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Effect of genetic factors on the interindividual variability of warfarin dosage requirements in Japanese patients after adjusting for renal function

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Renal function significantly influences the appropriate warfarin dosage. However, studies investigating the impact of genetic factors on warfarin dosage, considering renal function, are limited. This study aimed to assess the role of genetic polymorphisms in *VKORC1*, *CYP2C9*, *CYP2C19*, *CYP4F2*, *GGCX*, and *APOE* in warfarin dosage adjustment considering renal function. A total of 108 outpatients receiving warfarin treatment with controlled prothrombin time-targeted international normalized ratio (1.5–3.0) were included. Patient data, warfarin dosage, and laboratory results were collected from electronic medical records. Each SNP [*VKORC1* rs9923231, *CYP2C9* rs1057910, *CYP4F2* rs2108622, *CYP2C19*2* (rs4244285) and **3* (rs4986893), *GGCX* rs699664 and rs12714145, and *APOE* rs7421] was analyzed. Multiple regression analysis revealed estimated glomerular filtration rate as the most significant factor influencing warfarin dose ($p < 0.001$) ($\beta = -0.445$). *VKORC1* rs9923231 AA, *CYP4F2* rs2108622 CT/TT, *GGCX* rs12714145 CT/TT, and *CYP2C9* rs1057910 AC carriers were associated with warfarin dose ($p < 0.001$, 0.015, 0.020, 0.038 and $\beta = -0.317$, 0.191, –0.188, –0.162, respectively); however, other genes showed no significant association. In conclusion, after adjusting for renal function, genetic factors of *VKORC1* rs9923231, *CYP4F2* rs2108622, *GGCX* rs12714145, and *CYP2C9* rs1057910 were found to contribute to warfarin dosage, having impact in that order. In contrast, the contribution of other genes to warfarin dosage was absent or negligible.

1. Introduction

Warfarin is the most widely used oral anticoagulant (Barnes et al. 2015). In Japan, it is extensively used in the treatment of thrombosis and in the management and prevention of cardiogenic cerebral embolism associated with atrial fibrillation and artificial valve replacement. Despite the rise of direct oral anticoagulants as the mainstream option, warfare remains highly necessary due to its cost-effectiveness, broad indications, and the ability to monitor treatment efficacy through the prothrombin time-targeted international normalized ratio (PT-INR). The therapeutic range for warfarin typically falls within 1.5–3 with a target PT-INR. However, considerable interindividual variation in warfarin dosing exists, with maintenance doses ranging from 1 to 20 mg to attain the desired PT-INR target (Klein et al. 2009). Various factors contribute to these differences in warfarin dosing (Johnson et al. 2007; Mar et al. 2022).

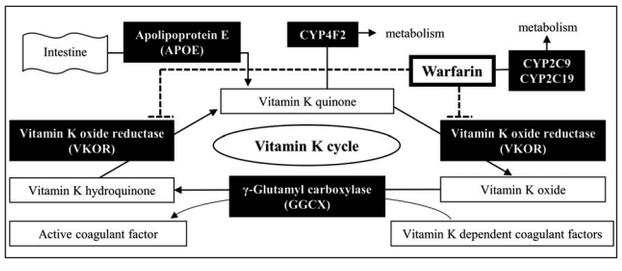

Fig. 1: Mechanism of warfarin and vitamin K cycle. VKOR is crucial for Vitamin K reduction; CYP2C9 is responsible for the metabolism of the S form of warfarin; CYP4F2 is a vitamin K-metabolizing enzyme; CYP2C19 is an enzyme that metabolizes the R form of warfarin; GGCX is an enzyme that uses reduced vitamin K to convert glutamate residues of vitamin K-dependent proteins to gamma-glutamyl; and APOE is an enzyme that converts vitamin K from the gut.

Several genetic variants linked to the vitamin K (VK) reductase necessary for the VK cycle and enzymes involved in warfarin metabolism significantly influence warfarin dosage (Fig. 1). Among these, *VKORC1* rs9923231, crucial for VK reduction, and *CYP2C9* rs1057910, responsible for metabolizing the highly active S form of warfarin, which consists of racemic forms, are particularly important and account for approximately 40% of individual differences in warfarin dosage (Jonas and McLeod 2009). Other genetic polymorphisms implicated in warfarin dosing include *CYP4F2*, an enzyme that metabolizes VK; *CYP2C19*, an enzyme that metabolizes the R form of warfarin; Gamma-glutamyl carboxylase (GGCX), an enzyme that activates coagulation factors while oxidizing VK to its epoxide form; and Apolipoprotein E (*APOE*), mediating VK uptake. Some reports on these genetic factors suggest a link (Danese et al. 2019; Li et al. 2022; Jiang et al. 2017; Rafiee et al. 2017; Yu et al. 2016; Liu et al. 2016), whereas others suggest no link (Galvez et al. 2018; Sun et al. 2015;

Tian et al. 2015; Hamadeh et al. 2016; Sconce et al. 2006), yielding inconsistent results. Other factors such as drug interactions, patient age, and body size have long been recognized (Jonas and McLeod 2009); however renal function emerges as a more influential factor. In a study by Limdi et al. (2009) on 980 patients, impaired renal function emerged as an important factor independent of genetic polymorphisms of *VKORC1* and *CYP2C9*, with daily doses lower by 19.1% and 9.5% in patients with estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2 compared with those with eGFR ≥60 mL/min/1.73 m^2 and eGFR 30–59 mL/min/1.73 m^2, respectively. Similarly, Tanaka et al. (2021) and Ichihara et al. (2015) found that renal function was independently associated with warfarin doses in Japanese patients, with a contribution comparable to that of the genetic polymorphisms of *VKORC1* and *CYP2C9*. However, comprehensive analyses adjusting for renal function have not been conducted for genetic variants other than *VKORC1* and *CYP2C9* in previous studies. Therefore, the inconsistent findings in studies on each genetic polymorphism regarding warfarin dosage may be attributed to the lack of adjustment for renal function. Adjusting for renal function in assessments could potentially clarify the contribution of other genetic polymorphisms (*CYP4F2*, *CYP2C19*, *GGCX*, and *APOE*) to warfarin dosage.

In this study, we analyzed the genetic variations in VKORC1, CYP2C9, CYP2C19, CYP4F2, GGCX, and APOE, which have been suggested to be associated with warfarin dose, and assessed the contribution of each genetic polymorphism to warfarin dosage by adjusting renal function.

2. Investigations and results

2.1. Patient characteristics

Table 1 displays the characteristics of 108 patients receiving warfarin. The mean age of patients was 65.7±16.3 years, with mean height and weight measuring 161.4±9.2 cm and 59.9±14.4 kg, respectively. The mean eGFR was 59.6±25.0 mL/min/1.73 m^2, with 60 patients (55.6%) exhibiting renal dysfunction (eGFR <60 mL/min/1.73 m^2). The average warfarin dosage was 3.04±1.27 mg/day, consistent with the typical dose among Japanese patients. The most prevalent indication for warfarin use was atrial fibrillation, observed in 59 (54.6%) patients.

Table 1: Patient characteristics

Variable	N = 108
Age, years	65.7 ± 16.3
Female	47 (43.5)
Height, cm	161.4 ± 9.2
Weight, kg	59.9 ± 14.4
BSA, m^2	1.62 ± 0.22
WBC, count/L	5.66 ± 1.70
Hemoglobin, g/dL	13.2 ± 1.93
AST, IU/L	25.5 ± 12.7
ALT, IU/L	20.6 ± 13.7
Creatinine, mg/dL	1.17 ± 1.21
eGFR, mL/min/1.73 m^2	59.6 ± 25.0
<60 mL/min/1.73 m^2	60 (55.6)
>60 mL/min/1.73 m^2	48 (44.4)
PT-INR	1.99 ± 0.34
Daily dose of warfarin, mg/day	3.04 ± 1.27
Disease history	
Atrial fibrillation	59 (54.6)
Valve replacements	29 (26.9)
Pulmonary thromboembolism	15 (13.9)
Others	11 (10.2)

Data are presented as mean±standard deviation or number (percentage). BSA, body surface area; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT-INR, prothrombin time-targeted international normalized ratio; eGFR, estimated glomerular filtration rate.

2.2. Genotype analysis

Table 2 presents gene polymorphisms and corresponding warfarin doses. The *VKORC1* rs9923231 AA and *GGCX* rs699664 TT genotypes were associated with lower warfarin doses (p = 0.013, 0.025), while the *CYP4F2* rs2108622 CT/TT genotype was associated with higher warfarin doses (p = 0.002). No deviation from the Hardy–Weinberg equilibrium was observed.

Table 2: Differences in warfarin dose by genotype

Genotype	N	Warfarin dose (mg/day)	p value
VKORC1 -1639G>A (rs9923231)			
GG/GA	0/20	3.68 ± 1.78	0.013
AA	88	2.90 ± 1.09	
A frequency	90.7%		
CYP2C9 1075A>C (rs1057910)			
AA	103	3.08 ± 1.28	0.198
AC/CC	5/0	2.33 ± 0.74	
C frequency	2.3%		
CYP4F2 1347C>T (rs2108622)			
CC	60	2.71 ± 1.12	0.002
CT/TT	36/12	3.40 ± 1.34	
T frequency	27.8%		
CYP2C19 *2 (rs4244285), *3 (rs4986893)			
RM/IM	44/58	3.03 ± 1.27	0.624
PM	6	3.29 ± 1.30	
GGCX 8016G>A (rs699664)			
CC/CT	59/43	3.11 ± 1.27	0.025
TT	6	1.92 ± 0.74	
T frequency	25.5%		
GGCX 214+597G>A (rs12714145)			
CC	54	3.28 ± 1.37	0.057
CT/TT	35/19	2.81 ± 1.13	
T frequency	33.8%		
APOE 472C>T (rs7412)			
CC	98	3.05 ± 1.30	0.621
CT/CT	9/0	2.83 ± 0.98	
T frequency	4.2%		

Warfarin doses are presented as mean ± standard deviation. CYP2C19 is a combination of *2 and *3 and is classified as a rapid metabolizer (RM) (*1/*1), intermediate metabolizer (IM) (*1/*2 or *1/*3), and poor metabolizer (PM) (*2/*2 or *2/*3 or *3/*3).

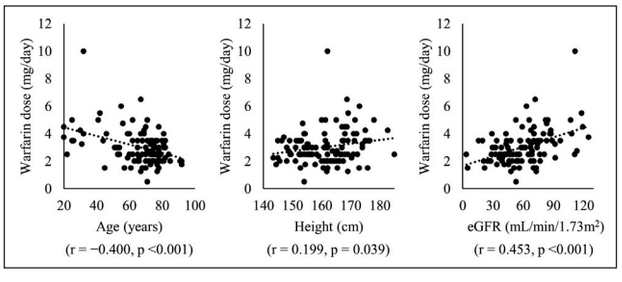

Fig. 2: Correlation between daily warfarin dose and age, height, and estimated glomerular filtration rate (eGFR). There was a positive correlation between height, eGFR, and warfarin dose and a negative correlation between age and warfarin.

2.3. Non-genetic factors

Table 3 displays sex and the doses of concomitant medications and warfarin. Regarding the relationship between warfarin dose and other non-genetic factors, age, height, and eGFR were significantly

Table 3: Differences in daily warfarin dose by sex, disease, and concomitant medications

Variable	N	Warfarin dose (mg/day)	p value
Sex			
Male	61	3.03 ± 1.21	0.941
Female	47	3.05 ± 1.36	
Disease			
Atrial fibrillation			
Yes	59	2.83 ± 1.02	0.067
No	49	3.26 ± 1.49	
Valve replacements			
Yes	29	3.23 ± 1.27	0.350
No	79	2.97 ± 1.27	
Pulmonary thromboembolism			
Yes	15	3.46 ± 2.09	0.174
No	93	2.98 ± 1.09	
Concomitant medications			
NSAIDs			
Use	10	3.28 ± 1.70	0.547
Non-use	98	3.02 ± 1.23	
Aspirin			
Use	24	2.92 ± 1.08	0.584
Non-use	84	3.08 ± 1.33	
Amiodarone			
Use	5	2.25 ± 0.66	0.154
Non-use	103	3.08 ± 1.28	
Sulfonylurea			
Use	4	2.75 ± 0.65	0.641
Non-use	104	3.05 ± 1.29	

Data are presented as mean±standard deviation. NSAIDs, non-steroidal anti-inflammatory drugs.

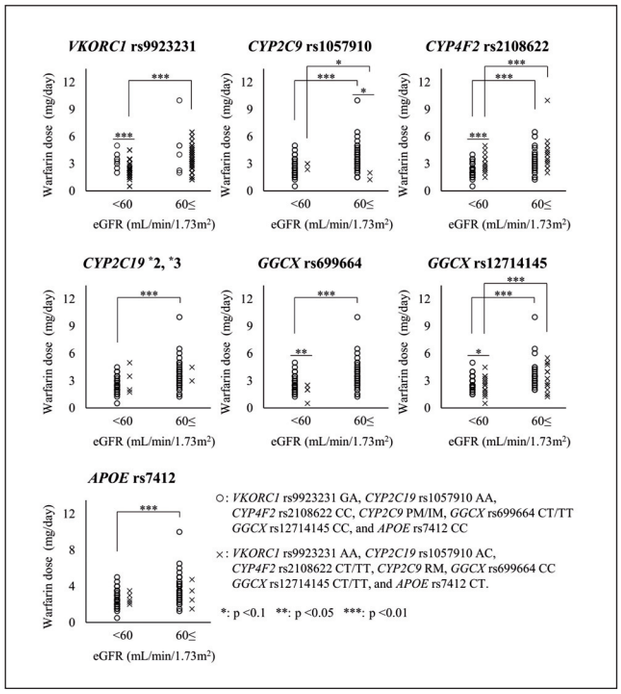

Fig. 3: Association between the presence of genetic polymorphisms in each estimated glomerular filtration rate (eGFR) category and warfarin dose.

correlated (p <0.001, 0.039, <0.001, respectively; Fig. 2), while no significant correlations were found with weight, body surface area (BSA), or other laboratory values (data not shown).

2.4. Effect of each genetic factor on renal function

Figure 3 illustrates the relationship between each genetic polymorphism and warfarin dose when eGFR is categorized as <60 and ≥60 mL/min/1.73 m^2, adjusted for renal function. Among patients with eGFR <60 mL/min/1.73 m^2, significant differences in *VKORC1* rs9923231 AA, *CYP4F2* rs2108622 CT/TT, and *GGCX* rs699664 TT genotypes were observed in relation to warfarin dose (p <0.01, <0.01, <0.05, respectively). Conversely, among patients with eGFR ≥60 mL/min/1.73 m^2, no individuals possessed *GGCX* rs699664 TT genotype, and no significant differences were noted between warfarin dose and *VKORC1* and *CYP4F2* polymorphisms. Additionally, within the group with the same genetic polymorphism, the warfarin dose was significantly lower in patients with eGFR <60 mL/min/1.73 m^2 compared with those with eGFR ≥ 60 mL/min/1.73 m^2.

2.5. Factors influencing individual differences in warfarin dosage

Multiple regression analysis results are depicted in Table 4. In model 1, which incorporated age, height, PT-INR, and each gene (*VKORC1*, *CYP2C9*, *CYP4F2*, *CYP2C19*, *GGCX*, and *APOE*), age, height, *VKORC1* rs9923231, and *CYP4F2* rs2108622 emerged as significant factors. The multiple correlation coefficient (R) was 0.595, with a coefficient of determination (R^2) of 0.354 for this regression equation. In model 2, eGFR was introduced as a new factor alongside those in model 1. Subsequently, eGFR, *CYP2C9* rs1057910, and *GGCX* rs12714145 were identified as new significant factors, with age being designated as a confounding factor. The regression equation for model 2 was R = 0.692, with R^2 = 0.479.

3. Discussion

This study is the first investigation to demonstrate the individual contributions of various genetic polymorphisms associated with warfarin dosage in the Japanese population, while adjusting for renal function. Consistent with previous research, the genetic polymorphisms of *VKORC1* rs9923231 and *CYP2C9* rs1057910 were found to influence individual differences in warfarin dosage, even after adjusting for renal function. While the status of other gene polymorphisms remains controversial, our renal function-adjusted analyses revealed that *CYP4F2* rs2108622 and *GGCX* rs12714145 exerted an impact on warfarin dosage. Conversely, *CYP2C19*2* and **3*, *GGCX* rs699664, and *APOE* rs7412 were found to lack a potentially significant effect on warfarin dosage.

Non-renal clearance has also been reported to be reduced when renal function is impaired, potentially altering the bioavailability of drugs predominantly metabolized in the liver and the response to these drugs (Leblond et al. 2001; Guévin et al. 2002). Additionally, it has been reported that reduced renal function may result in decreased VK, potentially reducing coagulation activity (Wuyts and Dhondt 2016; Grzejszczak and Kurnatowska 2021). These findings suggest that renal function may have a significant influence on warfarin dosage, thus contributing to individual variations. Numerous studies have reported renal function being associated with warfarin dosage independently of the well-established genetic factors such as *VKORC1* rs9923231 and *CYP2C9* rs1057910, both of which have demonstrated substantial contributions (Tanaka et al. 2021; Ichihara et al. 2015; Jun et al. 2015). However, prior studies did not simultaneously analyze genes other than *VKORC1* and *CYP2C9*, rendering the contribution of other genetic polymorphisms to renal function unexplored. In our study, besides *VKORC1* and *CYP2C9*, *CYP4F2* rs2108622 and *GGCX* rs12714145 emerged as significant genetic factors. *In vitro* studies suggest that individuals with the *CYP4F2* rs2108622 polymorphism exhibit reduced VK metabolic activity, potentially necessitating higher warfarin doses (McDonald et al. 2009). This trend towards elevated doses in patients with T alleles was confirmed in the present study, consistent with previous results. Conversely, while in-depth analysis of the *GGCX* rs12714145 polymorphism through in vitro studies is lacking, GGCX is a pivotal enzyme

Table 4: Factors affecting warfarin dose in multiple regression model

	Model 1				Model 2			
	Unstandardized coefficient B	Standardization coefficient β	p value	95% CI	Unstandardized coefficient B	Standardization coefficient β	p value	95% CI
(constant)	1.974		0.417	−2.838 to 6.786	−1.562		0.501	−6.152 to 3.029
VKORC1 rs9923231 (AA)	−0.837	−0.257	0.003	−1.385 to −0.289	−1.033	−0.317	<0.001	−1.535 to −0.531
CYP2C9 rs1057910 (AC)	−0.819	−0.136	0.113	−1.834 to 0.197	−0.977	−0.162	0.038	−1.897 to −0.057
CYP4F2 rs2108622 (CT or TT)	0.516	0.202	0.020	0.082 to 0.951	0.488	0.191	0.015	0.096 to 0.881
CYP2C19 (PM)	0.063	0.011	0.894	−0.871 to 0.998	−0.035	−0.006	0.935	−0.880 to 0.810
GGCX rs699664 (TT)	−0.247	−0.045	0.612	−1.212 to 0.717	−0.189	−0.034	0.668	−1.060 to 0.683
GGCX rs12714145 (CT or TT)	−0.384	−0.151	0.088	−0.826 to 0.059	−0.477	−0.188	0.020	−0.879 to −0.075
APOE rs7412 (CT)	0.137	0.030	0.721	−0.624 to 0.898	0.200	0.044	0.564	−0.487 to 0.888
Age, years	−0.027	−0.336	<0.001	−0.041 to −0.013	−0.005	−0.066	0.497	−0.021 to 0.010
Height, cm	0.027	0.195	0.025	0.004 to 0.051	0.036	0.258	0.001	0.014 to 0.058
PT-INR	0.354	0.096	0.266	−0.274 to 0.983	0.334	0.090	0.246	−0.234 to 0.902
eGFR, mL/min/1.73 m^2	-	-	-	-	0.023	0.445	<0.001	0.014 to 0.033

All genetic factors and non-genetic factors related to warfarin dose (age, height, and PT-INR) were entered into the multivariate analysis.
Model 1 shows the contribution of each factor with eGFR not included as a factor.
Model 2 shows the contribution of each factor with eGFR as a factor in Model 1.
PT-INR, prothrombin time-targeted international normalized ratio; eGFR, estimated glomerular filtration rate.

in blood coagulation factor synthesis, involved in the gamma-carboxylation of reduced VK, and is frequently implicated in warfarin dosage studies. To date, analyses conducted primarily in Caucasian and Chinese populations have yielded inconsistent results (Sun et al. 2015). However, in the present study, we observed significantly lower warfarin doses in patients with the *GGCX* rs12714145 AA genotype. The mean age of participants in our study was relatively older (65.7±16.3 years), and renal function exhibited considerable variability, with a coefficient of variation of 25.0. Prior studies have not considered renal function, but it is presumed that individual differences in renal function were significant, particularly in elderly populations, as evidenced by our findings. Our study results confirm that warfarin doses are significantly lower in patients with the same genotype but with eGFR <60 mL/min/1.73m^2, in addition to the primary genetic factors *VKORC1* rs9923231 and *CYP2C9* rs1057910, when accounting for renal function, *CYP4F2* rs2108622 and *GGCX* rs12714145 could be detected. To the best of our knowledge, the *GGCX* rs12714145 polymorphism was identified as an independent genetic factor for the first time in the Japanese population.

Multiple regression analysis revealed that renal function contributed the most to warfarin dosage, and the factors analyzed in our study allowed for the prediction of approximately 50% of warfarin dosage. In a previous study considering renal function in Japanese patients, Tanaka et al. (2021) performed multiple regression analysis of 176 eligible patients and reported an R^2 of 0.56 for the regression equation using BSA, age, changes in VK intake, aspartate aminotransferase levels, drinking habits, *VKORC1* and *CYP2C9* genotypes, and eGFR. Ichihara et al. (2015) also performed a multiple regression analysis of 137 eligible patients and reported an R^2 of 0.47 for the regression equation, with creatinine clearance, age, weight, PT-INR, and the genetic variant of *VKORC1* as variables. Despite our study's smaller cohort compared with Tanaka et al. (2021) and Ichihara et al. (2015) we were able to explain up to $R^2 = 0.482$ by including the genetic factors *CYP4F2* rs2108622 and *GGCX* rs12714145. However, our study did not account for the effects of VK intake and alcohol consumption, and it is crucial to include these and other relevant factors in future research to refine the predictive equation for warfarin dosage. The findings of our analysis, adjusted for renal function, suggest that *CYP2C19*2* and **3*, *GGCX* rs699664, and *APOE* rs7412 have either no effect or a minimal contribution to the variability of warfarin doses in the Japanese population.

This study has several limitations. First, patients with *VKORC1* rs9923231 GG and *CYP2C9* rs1057910 CC genotypes are rare in the Japanese population, and no applicable patients were identified in our study. In addition, the CYP2C9 rs1057910 CA genotype was present in only five patients; therefore, its contribution to warfarin dose may be underestimated. Second, the study spanned two hospitals and patients could not be stratified by disease due to the inter-institutional nature of our study. In the future, we aim to explore the underlying factors contributing to individual differences in warfarin dosage by conducting a more detailed analysis that incorporates factors such as drinking history and other variables that may influence the appropriate dose of warfarin, including blood levels of warfarin and VK.

In conclusion, after accounting for renal function as a factor influencing warfarin dosage, it was observed that in addition to *VKORC1* rs9923231 and *CYP2C9* rs1057910, *CYP4F2* rs2108622 and *GGCX* rs12714145 also contributed as genetic factors. Conversely, the contributions of *CYP2C19*2* and **3*, *GGCX* rs699664, and *APOE* rs7412 were either negligible or absent.

4. Experimental

4.1. Patient characteristics

This study included Japanese outpatients aged ≥20 years receiving warfarin treatment at the Department of Cardiology, Gunma University Hospital, and the Department of Internal Medicine, JCHO Gunma Central Hospital. Eligible participants included those who had received a fixed maintenance warfarin dose for at least three consecutive sessions and maintained a controlled PT-INR within the range of 1.5–3.0. Exclusion criteria included patients with untested PT-INR, patients with cirrhosis, and patients deemed unfit for study participation by their attending physician. Blood samples were collected for genetic polymorphism analysis, and basic patient information, warfarin dosage, and laboratory data were collected from electronic medical records. The research protocol was approved by the ethics committee of Gunma University (HS2020-170), Takasaki University of Health and Welfare (2338), and the JCHO Gunma Central Hospital (2022-009). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional or national research committee and with the ethical standards of the 1964 Declaration of Helsinki and subsequent amendments or equivalents. Written informed consent was obtained from all participants.

4.2. Genotyping of polymorphisms

Genomic DNA was extracted from whole blood using a QIAamp® Blood kit (Qiagen, Valencia, CA, USA). Single nucleotide polymorphisms (SNPs) were analyzed via the TaqMan genotyping assay (Applied Biosystems, Foster City, CA, USA) for eight SNPs: *VKORC1* (rs9923231), *CYP2C9* (rs1057910), *CYP4F2* (rs2108622), *CYP2C19**2 (rs4244285), *CYP2C19**3 (rs4986893), *GGCX* (rs699664, rs12714145), and *APOE* (rs7412). The TaqMan primer and probe set designed for each SNP allele was included in the kits. The following assay IDs were used: *VKORC1* rs9923231: C__30403261_20, *CYP2C9**3 rs1057910: C__27104892_10, *CYP4F2* rs2108622: C__16179493_40, *CYP2C19* rs4244285: C__25986767_70, *CYP2C19* rs4986893: C__27861809_10, *GGCX* rs699664: C__1036123_10, *GGCX* rs12714145: C__31839079_10, *APOE* rs7412: C___904973_10. CYP2C19 was categorized as rapid metabolizer (RM) (*1/*1), intermediate metabolizer (IM) (*1/*2 or *1/*3), and poor metabolizer (PM) (*2/*2 or *2/*3 or *3/*3) in combination with *2 and *3.

4.3. Statistical analysis

Differences in mean warfarin doses by genetic polymorphism, concomitant medication, disease, and sex were assessed using either Student's t-test or the Mann-Whitney U test, depending on data distribution. Genetic polymorphisms were categorized into two groups based on ANOVA analysis (post-hoc analysis): strongly related genotypes and others. Hardy–Weinberg equilibrium was tested using the Pearson goodness-of-fit chi-square test. Pearson's product-moment correlation analysis was employed to assess correlations between warfarin dose and continuous variables such as age, height, weight, eGFR, blood urea nitrogen, serum creatinine level, hemoglobin, white blood cell count, platelet count, serum Na level, and serum K level. Factors included in the multiple regression analysis comprised renal function, PT-INR, seven genetic polymorphisms, and non-genetic factors identified as significantly associated with warfarin dose in univariate analysis. Statistical analysis was conducted using SPSS software version 26.0 (SPSS, Inc., Chicago, IL, USA). A p value less than 0.05 was considered statistically significant.

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Conflicts of interest: The authors declare that they have no conflicts of interest.

Authors' contributions: Conceptualization and methodology, HN, AN, and KO; formal analysis and data curation, HN, AN, and HY; project administration, visualization, and writing–original draft preparation, HN, AN, YT, TA, and KO; supervision, KY and KO; investigation and resources, HY, YH, NS, HN, NT, NK, TN, YK, YO, TY, KI, MK, and KY; writing–review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

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References

Barnes GD, Lucas E, Alexander GC, Goldberger ZD (2015) National trends in ambulatory oral anticoagulant use. Am J Med 128: 1300-5.e2.

Danese E, Raimondi S, Montagnana M, Danese E, Raimondi S, Montagnana M, Tagetti A, Langaee T, Borgiani P, Ciccacci C, Carcas AJ, Borobia AM, Tong HY, Dávila-Fajardo C, Rodrigues Botton M, Bourgeois S, Deloukas P, Caldwell MD, Burmester JK, Berg RL, Cavallari LH, Drozda K, Huang M, Zhao LZ, Cen HJ, Gonzalez-Conejero R, Roldan V, Nakamura Y, Mushiroda T, Gong IY, Kim RB, Hirai K, Itoh K, Isaza C, Beltrán L, Jiménez-Varo E, Cañadas-Garre M, Giontella A, Kringen MK, Haug KBF, Gwak HS, Lee KE, Minuz P, Lee MTM, Lubitz SA, Scott S, Mazzaccara C, Sacchetti L, Genç E, Özer M, Pathare A, Krishnamoorthy R, Paldi A, Siguret V, Loriot MA, Kutala VK, Suarez-Kurtz G, Perini J, Denny JC, Ramirez AH, Mittal B, Rathore SS, Sagreiya H, Altman R, Shahin MHA, Khalifa SI, Limdi NA, Rivers C, Shendre A, Dillon C, Suriapranata IM, Zhou HH, Tan SL, Tatarunas V, Lesauskaite V, Zhang Y, Maitland-van der Zee AH, Verhoef TI, de Boer A, Taljaard M, Zambon CF, Pengo V, Zhang JE, Pirmohamed M, Johnson JA, Fava C (2019) Effect of CYP4F2, VKORC1, and CYP2C9 in influencing coumarin dose: A single-patient data meta-analysis in more than 15,000 individuals. Clin Pharmacol Ther 105: 1477–1491.

Galvez JM, Restrepo CM, Contreras NC, Galvez JM, Restrepo CM, Contreras NC, Alvarado C, Calderón-Ospina CA, Peña N, Cifuentes RA, Duarte D, Laissue P, Fonseca DJ (2018) Creating and validating a warfarin pharmacogenetic dosing algorithm for Colombian patients. Pharmgenomics Pers Med 11: 169–178.

Grzejszczak P, Kurnatowska I (2021) Role of vitamin K in CKD: Is its supplementation advisable in CKD patients? Kidney Blood Press Res 46: 523–530.

Guévin C, Michaud J, Naud J, Leblond FA, Pichette V (2002) Down-regulation of hepatic cytochrome p450 in chronic renal failure: role of uremic mediators. Br J Pharmacol 137: 1039–1046.

Hamadeh IS, Shahin MH, Lima SM, Oliveira F, Wilson L, Khalifa SI, Langaee TY, Cooper-DeHoff RM, Cavallari LH, Johnson JA (2016) Impact of GGCX, STX1B and FPGS polymorphisms on warfarin dose requirements in European-Americans and Egyptians. Clin Transl Sci 9: 36–42.

Ichihara N, Ishigami T, Umemura S (2015) Effect of impaired renal function on the maintenance dose of warfarin in Japanese patients. J Cardiol 65: 178–184.

Jiang NX, Xu YH, Xia JW, Jiang B, Li YS (2017) Impact of GGCX polymorphisms on warfarin dose requirements in atrial fibrillation patients. Turk J Med Sci 47: 1239–1246.

Johnson JA, Caudle KE, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, Lee MT, Gage BF, Kimmel SE, Perera MA, Anderson JL, Pirmohamed M, Klein TE, Limdi NA, Cavallari LH, Wadelius M (2017) Clinical pharmacogenetics implementation consortium (CPIC) guideline for pharmacogenetics-guided warfarin dosing: 2017 update. Clin Pharmacol Ther 102: 397–404.

Jonas DE, McLeod HL (2009) Genetic and clinical factors relating to warfarin dosing. Trends Pharmacol Sci 30: 375–386.

Jun M, James MT, Manns BJ, Quinn RR, Ravani P, Tonelli M, Perkovic V, Winkelmayer WC, Ma Z, Hemmelgarn BR (2015) The association between kidney function and major bleeding in older adults with atrial fibrillation starting warfarin treatment: population based observational study. BMJ 350: h246.

Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA (2009) Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 360: 753–764.

Leblond F, Guévin C, Demers C, Pellerin I, Gascon-Barré M, Pichette V (2001) Downregulation of hepatic cytochrome P450 in chronic renal failure. J Am Soc Nephrol 12: 326–332.

Li J, Chen T, Jie F, Xiang H, Huang L, Jiang H, Lu F, Zhu S, Wu L, Tang Y (2022) Impact of VKORC1, CYP2C9, CYP1A2, UGT1A1, and GGCX polymorphisms on warfarin maintenance dose: Exploring a new algorithm in South Chinese patients accept mechanical heart valve replacement. Medicine (Baltimore) 101: e29626.

imdi NA, Beasley TM, Baird MF, Goldstein JA, McGwin G, Arnett DK, Acton RT, Allon M (2009) Kidney function influences warfarin responsiveness and hemorrhagic complications. J Am Soc Nephrol 20: 912-21.

Liu R, Zhang K, Gong ZZ, Shi XM, Zhang Q, Pan XD, Dong R (2016) Association of apolipoprotein E (APOE) polymorphisms with warfarin maintenance dose in a northern Han Chinese population. Lipids Health Dis 15: 34.

Mar PL, Gopinathannair R, Gengler BE, Chung MK, Perez A, Dukes J, Ezekowitz MD, Lakkireddy D, Lip GYH, Miletello M, Noseworthy PA, Reiffel J, Tisdale JE, Olshansky B (2022) Drug interactions affecting oral anticoagulant use. Circ Arrhythm Electrophysiol 15: e007956.

McDonald MG, Rieder MJ, Nakano M, Hsia CK, Rettie AE (2009) CYP4F2 is a vitamin K1 oxidase: An explanation for altered warfarin dose in carriers of the V433M variant. Mol Pharmacol 75: 1337–1346.

Rafiee S, Rajabibazl M, Meshkani R, Daraei A, Zargari M, Sharafeddin F, Fazeli Z, Toffani Milani A, Taherkhani M (2017) Association of warfarin therapy with APOE and VKORC1 genes polymorphism in Iranian population. Iran J Pharm Res 16: 1230–1237.

Sconce EA, Daly AK, Khan TI, Wynne HA, Kamali F (2006) APOE genotype makes a small contribution to warfarin dose requirements. Pharmacogenet Genomics 16: 609–611.

Sun Y, Wu Z, Li S, Qin X, Li T, Xie L, Deng Y, Chen J (2015) Impact of gamma-glutamyl carboxylase gene polymorphisms on warfarin dose requirement: a systematic review and meta-analysis. Thromb Res 135: 739–747.

Tanaka T, Ihara M, Fukuma K, Yamamoto H, Washida K, Kimura S, Kada A, Miyata S, Miyata T, Nagatsuka K (2021) Influence of renal impairment and genetic subtypes on warfarin control in Japanese patients. Genes (Basel) 12: 1537.

Tian L, Zhang J, Xiao S, Huang J, Zhang Y, Shen J (2015) Impact of polymorphisms of the GGCX gene on maintenance warfarin dose in Chinese populations: Systematic review and meta-analysis. Meta Gene 5: 43–54.

Wuyts J, Dhondt A (2016) The role of vitamin K in vascular calcification of patients with chronic kidney disease. Acta Clin Belg 71: 462–467.

Yu WY, Sun X, Wadelius M, Huang L, Peng C, Ma WL, Yang GP (2016) Influence of APOE gene polymorphism on interindividual and interethnic warfarin dosage requirement: A systematic review and meta-analysis. Cardiovasc Ther 34: 297–307.