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ORIGINAL ARTICLE

Interpretation of the effect of CYP2C9, VKORC1 and CYP4F2 variants on warfarin dosing adjustment in Turkey

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Abstract

It was aimed to underline the importance and explain the meaning of genetic testing in warfarin dosing and investigate and evaluate the contributions of the CYP2C9, VKORC1, and CYP4F2 variants in a Turkish population. Two hundred patients were genotyped for CYP2C9 (rs1799853, rs1057910 and rs56165452), VKORC1 (rs9934438, rs8050894, rs9923231, rs7294 and rs2359612) and CYP4F2 (rs2108622), yet, only 127 patients were found suitable for further evaluation in terms of their personal response to warfarin due to long term usage and available INR and dose usage information. The DNA sequences were determined by the ABI PRISM 3100 Genetic Analyzer to 3130xl System (Applied Biosystems, Foster City, California). Warfarin dose application suggestions by warfaringdosing.org, FDA and MayoClinic were followed. Dose requirements in the Turkish population were found higher than the suggested doses by warfarindosing.org. The multivariate logistic regression analysis reveals the utilization of VCORC1 genetic evaluation is valuable in warfarin dosing (low and moderate vs. high) in this study ($p < 0.001$). The present study provides findings for clinicians to adapt the genetic data to the daily practice. We observed that the VKORC1 variant showed a more potent impact in warfarin dosing in this study.

Keywords Warfarin dosing · Drug sensitivity · Pharmacogenetics

Introduction

People are unique due to their genetic structure, and they respond differently to medicinal substances. Pharmacogenetics aims to foresee a possible drug response via interpreting the activation and detoxification ability of enzymes focusing on the polymorphic alterations affecting their activities. One of the prominent examples is the members of the cytochrome P450 (CYP) enzyme family, which are highly polymorphic and metabolizers of thousands of endogenous or exogenous compounds that usually result in inter-individual metabolism rate variations [1]. The value of pharmacogenetics is recognized worldwide and today drug companies test their products for how well they can be metabolized by the CYP enzymes.

Throughout the years, many studies have been conducted on the pharmacogenetics of warfarin, an anticoagulant agent highly efficient and low-priced, yet may be challenging to manage at first. Patients with valve replacements are regularly being prescribed warfarin following surgery or similar hypercoagulable states such as stroke, deep vein thrombosis (DVT) or pulmonary embolisms (PE) and also arrhythmic cases as atrial fibrillation or flutter. The two main complexities about warfarin dosing are determination of the proper dose right away and balancing the clearance (rate of elimination) with other variables such as BMI, diet, disease state, and drug interactions [2]. Nowadays clinicians acknowledge that greater part in warfarin dosing is attributed to the genetic foundation of patients with stable dietary habits.

Warfarin presents its anticoagulation effect by inhibiting the vitamin K epoxide reductase (VKOR) enzyme of the

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vitamin K cycle, responsible for the regeneration of vitamin K from its epoxide form (Fig. 1). Reduced or active form of vitamin K is in charge of the ɣ-carboxylation of the coagulation factors II, VII, IX and X, where also protein C and S, so as to activate and generate functional coagulation. Also, the ɣ-glutamyl carboxylase enzyme (GGCX) works with VKOR for the vitamin K regeneration [3], yet studies demonstrated that although it seems to be located at a critical spot, GGCX variations are negligible in terms of warfarin dosing adjustments in Caucasions [4, 5]. It has been shown that, the polymorphisms of the gene coding VKOR, VKORC1 cause coagulation factor defects and its variants are associated with warfarin resistance [6]. The clearance of warfarin is mainly dependent on the hepatic metabolism catalyzed by the CYP450 (Fig. 1). Warfarin is a racemic mixture with S-warfarin being the potent enantiomer and only inactivated by CYP2C9. The polymorphisms of CYP2C9 are associated with warfarin sensitivity [7]. Studies showed that warfarin dose variations are determined by approximately 13–34% VKORC1, and 5–20% CYP2C9*2 and *3 polymorphisms [8–10]. In other words, patients can be divided into groups of 'low dose', 'average dose' or 'high dose' requiring individuals based on their genotypes.

These polymorphisms and their effect on warfarin dosing are almost considered as common knowledge for pharmacogenetic researchers. Today, clinicians are encouraged to turn to genetic testing, since studies showed that pharmacogenetic testing reduces the hospitalization rates due to warfarin adverse effects [11]. It has been stated that one-third

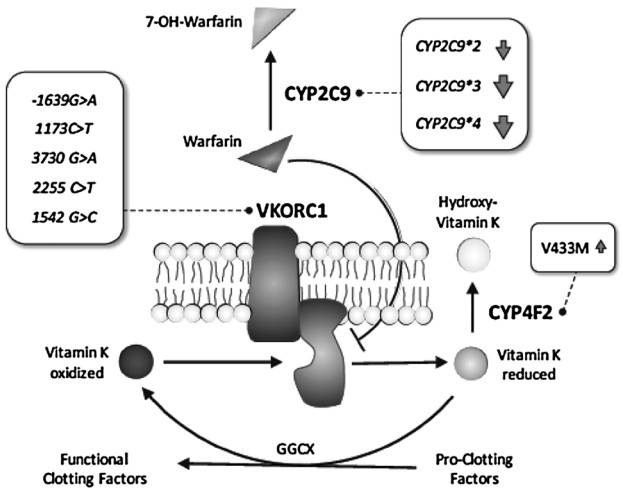

Fig. 1 Mechanism of action of warfarin. Warfarin inhibits the VKOR enzyme, which functions in the oxidation–reduction cycle of Vitamin K, essential for the activation of some clotting factors. The CYP2C9 is responsible for the clearance of warfarin, where as CYP4F2 is of reduced Vitamin K, a molecule partaking in the cycle. Gene variants previously associated with warfarin pharmacogenetics are displayed. Sizes of the arrows with the CYP enzymes indicate their impact on warfarin dosing

of adverse effect related hospitalizations in older people in the USA are due to warfarin [12]. Hospitalizations for adverse effects in the Medco–Mayo Warfarin Effectiveness Study, genotyped patients prior to the initiation of warfarin treatment had approximately 30% less hospitalizations for bleeding and thrombotic events, which was also supported by a meta-analyses by Eckman et al. with 32% [13]. The Food and Drug Association (FDA) in the United States updated the warfarin labeling by adding a warning for clinicians to take CYP2C9 and VKORC1 variants into consideration when adjusting dosage, in August 2007. In 2010, the FDA updated the label again with pharmacogenetic dosing information and how to interpret [14]. Furthermore, more recently, many study groups have been working on warfarin dosing algorithms specific to ethnicity in order to predict the required dose [15]. They generally only consider VKORC1 – 1639 G>A, CYP2C9*2 and *3 variants genetic wise and environmental factors such as BMI, age and other drugs. A relatively newer gene functioning in the elimination of reduced vitamin K is also included to warfarin pharmacogenetics, CYP4F2 [16, 17]. It has been shown on a Turkish population that CYP2C9, VKORC1 and CYP4F2 variant account for approximately 40% of the dose variations [18].

Furthermore, in a study that aimed to develop a population specific algorithm on Turkish patients [19] without taking CYP2C9 variants into account was unable to predict the required doses. Thus, even though VKORC1 is reported to have a greater impact on warfarin dosing, CYP2C9 genotyping was confirmed to be essential.

Many studies showed that a pharmacogenetic approach to warfarin treatment is beneficial. The aim of this study was to inform clinicians about warfarin pharmacogenetics and help them manage the treatment, and point out the population related variations. In other words, to evaluate the current perspective against warfarin genotyping in Turkey.

Patients and methods

Patient selection

Randomly selected 200 warfarin using patients from Department of General Surgery in Cerrahpasa Medical Faculty, Istanbul University and Clinic of Cardiology, Dr Siyami Ersek Thoracic and Cardiovascular Surgery Center Training and Research Hospital, Istanbul between the years 2013–2014 were included in this study. This study protocol was approved by the Local Ethical Committee of Istanbul University (approval number: A-18, date: 06th November 2012). Blood samples were obtained for DNA isolation with their informed consent. All procedures performed were in accordance with the ethical standards with the Helsinki

declaration and its later amendments or comparable ethical standards [20].

All 200 patients were genotyped for the determination of the population frequency, yet among these 200 patients, 127 were found suitable for further evaluation in terms of their personal response to warfarin. Patients were not included to dosing assessments due to short-term usage, insufficient retrospective dose, or INR data.

Genotyping

DNA purifications were performed using MasterPure™ Complete DNA and RNA Purification Kit, Epicentre, İllumina (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's manual.

PCR amplifications were carried out using the appropriate primers for CYP2C9 (rs1799853, rs1057910 and rs56165452), VKORC1 (rs9934438, rs8050894, rs9923231, rs7294 and rs2359612) and CYP4F2 (rs2108622) regions. Following amplification PCR products were purified from excess PCR components using PCR Rapid Kit, GeneDireX (GeneDireX, Nevada, USA) and BigDye® XTerminator™ Big Dye Terminator kit (Applied Biosystems, Foster City, California) and the Applied Biosystems GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California) was used for Cycle sequencing both according to the manufacturer's manual. The DNA sequences were determined by the ABI PRISM 3100 Genetic Analyzer to 3130xl System (Applied Biosystems, Foster City, California).

Dose requirement assessment

Dose application suggestions by warfaringdosing.org, FDA and Mayo Clinic were followed [11, 21, 22].

Statistical analyses

Demographic features, allele frequencies and algorithm related associations were evaluated using the IBM SPSS 20.0 program. Continuous variables are given as means ± standard deviation (Student's T test), while categorical variables as frequencies. Accordance to Hardy–Weinberg Equilibrium (HWE) was evaluated using the Chi square test. The multivariate logistic regression analysis was performed to assess the effects of CYP2C9 and VKORC1 on dosing.

Results

The study was carried out with 127 of these patients of whom 66.1% (n = 84) were women and 33.9% (n = 43) were men. Mean ages of both genders were similar ($p > 0.05$). Details of patient diagnoses are given in Table 1. Diagnoses were gathered under three main groups as valvular heart disease (aortic or mitral valve replacement etc.), heart rhythm or medical disorders (atrial fibrillation or flutter, idiopathic intracranial hypertension, coronary artery disease etc.), hypercoagulability (pulmonary embolism, deep vein thrombosis, etc.). Although the prevalence of some cardiovascular diseases differs among genders, overall warfarin usage is similar both in Western and Asian populations [24].

Allele frequencies of 200 warfarin user patients are given in Table 2. The VCORC1 and CYP genotypes and alleles and they were consistent to HWE ($p > 0.05$).

Data obtained from the FDA, Mayo Clinic and literature, we tried to predict the dose requirements taking into consideration all the VKORC1 polymorphisms studied on warfarin dosing in order to see if there was a difference. The VKORC1 variants were grouped as A—low dose and B—high dose haplotypes [8]. Since these variants are not fully linked, majority of variants indicating a certain group was decided accordingly as VKORC1 −1639G>A and 1173C>T being most predominant. For the CYP2C9 variants, dose requirements were evaluated according to the dose reduction percentages of a meta-analysis [23]. Dose requirements were determined based on the ranges given in Table 3.

Categorizations of the patients based on their genotypes according to Medco–Mayo Warfarin Effectiveness study

Table 1 Demographic features

	Overall	Women	Men
Mean age	59.87	57.95	58.45
Min–max	20–85	20–85	25–77
SE; SD	1.19; 13.46	1.91; 12.53	2.43; 13.10
Diagnosis	N (%)	N (%)	N (%)
Valvular heart disease	59 (46.5%)	42 (50%)	17 (39.5%)
Heart rhythm or medical disorders	52 (40.9%)	34 (40.5%)	18 (41.9%)
Hypercoagulability disorders	16 (12.6%)	8 (9.5%)	8 (18.6%)
Total	127 (100%)	84 (100%)	43 (100%)

Table 2 Allele frequencies in Turkish population

Gene	Variant	Prevalence (N)	Gene	Variant	Prevalence (N)
CYP2C9*2	TT	1.5% (3)	VKORC1 1173	CC	27.5% (55)
	TC	18.5% (37)		CT	50.5% (101)
	CC	80% (160)		TT	21.5% (43)
	T	10.75%		C	52.8%
	C	89.25%		T	47.2%
CYP2C9*3	CC	2% (4)	VKORC1 1542	GG	19% (38)
	AC	21.5% (43)		GC	51% (102)
	AA	76.5% (153)		CC	30% (60)
	C	12.75%		G	44.5%
	A	87.25%		C	55.5%
CYP4F2	CC	96% (192)	VKORC1 2255	CC	23.5% (47)
	CT	4% (8)		CT	57.5% (115)
	TT	–		TT	19% (38)
	C	98%		C	52.25%
	T	2%		T	47.75%
VKORC1 – 1639 G>A	GG	32.5% (65)	VKORC1 3730	GG	51% (102)
	GA	47% (94)		GA	39.5% (79)
	AA	20.5% (41)		AA	9.5% (19)
	G	56%		G	70.75%
	A	44%		A	29.25%

Table 3 Dose requirements suggested by the FDA and warfarindosing.org (mg/day)

VKORC1 variants (– *1639 G>A*)		CYP2C9 variants (**1/*2/*3*)											
		**1/*1*		**1/*2*		**1/*3*		**2/*2*		**2/*3*		**3/*3*	
Doses	→	FDA	WD	FDA	WD	FDA	WD	FDA	WD	FDA	WD	FDA	WD
↓	*GG*	5–7	6	5–7	5	3–4	4	3–4	4	3–4	3.5	0.5–2	3
	GA	5–7	5	3–4	4	3–4	3	3–4	3	0.5–2	2.5	0.5–2	2
	AA	3–4	3	3–4	2.5	0.5–2	2	0.5–2	2	0.5–2	2	0.5–2	1.5

*FDA: doses recommended by FDA [22]

*WD: doses recommended by http://www.warfarindosing.org for an average 65 year old Caucasian male patient [23]

Arrows indicate dose reduction tendencies. A similar chart was used to evaluate the patients in this study, yet our patients required higher doses than these patients. For example, a VKORC1 GG/CYP2C9 *1/*1 genotype carrier may need a starter dose of 7–7.5 mg/day and a VKORC1 AA/CYP2C9 *3/*3 genotype carrier < 1.5 mg/day

[11] are given in Table 4, and results of the multivariate logistic regression analysis are in Table 5. The logistic regression analysis reveals the utilization of VCORC1 genetic evaluation is valuable in warfarin dosing (low and moderate vs. high) in our study group ($p < 0.001$).

Discussion

Pharmacogenetic tests aim to discriminate patients, who may not benefit or experience severe adverse effects, to determine the most beneficial treatment for patients; in other words attaining 'personalized medicine'. Thus, with pharmacogenetics, patients will receive the most beneficial treatment for their personalized needs in no time instead of months of attempts and failures, next to preventing excessive unnecessary costs and adverse effects. In addition, the expenditures of treatments for redeeming health from severe adverse effects of nonspecific dosing may be much more costly than the actual treatment and simple genetic tests, if not fatal. Most importantly, for critical drugs with narrow therapeutic index, like warfarin, the safe and effective dose should be maintained rapidly, where simultaneously the clearance dose should be arranged by taking age, BMI, disease state, diet and drug interactions into consideration [2]. However, age, BMI, medical history, non-genetic factors account for only 60% of dose variables [25]. Therefore, genetics is thought to be in charge of up to 40% of warfarin dosing. By means

Table 4 Patient sensitivity characterizations according to the Medco–Mayo based on their genotypes

Mayo Clinic sensitivity classifications		CYP2C9			VKORC1 1639			VKORC1 1173			VKORC1 2255			VKORC1 3730			VKORC1 1542		
		1/1	1/2	2/2	2/3	GG (CC)	GA (CT)	AA (TT)	CC	CT	TT	CC (GG)	TC (AG)	TT (AA)	TT (AA)	CT (GA)	CC (GG)	GG	GC	CC
1	Very High	0	0	13	6	0	0	19	0	0	19	0	2	17	0	0	19	13	0	6
				68.4%	31.6%			100%			100%		10.5%	89.5%			100%	68.40%		31.6%
2	High	0	4	0	16	0	16	4	1	16	3	1	16	3	1	10	9	4	15	1
			20%		80%		80%	20%	5%	80%	15%	5%	80%	15%	1%	50%	45%	20%	75%	5%
3	Moderate	1	13	33	9	9	46	1	10	44	2	9	46	1	4	27	25	3	44	9
		1.8%	23.2%	58.9%	16.1%	16.1%	82.1%	1.8%	17.9%	78.6%	3.6%	16.1%	82.1%	1.8%	7.1%	48.2%	44.6%	5%	78.6%	16.1%
4	Mild	0	6	25	0	31	0	0	27	3	1	26	5	0	12	10	9	2	4	25
			19.4%	80.6%		100%			87.1%	9.7%	3.2%	83.9%	16.1%		38.7%	32.3%	29%	6.5%	12.9%	80.6%
5	Normal	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0
		100%					100%			100%			100%			100%			100%	
6	Less than normal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

of genetic testing, the safest and most effective warfarin dose range can be presented to the clinicians. Consequently, patients with warfarin resistance can receive higher doses, where warfarin sensitive patients can receive lower doses safely right away without experiencing severe, in some cases fatal, adverse effects.

Since polymorphisms decide on the genetic part of the variation, a vast part of the environmental effects is comprised of diet. Every population has its own habit when it comes to eating due to reasons as climate, geography, agriculture, socioeconomic circumstances etc. In the USA, although increasing, leafy green vegetable consumption is still very low [26]. On the other hand, in Turkey especially in the western side, as in other Mediterranean populations, greens are highly consumed where some greens such as parsley, which is a rich source of vitamin K, is considered 'indispensable' and are consumed in most meals and can be found in many different recipes. Thus, since greens are thought as 'safe food' or 'can do no harm' it may be hard to attract the attention of the caregivers. So much so that, they may insist on nourishing the patient with greens and vegetables so they believe patients will regain their strength, since they have heart conditions and greens are supposed to be good for your health, except, possibly not as continuous as to arrange the dose accordingly. What is missed is that foods rich with vitamin K, for instance salad greens, cucumbers, cabbage, scallions, parsley, spinach, broccoli etc., if not consumed in a particular custom will alter the required warfarin dose and may generate serious risks. On the other hand, animal liver, where vitamin K is stored in the body, is also a popular dish in Turkey, and some clinicians forget to inform their patients about it. Thus, genetic contribution aside, dose requirements may differ among populations [27, 28]. According to the predicted dose by http://www.warfarindosing.org average Caucasian's required dose seems too low for an average Turkish patient, where dietary traditions may be a big contributor.

Recently, various studies have been published that investigated the pharmacogenetic implementations in Europe or the USA [29, 30]. In the USA, clinicians nationwide were invited to participate in a pharmacogenetic testing related questionnaire in order to estimate the general approach to the matter [31]. Vast majority of the participants (97.6%) acknowledged the impact of genetic variations on drug response; however, only 12.9% have ordered a pharmacogenetic test the preceding 6 months and 26.4% indicated that they might order a test in the following 6 months. Most striking, only 10.3% believed that they were sufficiently informed about genetic testing. This clearly points out that clinicians need to be educated about the benefits of pharmacogenetic testing and how to interpret the reports they receive. This general picture seems to be similar in Turkey too. The commercial Labs in Turkey evaluate the CYP2C9*2 and *3,

Table 5 Evaluation of genotypes on dose requirements with multivariate logistic regression analysis

Dependant variable	Independent variables	P value	OR	95% CI
Doses Low and moderate vs. high	Age	0.250	0.004	(−) 0.003–0.01
	Gender	0.754	0.028	(−) 0.151–0.208
	CYP2C9	0.858	0.007	(−) 0.074–0.089
	VKORC1	0.001*	0.220	0.099–0.341

OR odds ratio, *CI* confidence Interval; (level of significance: $p < 0.05$)

and VKORC1 − 1639 G>A polymorphisms, yet they do not report the result in a way a clinician would really understand. Thus, firstly, clinicians should be informed about pharmacogenetic testing as an option in a part of a 'required dose discovery' and what the test results implicate. In the case of warfarin, reported genotype corresponds to a phenotype or an approximate dose range not a definite or permanent mg. In addition, what we encountered in our briefings was that clinicians were expecting these tests to sharply drop the frequency of INR monitoring. It is crucial to highlight that it is not what pharmacogenetic testing for warfarin is promising. The routine INR monitoring should be carried on as it is done even for a stable patient. Therefore, it can be understood that here we need to educate our clinicians about pharmacogenetic approaches in order to make them feel comfortable with utilizing it. For instance, generalizing post-graduate pharmacogenetic courses may be useful at this point. In this study it was also aimed to inform clinicians on how warfarin pharmacogenetics should be handled.

The CYP2C9 enzyme is responsible for the clearance of S-warfarin from the body; a decrease in the enzyme activity will extend the duration of elimination and thus increase the warfarin levels in stream. The CYP2C9 variants are named with *, where *1 correspond to the wild type. Most commonly found variants in Caucasians are *2 (430 C>T; Arg144Cys) (rs1799853) with the frequency of 11% and *3 (1075A>C; Ile359Leu) (rs1057910) with 7% and they downgrade the enzyme efficiency approximately by 30–40% and 80–90%, respectively [32]. In this study, coherent with other studies conducted on the Turkish population [33–35] the CYP2C9*2 allele frequency was found 10.75%. Likewise, frequency of CYP2C9*3 was previously demonstrated as 9–10% in the Turkish population [33–35] and the allele frequency was found 12.75% in this study. In homozygote CYP2C9*3 allele carriers compared to wild type carriers, warfarin clearance decreases up to 90% where both Vmax and Km values are effected [36]. Another genetic addition of these variants is that more time is needed to establish the targeted stable INR [21].

The catalytic activity of CYP2C9*4 (107T>C; Ile-359Thr) (rs56165452) is similar to *3 but it is very rare [37]. In a study on Turkish population 3 of 205 patients were found heterozygote, yet in this study, we did not encounter this variant [35].

As a vitamin K antagonist, warfarin targets the VKORC enzyme, which is responsible for vitamin K regeneration. Therefore, any alterations in enzyme activity will affect the required warfarin dosage accordingly. The − 1639G>A (3673) (rs9923231) variant is located in the promoter region and it has been reported that homozygote A allele carriers require 50% less warfarin dosage compared to homozygote G carriers [38]. Thus, GG genotype carriers are interpreted as resistant. Concordant with a previous study [36], the allele frequency was found 44%. The 1173C>T (6484) (rs9934438) variant is located in the first intron of VKORC1 and highly linked with the − 1639G>A variant. The T allele is associated with sensitivity or lower dose requirements [39]. The T allele is reported in 42% of Caucasians [40] and 91% in the Japanese population [41]. There are no other studies conducted on Turkish population on this variant. In this study, the allele frequency was found similar to Caucasians with 47.2%.

Since these two variants are not fully linked, we evaluated if patients with different alleles needed a different dosage approach. Among 200 patients, only 12 patients' − 1639G>A and 1173C>T genotypes were observed different. Of the 12 patients only 1 patient's − 1639G>A and 1173C>T genotype was converse in terms of dose requirements (1639G (high dose) and 1173T (low dose)), where 11 patients were if one was homozygote the other was heterozygote. All 3 patients with − 1639 G and 1173 CT (4th patient was not further evaluated due to insufficient warfarin usage data) both algorithm and our literature based dose prediction was higher than what patients took. Certainly, this small number of patients is not enough for a statistical significance, besides environmental factors are not even in the picture. Recent studies only includes the − 1639G>A variant as a marker of VKORC1 activity, where others were discarded in time due to their high linkage or insufficiency.

Previously, the 2255 C>T (7566) (rs2359612), 3730 G>A (9041)(rs7294) and 1542 G>C (6853)(rs8050894) variants were also evaluated for their relationship with VKOR activity or in other words warfarin dosing [8, 39, 42, 43]. However, in time they lost their importance. We also investigated these variants to detect even a minor contribution on dosing for finer tune, independently and together, yet, no significant relationship was found (data not shown).

Another gene that attracted attention recently is CYP4F2, which is a member of the cytochrome P450 enzyme family participating in the omega-hydroxylation of vitamin E and arachidonic acid [44, 45]. The CYP4F2 rs2108622 polymorphism is suggested to alter the vitamin K1 (VK1) oxidation activity, where T allele carriers have higher VK1 levels thus need higher warfarin doses [44, 45]. In addition, it has been shown that homozygote C carriers need a 1 mg higher daily warfarin dose than homozygote T carriers [46]. In this study, the allele frequency is found 2% in the Turkish population. Its effect on dosing requirement could not be demonstrated since there were only three heterozygote carriers in the further evaluated group.

Most recently, a large Turkey-wide clinical study, named Warfarin-TR study, was conducted on 4987 Turkish warfarin users in which therapy efficiency, adverse effects, diseases and patient awareness were evaluated [47]. They reported that 20% of all patients experienced bleeding events with 3.2% being major within a year following initiation. Moreover, 70.9% had INRs over the targeted range, 4.6% under and only 24.6% of the patient were in the therapeutic targeted range. These statistics clearly show us that in warfarin therapy, INR-dose pertinence needs reconsideration. Time in therapeutic range (TTR) corresponds to decreased adverse effect risk [48]. In the warfarin-TR study although awareness or the knowledge of food–drug interactions were low (55%) TTR was higher in these patients. This study also explains the INR inconsistencies and limitations we experienced in our study. In addition to patients' approaches to the treatment, in most cases, lack of awareness, the clinicians should also be reminded about these interactions. More importantly, what we observed is clinicians mostly are not aware of the genetic impact on this manner. Furthermore, unfortunately, they cannot spare much time to an individual patient, which results in irregular dose adjustments that are mostly are not adequately considered.

In this study, we tried to foresee the possible dose requirements of the patients only taking into consideration the genetic data that comprises 5 VKORC1 variants, 2 CYP2C9 variants (other variants were not encountered sufficiently for an evaluation—CYP2C9*4 and CYP4F2 C>T). These VKORC1 variants (2255 C>T, 1542 G>C, 3730 G>A) indicated no adaptable contribution to warfarin dosing in our population.

In our study, estimation with only taking genetic pattern into account, we were able to foresee 58.3% of the doses. Which indicates the value of utilization of genetic evaluation in warfarin dosing. Performed regression analysis revealed that (Table 5) VKORC1 shows significance, which is compatible with the results of Ozer et al. [18]. It should be reminded that this does not mean VKORC1 alone is responsible for dosing differences, as shown in the study of Karaca et al.

Accurate warfarin dosing in clinic, may bear hesitations, all around the world. In this study, it was aimed to merge the conventional dosing adjustments with pharmagenetic information by taking into account the studies conducted in the USA and Europe. Prominent variants that were suggested to alter warfarin metabolism were evaluated on the Turkish population. Various studies pointed out that awareness in this manner is needed to be raised, which is what we also aimed. Our findings, besides displaying the population frequencies, demonstrated the contribution of the correct interpretation of the major variants together, which play roles in warfarin pharmacogenetics.

Unfortunately, the limitation of this study was that we were unable to gather sufficient patient clinical data to develop an algorithm to foresee the required doses for the patients. This study should be carried on with a larger patient group with standard dietary habits, and a more controlled and comprehensive INR-dose data to do so. Therefore, a prospective rather than a retrospective approach may be more suitable. In this study, the effects of the CYP2C9, VKORC1 and CYP4F2 variants were evaluated. This study also could be expanded with more genes taken into account, somehow related to warfarin metabolism, such as APOE and EPHX1. In our future studies we aim to develop an algorithm specific to Turkish patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no any financial or non-financial conflict of interests.

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