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Prevalence of Warfarin Genotype Polymorphisms in Patients with Mechanical Circulatory Support

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Polymorphisms for *VKORC1* and *CYP2C9* are associated with increased warfarin sensitivity. The prevalence of these polymorphisms in patients with mechanical circulatory support (MCS) is unknown. Polymorphisms for *VKORC1* and *CYP2C9* were determined in 65 patients undergoing MCS surgery. Postoperative warfarin dose, international normalized ratio (INR), and bleeding events were measured until discharge, 6 months, or composite end point (in-hospital MCS recovery, heart transplant, or death). A total of 67.7% (44/65) had at least one polymorphism: *VKORC1* (44.6%), *CYP2C9*2* (7.7%), *CYP2C9*3* (4.6%), *CYP2C9*2* and *VKORC1* (3.1%), or *CYP2C9*3* and *VKORC1* (7.7%). At discharge or before composite end point, patients with any polymorphism received a lower mean warfarin dosage than patients having no polymorphism (3.21 ± 1.47 *vs.* 5.57 ± 3.72 mg, $p = 0.015$) and achieved a similar mean INR (2.20 ± 0.67 *vs.* 2.19 ± 0.69, $p = 0.96$). There was no significant difference in bleeding rates within 6 months or before composite end point (6.13 *vs.* 8.02 events/patient-year, $p = 0.13$). One or more polymorphisms for *VKORC1* or *CYP2C9* (associated with warfarin sensitivity) were found in 67.7% of MCS patients. By using a warfarin genotype-guided approach, MCS patients with polymorphisms received a lower warfarin dosage to achieve a similar INR, with similar bleeding rates, in comparison with no polymorphisms. A warfarin genotype-guided approach avoided excessive anticoagulation and its attendant bleeding risks. *ASAIO Journal* 2015; 61:391–396.

Key Words: warfarin, *VKORC1*, *CYP2C9*, mechanical circulatory support, genotype, bleeding

Warfarin is a coumarin-derived anticoagulant that is frequently administered to patients with mechanical circulatory support (MCS) to prevent pump thrombosis and thromboembolic complications, but may cause bleeding. The risk factors contributing to warfarin-associated bleeding events include age, female gender, atrial fibrillation, heart disease and hypertension, hepatic and renal insufficiency, and alcohol ingestion.[1] Approximately 50% of variability in warfarin dosage has been linked to age, body size, and genotype polymorphism of at least 30 genes, of which 2 are the most important.[2,3] These two genes, *VKORC1* and *CYP2C9*, in combination seem to contribute to a significant proportion (~30%) of warfarin-dosage variability,[2] whereas stepwise regression analysis showed that mutations in these two genes reflected 21 and 6% of variance in warfarin dosage, respectively.[4] Polymorphisms (or mutations) in both genes have been associated with an increased sensitivity to warfarin.[1]

VKORC1 is a gene on chromosome 16 that encodes vitamin K epoxide reductase complex subunit 1. *VKORC1* is responsible for reducing vitamin K, which becomes re-oxidized by the gamma-glutamyl carboxylase protein, thereby activating the clotting factors II, VII, IX, and X.[1] Warfarin acts as an anticoagulant by inhibiting the protein product of the *VKORC1* gene, thereby rendering the clotting factors inactive.[1] *CYP2C9* is a gene on chromosome 10 that encodes cytochrome P450, family 2, subfamily C, polypeptide 9, which is necessary for metabolism and clearance of warfarin in hepatocytes.[1] Mutations in the *CYP2C9* gene, the two common ones of which are *CYP2C9*2* and *CYP2C9*3*, confer low warfarin dosage requirement,[5] because as the gene loses its functionality, warfarin will not be metabolized but will remain in circulation for a longer time.

Genetic polymorphisms for *VKORC1* and *CYP2C9* were determined in patients who underwent MCS surgery at our medical center so that a warfarin dosage could be estimated. We found that a large number of the MCS patients had polymorphisms (mutations) in the *VKORC1* and *CYP2C9* genes. The high frequency of polymorphisms in this population directed us to perform a retrospective analysis of these patients to compare the presence or absence of warfarin polymorphisms, the warfarin dosage, international normalized ratio (INR) reached, and bleeding event rate. We also examined any association between patient characteristics and warfarin polymorphisms.

Methods

A total of 65 adult patients received mechanical circulatory support (MCS) consisting of a ventricular assist device (VAD) or total artificial heart (TAH) from February 2011 to July 2013 at Cedars Sinai Medical Center, Los Angeles, California. Of these, there were 22 patients with biventricular assist device (Thoratec-PVAD, Thoratec Corp., Pleasanton, California), 33 with left ventricular assist device (6 HeartWare (HeartWare Corp., Framingham, Massachusetts) and 27 HeartMate II, Thoratec Corp., Pleasanton, California), 1 with right ventricular assist

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device, and 9 with TAH (SynCardia Systems, Inc.; Tucson, Arizona). These patients received genotype-guided warfarin dosing (lower doses in patients with a polymorphism) and were then closely monitored based on their INR. We collected data on the genetic polymorphisms of the *VKORC1* and *CYP2C9* genes. We examined the *VKORC1* 1639 G>A mutation, which was located in the nucleotide position 1639 of the DNA sequence as a result of a guanine to adenine point mutation.[1] We also examined the *CYP2C9*2* (430 C>T; cytosine to thymine) and the *CYP2C9*3* (1075 A>C; adenine to cytosine) mutations.[1]

We collected data on the INR level achieved and the most representative warfarin dosage of the patients at discharge or before in-hospital MCS recovery, heart transplant (HTx), or death. In addition, we gathered data on the bleeding events for up to 6 months after MCS implant or before in-hospital MCS recovery, HTx, or death. The study was approved by the institutional review board of the medical center.

We divided the patients into two groups for analysis: patients with abnormal warfarin polymorphisms and patients without any polymorphisms. We determined the mean warfarin dosage, mean reached INR, and the rate of bleeding events per patient-year and compared the two groups for these measures.

We collected de-identified demographic and historical data on age, gender, height, weight, race, obesity, diabetes, hypertension, cardiomyopathy type, and MCS type. Binary variable analysis was used to determine any association between polymorphism (mutation) presence and any of these variables. We also examined any association between the mutation presence and the numerical variables of age, height, weight, and body mass index (BMI). Furthermore, multivariable analysis was performed to identify any significant predictors for the presence of any of the analyzed polymorphisms.

Continuous variables were summarized by mean ± standard deviation. Normally distributed continuous variables were compared across the two patient groups by the independent samples t-test. Nonnormally distributed numerical variables were compared across the two groups by the Wilcoxon rank sum test. Categorical variables were summarized by frequency (%). Categorical variables were compared across groups by the Fisher exact test. A two-sided 0.05 significance level was used throughout. SPSS version 18.0 (SPSS, Chicago, IL) and SAS version 9.2 (SAS Institute, Cary, NC) were used for statistical analysis.

Results

Table 1 shows the genotype polymorphism profile among the 65 MCS patients in the study. Mutation in *VKORC1* was the most frequent (44.6%) abnormality among the warfarin genotype polymorphisms. Furthermore, some patients showed combinations of the abnormal mutant genotype in *VKORC1* and either *CYP2C9*2* or *CYP2C9*3*. The small sample size for each of the separate polymorphisms limited the ability to do meaningful statistical comparison between patients in each group; therefore, the polymorphisms were aggregated for statistical analysis. Overall, 44 (67.7%) of 65 patients had one or more abnormal warfarin genotypes (polymorphisms), and these patients were compared with those with no polymorphisms.

Table 1. Warfarin Polymorphism Genotype Profile of Patients in the Study

Genotype Polymorphism	N (%)
No mutation	21 (32.3)
*CYP2C9*2* only	5 (7.7)
*CYP2C9*3* only	3 (4.6)
VKORC1 only	29 (44.6)
VKORC1 + *CYP2C9*2*	2 (3.1)
VKORC1 + *CYP2C9*3*	5 (7.7)
Total	65 (100)

There is a high prevalence (67.7%) of abnormal *VKORC1* and *CYP2C9* mutations in patients with mechanical circulatory support.

Table 2 shows the differences in INR, warfarin dosage, and bleeding events between the groups with and without one or more polymorphisms (mutations or abnormal warfarin genotypes). Patients with one or more polymorphisms received a lower mean daily warfarin dosage compared with patients without a polymorphism (3.21 ± 1.47 *vs*. 5.57 ± 3.72, $p = 0.015$). The warfarin dosage at discharge or before in-hospital MCS recovery, HTx, or death was missing in four patients (two with and two without a polymorphism).

By using genotype-guided dosing (approximately half dose in heterozygous and one-fourth dose in homozygous patients with a polymorphism), patients with a polymorphism reached similar mean INR values compared with patients without a polymorphism (2.20 ± 0.67 *vs*. 2.19 ± 0.69, $p = 0.96$). International normalized ratio values were available in all patients at discharge or before in-hospital MCS recovery, HTx, or death.

Similarly, the percentage of patients with bleeding events during 6 months or before MCS recovery, HTx, or death did not statistically differ between both groups (79.5 *vs*. 81.0%, $p > 0.99$). In addition, patients with a polymorphism had similar linearized bleeding event rates per year (which accounts for multiple bleeding events in a given patient) when compared with patients without a polymorphism (6.13 *vs*. 8.02 events/patient-year, $p = 0.13$). Accordingly, with genotype-guided dosing, patients with a warfarin polymorphism used a lower

Table 2. Clinical Outcomes of Patients in the Mutant (Abnormal Genotype) and Wild-Type (Normal Genotype) Groups

Variable	Genotype Polymorphism: Mutant (n = 44)	Genotype Polymorphism: Wild Type (n = 21)	*p* Value
Warfarin daily dosage (mg)*	3.21 ± 1.47	5.57 ± 3.72	0.015
INR reached	2.20 ± 0.67	2.19 ± 0.69	0.96
Number of patients with bleeding events (%)	35 (79.5)	17 (81.0)	>0.99
Bleeding event rate (event/patient-year)	6.13 (4.91–7.55)	8.02 (5.93–10.61)	0.13

When compared with patients without genotype mutation, patients with mutations in the *VKORC1* and *CYP2C9* genes required a lower mean warfarin daily dosage to reach the same mean INR levels at discharge or before in-hospital mechanical support recovery, cardiac transplantation, or death. Patients in both groups had similar bleeding event rates for up to 6 months or before mechanical support recovery, cardiac transplantation, or death.

Data are represented as mean ± standard deviation, frequency (%), or rate (95% confidence interval).

*Frequency missing = 4.

INR, international normalized ratio.

warfarin dosage to reach similar INR levels and bleeding event rates compared with patients without a polymorphism.

It is worth noting that the location of the bleeding did not seem to differ between patients with and without warfarin polymorphisms: 59.1% (52/88) and 55.1% (27/49) were chest bleeding, 33.0% (29/88) and 32.6% (16/49) were gastrointestinal bleeding, and 1.1% (1/88) and 0% (0/49) were brain bleeding, respectively.

Table 3 shows no association between the presence of a polymorphism in the *VKORC1* and *CYP2C9* genes and age, height, weight, or BMI in MCS patients. In multivariable analysis, there were no significant associations between polymorphism presence and any of the potential predictors including age, BMI, obesity, gender, race, cardiomyopathy type, or MCS type (data not shown; $p > 0.05$ for all).

Table 4 shows the binary analysis of the characteristics of patients with any warfarin polymorphism. There were no preoperative features associated with the presence of a polymorphism. There seemed to be an association with operation for TAH ($p = 0.048$); however, only 9 of 65 patients had TAH, 3 (33.3%) of which had a polymorphism.

Discussion

Our analysis showed that MCS patients have a high prevalence (67.7%) of one or more of the warfarin polymorphisms (mutations) in the *VKORC1* and *CYP2C9* genes. When examined separately, 15 of 65 patients (23.1%) had a *CYP2C9* mutation and 36 of 65 patients (55.4%) had a *VKORC1* mutation, with some patients having both. In previous studies, the prevalence of *VKORC1* mutations (homozygous or heterozygous) ranged from 51.8% to 66.4%, with an average of 65.5%.[2,6–10] The prevalence of *CYP2C9* mutations (homozygous or heterozygous for *CYP2C9*2* or *CYP2C9*3*) ranged from 24.8% to 39%, with an average of 27.8%.[2,6,8,10,11] The frequency of polymorphisms reported in this study of MCS patients for the *VKORC1* and *CYP2C9* genes fall within or close to the ranges given by other studies of patients receiving warfarin therapy to treat atrial fibrillation, thrombosis, or embolism.

However, it is not appreciated that the aggregate frequency for the presence of any warfarin polymorphism in the MCS population can be as high as 67.7%. Given that two-thirds of MCS patients have a warfarin polymorphism, this high prevalence could influence warfarin dosing and bleeding rates if warfarin dosing is performed without knowledge of the presence or absence of the polymorphism. This study examined whether a strategy of knowledge of the polymorphism could influence warfarin dosing and avoid excessive bleeding rates in MCS patients.

Our investigation indicated no association between the presence of genotype abnormality in either of the two genes (*VKORC1* and *CYP2C9*) and patient characteristics such as age, height, weight, or BMI. We also found no significant potential predictors of polymorphism presence among gender, race, or type of cardiomyopathy.

In larger studies of warfarin-treated patients, it has been shown that the presence of mutations in *VKORC1* and *CYP2C9* differ between races.[12] In a review by the Mayo Clinic group, averages of 36% of 1,759 Caucasians in 9 studies, 4% of 334 Asians in 2 studies, and 5% of 226 Africans in 1 study had at least a single mutation in the *CYP2C9* gene.[1] Averages of 63% of 1,056 Caucasians in 6 studies, 99% of 228 Asians in 3 studies, and 24.6% of 254 Africans in 2 studies had at least a single mutation in the *VKORC1* gene.[1] A study containing only Caucasians reported that 36.7% of 668 individuals and 60.4% of 671 individuals had at least a single mutation in the *CYP2C9* and *VKORC1* genes, respectively.[2] We calculated percent averages from a recent meta-analysis and concluded that 34.4% on average of Caucasians in 12 Caucasian-only studies and 10% on average of Asians in 3 Asian-only studies had at least a single mutation in the *CYP2C9* gene.[12] By using the same percent average calculations, we concluded that 64.0% of Caucasians in six Caucasian-only studies had at least a single mutation in the *VKORC1* gene. In terms of allelic frequencies, the same meta-analysis showed that 35–45% of

Table 3. Patient Demographics to Examine any Associations with Mutation Presence

Variable	Genotype Polymorphism		*p* Value
	Mutant (n = 44)	Wild Type (n = 21)	
Age (yr)	50.2 ± 16.0 (20.0, 75.0)	53.3 ± 14.3 (18.0, 73.0)	0.44
Height (cm)	175.2 ± 9.7 (152.4, 189.2)	175.6 ± 9.1 (152.4, 203.2)	0.87
Weight (kg)	81.0 ± 23.4 (44.9, 155.4)	81.6 ± 22.6 (52.4, 153.4)	0.91
BMI (kg/m^2)	26.2 ± 6.4 (19.0, 46.8)	26.4 ± 6.0 (17.3, 48.1)	0.90

There was no association between any of the patient demographics and mutation presence.

Data are represented as mean ± standard deviation. (minimum, maximum)

BMI, body mass index.

Table 4 shows the binary analysis of the characteristics of patients with any warfarin polymorphism. There were no preoperative features associated with the presence of a polymorphism. There seemed to be an association with operation for TAH ($p = 0.048$); however, only 9 of 65 patients had TAH, 3 (33.3%) of which had a polymorphism.

Feature	Feature Present	Feature Absent	*p* Value
Female	60.0 (6/10)	69.1 (38/55)	0.72
Hypertension	69.2 (27/39)	65.4 (17/26)	0.79
Obese	63.4 (7/11)	68.5 (37/54)	0.74
Diabetes	73.9 (17/23)	64.3 (27/42)	0.58
Race/ethnicity			0.53
Caucasian	70.6 (36/51)	57.1 (8/14)	
Asian/Pacific Islander	66.7 (2/3)	67.7 (42/62)	
African American	54.6 (6/11)	70.4 (38/54)	
Cardiomyopathy			0.21
Nonischemic	62.0 (26/42)	78.3 (18/23)	
Ischemic	78.9 (15/20)	63.0 (29/45)	
Mixed	100 (3/3)	66.1 (41/62)	
Mechanical circulatory device			
BIVAD	72.7 (16/22)	65.1 (28/43)	0.59
LVAD	72.7 (24/33)	62.5 (20/32)	0.43
RVAD	100 (1/1)	67.2 (43/64)	> 0.99
TAH	33.3 (3/9)	73.2 (41/56)	0.048

There was no association between mutation presence and any of the preoperative variables.

Data are represented as percent (no. of patients with mutation/no. of patients with or without a feature).

BIVAD, biventricular assist device; LVAD, left ventricular assist device; RVAD, right ventricular assist device.

Caucasians and 90–95% of Asians had the *VKORC1* abnormal allele.[12] Another study calculated *VKORC1* haplotype frequencies from two different studies and showed that 14–21% of African Americans, 37–42% of Caucasian non-Hispanics, and 85–89% of Asians had the abnormal *VKORC1* haplotype.[13]

Accordingly, *VKORC1* polymorphism (mutation) is most common in Asians, followed by Caucasians, and least found in Africans. These conclusions are supported by several reports stating that on average, Asians require a lower warfarin dosage (because of a higher polymorphism rate), Caucasians an intermediate dosage, and Africans a higher dosage (because of a lower polymorphism rate).[4] Moreover, *CYP2C9* polymorphism seems to be most common in Caucasians and lower in Asians and Africans. Our analysis did not recognize any association between race and polymorphism presence; however, type 2 statistical error cannot be ruled out because of the smaller sample size analyzed (n = 65), most of whom (78.5%; n = 51) were Caucasians, with 11 Africans (16.9%) and 3 Asians (4.6%).

Our findings illustrated that MCS patients with one or more *VKORC1* or *CYP2C9* polymorphisms required a lower warfarin dosage to reach the same target INR range, with similar bleeding event rates, compared with those without a polymorphism, when a warfarin genotype-guided strategy was used.

Previous studies have shown that patients carrying the *VKORC1*, *CYP2C9*2*, or *CYP2C9*3* polymorphisms required a lower warfarin dosage to reach the therapeutic INR range.[1,4,11,12,14,15] *CYP2C9* is a gene that encodes the hepatic microsomal enzyme responsible for the metabolism of the three to five times more potent S-enantiomer of warfarin into inactive metabolites.[1,3,6,11] *CYP2C9*1* is the wild-type form of the gene, whereas *CYP2C9*2* and *CYP2C9*3* are mutated variants (polymorphisms) of the gene.[1] *CYP2C9*1/*1* wild-type homozygous patients required a higher mean warfarin daily dosage than patients with the *CYP2C9*2* and *CYP2C9*3* variants, whereas both mutant alleles have been associated with a lower warfarin dosage.[1,3,11] A reduced *CYP2C9* enzymatic activity because of mutation causes a reduced clearance rate of warfarin, which potentially can lead to overanticoagulation with a normal warfarin dosage.[16] Therefore, as our results confirmed, patients with *CYP2C9* polymorphism should be given a lower warfarin dosage to reach the same INR range as patients without a polymorphism.

VKORC1 is a gene that encodes the reductase enzyme targeted by warfarin.[1,3] *VKORC1-B* (wild-type *VKORC1*) homozygous patients required a higher mean warfarin daily dosage than patients who are either *VKORC1-AA* (mutant) homozygotes or *VKORC1-AB* mutant heterozygotes.[1] This *VKORC1* variant allele has been associated with a decreased warfarin dosage in Caucasians and Asians.[17] The mutant variant of *VKORC1* results from a polymorphism within the promoter region by a conversion of guanine into adenine, thus reducing *VKORC1* enzyme activity.[1] This reduction in *VKORC1* activity reduces the levels of activated vitamin K needed for coagulation. Therefore, a lower dosage of warfarin should be given to patients with *VKORC1* genotype abnormality to reach the same INR range as patients without a polymorphism.

In this study, the *VKORC1* and *CYP2C9* genotype was known before MCS treatment, and patients were treated with lower dosages of warfarin when a polymorphism was present. Achievement of a similar INR and the occurrence of similar bleeding rates was confirmation that genotype-guided warfarin therapy led to avoidance of higher bleeding event rates in MCS patients with a polymorphism. Given that two-third of patients had one or more warfarin polymorphisms leading to warfarin sensitivity, warfarin genotype-guided treatment may mitigate the risk of overanticoagulation and excessive bleeding in the majority of MCS patients.

There is growing evidence that polymorphisms in the *VKORC1* and *CYP2C9* genes are associated with major adverse events. A meta-analysis study concluded that the *CYP2C9*2* and *CYP2C9*3* variants lead to a higher frequency of overanticoagulation within the first 30 days of warfarin treatment; however, only *CYP2C9*3* or both mutations combined conferred a higher risk of overanticoagulation after 30 days.[12] In addition, both *CYP2C9* mutations were associated with an increased risk of hemorrhage complications.[1,11,12,15] Having one mutation in the *CYP2C9* gene conferred an increased risk of INR above therapeutic range compared with those with wild-type gene, and patients with *CYP2C9* polymorphism required more time to achieve a stable warfarin dosage.[11] Conversely, the *VKORC1* polymorphism was found to be associated with overanticoagulation within 30 days of treatment, but not hemorrhagic complications.[12] Earlier studies showed that the effect of *VKORC1* mutation seemed to be greater than that of the *CYP2C9* mutation.[18] Although the findings of the meta-analysis suggest that *VKORC1* mutation confers a higher sensitivity to warfarin, *CYP2C9* mutations may confer a long-lasting effect of warfarin sensitivity.[12] Results on the relative effects of *VKORC1* and *CYP2C9* mutations are conflicting, with *VKORC1* having a greater effect than *CYP2C9* mutation in six studies, *CYP2C9* having a greater quantitative contribution in two studies, and both mutations had the same effect in one study.[3]

Warfarin has a narrow therapeutic index,[1,3,11] which increases the risk of overanticoagulation and bleeding (supratherapeutic INR) or underanticoagulation and clotting (subtherapeutic INR) in warfarin-treated patients.[1,2,6,11,16] Warfarin dosing is an individualized therapy with a defined therapeutic INR range as a measurement of anticoagulation status.[2,11,16] Knowledge of the warfarin genotype may be necessary to determine proper warfarin dosing.

Pharmacogenetics-based warfarin-dosing algorithms have been created and used to safely prescribe warfarin to patients with *CYP2C9* and *VKORC1* mutations. A Caucasian-based study on patients suffering from thromboembolism analyzed the outcomes of *CYP2C9* genome-based warfarin-dosage algorithm applied to these patients who were treated with standard warfarin therapy.[2] For patients with a mean INR > 4 after standardized warfarin therapy, the algorithm predicted a lower warfarin dose by a median of 4.2 mg; however for patients with a mean INR < 2 after standardized therapy, the algorithm predicted a similar warfarin dose. The study concluded that genotype-based dosage prevents overanticoagulation.[2] The International Warfarin Pharmacogenetics Consortium algorithms study analyzed 4,043 patients using a pharmacogenetics-based algorithm and a clinical-based algorithm.[8] The genotype-based or pharmacogenetics-based algorithms more accurately predicted a practical warfarin dosage compared with clinically administered standardized warfarin treatment.[8] Our results seem to be consistent with these results, because patients with abnormal mutation in *VKORC1* and *CYP2C9* received a lower warfarin dosage to reach the same

INR with similar bleeding event rates as patients without a polymorphism.

Of the three clinical trials published in 2013, only one showed a positive impact on clinical practice using pharmacogenetics-guided warfarin therapy. The three trials were the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial using warfarin, the Clarification of Optimal Anticoagulation through Genetics (COAG) trial using warfarin, and the EU-PACT trial using acenocoumarol and phenprocoumon.[6,9,10]

The EU-PACT trial using warfarin randomized patients to genotype-guided or standardized clinically-guided warfarin-dosing groups.[6] When compared with patients in the standardized group, patients in the genotype-based group had fewer incidences of overanticoagulation, reached the therapeutic INR range sooner, and spent more time in the therapeutic INR range during the first 4 weeks and throughout the study period of 12 weeks. There was no difference in the overall bleeding events between both groups. Using pharmacogenetics for warfarin dosing proved to have better outcomes compared with standard warfarin-dosing techniques. Similar to the EU-PACT trial for warfarin, patients who received pharmacogenetics-based warfarin dosing in another study had a lower frequency of out-of-therapeutic range INR at 1 month after warfarin initiation.[7] At 3 months, they had fewer occurrences of INR ≥ 4 and ≤1.5 and fewer serious adverse events. Patients receiving standard warfarin dosing had more hemorrhagic events, thromboembolic events, death, and serious adverse events within 90 days after warfarin initiation compared with patients with pharmacogenetics-guided warfarin treatment.[7] Furthermore, another study showed that genotype-based treated patients reached the target INR earlier than patients treated with a stable dose.[19] These results illustrate that genotype-guided warfarin dosing was safer for patients on warfarin therapy.

Conversely, the COAG group randomized patients into two different algorithms for warfarin treatment: a clinical-containing and a clinical- and genotype-containing algorithm, with a primary outcome of the percentage of time spent in the therapeutic INR range during the first 4 weeks of treatment.[9] The mean percentage of time spent in the therapeutic INR range was similar between both groups. Adverse events included INR ≥ 4, major bleeding, and thromboembolism, none of which differed between both groups.[9] Similarly, the second EU-PACT trial randomized patients to a genotype-dosing and a clinical-dosing algorithm group for treatment of the coumarin-derived anticoagulants, acenocumarol and phenprocoumon, with a primary outcome of the percentage of time spent in the therapeutic INR range during the first 12 weeks of treatment.[10] There was no difference between the mean percentages of time spent in the therapeutic INR range during the 12-week period. However, during the first 4 weeks, patients in the genotype-dosing group spent more time in the therapeutic INR range compared with patients in the clinical-dosing group.[10] There was no difference between the frequencies of bleeding and the thromboembolic events in both groups.[10]

The patients in these trials received anticoagulation therapy because of atrial fibrillation, atrial flutter, thrombosis, or embolism.[6,9,10] These patient populations were different from the MCS patient population in our study. These trials analyzed the initiation of anticoagulation and not intermediate or long-term anticoagulation therapy.[16] MCS patients in this study received intermediate- or long-term anticoagulation therapy until they either underwent HTx or were bridged to MCS recovery. Sustained anticoagulation therapy for MCS patients was beyond the scope of the aforementioned trials.

Furthermore, the primary end point of these trials was the percentage of time in the therapeutic INR range, whereas another end point (rate of bleeding events) was not the primary focus of the trials.[16] For MCS patients, an important end point is the rate of bleeding events because these patients are at a higher risk of mediastinal bleeding compared with the normal patient population receiving anticoagulation therapy (*e.g.*, for atrial fibrillation or flutter, thrombosis, or embolism), because of recent major cardiac surgery with sternotomy or thoracotomy in the MCS patients. Furthermore, MCS devices with VAD support have high-speed rotors that cause a high shear stress that affects high-molecular-weight multimers of the von Willebrand factor responsible for platelet adhesion and aggregation,[20] and an acquired von Willebrand syndrome has been described in MCS patients,[21] which can affect bleeding tendency.

Based on the study design of each of the trials, this current study is closer to the EU-PACT trial using warfarin because the trial followed patients for up to 12 weeks, which was longer than the 4 week follow-up period of the COAG trial. As for the second EU-PACT trial, it did not use warfarin as an anticoagulant, which makes its findings difficult to compare with the current MCS study. Accordingly, the findings of the clinical trial closest to our study (EU-PACT trial with warfarin) seem to support this study, and the impression that genotype-guided warfarin therapy should be used for the initiation of anticoagulation therapy in MCS patients.

In 2007, the Food and Drug Administration approved adding pharmacogenetics data to the warfarin label; however, no recommendations about using the procedure to prescribe a warfarin dosage were specified.[8] Furthermore, the guidelines do not require genotyping for *CYP2C9* and *VKORC1* when prescribing the warfarin anticoagulant.[6] This could be partly because of the cost of the genotyping procedure. Medical insurance companies may not cover the cost of the genotyping.[4,9] However, based on the fact that MCS patients are at a higher risk of mediastinal bleeding because of the recent sternotomy or thoracotomy, the benefit of performing the genotyping procedure may outweigh its cost, especially because bleeding events in MCS patients may require a longer in-hospital stay or return to the operating room. Of note, the EU-PACT trial using warfarin showed that patients in the genotype-guided warfarin-dosing group required only 21 days to reach therapeutic INR levels *versus* 29 days in the standardized warfarin-dosing group.[6] The longer time to reach therapeutic INR in the EU-PACT trial would likely translate into a longer in-hospital stay after MCS placement, but this requires confirmation in a larger study.

Limitations to this study are that it was a single-centered, retrospective analysis of only 65 MCS patients. The small sample size prevented the identification of possible associations of patient characteristics (such as race or ethnicity) with warfarin genotype polymorphisms. There was no control group receiving only clinically guided warfarin dosing without genotype-guided therapy. This was a single-centered retrospective analysis. Future directions include larger, double-blinded, randomized, and controlled clinical trials comparing between

genotype-guided and clinically guided warfarin therapy in patients with mechanical support.

In conclusion, our analysis showed that two-thirds (67.7%) of MCS patients have polymorphisms (mutations) in one or more of the *VKORC1* and *CYP2C9* genes, which affect warfarin sensitivity. This high prevalence, while similar to the prevalence in other (nonsurgical) patient populations, is not well appreciated in MCS patients who may be at higher risk of mediastinal bleeding because of recent sternotomy or thoracotomy. This study showed that patients with at least one polymorphism in either gene required a lower average daily warfarin dosage to reach the same INR levels, while experiencing similar bleeding event rates, compared with patients without any polymorphism.

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