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Review article

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PHARMACOGENETIC CONSIDERATIONS OF ANTICOAGULANT MEDICATION

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Predicting the clinical consequences of anticoagulant therapy by identifying gene variants could help in the risk assessment of thrombosis or bleeding before and after surgery and may result in choosing more beneficial therapy. This work provides an overview of pharmacogenetic data of commonly used anticoagulant medication. The review focuses on polymorphisms influencing the efficacy and safety of the parenteral and oral anticoagulants. There is evidence that heparin resistance and heparin-induced thrombocytopenia could be genetically determined but it does not mean that the risk of bleeding or thromboembolism is related to mutations in general. *CYP2C9* and *VKORC1* polymorphisms are essential determinants in the genotype-guided dosing of warfarin and may distinguish patients who would benefit from switching to direct oral anticoagulants (DOACs). Further multi-ethnic studies associating genes of enzymes metabolizing DOACs with primary clinical endpoints are necessary. Pharmacogenetics-based dosing of anticoagulant medication should point towards the subpopulation of patients.

Key words: *anticoagulants, heparin, pharmacogenetics, polymorphism, warfarin, direct oral anticoagulants, vitamin K antagonists, heparin*

INTRODUCTION

Multiple clinical trials have demonstrated anticoagulant therapy to be highly efficacious in the prevention and treatment of thrombosis. Warfarin and unfractionated heparin (UFH), approved by the US Food and Drug Administration (FDA) for clinical use in the 1950s and 1930s, respectively, are still the most widely-used anticoagulants. Warfarin and other vitamin K antagonists (VKAs), *e.g.*, acenocoumarol and phenprocoumon, interfere with the cyclic conversion of vitamin K and lead to the production of vitamin K-dependent coagulation factors X, VII, IX, and thrombin with reduced coagulant activity (1). VKAs were the only approved oral anticoagulants for more than 50 years. The newer DOACs (direct oral anticoagulants) are target-specific agents directly inhibiting thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban, edoxaban) (2). DOACs have been approved by the US FDA in the last few years. UFH and its newer low-molecular-weight derivatives LMWHs, (low-molecular-weight heparins), and fondaparinux belong to a group of parenteral anticoagulants. They increase the antithrombin (AT) activity which leads to inhibition of various coagulation factors (thrombin, IXa, Xa, XIa, and XIIa in case of UFH; mainly Xa in case of LMWHs, specifically Xa in case of fondaparinux) (3). Despite the recent advances in the anticoagulant therapy (4), it still has limitations, *e.g.*, insufficient or pronounced therapeutic responses. Unfavorable effects of anticoagulants, *e.g.*, bleeding, can be reversed by several specific antidotes which are approved (5-7) or in development (4, 8-10).

The individual risk of adverse effects or lack of efficacy could be potentially predicted based on the genetic polymorphism (11, 12). The interest in the role of polymorphism in the pathophysiology and the therapy of cardiovascular disease is increasing (13). Many identified polymorphisms, but also those yet to be identified, could be connected with the unexpected complications (14). The genetically induced hypercoagulation, followed by developing thrombosis is often the direct cause of coronary heart disease or embolic stroke (15, 16).

Predicting the clinical consequences of therapy by the identification of gene variants enables the choice of the most appropriate anticoagulation method, making the treatment more efficient and safe. The genotyping will enable the identification of high-risk patients requiring specific therapy. The present literature review summarizes current knowledge on the pharmacogenomics of commonly used anticoagulants (graphically presented in *Fig. 1*) and confronts new findings with current clinical practice. It gives a perspective of what has been achieved to date and what further research needs to be done in this field.

VITAMIN K ANTAGONISTS

Oral anticoagulants antagonizing vitamin K production improved the compliance in the therapy for cardiovascular diseases accompanied by thromboembolic disorders in comparison to heparins. The inter-individual variability in dose requirements and their narrow therapeutic index often lead to serious adverse reactions, over-dosage or bleeding. However, VKAs used at the appropriate time in the therapeutic range (TTR), effectively prevent thrombotic complications, especially in patients with atrial fibrillation (AF), for whom oral anticoagulant therapy is strongly recommended (17, 18). VKAs

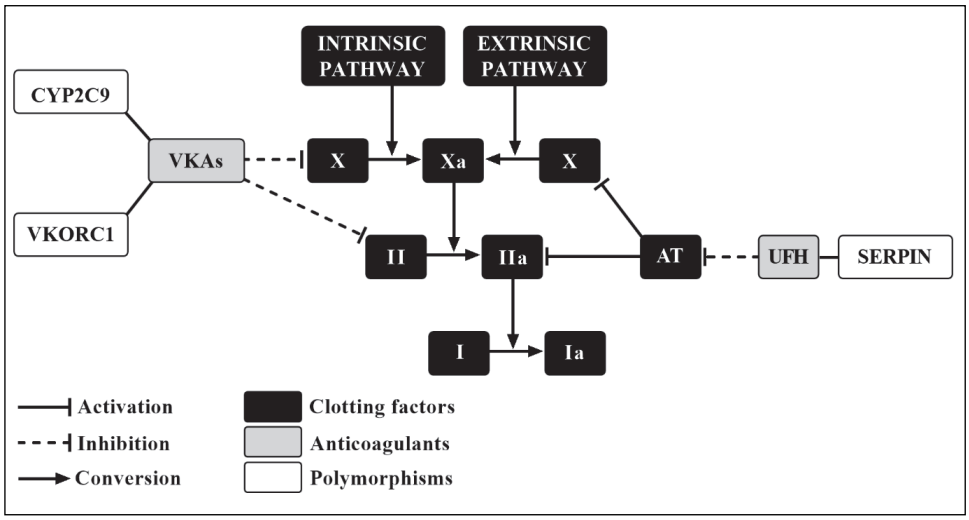

Fig. 1. Pharmacogenetic polymorphisms affecting the activity of agents inhibiting the coagulation cascade.
Abbreviations: SERPIN, serine protease inhibitor-antithrombin; UFH, unfractionated heparin; VKAs, vitamin K antagonists; VKORC1, vitamin K epoxide reductase complex subunit 1.

Table 1. Pharmacogenetic of anticoagulant medication.

	POLYMORPHISM	**PHENOTYPE**
VITAMIN K ANTAGONISTS		
Cytochrome P450	*CYP2C9*2,*3,*5, *6, *8, *11*	poor metabolizers
	CYP2C rs12777823	poor metabolizers
	*CYP4F2*3*	warfarin resistance
Vitamin K epoxide reductase	*VKORC1*-1639G>A	warfarin sensitivity
HEPARINS		
Antithrombin	*SERPINC1* rs2227589	heparin resistance
Platelet Fcγ-receptor	FcγRIIa-H131R	thrombosis risk in HIT
	FcγRIIIa-V158F	thrombosis risk in HIT

HIT, heparin-induced thrombocytopenia

are preferred for patients ineligible for DOAC therapy and with moderate or severe mitral stenosis or mechanical valvular prosthesis (18). The RE-ALIGN trial, tested DOACs in patients with mechanical heart valve prostheses but concluded that dabigatran has demonstrated an unacceptable risk/benefit ratio, thus VKAs remain the only drugs with established safety for this patient group (17).

Although DOACs are strongly recommended in nonvalvular AF (18), VKAs therapy for patients after heart valves replacement (HVR) will be still recommended. The clinical data about VKAs pharmacogenetics is obscure in this patient group and bears closer analysis. Almost half of the patients with aortic valve replacements have received the appropriate dosage based on the Gage *et al.* (19) algorithm (20). The carriers of vitamin K epoxide reductase complex subunit 1 (*VKORC1*) and/or cytochrome *P-450 2C9* (*CYP2C9*) polymorphisms required about 20% lower dosage than the wild-type. It is consistent with previous observations that carriers of allelic *CYP2C9* variants after HVR might be exposed to increased risk of unstable anticoagulation (21). The polymorphisms affected TTR, international normalized ratio (INR) values and warfarin metabolism in heart valve recipients, regardless of comorbidities and medications (21, 22). Notwithstanding, there were no associations identified between polymorphisms and bleeding or thrombotic complications (20). As yet there are no clear results related to acenocoumarol, which is still commonly used in Europe. The polymorphisms have different effects on acenocoumarol and warfarin efficacy. The process of genotyping might improve the dosage regimen among patients after HVR, especially for elderly patients, requiring ultra-stable and safe therapy (21-23). The current position however, is that routine genotyping of patients with valvular heart defects undergoing VKAs treatment, is not recommended, due to the lack of convincing clinical benefits and cost concerns (24, 25).

Warfarin

Racemic warfarin is broadly used to treat venous thromboembolism (VTE) and to prevent the thrombotic events in AF (26). However, the S-enantiomer is five times more potent and predominantly metabolized by CYP2C9 enzymes (27). Warfarin acts through the inhibition of vitamin K epoxide reductase (VKOR), thus blocks the production of functional coagulation factors II, VII, IX, and X and proteins C and S (1). In addition to nongenetic factors, the variability of warfarin anticoagulation is primarily determined by the human pharmacogenetic profile (28, 29). Common genetic variants in *CYP2C9*, *VKORC1*, *CYP4F2* and the *CYP2C* cluster (rs12777823) presented in *Table 1*, as well as environmental factors, account for 45 – 55% of the variability in dosing

requirements; about 30 – 40% of them could be attributed to *VKORC1* and *CYP2C9* polymorphisms (19, 30-38). Mutations in genes: *CYP1A2*, *CYP3A4*, *apolipoprotein E* (*ApoE*), and *gamma-glutamyl carboxylase* (*GGCX*; rs699664) have also been associated with different metabolism and pharmacologic response to warfarin (34, 39). Therefore, the determination of the optimum dosage of warfarin based on the INR remains a challenge to achieve.

1. CYP2C9

A meta-analysis of 22 studies showed that the *VKORC1* variant contributes to over-anticoagulation, but it is not the main genetic factor of warfarin hemorrhagic complications for which *CYP2C9*2* (c.430C>T; p.R144C; rs799853) and *CYP2C9*3* (c.1075A>C; p.I359L; rs1057910) are responsible (40). About 31% of predominantly European patients have at least one variant of the *CYP2C9* allele responsible for the reduced activity of warfarin metabolizing enzyme (41-43). Carriers of one or more *CYP2C9*2* and *CYP2C9*3* variants are characterized by the slowed clearance of S-enantiomer and thus the elongated elimination half-life of the drug compared to the wild-type genotype (*CYP2C9*1/*1*) (41, 42, 44, 45). Patients with this type of genetic variability whose INR is more likely to be above the target therapeutic range, will require lower doses as they are at higher risk of over-anticoagulation during initial therapy (42-45). A meta-analysis of 9 studies showed that just one copy of the *CYP2C9*2* or *CYP2C9*3* allele reduces the daily dose of warfarin by 17% or 37%, respectively, compared to the *CYP2C9*1* allele (34). Other studies have confirmed these results (46, 47).

The frequency of *CYP2C9*2* and *CYP2C9*3* alleles is much higher in Caucasian Americans than in African and Asian Americans. The pharmacokinetic and *in vitro* data on *CYP2C9*5* (c.1080C>T; p.D360E, rs28371686), **6* (c.818delA; rs9332131), **8* (c.449G>A; p.R150H; rs7900194) and **11* (c.1003C>T; p.R335W; rs28371685) alleles indicate that they could increase the accuracy of warfarin dosing, when included in standard algorithms in African descent (35, 48-53).

About 12% of African Americans have reduced S-warfarin clearance induced by the *CYP2C9*8* allele as confirmed *in vitro* and *in vivo* (54). Subsequent studies provided new information on the **5*, **6*, and **11* alleles and their dose-effect (55, 56). The adaptation of black-specific genotypes in the dosing warfarin algorithm has been described by Hernandez *et al.* (57) and Ramirez *et al.* (49), but only the former included all described polymorphisms. The percentage time in therapeutic range (PTTR) did not improve in the genotype-guided dosing group in the Clarification of Optimal Anticoagulation through Genetics (COAG) trial, because the difference in polymorphism of Africans being 1/3 of all patients (58, 59). The *CYP2C9*4* (c.1076T>C; p.I359T) variant was found in Japanese individuals (60). Additionally, 16 novel polymorphisms in the *CYP2C9* gene were identified in Asians subjects, but their role in the warfarin dose adjustment is unclear (61).

2. CYP2C rs12777823

The rs12777823 is a single nucleotide polymorphism (SNP) associated with a significant effect on warfarin dosage through alteration of warfarin clearance (62). The genome-wide association study (GWAS) first identified as heterozygous or homozygous the rs12777823 in African Americans, who required a dose reduction of 7 or 9 mg/week, respectively (62). Consideration of this SNP in therapy improves the warfarin dosing algorithm published by the International Warfarin Pharmacogenetics Consortium (IWPC). Further studies also demonstrated its importance in African Americans, but not in Egyptians (63, 64). Although the rs12777823 variant is common among various ethnic populations, it is associated with warfarin clearance only in African Americans (62, 64).

3. CYP4F2

CYP4F2 is a primary liver vitamin K oxidase responsible for the metabolism of vitamin K to hydroxy-vitamin K1 (65). The variant *CYP4F2*3* (c.1297G>A; p.V433M; rs2108622) was associated with the required dose of warfarin, which was confirmed in 3 independent white cohorts. The difference in dosage between homozygous patients for the variant allele and homozygotes for the wild-type allele was 1 mg/day (64-67). The improvement in the accuracy of warfarin dose prediction was possible by understanding the clinical consequences of *CYP4F2* variant presence (66, 68). Thus, the new polymorphism has been incorporated in the dosing algorithm offered at www.WarfarinDosing.org and has become part of the latest Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines (64). Its effect varies depending on race, which should be kept in mind when adjusting the dose. European Americans (38, 65, 66, 69, 70-74), Asians (75-78), and Hispanics (79) require higher doses but not Brazilians (72) or African Americans (79, 80). Two large meta-analyses suggest a statistically significant (8 – 11%) increase of warfarin dosage is necessary to achieve therapeutic INR and stable treatment in carriers of *CYP4F2*3* with European and Asian ancestry (38, 69, 81). The *CYP4F2*3/*3* genotype did not affect the time needed to reach the target INR, stable dosage and PTTR among African Americans (82).

4. Vitamin K epoxide reductase complex

The significance of VKORC1 in the variance of warfarin dosing is much higher than the *CYP2C9* polymorphism (36, 83-85). Warfarin inhibits this enzyme and the production of active vitamin K and as a result also affects the formation of key coagulation factors (83, 84). The most common SNP in the promoter region of *VKORC1* (c.-1639G>A, rs9923231) serves as a marker for the detection of warfarin sensitivity or resistance (86). The A allele is associated with a significantly lower gene expression, which in clinical practice results in a lower dose of warfarin compared to the G allele (33). The meta-analysis found that the Caucasian carrying one A allele needed a 25% lower dose, while patients with two alleles required a 50% dose reduction, compared to noncarriers. A similar but smaller effect was observed in Asians (87, 88). In a prospective study (297 patients) conducted by Schwarz *et al.* the -1639A/A genotype reached ($P = 0.02$) significantly faster and exceeded ($P = 0.003$), the therapeutic INR range (47).

According to the HapMap data (http://hapmap.ncbi.nlm.nih.gov), the frequencies of the -1639A/A for Europeans and African Americans are 19% and 6% respectively. Average frequencies of -1639G>A based on the reported frequencies in one or multiple studies were: 88% and 15% for East and South/Central Asians (data from https://www.pharmgkb.org/page/vkorc1RefMaterials). Because the -1639G/G homozygotes represent the higher expression of the enzyme, African Americans require higher doses of warfarin for effective therapy (31, 33).

At least several alleles of the *VKORC1* gene, regulate its expression. Geisen and colleagues distinguished 3 haplotypes in the Europeans: *VKORC1*2* (containing the following SNPs: rs8050894, rs2359612, rs9923231, rs9934438), *VKORC1*3* (rs7294) and *VKORC1*4* (rs17708472) (89). *VKORC1*2* is responsible for a reduction of about 50% of *VKORC1* gene

expression, thus require a smaller amount of enzyme and warfarin dose (90). The *VKORC1*3* and *VKORC1*4* haplotypes require higher doses of warfarin to obtain a therapeutic INR value. In the American population, Rieder *et al.* proposed a nomenclature based on five haplotypes divided into two groups: A - sensitive and B - resistant haplotypes (33). Haplotype A corresponds to *VKORC1*2*, and haplotype B corresponds to the haplotype *VKORC1*3* and *VKORC1*4* (90).

5. Vitamin K dependent factors

The role of vitamin K dependent clotting factors polymorphism in response to VKAs is unclear. Some authors published cases showing that mutations at Ala-10 (Ala-10Thre and Ala-10Val) in the factor IX precursor might lead to the impairment of its production and prolongation of activated partial thromboplastin time in patients taking warfarin at the same time (91). Changes in dosing were also reported in patients with prothrombin (PT-G20210A) and factor VII polymorphism (G401T and G402A) (92). However, present data is still insufficient to have any effect on the VKAs dosage changes.

Acenocoumarol

Warfarin is the only VKA used in the US and Canada, while Europeans commonly use acenocoumarol or phenprocoumon (93). Patients polymorphic in *CYP2C9* showed a higher sensitivity to acenocoumarol and were exposed to major bleeding (94). Carriers of *CYP2C9*3* allele required a 19 – 29% reduction of mean dose (95), while carriers of *CYP2C9*2* allele required a 13 – 15% reduction of mean dose (94, 96). Comparison of genotyping results by Tassies *et al.* suggests that a lower dose is needed to maintain a target INR (2.0 – 3.0) in these patients. The presence of the *CYP2C9*3* allele resulted in a dangerous excess of anticoagulation especially at the initiation of therapy (INR>4.5) (96). The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trial to some extent assessed the effect of the initial dose of acenocoumarol and phenprocoumon estimated by two algorithms. The first one combined both clinical and genetic factors: *VKORC1* and *CYP2C9* genotypes, age, gender, weight, height and amiodarone use. The second algorithm included only clinical factors (97, 98). Patient groups did not differ significantly in most of the outcomes: TTR, the percentage of patients with an INR ≥ 4, the percentage of time with an $4 \leq \text{INR} < 2$, the median time to reach a therapeutic INR, and the percentage of patients with a stable dose during the first 12 weeks of therapy (98).

SNP in a promoter region (*VKORC1* c.-1639G>A, rs17878363) is highly prevalent among Europeans (40%) (89). It has been shown that in the Polish cohort (57 patients) treated mainly with acenocoumarol (n = 50), *VKORC1* -1639G>A polymorphism might be responsible for a partially reduced response to the anticoagulant. Carriers of the G allele (n = 49) required a greater need for acenocoumarol to achieve a therapeutic INR (5.9 ± 1.9 mg per day) compared to the 8 noncarriers (4.1 ± 3.3 mg) (99). The *VKORC1*3* and *VKORC1*4* haplotypes were detected in 92% of the Polish patients (n = 35) requiring an increased dose of warfarin or acenocoumarol (100).

Phenprocoumon

Phenprocoumon together with warfarin as a long-acting VKA provide more stable anticoagulation in comparison to short half-life acenocoumarol (101). Phenprocoumon proved to be the most stable in relation to genetic polymorphisms in *CYP2C9*. No correlation between the drug clearance and the presence of *CYP2C9* variants has been evidenced (102). However, Schalekamp and colleagues observed a 22 – 25% dose reduction in *CYP2C9* variant carriers (103). In comparison to warfarin, the CYP2C9 enzyme has less influence on the phenprocoumon pharmacokinetics and therefore on the estimation of the appropriate dose. It is also probable that the effect of pharmacogenetically guided dosing will be less important (98). Given slight discrepancies in dose requirements, some authors point to phenprocoumon as an alternative to other poorly metabolized VKAs (104, 105).

Clinical relevance

The Medco-Mayo Warfarin Effectiveness Study was important in the introduction of genotype-guided dosing in the treatment of warfarin. The project assessed hospitalization rate within six months from the begining of outpatient anticoagulation in patients with confirmed warfarin sensitivity based on the genotyping for polymorphisms in *CYP2C9* and *VKORC1*. Similarly selected patients starting therapy for the first time during the preceding year served as the historical control group. Genotyping decreased hospitalization by 31%, with a 28% reduction in hospitalization induced by bleeding or thromboembolism. However, the study had several weak points, such as no randomization, the algorithm was unconstrained by the study protocol and the study protocol did not take into account the range of possible interventions required by physicians (106). So, further randomized clinical trials were needed.

Mindful of the shortcomings of the Medco-Mayo design, the pharmacogenetics of warfarin was subsequently compared and tested against standard medical care. The experience from the CoumaGen I study (Applying Pharmacogenetic Algorithms to Individualize Dosing of Warfarin) helped to design a new improved project - CoumaGen II (107, 108). Despite failing to achieve endpoints and satisfactory results in the CoumaGen I trial, a safer determination of a stable maintenance dose based on the pharmacogenetic information about *CYP2C9* and *VKORC1* genes were showed in CoumaGen II (107, 108). The second study compared the clinical effects between three groups of patients initiating warfarin therapy: two different pharmacogenetic strategies and a control group (108). The first genetic approach relied on the CoumaGen I trial and used the IWPC algorithm (taking into account *CYP2C9*2/*3* and *VKORC1* -1639G>A polymorphisms) including Gage *et al.* corrections. The second and more complex strategy included two modifications. It omitted the *CYP2C9* polymorphisms in the initial phase of anticoagulation and applied a dose-revision equation for predicting the daily maintenance dose on day 4 or 5. In both trials, the effectiveness index was the percentage of out-of-range as well as TTR. The second, modified pharmacogenetic algorithm did not show superiority over the scheme used in CoumaGen I. Nevertheless, each of them had an advantage in the effectiveness and safety in comparison to standard care (107, 108).

The Genetic Informatics Trial of Warfarin Therapy to Prevent Deep Vein Thrombosis (GIFT) (completed November 2016) was the first trial quantifying the impact of pharmacogenetics on the safety and control of initial warfarin therapy. If compared with previous studies, the GIFT trial was considerably larger and added another gene (*CYP4F2*). The genotype-guided dosing reduced adverse events from 14.7% to 10.8%, giving an example of how warfarin may act effectively and safely with the INR staying in a target range. There were no deaths during the trial, so it was not possible to assess whether the pharmacogenetic algorithm reduced mortality (109, 110). This study also had some limitations, such as a non-blinded dosage of warfarin and involved the participation at large medical centers of patients over 65 years old with a consequently

Table 2. Clinical, genetic and sociodemographic characteristics of participants in the warfarin pharmacogenetics trials.

Genotype-guided group	**Medco-Mayo (106)**	**CoumaGen I (107)**	**CoumaGen II (108)**	**COAG (59,111-115)**	**EU-PACT (97,116,117)**	**GIFT (109,110)**
n	896	101	504	514	227	808
Age, years, mean or median (SD or range)	65.2 (8.3)	63.2 (25 – 86)	60.6 (18 – 90)	59 (48 – 70)	67.8 (23.7 – 90.2)	72.2 (5.3)
Male sex, %	60.5	49.5	46.9	53	64.2	35.4
Indication, %						
Atrial fibrillation	41.1	12.9	10.6	23	72.2	no data
Pulmonary embolism	11.8	18.8	29.6	21	no data	no data
Vein thrombosis	25.8	18.8	29.6	29	27.8	0.7
Pulmonary embolism/vein thrombosis	37.6	18.8	29.6	56	27.8	no data
Orthopaedic	no data	65.3	55.8	no data	no data	100
History, %						
Diabetes	11.6	22.8	16.9	23	no data	14.4
Smoking	no data	6.9	8.0	15	10.3	2.6
Hypertension	54.2	63.5	47.2	54	no data	no data
Medications, %						
Amiodarone	3.2	no data	no data	3	no data	0
Statin	17.0	no data	no data	no data	no data	45.2
Sulfamethoxazole	5.2	no data	no data	no data	no data	0.5
Fluconazole	2.6	no data	no data	no data	no data	0.4
Race, %						
Black	no data	no data	no data	27	1.3	6.4
White	no data	94.1	95.4	no data	98.2	91.0
Asian	no data	no data	no data	no data	0.4	2.0

COAG, Clarification of Optimal Anticoagulation Through Genetics; EU-PACT, European Pharmacogenetic Approach to Coumarin Anticoagulant Therapy; GIFT, Genetics Informatics Trial of Warfarin to Prevent Deep Venous Thrombosis; n, the number of patients with warfarin dosing by genotype-guided; SD, standard deviation.

higher risk of bleeding. Clinical, genetic and sociodemographic characteristics of patients in GIFT and other trials are presented in *Table 2*.

COAG trials did not show differences in PTTR, the rate of episodes with INR ≥ 4, bleeding or thromboembolism events between the genotype-guided and clinically predicted dosage of warfarin. In a racially diverse cohort, the genetic algorithm was better in non-blacks (59). The polymorphisms included in the pharmacogenetic algorithms were *CYP2C9*2*, *CYP2C9*3* and *VKORC1* -1639 G>A (rs9923231). More common variants in African patients (*CYP2C9*5*, **6*, **8*, **11* and rs12777823) were not genotyped in the COAG trial and it is likely this omission led to significant overdosing (59, 111-115).

The EU-PACT trial showed that genotype-guided warfarin dosing improved PTTR and reduced incidences of INR ≥ 4 (116). Before starting therapy all patients were genotyped for *CYP2C9*2*, *CYP2C9*3* and *VKORC1* -1639G>A (rs9923231) polymorphisms. The EU-PACT trial successfully implemented a slightly modified version of the pharmacogenetic loading dosage approach developed by Avery *et al.* (97, 116, 117). In COAG, *CYP2C9* variants were not considered for the initial dose, providing a small loading dosage on the first day (59). Perhaps differences in loading dose strategies between the EU-PACT and COAG trials contributed to different results. *Table 3*. summarizes the most important information about the studies described above, such as study duration or algorithms used.

The guidelines for the management of pharmacogenetics

The US FDA added a pharmacogenomics section in the package insert of warfarin (36), and also approved the detection and genotyping test for *CYP2C9*2* and *CYP2C9*3* variants and polymorphism of the *VKORC1* (118). Also, two algorithms basing on the clinical and genetic data have now been developed, Gage *et al.* and the IWPC has established an estimate for stable warfarin dosage (19, 31). The pharmacogenetics-based algorithms turned out to be better in dose prediction than non-genetic clinical algorithms or fixed-dose approaches for warfarin, which indicates the need to include warfarin pharmacogenetics in the stable control of oral anticoagulation. Based on the current clinical trials (31, 107, 119) the CPIC guidelines strongly recommend calculating doses using a pharmacogenetic algorithm in patients with non-African ancestry (19, 31, 64). Gage *et al.* and the IWPC algorithms do not include *CYP4F2*, *CYP2C9*5*, **6*, **8*, **11* or rs12777823, which occur in African patients. If these genotypes are known, warfarin dosage should be calculated by a validated pharmacogenetic algorithm (19, 31, 59, 64, 120), or optionally the dosage should be decreased by 15 – 30% per variant or warfarin could be replaced by an alternative anticoagulant. Further reduction may be required in homozygotes (20 – 40%). Because of the lack of data rs12777823 should not be used in dosing algorithms in non-African Americans, whereas the CPIC moderately recommends a dose reduction of 10 – 25% for African Americans. The detection of *CYP4F2*3* should result in the increase of the dose by 5 – 10% (64).

The implementation of pharmacogenetics data according to CIPC guidelines improves patient care by reducing the risk of adverse events and the planned days required to achieve the target daily warfarin dose. This recommendation is also underlined in the guidelines recently released by the European Medicines Agency (121).

The current Guidelines for AF management developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS) in 2016 do not recommend the use of genetic tests before the start of VKAs treatment (18). This conclusion is based on the meta-analysis of randomized controlled trials that did not show a clinically significant advantage of genotype-guided dosing algorithm over the clinical guided dosing algorithm (122). In one of the trials Jonas *et al.* observed only a trend towards a decrease in emergency department visits, hospitalization, bleeding, thromboembolism or death. The main weakness of this study was the small sample size (n = 109), which is likely to have limited the potential to detect small differences between groups and draw strong conclusions (123). In turn, a case-control study conducted by

Table 3. Summary of the key warfarin pharmacogenetics clinical trials.

Trial	N	Design	Study duration	Genes	Algorithms	Genotype-guided dosing duration	Primary endpoint	Conclusion
Medco-Mayo (106)	3584	nonrandomized study	6 months	*CYP2C9* *VKORC1*	usual care supplemented by genetic testing without mandated interventions (unconstrained by study protocol)	the time between therapy initiation and delivery of the genotype results ranged 11 – 60 days (Median = 32)	incident hospitalization rate (measured as event-free time)	PG dosing reduced the risk of hospitalization in outpatients initiating therapy
Coumagen I (107)	200	blinded, randomized clinical trial	3 months	*CYP2C9* *VKORC1*	IWPC algorithm + supplemental data from Gage *et al*.	1	OOR	PG dosing no reduced OOR
Coumagen II (108)	2415	blinded, randomized clinical trial	3 months	*CYP2C9* *VKORC1*	(PG-1) IWPC algorithm + supplemental data from Gage *et al*.; (PG-2) IWPC algorithm + supplemental data from Gage *et al*. + 2 IWPC modifications: (1) the first 2 days-ignore *CYP2C9* status (2) special dose-revision algorithm based on a day 4 – 5 INR after 3 – 4 doses	1 – 8 days	OOR; TTR	PG-1 and PG-2 are comparable for OOR and TTR; PG dosing reduced OOR and increased TTR
COAG (59,111-115)	1015	double-blind, randomized clinical trial	6 months	*CYP2C9* *VKORC1*	Gage *et al*. dose-initiation algorithm (non incorporated CYP2C9); Gage *et al*. dose-revision algorithm	1 – 4 or 5 days	TTR	PG dosing no increased TTR
EU-PACT (97,116,117)	455	single-blind, randomized clinical trial	3 months	*CYP2C9* *VKORC1*	modified IWPC loading-dose-initiation algorithm; modified Gage *et al*. dose-refinement algorithm	1 – 4 or 5 days	TTR	PG dosing increased TTR
GIFT (109,110)	1598	partially blinded, randomized clinical trial	3 months	*CYP2C9* *VKORC1* *CYP4F2*	algorithms guided by a web application (www.WarfarinDosing.org) with incorporated clinical variables	1 – 11 days	adverse events	PG dosing reduced adverse events

COAG, Clarification of Optimal Anticoagulation Through Genetics; EU-PACT, European Pharmacogenetic Approach to Coumarin Anticoagulant Therapy; GIFT, Genetics Informatics Trial of Warfarin to Prevent Deep Venous Thrombosis; INR, international normalized ratio; IWPC, International Warfarin Pharmacogenetics Consortium; N, the number of patients in trial; OOR, percentage of out-of-range INRs; PG, pharmacogenetics; TTR, percentage of time in therapeutic range INRs.

Roth *et al.* marginalized the importance of *VKORC1* and *CYP2C9* polymorphisms in the severity of major hemorrhages in the initial and chronic treatment of AF (124).

Incorrect handling of genetic information when establishing dosage control may introduce a state of false security and insufficient monitoring during pharmacotherapy. Genetic-guided dosing may increase the risk of under/over dosing, especially in individuals who carry rare or untested variants and are assigned as wild-type by default (31, 59). The genotyping must be performed in certified laboratories by qualified personnel to be reliable. In the case of an error, personalized medicine can become very dangerous. The result of the genetic test will probably accompany the patient for life, and any associated error may have long-term adverse health effects. It is equally important that only appropriately trained and experienced physicians are charged with the interpretation of the results of such prediction tests. Besides the usefulness of polymorphisms, the importance of the pharmacoeconomic aspect is increasing (125). Increasing attention is paid to the cost-effectiveness of pharmacogenetic testing, the savings from the reduction of side effects and the rapid achievement of the intended purposes of therapy (126). The high costs of genetic tests, as well as the time required for genotyping, could raise financial concerns from health insurers which in some part could limit the use of pharmacogenetics on a daily basis. Additional studies on the cost-effectiveness and clinical relevance could identify groups of patients for whom genotyping will be the most beneficial. According to Patrick *et al.* the preliminary genotyping of patients before therapy reduces out of range INR values by more than 5 – 9% compared to the standard care of AF patients (127). Pharmacogenomic tests could also be preferred in newly diagnosed patients and in those with a high risk of bleeding compared to patients with nonvalvular AF (126, 128).

OTHER ANTICOAGULANTS

Heparin anticoagulants

The inter-individual variability of response to some anticoagulants seems to be difficult to determine, especially if they are used as emergency treatment. This applies to heparins intravenously administered in the acute coronary syndrome, AF, hemodialysis or during cardiac surgery to suppress the formation of blood clots in devices used in extracorporeal circulation (3, 5). In these cases, well-known polymorphisms (*Table 1*) can modify the action of heparins, making patients resistant to their effect or increase the incidence of serious side effects, for example, heparin-induced thrombocytopenia (HIT). The patients with high risk of HIT determined by genotyping could receive intensive care or alternative anticoagulant treatment.

1. Heparin resistance

In many patients, the same dose of UFH does not provide a reproducible therapeutic response. The changes in the

anticoagulation level can be monitored by activated clotting time (ACT) (129). During cardiac surgery, the resistance to UFH or insufficient anticoagulation is defined as ACT < 400 seconds and may be explained by the abnormalities in AT activity and mutation in its gene (*SERPINC1*) (130-132). More than 200 different mutations in *SERPINC1* have been detected (133-135). One of the SNPs, rs2227589 (786G>A) in the intron 1 results in lower levels of AT as well as reduced anticoagulant activity (135). The prevalence of AT deficiencies is 0.02 – 0.05% in the general population and 0.5 – 5% among patients with VTE, in which it can be considered as a high-risk factor for morbidity (136-138).

Wypasek *et al.* conducted a genetic analysis in a small group of patients with AT deficiency. The AT deficient group (n = 35) predominantly had mutations located in exon 2 and 7 of the *SERPINC1* gene. About 31% of patients were characterized by type II AT deficiency, partly connected with heparin affinity defects. The AT activity levels in patients with mutation ranged between 38 – 67%, and without the mutation between 49 – 78%. There was no mutation in patients with AT activity higher than 70%, but the mutation detection rate was 90% when AT activity was below the threshold. It seems that in the Polish population, a genetic analysis of the *SERPIC1* gene could be useful in patients with AT activity lower than 70% (139).

Heparin resistance in homozygous AT deficiency may be overcome by AT infusion in combination with UFH, which restores UFH responsiveness in patients undergoing cardiac surgery (140, 141) but AT concentrates are expensive and require an intravenous administration (142). Rivaroxaban may be considered as another option for the management of heparin resistance, as its effects are independent of AT (142). AT deficiency is associated with at least a 50% risk of VTE, and should be chronically treated with VKA, rivaroxaban or dabigatran (143).

2. Heparin-induced thrombocytopenia

Pharmacogenetic considerations of heparins mostly concern the immunological side effect-HIT, manifested by thrombocytopenia and limb or life threatening thrombosis (144). Heparin and platelet factor 4 (PF4) complexes induce an immune response with IgG antibodies activating platelets *via* the platelet Fcγ receptor type IIa (FcγRIIa) (145, 146). Although clinical management with LMWH is more predictable and safe, the prevalence of HIT after LMWH and UFH is comparable, probably because both heparins form similar stable complexes with PF4 regardless of the molecule size (147). HIT remains a serious clinical problem difficult to diagnose and exclude. A high percentage of hospitalized patients produce antibodies after contact with heparins asymptomatically (148). HIT is manifested by thromboembolic complications only in some seropositive patients, and the mortality rate varies between 10 and 20% (149, 150). Delayed diagnosis and treatment increase the risk of thrombosis and death, but misdiagnosis exposes patients to unnecessary anticoagulant therapy and the risk of bleeding (147). Identification of polymorphisms before UFH or LMWH administration could help to determine the risk group of patients.

The FcγRIIa-H131R (G507A; rs1801274) and FcγRIIIa-F158V (G559T; rs396991) polymorphisms have been the most extensively investigated, but there is no consistent evidence for their role in HIT (147, 151). Arepally *et al.* showed no significant differences in the distribution of FcγRIIa-H131R genotypes among HIT patients (n = 36) without or with thrombotic complications (n = 23) (149). The next study was conducted on 389 HIT patients and the control group composed of healthy blood donors and patients presenting thrombocytopenia or thrombosis without the antibodies (152). The FcγRIIa-131RR genotype was more frequent in HIT patients and correlated with the clinical symptoms (152). The significantly higher frequency of FcγRIIa-131RR was found in patient cohorts who manifested thrombotic complication related with HIT, suggesting that this association may be HIT-specific (152). Carlsson *et al.* hypothesized that immunogenic complex might be less efficiently removed from the circulation of FcγRIIa-131RR homozygotes, thereby prolonging cell activation and increasing the risk of thrombosis (152). On the other hand, severe thrombocytopenia was reported in homozygous FcγRIII-158VV patients with high levels of antibodies (148). The FcγRIIIa-F158V and FcγRIIa-H131R genotypes were determined in the HIT group (n = 102) and in seropositive and seronegative patients treated with heparin. The FcγRIIa-H131R distribution did not statistically differ between 3 groups (148). Among the patients with high titers of antibodies, the FcγRIIIa-158VV homozygotes appeared more frequently in HIT cases (21.5% versus 9.5%; P = 0.02). Rollin *et al.* tried to explore why the FcγRIIa-131RR homozygotes have a higher risk of thrombosis in HIT patients (n = 89), and whether IgG antibodies modulate the platelet response according to the FcγRIIa polymorphism. There was no significant correlation between antibody levels and the FcγRIIa-H131R polymorphism (153). The association was observed in homozygous FcγRIIa-131RR patients with HIT-related thromboembolic complications compared to cases without thrombosis (34.5% versus 8.5%, P = 0.008). They postulated that the receptor polymorphism was controlling platelet responsiveness, independent of the complex clearance (153).

Some of the polymorphisms may cause hypercoagulability, and for this reason, their prevalence has been examined in HIT patients. Scarparo *et al.* did not support the more frequent occurrence of the FcγRIIIa-F158V genotype, although they observed a significant association between the FcγRIIa-131RR genotype and thrombosis in HIT, but only in the presence of the HPA-1a/b or PECAM1-V/V125 gene polymorphism (154). There is no correlation between platelet glycoprotein receptor polymorphisms and thrombosis (155). Recent GWAS conducted on HIT cohort (n = 67) and the heparin-exposed group had investigated multiple SNPs within the FcγRIIa gene, excluding the FcγRIIIa-F158V polymorphism (156). Results suggest that the FcγRIIa-H131R polymorphism is not a genetic risk factor for HIT (156). Witten *et al.* explored the genetic predisposition by conducting a GWAS within a case-control pharmacovigilance study program. The study did not support a role for previously implicated polymorphisms such as FcγRIIa-H131R (P = 0.83) for genetic HIT predisposition. Nevertheless, findings on chromosome 5 and new candidate genes provide a basis for future studies aiming to identify and characterize genetic susceptibility factors for HIT (157). Meta-analysis of 6 studies suggests that there is no direct relationship between the development of HIT and SNPs in the gene coding for FcγRIIa receptors (151). The results of recent studies have confirmed this conclusion, but also have shown that the frequencies of the homozygous genotypes are significantly higher in patients with thromboembolic complications and at higher risk of symptomatic HIT (148, 153, 154).

The potential reasons for the inconsistency between the results of different studies can be related to complex and multifactorial pathogenesis or delayed diagnosis of HIT. The functional tests for HIT detection may have some advantage over standard laboratory analysis of coagulation (158). It is recommended to assess the platelet aggregation and to perform serotonin release assay (145). The monitoring of thromboxane A2 metabolites which are produced during the platelet activation could be also helpful (159). Nevertheless, we still do not have

perfect diagnostic tools which would ensure an accurate diagnosis. Low frequency and a different time in the occurrence of HIT makes it difficult to collect a sufficient study group for the detection of differences. Often HIT was retrospectively identified, which could lead to some misclassification of patients. Also, the duration of anticoagulation is different, and many patients may not have been treated for a sufficient time to develop HIT. Despite the development of genetic engineering, there is still a lack of perfect experimental animal models reflecting the human Fc receptor system, making it impossible to examine the complexity of thrombocytopenia pathogenesis (147).

Direct oral anticoagulants

VKAs have been the most widely used anticoagulants for more than 50 years. However, the high risk of major bleeding and numerous interactions contributed to their replacement by a new generation of drugs. DOACs have been used in the VTE prophylaxis undergoing total hip and knee replacement surgery (160, 161). DOACs are also preferred in AF patients initiating oral anticoagulation (18). The recent extensive genetic analysis indicated the influence of *VKORC1* and *CYP2C9* variants on bleeding events in AF patients treated with warfarin. Fewer patients treated with edoxaban displayed early complications compared to those treated with warfarin. The study suggested that clinical response to edoxaban is independent of these 2 gene polymorphisms (162). Clinical application of the pharmacogenetics for DOACs therapy is currently being investigated. The genotyping could be applied not only to predict the dosage scheme but alternatively also to distinguish patients who would benefit from switching to DOACs. According to the CPIC guidelines, patients who are poor metabolizers, or who may also display increased sensitivity, oral anticoagulant could be considered (64).

1. Dabigatran

Dabigatran etexilate is the longest-standing synthetic reversible direct thrombin inhibitor on the pharmaceutical market. Thus, its pharmacokinetics and pharmacodynamics have been better studied than other DOACs (161, 163-167). As a prodrug, it must be transformed into a pharmacologically active metabolite dabigatran by intestinal carboxylesterase 2 and hepatic carboxylesterase 1 (CES1) to directly inhibit thrombin (168). The inter-individual variability in metabolism causes a fluctuation in dabigatran plasma concentration (161, 163-167), which may lead to a different response to treatment. The GWAS study conducted in Randomized Evaluation of Long-term Anticoagulation Therapy cohort (n = 2944) identified three polymorphisms that influenced the plasma level of dabigatran: rs8192935, rs2244613 of *CES1* and rs4148738 of *ABCB1* gene coding for p-glycoprotein (P-gp) (163). Pare *et al.* monitored trough concentration within 10 to 16 hours after the previous dabigatran etexilate dose, and peak concentration within 1 to 3 hours after administration (163). Patients affected by the SNPs: rs8192935 of *CES1* and rs4148738 of *ABCB1* presented, a 12% decrease or increase in peak plasma concentrations of dabigatran per minor allele detected, respectively. Other influential *CES1* SNP rs2244613 was associated with a 15% reduction in trough concentration and a decreased risk of any bleeding (163). However, in the stable AF patients group (n = 92) no significant relation was found, except significant positive correlation between *CES1* SNP rs8192935 and decreased drug trough levels (169). Polymorphisms cannot be considered as a predictive marker in determining the optimal dose in non valvular AF patients group (n = 52) treated with dabigatran (170). The remaining *ABCB1* SNPs: rs2032582 and rs1045642 which reduce the P-gp activity *in vivo* and *in vitro* were examined in healthy male volunteers (n = 60). Gouin-Thibault and colleagues demonstrated that neither of the SNPs induced clinically significant changes in dabigatran and rivaroxaban pharmacokinetics (171). In addition, the plasma concentration of dabigatran depends on the gender as shown in the clinical studies. The predisposition of women to higher plasma levels has not been fully investigated (164, 166).

2. Rivaroxaban

Rivaroxaban is the first representant of DOACs group inhibiting factor Xa (172). Similarly to dabigatran, it is a substrate for the P-gp transporter (173). It seems that *ABCB1* polymorphism is not significantly responsible for the pharmacokinetics fluctuation of both anticoagulants (168, 171). Absorption and excretion of rivaroxaban are also mediated by the breast cancer resistance protein (BCRP) encoded by the *ABCG2* gene. The genetic variants of this gene, including the most common Q141K, are responsible for the reduced transport of BCRP substrates (172). Rivaroxaban is mainly metabolized by CYP enzymes: CYP3A4/5 and CYP2J2 (174). At the moment, there is a lack of analysis performed on patients taking rivaroxaban with regard to the above genes polymorphisms. A larger cohort is necessary to assess the potential impact on the plasma drug concentration or clinical outcome (172).

3. Apixaban

Apixaban is another direct factor Xa inhibitor as well as a P-gp and BCRP substrate (172). The clinical relevance of *ABCB1* polymorphism has not been proven in dabigatran and rivaroxaban therapy, but the modulatory effect of SNP rs4148738 was found for apixaban plasma concentrations. About 6% of the difference in response to the anticoagulant is attributed to rs4148738 variations. Among 80 Caucasian patients, the peak concentrations were decreased by 26% and 32% in heterozygotes and homozygotes, respectively. In contrast, changes in trough apixaban concentration was not statistically significant in heterozygous and homozygous genotypes (175). Apixaban is primarily metabolized by CYP3A4, with the minor role of CYP2J2, CYP2C8/9/19, and CYP1A2. Pharmacogenetic considerations of apixaban might focus not only on cytochrome related genes but also on *SULT1A1* and *SULT1A2* genes which regulate the formation of the first nonreactive metabolite: O-demethyl-apixaban sulfate (176).

4. Edoxaban

Edoxaban is the last registered anticoagulant from the DOACs group directly inhibiting factor Xa. Currently, there are no studies showing that any of the SNPs can modify the activity of edoxaban. It is possible that genes coding CES1 and CYP3A4/5 which are involved in biotransformation and the gene for P-gp transporter could help in estimating the personalized dose of edoxaban. However, as with the other DOACs, edoxaban requires large, multi-ethnic studies with dosing determined by genotype and with hard primary endpoints. Nonetheless, it is challenging to fund such trials, thus the observational studies may also be helpful (172, 176).

Conclusions

There is a growing interest about pharmacogenetics as a reason for a change in bioavailability of anticoagulants. A great deal of data about polymorphisms affecting response to VKAs,

especially warfarin, encourages belief in the significant potential benefits of incorporating pharmacogenetic information into routine clinical care. There are available pharmacogenetic sections in product characteristics of warfarin as well as dosing based on pharmacogenetic algorithms. CPIC guidelines for *CYP2C9*, *VKORC1*, *CYP4F2*, and rs12777823 genotype-guided warfarin dosing when clinical genotype results are known. Despite the evidence from the published literature, clinicians do not yet recognize the potential scale of such benefits, and there are questions concerning cost efficiency. According to the ESC/EACTS guidelines routine genotyping is not recommended in patients before initiating VKAs treatment. *CYP2C9* and *VKORC1* polymorphisms are important determinants in the genotype-guided dosing of warfarin, and they may distinguish patients who would benefit from switching to DOACs. When starting warfarin therapy based on the pharmacogenetic tests the race of patient should be taken into account. The bioavailability of DOACs is potentially susceptible to pharmacogenetic variation. Researchers have identified gene polymorphism of proteins that have the potential to impact their metabolism. However further multi-ethnic studies with primary clinical endpoints are necessary. There is evidence that heparin resistance and HIT could be genetically determined although it does not mean that the risk of bleeding or thromboembolism is related to mutations in general.

Further development work is clearly still necessary in order to devise and implement pharmacogenetics-based treatment protocols and the future research goals related to cardiovascular pharmacogenomics should be identified.

Abbreviations: ACT, activated clotting time; AF, atrial fibrillation; AT, antithrombin; CES1, hepatic carboxylesterase 1; COAG, Clarification of Optimal Anticoagulation through Genetics trial; CPIC, Clinical Pharmacogenetics Implementation Consortium; DOACs, direct oral anticoagulants; EU-PACT, European Pharmacogenetics of Anticoagulant Therapy trial; GIFT, Genetic Informatics Trial of Warfarin Therapy; GWAS, genome-wide association study; HIT, heparin-induced thrombocytopenia; HVR, heart valves replacement; INR, international normalized ratio; IWPC, International Warfarin Pharmacogenetics Consortium; LMWH, low-molecular-weight heparins; PTTR, percentage time in therapeutic range; SNP, single nucleotide polymorphism; TTR, time in therapeutic range; UFH, unfractionated heparin; US FDA, United States Food and Drug Administration; VKAs, vitamin K antagonists; VKORC1, vitamin K epoxide reductase complex subunit 1; VTE, venous thromboembolism.

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