Research Article

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Pharmacogenomics

International warfarin genotype-guided dosing algorithms in the Turkish population and their preventive effects on major and life-threatening hemorrhagic events

Aim: To determine the accuracy of international warfarin pharmacogenetic algorithms developed on large multiethnic cohorts (comprising more than 1000 subjects) to predict therapeutic warfarin doses in Turkish patients. **Materials & methods:** We investigated two Turkish warfarin-treated cohorts: patients with no history of hemorrhagic or thromboembolic event and patients with major and life-threatening hemorrhagic events. **Results:** International pharmacogenetic algorithms showed good performances in predicting the therapeutic dose of patients with no history of bleedings, but they did not significantly detect the incorrect warfarin dose of patients with major and life-threatening hemorrhagic events. **Conclusion:** Although genetic information can predict the therapeutic warfarin dose, the accuracy of the international pharmacogenetic algorithms is not sufficient to be used for warfarin screening in Turkish patients.

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Keywords: drug response • ethnicity • pharmacogenetic algorithms • Turkey • validation study

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Background

Warfarin is the most widely used oral anticoagulant prescribed to treat and prevent thromboembolism [1]. Anticoagulant therapy is prescribed for different indications (e.g., deep vein thrombosis, pulmonary embolism and prevention of systemic embolism or stroke in patients with prosthetic heart valves or atrial fibrillation) [2]. Among them, atrial fibrillation is the most frequent indication since it has a prevalence of approximately 2% in the developed countries [3]. Warfarin therapy can reduce the risk of stroke and systemic embolism by about 60% in patients with atrial fibrillation [4]. Relevant risk reductions are also observed for the other warfarin indications [5]. Although it has a significant thromboembolism risk reduction, incorrect warfarin dosing can be associated with thromboembolic events (in the case of underdosing) or bleeding complications (in the case of overdosing) [6]. Warfarin is the first cause of emergency room visits for adverse drug events in older adults in the USA [7]. This is mainly due to the interindividual (i.e., differences among patients) and intraindividual (i.e., differences over time within the same patient) variability in the warfarin dose-response [2]. To monitor the anticoagulant effect of warfarin treatment, it is possible to measure the prothrombin time expressed as the International Normalized Ratio (INR). Specific INR ranges are used for the warfarin indications [8]. Genetic studies also contributed to improve warfarin dosing. Several polymorphisms have been associated with warfarin response. Among them, two loci play a relevant role in determining large variation in dose requirements: *VKORC1* (~30%) and *CYP2C9* (~10%) [2]. Numerous research groups developed different dosing algorithms based on genetic, clinical and anthropometric characteristics [9] on the basis of different statistical approaches [10,11]. Among

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these research groups, the International Warfarin Pharmacogenetics Consortium and few other research groups developed their algorithms in large multiethnic cohorts (comprising more than 1000 subjects) [12–15]. These large efforts could provide reliable indications that could help clinicians define the warfarin dose on the basis of patient characteristics. However, human genetic diversity can significantly affect the reliability of these warfarin pharmacogenetic algorithms [16,17], as has been demonstrated in other pharmacogenomics contexts [18–20]. In particular, some human populations with peculiar genetic features, such as the Turkish population, have not been included in these large multiethnic investigations, and no information is available about the effectiveness of these warfarin pharmacogenetic algorithms in these human groups. The genetic background of the Turkish population is an admixture of European, Middle Eastern and Central Asian ancestries [21]. Although Turkish people share a relevant percentage of their genetic background with Europeans, significant differences are present between Turkish and North European populations, partially explaining the health disparities of Turkish communities in Northern Europe [22,23]. To our knowledge, three previous studies investigated the impact of *VKORC1* and *CYP2C9* polymorphisms on warfarin dosing in the Turkish populations [24–26]. However, no studies investigated the reliability of international algorithms on Turkish patients with no indication of the usefulness of warfarin dosing in Turkey.

The aim of the present study is to validate the reliability of international pharmacogenetic algorithms developed on large multiethnic cohorts (comprising more than 1000 subjects) using the data related to patients with a warfarin-stable dose and no hemorrhagic or thromboembolic event and patients without a controlled therapy admitted to emergency with a major or life-threatening hemorrhagic event. Specifically, we verified whether these international algorithms showed the performance expected by an optimal warfarin genotype-guided dosing algorithm: the predicted dose is close to the correct dose (i.e., warfarin dose in controlled patients) and is significantly different from warfarin dosage associated with bleedings (i.e., warfarin dose in the uncontrolled sample). We also develop a model based on Turkish controlled sample to compare its performance to those of the international algorithms.

Materials & methods

Study subjects

Prior to the study, appropriate institutional ethics committee approval was obtained, and the principles outlined in the Declaration of Helsinki for human experimental investigations were followed. After obtaining written consent, a total of 189 Turkish patients who were on warfarin treatment with a stable dose schema for at least 3 weeks were selected. 97 patients without hemorrhagic or thromboembolic events were defined as controlled patients and enrolled during their routine follow-up for warfarin use (i.e., they underwent a regular control). 92 patients were admitted to emergency rooms with a major or life-threatening hemorrhagic event. These subjects used a stable warfarin dose schema for at least 3 weeks without coming to regular controls. Accordingly, they were defined as uncontrolled patients. Major hemorrhage and life threatening/fatal hemorrhage were defined in accordance with the study of Fihn and colleagues [27].

Demographic, anthropometric and clinical data were collected, and INR levels were measured for all the subjects. Body surface area (BSA) was calculated using the Du-Bois formula. In this study, the upper limit of INR for cases with a prosthetic heart valve indication was 4.5, while it was 4.0 for other indications. These limits were defined in accordance with the guidelines considered during the enrollment of the patients [28,29]. INR values higher than upper limit of therapeutic INR range were considered 'suprathrapeutic'. $CHADS_2$ scores were calculated for both controlled and uncontrolled patients [30].

Genotyping

Genomic DNA was isolated from periferal blood samples using commercial DNA isolation kit (Macherey-Nagel, NucleoSpin®, Düren, Germany). Sequenom RealSNP software was used to design sequence and allele-specific PCR primers for *CYP2C9*1* (no variants), *CYP2C9*2* (rs1799853), *CYP2C9*3* (rs1057910) and *VKORC1* -1639G>A (rs9923231) variants (Metabion, Germany). All laboratory procedures were performed according to the manufacturer's protocol of the MassArray platform (Sequenom Inc., CA, USA). Detailed information about the genotyping pipeline is reported in our previous study [22].

Statistical analysis

Appropriate statistic tests were used to compare the characteristics of controlled patients and uncontrolled patients, to evaluate the impact of genetic and nongenetic factors on warfarin doses in controlled patients, and to investigate the effects of genetic and nongenetic factors on the treatment response index in uncontrolled patients. We also calculated the treatment response index was defined as ln(INR/ daily warfarin dose). This metric index is an exponential-decay pharmacokinetic model used to describe the relationship between INR and warfarin dose [15]. A logarith-

mic transformation of the continuous variables was applied when necessary. Parametric tests (i.e., t test and ANOVA) were used for variables with normal distribution, whereas nonparametric tests (i.e., Pearson's correlation test and Mann–Whitney test) were used for variables with non-normal distribution. Hardy–Weinberg equilibrium of the investigated loci was evaluated using Fisher's exact test.

Regression analysis was used to evaluate the effect of genetic, clinical and anthropometric traits on warfarin dose in controlled patients and to evaluate the treatment response index in uncontrolled patients. To define the best predictive model for warfarin dose in our controlled samples, we performed a univariate regression analysis of each genetic or nongenetic parameter with respect to the actual warfarin dose, and selected those parameters with a p-value < 0.2. Then, we used a multivariate regression approach, the stepwise model selection via exact Akaike Information Criterion, to define the best model using these parameters. As numerous previous studies [2], in the model development we also considered the warfarin indications as they have a relevant role in determining the correct warfarin dosage. The model developed was used to validate the effectiveness of previous warfarin pharmacogenetic algorithms. On the basis of a recent review [2], we selected those algorithms developed on large cohorts (comprising more than 1000 subjects; Supplementary Table 1). To estimate the accuracy of each algorithm, we calculated the mean absolute error (MAE) and the percentage of patients whose predicted dose fell within 20% of the actual stable therapeutic dose. To determine the effects of the tested algorithms to prevent major and life-threatening hemorrhagic events, we evaluated the differences between the predicted dose of each algorithms and the actual dose of the uncontrolled patients

Table 1. Demographic, anthropometric, clinical and genetic characteristics of the controlled and uncontrolled patients.

Characteristics	Controlled patients (n = 97)	Uncontrolled patients (n = 92)	p-value
Females, n (%)	50 (51.5)	45 (48.9)	NS
Age, mean ± SD	61 ± 12.6	66.5 ± 12.1	0.003
BSA, mean ± SD	1.80 ± 0.29	1.77 ± 0.16	NS
Warfarin indication, n (%)			
Atrial fibrillation	38 (39,2)	21 (22.8)	NS
Prosthetic heart valve	28 (28.9)	24 (26.0)	
Cerebrovascular incident	14 (14.4)	19 (20.7)	
Comorbidity			
$CHADS_2$ score, median (minimum–maximum)	1 (0–5)	1 (0–6)	NS
Treatment duration, n (%)			
3 weeks–3 months	21 (21.6)	24 (26.1)	
3 months–6 months	10 (10.3)	14 (15.3)	NS
6 months–1 year	17 (17.5)	7 (7.6)	
>1 year	49 (50.5)	47 (51.0)	
Supratherapeutic INR, n (%)	7 (7.2)	81 (88.0)	$<10^{-4}$
INR, mean ± SD	2.56 ± 1.16	7.36 ± 2.23	$<10^{-4}$
***VKORC1*, n (%)**			
GG	22 (24)	10 (11)	
GA	46 (49)	50 (56)	NS
AA	25 (27)	30 (33)	
***CYP2C9*, n (%)**			
*1/*1	72 (74)	63 (68)	
*1/*2	24 (25)	21 (23)	NS
*1/*3	1 (1)	8 (9)	0.014

NS: Not significant (p > 0.05).

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and compared the performance of the pharmacogenetic algorithms in the uncontrolled patients to those observed in the controlled ones.

Results

Demographic, anthropometric, clinical and genetic characteristics of the controlled and uncontrolled patients are reported in Table 1. Additional information about the investigated patients is reported in Supplementary Tables 2 & 3. No deviations from the Hardy–Weinberg equilibrium were observed. The frequencies of *VKORC1* and *CYP2C9* polymorphisms are within the ranges previously reported for the Turkish population [26], and they are also consistent with the data reported for Middle East and Central Asia populations available in the Human Genome Diversity Project. Significant differences between controlled and uncontrolled patients are present for age, *CYP2C9* and INR. Specifically, uncontrolled patients are older and have a higher frequency of *CYP2C9*3* alleles and higher INR values than the controlled ones ($p = 0.003$, $p = 0.017$ and $p < 10^{-4}$, respectively). No significant differences are observed for the other parameters investigated. Considering the controlled cohort, univariate regression analysis identified *VKORC1* genotypes ($R^2 = 29.12\%$), age ($R^2 = 8.83\%$), BSA ($R^2 = 5.42\%$) and the presence of aortic valve replacement (AVR, $R^2 = 4.93\%$) as significant predictors of warfarin dose in controlled patients (Table 2). Considering the predictors with p-value < 0.2, the stepwise model selection by exact Akaike Information Criterion identified the following model ($R^2 = 46.36$, $p < 10^{-4}$):

Daily warfarin dose = EXP[2.292 + 0.1504*(sex) - 0.09932*(age) + 0.3679*(AVR) - 0.3227*(*VKORC1*) + 0.1397*(concomitant drugs),

where the parameters are codified as: sex (female = 1, male = 0), age (decades), AVR (presence = 1, absence = 0), *VKORC1* (GG = 0, GA = 1, AA = 2), and concomitant drugs (aspirin plus nonsteroidal anti-inflammatory drugs = 1, other drug condition = 0).

Table 2. Univariate regression analysis of clinical, demographic, anthropometric and genetic parameters respect to the warfarin dose in the controlled patients.

Parameters	R^2 (%)	p-value
VKORC1 genotype	29.12	**$<10^{-4}$**
Age (decades)	8.83	**0.003**
BSA (log-transformation)	5.42	**0.022**
Aortic valve replacement (AVR)	4.93	**0.029**
Sex (female)	3.84	0.054
Nonsteroidal anti-inflammatory (NSAI) drugs	2.47	0.124
AVR+MVR	2.28	0.14
Aspirin plus NSAI	2.06	0.161
Deep vein thrombosis	1.53	0.227
Atrial fibrillation	1.1	0.306
Pulmonary thromboembolism	0.95	0.343
CYP2C9 phenotype	0.59	0.455
Aortocoronary saphenous vein bypass grafts	0.5	0.492
*CYP2C9*2* allele	0.48	0.499
Thromboembolic cerebrovascular events	0.29	0.598
Mitral valve replacement (MVR)	0.18	0.679
Comorbidity (presence)	0.14	0.716
Hematological diseases	0.12	0.733
*CYP2C9*3* allele	0.12	0.733
Comorbidity (n)	0.04	0.855
Supratherapeutic status	<0.01	0.989
Aspirin	<0.01	0.997

CYP2C9 phenotype is defined as: *1/*1 (normal phenotype), *1/*2 and *1/*3 (intermediate phenotype).
In bold $p < 0.05$.

The stepwise method selected five of eight parameters tested to be included in the model, also excluding BSA that was nominally significant ($p < 0.05$) in the univariate regression analysis.

Comparisons of our model to the previously developed international algorithms in controlled and uncontrolled patients are shown in Table 3. As expected, in controlled patients our model achieved the best performance since it was developed on the same cohort. In controlled patients, the pharmacogenetic algorithms proposed by IWPG [13] and Wadelius and colleagues [14] showed good performances, considering the differences between predicted (IWPG: 3.81 ± 2.13 mg/day; Wadelius: 4.48 ± 2.28 mg/day) and actual warfarin dosages (4.84 ± 2.25), MAE (IWPG: 1.70 ± 0.19 mg/day; Wadelius: 1.69 ± 0.15 mg/day) and the percentage of patients with a predicted dose within 20% of the actual dose (IWPG: 33%; Wadelius: 34%). Our model showed a consistent reduction in its performance in the uncontrolled cohort with respect to the results observed in the controlled one: mean differences (controlled cohort $p > 0.05$; uncontrolled cohort $p = 0.025$), correlation, MAE (controlled cohort MAE: 1.21 ± 0.14 mg/day; uncontrolled cohort MAE: 1.29 ± 0.09 mg/day) and percentage of patients with the predicted dose within 20% of the actual dose (controlled cohort 'within 20%': 48%; uncontrolled cohort 'within 20%': 31%). Conversely, the performance of international algorithms in the controlled and uncontrolled patients did not show relevant differences.

Considering the treatment response index in relation to the parameters nominally significant in regression analysis that are included in the Turkish model, we observed that this parameter is significantly affected by supratherapeutic status and *VKORC1* genotype in the controlled and uncontrolled patients ($p < 10^{-4}$). In the uncontrolled patients, the treatment response index is also significantly affected by age ($p = 0.001$), and the presence of aortocoronary saphenous vein bypass grafts ($p = 0.004$). Table 4 reports the differences of treatment response index between controlled and uncontrolled patients, also considering the most significant parameters (i.e., therapeutic status and *VKORC1* genotype).

Discussion

Genetic studies uncovered and confirmed that *VKORC1* and *CYP2C9* play important roles in the response to warfarin. A number of algorithms have been developed to predict the optimal warfarin dose for each patient on the basis of *VKORC1* and *CYP2C9* genotypes and other nongenetic factors [2,9]. Some of these algorithms have been developed on large cohorts and can account for more than half of the variation in warfarin dose requirements [12–15]. Although these algorithms can be used in clinical practice to predict the optimal warfarin dose before treatment initiation, their effectiveness has not completely demonstrated in clinical practice [31,32]. Moreover, human genetic variation could alter the accuracy of these algorithms when used in populations with genomic backgrounds different from those populations used for algorithm development and validation [16,17]. The Turkish population is a good example of a human group with peculiar genetic features that may affect the accuracy of the international warfarin algorithms. To our knowledge, three previous studies investigated the role of *VKORC1* and *CYP2C9* genotypes in warfarin dose requirements [24–26]. However, these studies developed warfarin algorithms on small samples, and no large warfarin pharmacogenetic studies are available about Middle East populations. Furthermore, no study investigated the effectiveness of the international algorithm in predicting the optimal warfarin dose or evaluated the usefulness of these algorithms preventing major and life-threatening hemorrhagic events related to incorrect warfarin dosing in Turkish patients. In this study we investigated a cohort of patients with a warfarin stable dose and no hemorrhagic or thromboembolic event (i.e., controlled patients) and a cohort with a warfarin-stable dose admitted to emergency rooms with a major or life-threatening hemorrhagic event (uncontrolled patients). Specifically, we analyzed the capability of genetic and nongenetic factors to predict the optimal warfarin dose in controlled patients, evaluated the accuracy of international pharmacogenetic algorithms and assessed the ability to detect the incorrect dosage of uncontrolled patients.

The comparison analysis between controlled and uncontrolled patients showed significant differences in age, *CYP2C9*3* allele and INR. The higher INR values and supratherapeutic status of uncontrolled patients indicates that an inadequate monitoring of warfarin response caused the drug side effects. Warfarin therapy requires frequent monitoring to assess the optimal drug dose during the therapy initiation and to identify potential response variation over time (which could cause INR status alteration) [19,33]. The older age of the uncontrolled patients could correspond to the more complex clinical picture and the reduced therapy adherence of elderly people [7,34,35]. Although older people are more likely to bleed [36], the bleeding events are likely to be mainly due to the incorrect warfarin dose since there is a high percentage of subjects with supratherapeutic INR (i.e., 88%). The higher number of *CYP2C9*3* alleles in uncontrolled patients is in line with recent studies that indicated an increased bleeding risk of *CYP2C9*3* carriers [37,38]. However, *CYP2C9* alleles (*2 and *3) are not associated with the therapeutic warfarin doses

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Table 3. Performance comparisons of Turkish model and international pharmacogenetic algorithms in controlled and uncontrolled patients.

Algorithm	Daily warfarin dose (mean ± SD)	MAE (mean ± SE)	Pearson's r	Within 20%	Ref.
Controlled patients					
Controlled dose	4.84 ± 2.25	–	–	–	
Turkish model	4.60 ± 1.38	1.21 ± 0.14	**0.634**	48%	
Cage *et al.* (2008)	**6.60 ± 2.40**	**2.25 ± 0.23**	**0.558**	**29%**	[12]
IWPG (2009)	3.81 ± 2.13	1.70 ± 0.19	**0.586**	33%	[13]
Wadelius *et al.* (2009)	4.48 ± 2.28	1.69 ± 0.15	**0.569**	34%	[14]
Horne *et al.* (2012)	**1.91 ± 0.31**	**2.94 ± 0.22**	**0.604**	**5%**	[15]
Uncontrolled patients					
Uncontrolled dose	4.44 ± 1.53	–	–	–	
Turkish model	**4.11 ± 1.29**	1.29 ± 0.09	**0.426**	31%	
Cage *et al.* (2008)	**5.74 ± 1.38**	1.65 ± 0.12	**0.417**	36%	[12]
IWPG (2009)	**3.53 ± 1.38**	1.26 ± 0.10	**0.552**	34%	[13]
Wadelius *et al.* (2009)	4.32 ± 1.41	1.11 ± 0.09	**0.559**	46%	[14]
Horne *et al.* (2012)	**1.73 ± 0.25**	**2.74 ± 0.14**	**0.558**	**2%**	[15]

For daily warfarin dose and Pearson's correlation analyses, we analyzed the therapeutic/actual dose respect (for controlled and uncontrolled patients, respectively) to the predicted doses. For the mean absolute error and 'within 20' analyses, we compared the result obtained by Turkish model with those of the international algorithms.
In bold comparisons with $p < 0.05$.

in our controlled patients. This is probably due to the sample size, and to the absence of *CYP2C9* low-activity genotypes (i.e., *2/*2, *3/*3 and *2/*3). Accordingly, previous studies with similar sample sizes failed to replicate the *CYP2C9* effect on a warfarin dose [39]. Nevertheless, since the *VKORC1* genotype has a greater effect on the warfarin dose than those reported for CYP2C9 (30 vs 10%), and the *VKORC1* minor allele frequency is high in the Turkish population, we replicated the *VKORC1* finding in our controlled patients (R^2 = 29.12%). Furthermore, we observed that age, BSA and warfarin indications (i.e., AVR) are also significant predictors of therapeutic doses in controlled patients. These results are in agreement with the previous studies about the role of anthropometric and clinical factors in warfarin dosing [2]. Accordingly, the best model developed for our controlled cohort shares several parameters (i.e., *VKORC1*, age, BSA and concomitant drugs) with the previous warfarin pharmacogenetic algorithms [37]. Considering the accuracy of our model to evaluate the international warfarin pharmacogenetic algorithm, we observed good performance of the algorithms of the IWPG [13] and Wadelius and colleagues [14]. We also observed a poor performance of the algorithms of Gage and colleagues [12] and Horne and colleagues [15]. These differences may be explained by the ethnic background of the samples used to develop the algorithms. The Gage-, Horne- and IWPG-algorithms were developed on multiethnic cohorts that included subjects with European, African and Asian ancestries, whereas the Wadelius-algorithm was developed on a Swedish-majority cohort. Although it is difficult to derive the percentage of Middle East samples in these cohort, relevant differences in the Middle Eastern subjects included in the European class could plausibly account for the observed performance variability. Other reasons can be present, such as the higher adherence of our data to IWPG- and Wadelius-algorithms. However, since we collected detailed information about the investigated patients, the putative poor adherence of our data to Gage- and Horne- algorithms may indicate that these algorithms require information not usually collected during routine screenings for warfarin treatments.

Regarding the treatment response index, we observed that the INR therapeutic status and *VKORC1* genotype have a relevant effect both in controlled and uncontrolled patients. This confirms the significant role of the INR monitoring and *VKORC1* genotype in determining the dose-response relationship of the treated patients. Furthermore, age and warfarin indication (i.e., aortocoronary saphenous vein bypass grafts) significantly contributed to the dose-response relationship in uncontrolled patients. Therefore, both these factors may contribute to the uncontrolled warfarin therapy together with the poor INR monitoring and the *VKORC1* genotype. The age increases probability

Table 4. Differences of the treatment response index (i.e., ln [INR/daily warfarin dose]) between controlled and uncontrolled patients considering therapeutic status and *VKORC1* genotype.

Controlled patients			**Uncontrolled patients**		
ln(INR/dose) **-0.632 ± 0.060**			ln(INR/dose) **0.516 ± 0.055**		
Supratherapeutic		**Therapeutic**	**Supratherapeutic**		**Therapeutic**
0.266 ± 0.172		-0.702 ± 0.057	0.623 ± 0.049		-0.274 ± 0.123
***VKORC1* GG**	***VKORC1* GA**	***VKORC1* AA**	***VKORC1* GG**	***VKORC1* GA**	***VKORC1* AA**
-0.943 ± 0.226	-0.703 ± 0.075	-0.206 ± 0.120	0.084 ± 0.226	0.430 ± 0.060	0.807 ± 0.086

Highly significant difference is present between controlled and uncontrolled patients in treatment response index ($p < 10^{-4}$). Both therapeutic status and *VKORC1* genotype highly significantly affect the treatment response index in both controlled and uncontrolled patients ($p < 10^{-4}$).

of the complex clinical picture and poor therapy adherence [7,34,35], and the aortocoronary saphenous vein bypass grafts may interact with warfarin therapy in the determining the increased risk of bleeding events.

Finally, the algorithm accuracy analysis showed that our model decreased in accuracy in the uncontrolled cohorts, while the international algorithms showed similar performances in both the cohorts. The results of our model can be explained by two scenarios: our model is specifically defined for Turkish patients and it can detect the incorrect dosing of uncontrolled patients; since our model is designed with controlled patients, the performance reduction in the uncontrolled patients could due to the replication of our model in an independent cohort. The findings obtained about the international algorithms confirmed that the accuracy of the pharmacogenetic-guided warfarin dosing is not high enough to effectively identify the optimal warfarin dose and detect incorrect warfarin dosing. Indeed, they showed the same good performances in predicting both therapeutic and incorrect warfarin doses. Although genetic factors can explain a large variation of warfarin response, further investigations are needed to improve the warfarin algorithm through the development of population-specific algorithms or more complex algorithms based on additional molecular factors.

Conclusion

Our study provided novel data about the effectiveness of international warfarin pharmacogenetic algorithms in Turkish patients, and their accuracy in populations with a genomic background different from those populations on which the algorithms were developed. Our accuracy analysis indicated that international pharmacogenetic algorithms are able to predict the therapeutic warfarin dose of controlled patients, but they are not sufficiently accurate to distinguish the incorrect dosing of uncontrolled patients. This strongly indicates that the use of genetic information to assess warfarin therapeutic doses still needs further improvements. Our investigation also confirmed some warfarin knowledge in the Turkish population: the *CYP2C9*3* allele is associated with major and life-threatening hemorrhagic events in warfarin-treated patients and the *VKORC1* genotype is significantly involved in the warfarin dose-response relationship.

Future perspective

Since *VKORC1* and *CYP2C9* play a relevant role in determining warfarin individual response, a number of pharmacogenetic algorithms has been developed in recent years. Although some of these algorithms can explain more than 50% of the warfarin dose requirements, they are not sufficiently accurate to be used in routine screenings. Current research on warfarin pharmacogenetics focuses on the development of population-specific algorithms to reduce the altering effects of population genetic background. However, future perspective of warfarin investigation could be based on the integration of multiple molecular data (i.e., genetics, epigenetics and metabolomics) that can improve our understanding of the inter- and intraindividual variability of warfarin dose-response relationship.

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Financial & competing interests disclosure

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- Warfarin is the most widely used oral anticoagulant prescribed to treat and prevent thromboembolism.
- Although it has a significant thromboembolism risk reduction, incorrect warfarin dosing can be associated with thromboembolic events (in the case of underdosing), or bleeding complications (in the case of overdosing).
- *VKORC1* and CYP2C9 play a relevant role in determining large variation in dose requirements, and numerous research groups developed different dosing algorithms based on genetic, clinical and anthropometric characteristics.
- Although some of these pharmacogenetic algorithms can predict more than 50% of warfarin dose requirements, their effectiveness in determining the optimal warfarin dose has yet to be demonstrated.

Aim

- We investigated two independent Turkish warfarin-treated cohorts to determine the accuracy of international warfarin pharmacogenetic algorithms in predicting therapeutic and incorrect warfarin doses in Turkish patients.

Results

- CYP2C9*3 allele frequency is significantly higher in patients with major and life-threatening hemorrhagic events than in those with no history of hemorrhagic or thromboembolic events.
- *VKORC1* genotypes, age, BSA and aortic valve replacement (AVR, R^2 = 4.93%) are significant predictors of warfarin dose in Turkish patients with no history of hemorrhagic or thromboembolic event.
- International pharmacogenetic algorithms predicted the therapeutic dose of patients with no history of bleedings with a high degree of accuracy, but they did not significantly distinguish the incorrect warfarin dose of patients with major and life-threatening hemorrhagic events.

Conclusion

- We provided novel data about the effects of genetic and nongenetic factors on warfarin dose-response relationship in the Turkish population.
- Although genetic information can be considered to predict the therapeutic warfarin dose, the accuracy of the international pharmacogenetic algorithms is not sufficient to be used for warfarin screening in Turkish patients.

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