based on both separate and combined administration of pure warfarin enantiomers. Moreover, an uncommon *CYP2C19* SNP, rs3814637, has been reported to be significantly associated with elevated INR values during the first week of treatment, stable dose, and warfarin sensitivity [9, 21]. This SNP was subsequently included in a population PK model and correlated to reduced R-warfarin clearance [22]. In that study, the SNP rs3814637 was not in LD with *CYP2C19*2* and it is unclear whether this SNP is functional or acts as a marker for additional unknown functional SNPs. This SNP was not analyzed in the present study due to its low frequency (6 %) and high LD with *CYP2C9*3* [9, 21].

In conclusion, the clearance of R-warfarin was approximately 30 % higher in Italian warfarin-treated patients carrying *CYP2C19*17* compared to *CYP2C19*2*. *CYP2C19* genotypes also contributed significantly to the variability of warfarin INR/daily dose, indirectly indicating a clinical relevance of the R-enantiomer of warfarin. *VKORC1* and *CYP2C9* genotypes, however, remained the major genetic determinants of INR in relation to daily dose. Further studies are required to assess if adding *CYP2C19* in genotype-based dosing algorithms would improve the prediction of warfarin dose requirement.

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Conflict of interest The authors declare that they have no conflict of interest.

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