Farmakogenetika varfarina u kliničkoj praksi

The pharmacogenetics of warfarin in clinical practice

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Sažetak

Varfarin je najpropisivaniji oralni antikoagulans. Postoje velike interindividualne razlike (i do 20 puta) u potrebnim dozama lijeka, što je rezultat genetičkih i okolišnih čimbenika. Podaci dobiveni pomoću farmakogenomike, tj. ispitivanja interakcija osobnog genotipa i odgovora na lijek, mogu pomoći u postizanju optimalne učinkovitosti lijeka i svođenju štetnih reakcija na lijek na najmanju moguću mjeru. Podaci dobiveni analizom dvaju gena, enzima za metabolizam varfarina CYP2C9 i ciljnog enzima za varfarin, podjedinice 1 vitamin K ovisne epoksid reduktaze (VKORC1), potvrdili su njihov učinak na dozu održavanja varfarina. Ispitivanjem udruženosti na razini genoma također je utvrđen slab učinak *CYP4F2*. Prisutnost varijantnih alela *CYP2C9*2* ili *CYP2C9*3*, koje rezultiraju smanjenom enzimskom aktivnošću, udružena je sa značajnim smanjenjem srednje doze varfarina. Jednonukleotidni polimorfizmi *VKORC1* objašnjavaju velik dio interindividualnih razlika u dozi varfarina, a *VKORC1* ima otprilike tri puta jači učinak od *CYP2C9*. Stanje nositelja kombinacije polimorfizama *VKORC1* i *CYP2C9* udruženo je s teškom prekomjernom antikoagulacijom. Vrijeme do postizanja stabilnosti doze uglavnom je povezano s genotipom *CYP2C9*. Otpornost na varfarin vezuje se za nekoliko *missense* mutacija u genu *VKORC1*. Algoritmi koji uključuju genetičke (*CYP2C9* i *VKORC1*), demografske i kliničke čimbenike za procjenu doze varfarina mogli bi smanjiti rizik od predoziranja za vrijeme uvođenja varfarina.

Ključne riječi: farmakogenomika; varfarin; VKORC1; CYP2C9; algoritam doziranja

Abstract

Warfarin is the most widely prescribed oral anticoagulant. It shows great (up to 20-fold) interindividual variability in dose requirement because of both, genetic and environmental factors. Information from pharmacogenomics, a study of the interaction of the individual's genotype and drug response, can help optimize drug efficacy and minimize adverse drug reactions. Genotyping data on two genes, the warfarin metabolic enzyme CYP2C9 and warfarin target enzyme, vitamin K epoxide reductase complex 1 (VKORC1), confirmed their influence on warfarin maintenance dose. Genome-wide association study also found a weak effect of CYP4F2. The presence of *CYP2C9*2* or *CYP2C9*3* variant alleles, which results in decreased enzyme activity, is associated with a significant decrease in the mean warfarin dose. *VKORC1* single nucleotide polymorphisms (SNPs) explain a large fraction of the interindividual variation in warfarin dose, and *VKORC1* has an approximately three-fold *CYP2C9* effect. Carrier state of a combination of *VKORC1* and *CYP2C9* polymorphisms, rather than of one of these polymorphisms is associated with severe overanticoagulation. The time to achieve stability is mainly associated with the *CYP2C9* genotype. Warfarin resistance has been related to several missense mutations in the *VKORC1*. Algorithms incorporating genetic (*CYP2C9* and *VKORC1*), demographic, and clinical factors to estimate warfarin dosage could potentially minimize the risk of overdose during warfarin induction.

Keywords: pharmacogenomics; warfarin; VKORC1; CYP2C9; dosing algorithm

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Uvod

Godine 2007. je Uprava za hranu i lijekove (FDA) objavila kako će upute za varfarin sadržavati nove podatke o ulozi genetike u doziranju lijeka. Prema tim uputama niže početne doze varfarina „treba razmotriti kod bolesnika s od-

Introduction

In 2007, the Food and Drug Administration (FDA) announced that warfarin's label will carry new information describing the role of genetics in drug dosing. The label states that a lower initial warfarin dose "should be considered

ređenim genskim varijacijama". Za uspostavu algoritma doziranja preporuča se genotipiziranje *CYP2C9* i vitamin K epoksid reduktaza kompleksa 1 (*VKORC1*) (1). Ovo je prva preporuka FDA koja govori o genetičkom ispitivanju pri započinjanju terapije često propisivanim lijekom i to bi mogao biti presedan za buduću primjenu genskih tehnologija u kliničkoj praksi. Mnogo se napora ulaže u uvođenje ove tehnologije u praksu, pričem tvrtke nude testove, akademske ustanove nastoje ići ukorak s najnovijim saznanjima, dok bolesnike zanimaju mogućnosti personalizirane medicine.

Zašto varfarin? Nekoliko je razloga koji ovaj lijek čine privlačnom metom personalizirane medicine. Varfarin je antikoagulans koji se najviše primjenjuje; propisuje se za više od 2 milijuna novih bolesnika na godinu. Varfarin se često rabi kao doživotna terapija radi sprječavanja sustavne embolije u bolesnika s atrijskom fibrilacijom, bolešću srčanih zalistaka, te za primarnu i sekundarnu prevenciju venske tromboembolije. Varfarin se također primjenjuje za sprječavanje tromboembolijskih ispada u bolesnika s akutnim infarktom miokarda i pektoralnom anginom, u bolesnika s biološkim srčanim zaliscima, te nakon nekih ortopedskih operacija. Klinički tretman je zahtjevan zbog uskog terapijskog raspona i znatnih razlika među bolesnicima. Kombinirani genetički i negenetički čimbenici dovode do 20-strukih razlika u dozi varfarina potrebnoj za postizanje uobičajene terapijske razine antikoagulacije mjerene međunarodnim normaliziranim omjerom (INR) za protrombin. Optimalan omjer INR je 2 do 3, pričem omjeri < 2 znače povećanje trombotskih ispada, a oni > 4 povećanje hemoragijskih ispada (2,3). Nekoliko čestih genskih varijanata djeluje na metabolizam i aktivnost varfarina (4,5). U nedostatku podataka dobivenih genetičkim pretragama ili kliničkih podataka za predviđanje potrebne doze varfarina kod svakog pojedinog bolesnika, početne propisane doze mogu biti preniske, čime se povećava rizik od tromboze, ili pak previsoke, što dovodi do rizika od prekomjerne antikoagulacije i teškog krvarenja. U Sjedinjenim Državama na godinu ima do 800 štetnih događaja udruženih s primjenom varfarina koji su obuhvaćeni propisom o prijavljivanju (6).

Opasnost od ozbiljnih nuspojava povezanih s varfarinom, njegov uzak terapijski raspon i velike interindividualne razlike u doziranju zahtijevaju izradu algoritama kako bi se doza potrebna u početnom stadiju (ima) liječenja mogla što točnije predvidjeti.

Uloga CYP2C9 u metabolizmu varfarina

CYP2C9 je pretežito eksprimiran u jetri, gdje čini oko 20% jetrenog sadržaja CYP. CYP2C9 metabolizira više od 20% svih lijekova, kao i brojne endogene spojeve. Klinički važni lijekovi-supstrati uključuju antagonist angiotenzina 2, nesteroidne protuupalne lijekove (NSAR), oralne antifor patients with certain genetic variations". Genotyping of *CYP2C9*, and vitamin K epoxide reductase complex C1 (*VKORC1*) is recommended for dosing algorithm (1). This is the first FDA recommendation to consider genetic testing when initiating a commonly prescribed medication and may set a precedent for the future use of genetic technologies in clinical practice. Many efforts are driving this technology into practice with companies offering tests, academic institutions trying to be on the cutting edge of clinical medicine, and patients interested in the potential of personalized medicine.

Why warfarin? There are several reasons that make this drug attractive target of personalized medicine. Warfarin is the most widely used anticoagulant, prescribed to more than 2 million new warfarin patients *per* year. Warfarin is commonly used as life-long therapy in the prevention of systemic embolism in patients with atrial fibrillation, valvular heart disease, and in the primary and secondary prevention of venous thromboembolism. It is also used for the prevention of thromboembolic events in patients with acute myocardial infarction and with angina pectoris, in patients with biological heart valves, and after some types of orthopedic surgery. Clinical management is difficult because of a narrow therapeutic range and considerable interpatient variability. A combination of genetic and non-genetic factors is responsible for up to 20-fold variation in the warfarin dose required to achieve the usual therapeutic level of anticoagulation as measured by the prothrombin international normalized ratio (INR). The optimal INR range is 2 to 3, with ratios < 2 increasing thrombotic events and those > 4 increasing hemorrhagic events (2,3). Several common genetic variants affect warfarin metabolism and activity (4,5). In the absence of genetic testing data or clinical information to predict the warfarin dose required in each individual patient, the initial doses prescribed may be too low, increasing the risk of thrombosis, or too high, which may be associated with the risk of overanticoagulation and severe bleeding. Up to 800 reportable adverse drug events associated with warfarin usage *per* year occur in the United States (6).

The warfarin risk of serious side effects, narrow therapeutic range, and wide interindividual variation in warfarin dose have imposed the need of algorithms to better predict the dose in the initial stage(s) of treatment.

The role of CYP2C9 in warfarin metabolism

CYP2C9 is predominantly expressed in the liver, representing about 20% of the hepatic CYP content. It metabolizes more than 20% of all therapeutic drugs as well as a number of endogenous compounds. Drug-substrates of large clinical importance include angiotensin-2 antagonist, nonsteroid antiinflammatory drugs (NSAIDs), oral antidiabetics, antimicrobials, antiepileptics, and oral anticoagulants (Table 1) (7,8). Warfarin is a racemic mixture and

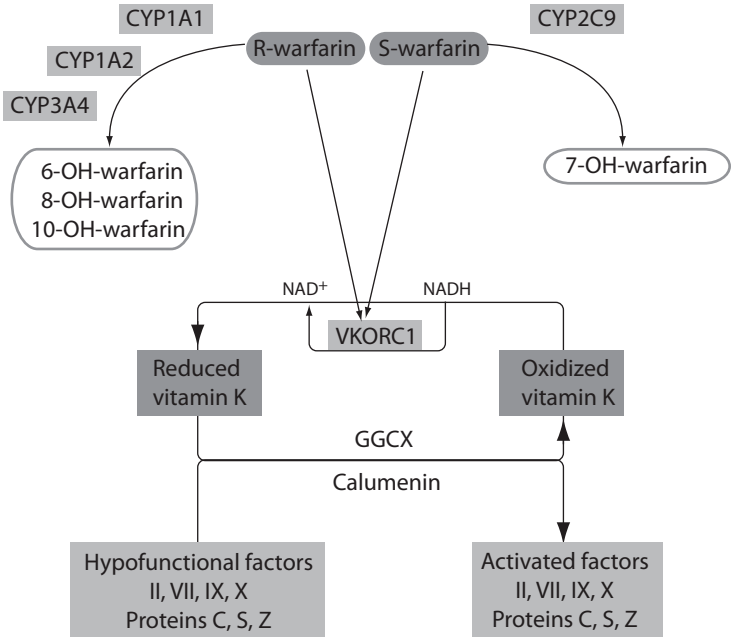
Tablica 1. Ostali važniji lijekovi koji su supstrati za CYP2C9 i mogu stupiti u interakciju s oralnim antikoagulansom varfarinom

Table 1. Other important drugs that are CYP2C9 substrates and can interact with the oral anticoagulant warfarin

Drug group	Drug
Angiotensin II receptor blockers	Losartan, irbesartan, valsartan
Antidiabetics	Tolbutamide, glipizide
Anticoagulants	Acenocoumarol
Anticonvulsants	Phenytoin
Antimicrobials	Metronidazole, sulfamethoxazole
Nonsteroid antiinflammatory drugs	Celecoxib, diclofenac, ibuprofen, indomethacin, naproxen, tenoxicam
Statins	Fluvastatin

dijabetike, antimikrobne lijekove, antiepileptike, te oralne antikoagulanse (Tablica 1.) (7,8). Varfarin je racemična smjesa, a CYP2C9 metabolizira S-varfarin, koji je najaktivniji enantiomer (Slika 1.). Opisani su genski polimorfizmi *CYP2C9*, i pronađeno je više od 34 alela (vidi: http://www.cypalleles.ki.se/). Aleli *CYP2C9*2, *3, *4,*5* i **30* kodiraju proteine s promjenama u aminokiselinskom slijedu i

CYP2C9 metabolizes S-warfarin, which is the most active enantiomer (Fig. 1). Genetic polymorphisms of *CYP2C9* have been reported; more than 34 alleles have been found (see http://www.cypalleles.ki.se/). The *CYP2C9*2, *3, *4,*5,* and **30* alleles encode for protein variants with amino acid replacement and have been reported to reduce catalytic activities *in vitro* and/or *in vivo* (9,10). There



OH - hydroxy; NAD+ - oxidized form of nicotinamide adenine dinucleotide; NADH - reduced form of NAD; GGCX - γ-glutamyl carboxylase.

Slika 1. Farmakokinetski i farmakodinamski put varfarina. Varfarin se daje kao racemična smjesa R- i S-enantiomera. Snažniji S-enantiomer se uglavnom metabolizira pomoću citokroma P450 (CYP2C9). Farmakološki učinak varfarina je posredovan inhibicijom vitamin K epoksid reduktaza kompleksa 1 (VKORC1). To dovodi do smanjenih koncentracija aktiviranih faktora zgrušavanja (II., VII., IX. i X.) i rezultira terapijskom antikoagulacijom (prema: www.medscape.com; 2008 Pharmacotherapy Publications).

Figure 1. Warfarin pharmacokinetic and pharmacodynamic pathway. Warfarin is administered as a racemic admixture of R- and S-enantiomers. The more potent S-enantiomer is metabolized principally by cytochrome P450 (CYP) 2C9. The pharmacological effect of warfarin is mediated by the inhibition of vitamin K epoxide reductase complex 1 (VKORC1). This results in decreased concentrations of activated clotting factors (II, VII, IX and X) producing therapeutic anticoagulation (according to: www.medscape.com; 2008 Pharmacotherapy Publications).

za njih je objavljeno da smanjuju katalitične aktivnosti *in vitro* i *in vivo* (9,10). U populaciji postoje velike interindividualne razlike u aktivnosti CYP2C9, što dovodi do interindividualnih razlika u odgovoru na lijek, pa i štetnim događajima. Učestalost fenotipa slabog metabolizatora je 3-5% kod bijelaca (11,12). Najčešće varijante su *CYP2C9*2* (rs4917639, p.R114C) i *CYP2C9*3* (rs1057910, p.I359L) aleli. Naročito su zanimljivi lijekovi-supstrati uskog terapijskog raspona, kao što su S-varfarin, tolbutamid i fenitoin, gdje bi poremećena metabolična aktivnost CYP2C9 mogla otežati prilagodbu doze i uzrokovati toksičnost (13,14). Polimorfizmi *CYP2C9* mogu isto tako biti udruženi s povećanom stopom štetnih događaja izazvanih primjenom NSAR (15).

Uz genske polimorfizme, čimbenici okoline mogu također uzrokovati interindividualne razlike u aktivnosti CYP2C9 (16). Pokazalo se da razni ksenobiotici poput fenobarbitala, rifampicina i hiperforina izazivaju povećanu transkripciju *CYP2C9* u primarnim ljudskim hepatocitima i povećavaju metabolizam supstrata CYP2C kod čovjeka *in vivo* (17,18). To može rezultirati interakcijama lijekova, ne/podnošljivosti lijeka i terapijskim neuspjehom. Nekoliko lijekom aktiviranih jezgrenih receptora uključujući receptor konstitutivnog androgena (CAR) te receptor za pregnan-X (PXR) prepoznaju elemente odgovora na lijek unutar 5'-promotorske regije gena za *CYP2C* kako bi posredovali transkripcijsku prilagodbu ovih gena u odgovoru na ksenobiotike i steroide (19). Moguća je i inhibicija CYP2C9, kako se pokazalo *in vitro* za sintetske konzervanse kao što je benzetonij klorid (20). Klinički značajna inhibicija može nastupiti kod istodobnog uzimanja amjodarona, flukonazola, fenilbutazona, nekih sulfonamida i oralnih kontraceptiva (17,21).

Učinci genskih polimorfizama na opseg metabolizma lijekova dobro su poznati, ali ostaje nejasno kako ovi polimorfizmi utječu na sklonost ka interakcijama lijekova. Što se tiče interakcija varfarina s drugim lijekovima, osobito je nejasno podliježu li proteini sa smanjenom funkcijom (npr. *CYP2C9*3*) inhibiciji *in vivo* u istoj mjeri kao i proteini kodirani alelima divljeg tipa (npr. *CYP2C9*1*). Farmakogenetičke razlike metaboličkih enzima i prijenosnika lijekova mogu imati važnu ulogu u interakcijama lijekova s oralnim antikoagulansima. To je osobito važno za lijekove - supstrate poput NSAID, fluvastatina, tolbutamida, fenitoina. Zbog moguće inhibicije ovisne o genotipu, genotip treba uzeti u obzir kad se nastoje predvidjeti moguće interakcije lijekova (22,23). Studije potvrđuju kako genski polimorfizam CYP2C9 doprinosi različitosti u potrebnom doziranju varfarina u prisutnosti interakcija lijek-bolest i lijek-lijek (24).

VKORC1 – meta za oralne antikoagulanse

Za oralne antikoagulanse ciljno mjesto djelovanja je podjedinica 1 vitamin K ovisne epoksid reduktaze (VKORC1)

is a large interindividual variation in CYP2C9 activity in the population with resulting interindividual variations in drug response and also in adverse effects. The poor metabolizer phenotype has a frequency of 3%-5% in Caucasians (11,12). The most frequent variants are *CYP2C9*2* (rs4917639, p.R114C) and *CYP2C9*3* (rs1057910, p.I359L) alleles. Of special interest are drug substrates with a narrow therapeutic range, such as S-warfarin, tolbutamide and phenytoin, where impaired CYP2C9 metabolic activity might cause difficulties in dose adjustment as well as toxicity (13,14) *CYP2C9* polymorphisms can also be associated with an increased rate of NSAID-induced adverse events (15).

Besides genetic polymorphisms, interindividual variability in CYP2C9 activity can also be caused by environmental factors (16). A variety of xenobiotics such as phenobarbital, rifampicin, and hyperforin have been shown to induce transcriptional expression of *CYP2C9* in primary human hepatocytes and to increase the metabolism of CYP2C substrates *in vivo* in man (17,18). This induction can result in drug-drug interactions, drug tolerance, and therapeutic failure. Several drug-activated nuclear receptors including constitutive androgen receptor (CAR) and pregnane X-receptor (PXR) recognize drug responsive elements within the 5' flanking promoter region of *CYP2C* genes to mediate the transcriptional up-regulation of these genes in response to xenobiotics and steroids (19). Inhibition of CYP2C9 is also feasible, as was shown *in vitro* for synthetic preservative substances such as benzethonium chloride (20). Clinically significant inhibition may occur with coadministration of amiodarone, fluconazole, phenylbutazone, certain sulfonamides and oral contraceptives (17,21).

The effects of genetic polymorphisms on the rates of drug metabolism are well known, but how these polymorphisms influence the susceptibility to drug-drug interactions is less clear, in particular considering warfarin-drug interactions, whether reduced function proteins (e.g., *CYP2C9*3*) are inhibited to the same extent in vivo as wild-type proteins (e.g., *CYP2C9*1*). Pharmacogenetic variations of metabolic enzymes and drug transporters can have a major role in drug interactions with oral anticoagulants. This is of special importance for drug substrates such as NSAIDs, fluvastatin, tolbutamide, and phenytoin. Genotype should be considered when attempting to predict the potential CYP2C9 drug-drug interactions because of the possible genotype-dependent inhibition (22,23). Studies confirmed that genetic CYP2C9 polymorphism contributed to the variability in warfarin dosage requirements in the presence of drug-disease and drug-drug interactions (24).

VKORC1 – a target of oral anticoagulants

The target of oral anticoagulants is the protein vitamin K reductase complex subunit 1 (VKORC1) encoded by the

koju kodira homonimni gen *VKORC1*. Vitamin K ovisna epoksid reduktaza (VKOR) reducira vitamin K 2,3-epoksid u biološki aktivni vitamin K hidrokinon koji katalizira proizvodnju karboksiliranih čimbenika zgrušavanja II., VII. i IX. (Slika 1.). Kumarinski antikoagulansi djeluju inhibirajući aktivnost VKOR. *VKORC1* je identificiran 2004. godine (25,26). Rost i sur. su identificirali gen *VKORC1* u četirima neovisnim obiteljima članovi kojih su bili otporni na varfarin. Jedan bolesnik je trebao više od 17 mg/dan varfarina kako bi postigao dostatnu antikoagulaciju, dvoje drugih je trebalo oko 40 mg/dan, dok četvrti bolesnik nije odgovarao ni na koju dozu varfarina. Analiza je potvrdila da su oni nositelji rijetkih mutacija *VKORC1,* tj. *g.85G>T* (p.V29L), *g.134T>C* (p.V45A), *g.172A>G* (p.A58G) i *g.3487T>G* (p.L128A) (25). Druge istraživačke skupine izvještavaju o tome da su česti jednonukleotidni polimorfizmi (SNP) *VKORC1* snažno udruženi s osjetljivošću na oralne antikoagulanse (4,27-30). Genski polimorfizam *VKORC1* utječe slično na sve oralne antikoagulanse (4,31). U kliničkoj praksi obično se genotipiziraju dva SNP, tj. -1639G>A i 1173C>T, za identificiranje haplotipova *VKORC1*. Dokazi ukazuju na to da promotorni polimorfizam -1639G>A smanjuje jetrenu ekspresiju VKORC1 i tako snižava potrebnu dozu lijeka (29,30). Alel -1639G je prisutan u haplotipovima *VKORC1*1, *3* i **4* i on je udružen s „normalnom" dozom varfarina (32,33). Suprotno tome, alel -1639A, koji je u velikoj veznoj neravnoteži (engl. *linkage disequilibrium*) sa alelima 6484C>T, 6853G>C, 7566C>T i 1173C>T, prisutan je u haplotipu *VKORC1*2* i predisponira osjetljivost za varfarin, kao i za niže doze lijeka (5,27,34,35). Njemačka skupina autora je na uzorku od 200 dobrovoljaca identificirala 28 SNP u genomskom slijedu *VKORC1* i utvrdila kako je šest od tih SNP u potpunoj međusobnoj veznoj neravnoteži, i to za tri glavna haplotipa *2, *3 i *4 (32,36). Kod bijelaca je procijenjena učestalost: *VKORC1*2*, 42%; *VKORC1*3*, 38%; *VKORC1*4*, 20%. Postoje velike međuetničke razlike u frekvenciji alela *VKORC1*. Kod Azijaca se učestalost *VKORC1* genotipa -1639AA (~83%) značajno razlikuje od one zabilježene u bijelaca (14%) (4,37). Haplotip *VKORC1*2* je prilično nizak među osobama afričkog podrijetla (0,15), visok u bijelaca (0,42) te iznimno čest među Azijcima (0,95) (29,32,38,39).

Prema podatcima iz farmakogenetičkih studija udruženosti *CYP2C9* i *VKORC1*, zabilježeno je nekoliko klinički značajnijih posljedica na farmakokinetiku-farmakodinamiku oralnih antikoagulansa.

Polimorfizmi *VKORC1* i *CYP2C9* i potrebne dnevne doze

Nositelji alela koji kodiraju smanjenu enzimsku aktivnost CYP2C9 i/ili VKORC1 trebaju niže doze varfarina i kod njih je teže titrirati lijek do stabilne doze održavanja negoli kod onih koji trebaju veće doze. Relativni doprinos

homonymous gene *VKORC1*. Vitamin K epoxide reductase (VKOR) reduces vitamin K 2,3-epoxide to the biologically active vitamin K hydroquinone, which catalyzes the production of carboxylated blood-clotting proteins II, VII, IX and X (Figure 1). Anticoagulants of the coumarin type act by inhibiting VKOR activity. *VKORC1* was identified in 2004 (25,26). Rost *et al*. identified the *VKORC1* gene from four independent families whose members were resistant to warfarin. One patient needed more than 17 mg/day of warfarin to achieve adequate anticoagulation, two others about 40 mg/day, and the fourth did not respond to any dose of warfarin. Analysis confirmed they carried rare mutations of *VKORC1, g.85G>T* (p.V29L), *g.134T>C* (p.V45A), *g.172A>G* (p.A58G) and *g.3487T>G* (p.L128A) (25). Other groups described the common *VKORC1* SNPs to be strongly associated with oral anticoagulant sensitivity (4,27-30). *VKORC1* genetic polymorphism has a similar effect on oral anticoagulants (4,31). In clinical practice, two SNPs, -1639G>A and 1173C>T, are usually genotyped to identify *VKORC1* haplotypes. Evidence suggest that -1639 G>A promoter polymorphism reduces hepatic VKORC1 expression and therefore decreases drug dose requirements (29,30). The -1639G allele is present in the *VKORC1*1, *3, and *4* haplotypes and is typically associated with a 'normal' warfarin dose (32,33). In contrast, the -1639A allele, which is in strong linkage disequilibrium with 6484C>T, 6853G>C, 7566C>T and 1173C>T, is present in the *VKORC1*2* haplotype and predisposes to warfarin sensitivity and lower drug doses (5,27,34,35). In a sample of 200 volunteers, a German group identified 28 SNPs within the *VKORC1* genomic sequence and found six of these SNPs in complete linkage disequilibrium with each other, forming three main haplotypes *2, *3, *4 (32,36). The estimated distribution in Caucasians is as follows: *VKORC1*2*, 42%; *VKORC1*3*, 38%; and *VKORC1*4*, 20%. There are large interethnic differences in the allelic frequency of *VKORC1*. In Asians, the frequency of the VKORC1 -1639 AA genotype (~83%) is significantly different from that recorded in Caucasians (14%) (4,37). The *VKORC1* *2 haplotype was found to be rather low among subjects of African origin (0.15), high in Caucasians (0.42), and extremely frequent among Asians (0.95) (29,32,38,39).

According to data from pharmacogenetic association studies of *CYP2C9* and *VKORC1*, several major clinical consequences on the pharmacokinetics-pharmacodynamics of oral anticoagulants have been observed.

VKORC1 and *CYP2C9* polymorphisms and daily dose requirements

Carriers of alleles coding for reduced CYP2C9 and/or VKORC1 enzyme activity require lower warfarin doses and have been observed to be more difficult to titrate to a stable maintenance dose than those needing higher doses. The relative contribution of each polymorphism

svakog polimorfizma toj razlici u potrebnoj dozi varfarina treba razmotriti u odnosu na učestalost polimorfizama. Tako će *VKORC1* vjerojatno tome doprinositi više od *CYP2C9,* zbog znatno više učestalosti nekih ispitivanih varijanata (1173C<T, -1639G>A) u usporedbi s varijantama *CYP2C9*2* i *CYP2C9*3*. Međutim, kod pojedinih bolesnika za očekivati je da će varijanta *CYP2C9*2* te poglavito varijanta *CYP2C9*3* znatno utjecati na potrebnu dozu varfarina (40,41). U usporedbi s homozigotnim nositeljima *CYP2C9*1*, procijenjeno je da bolesnici homozigotni za *CYP2C9*3* trebaju 3,3 puta niže srednje doze varfarina kako bi postigli isti INR, dok su nositelji alela *2 i heterozigotni bolesnici svrstani između tih vrijednosti (42). U dvjema studijama u kojima je analizirano prvih nekoliko tjedana liječenja varfarinom udio bolesnika s vrijednostima INR iznad 3 bio je veći među nositeljima alela CYP2C9*2 ili *3 nego među bolesnicima koji nisu bili nositelji ovih alela (43,44). U studiji na bolesnicima koji su započinjali liječenje varfarinom glavni je nalaz ukazao na to da je rani odgovor na varfarin izmijenila genska varijacija u *VKORC1,* a ne u *CYP2C9* (45). Međutim, i haplotip *VKORC1* i genotip *CYP2C9* imaju značajan utjecaj na dozu varfarina nakon prva dva tjedna. Utvrđeno je kako genotip *VKORC1* određuje i do 40% potrebne doze kumarina u pojedine osobe (4,5,33,46,47). Kod bijelaca je polimorfizam 1173 C>T bio udružen s nižim potrebnim dozama varfarina (27); srednja doza varfarina bila je viša (6,2 mg/dan) u bolesnika s VKORC1 genotipom 1173 CC nego kod onih s CT genotipom (4,8 mg/dan; P = 0,002) ili TT genotipom (3,5 mg/dan; P < 0,001). Rezultati studije potvrdili su da je *VKORC1*2* najvažniji haplotip za doziranje varfarina (33). Bolesnici s haplotipom *VKORC1*2* češće su dolazili na kontrole od bolesnika s haplotipom *VKORC1*3* ili **4*, imali su viši koeficijent varijacije protrombinskog vremena (INR) i viši postotak vrijednosti INR izvan terapijskog raspona u usporedbi s bolesnicima s haplotipom *VKORC1*3* ili **4*. Uz to, utvrđena je statistički značajna razlika između haplotipa *VKORC1*2* te *VKORC1*3* ili **4* u dozi varfarina (P < 0,001) i koncentraciji R-varfarina u plazmi (P < 0,01). Bolesnici s haplotipom *VKORC1*2* su trebali znatno niže doze varfarina od ostalih bolesnika. Kombinacija genotipa i kliničkih čimbenika objašnjava otprilike 50-60% razlike u potrebnim dozama varfarina kod bijelaca i Azijaca, ali samo 35-40% među Afroamerikancima. Rasne razlike u povezanosti genotipa i bolesnikova odgovora na liječenje varfarinom mogle bi biti rezultat razlika u učestalosti varijantnih alela *CYP2C9* i *VKORC1* i/ili pak utjecaja negenetičkih čimbenika.

Otpornost na varfarin

Otpornost na varfarin je rijetka pojava u ljudi, a povezuje se s nekoliko *missense* mutacija u genu *VKORC1*. Rezultati što su ih objavili Rost i suradnici govore u prilog tome da je gen za *VKORC1* glavna meta za spontane muta-

to the variation in warfarin dosage requirement must be considered in relation to the frequencies of the polymorphisms. Consequently, *VKORC1* is likely to contribute more than *CYP2C9* due to the markedly higher frequency of some tested variants (1173C<T, -1639G>A) as compared to the *CYP2C9*2* and *CYP2C9*3* variants. However, in individual patients, the *CYP2C9*2* and especially *CYP2C9*3* variant will be expected to markedly influence the warfarin dosage requirement (40,41). Compared with homozygous carriers of *CYP2C9*1*, patients homozygous for *CYP2C9*3* were estimated to need 3.3-times lower mean doses of warfarin to achieve the same INR, with *2 carriers and heterozygous patients in-between (42). Two studies analyzing the first few weeks of warfarin treatment showed the proportion of patients with INR values of more than 3 to be higher among carriers of the *CYP2C9*2* or **3* allele than in patients that were not carriers of these alleles (43,44). In a study of patients starting warfarin therapy, a major finding was that genetic variation in *VKORC1* but not in *CYP2C9* modulated the early response to warfarin (45). However, both the *VKORC1* haplotype and the *CYP2C9* genotype had a significant effect on the warfarin dose after the first 2 weeks. *VKORC1* genotype was found to determine up to 40% of individual coumarin dose requirement (4,5,33,46,47). In Caucasians, the 1173 C>T polymorphism was associated with lower warfarin dosage requirements (27): the mean warfarin dose was higher (6.2 mg/day) in patients with the VKORC1 1173 CC genotype than in those with the CT genotype (4.8 mg/day; P = 0.002) or TT genotype (3.5 mg/day; P < 0.001). Study results confirmed *VKORC1*2* to be the most important haplotype for warfarin dosage (33). Patients with *VKORC1*2* haplotype had more frequent visits to clinic than patients with *VKORC1*3* or **4* haplotypes, higher coefficient of variation of prothrombin time-INR and higher percentage of INR values outside the therapeutic interval than patients with *VKORC1*3* or **4* haplotypes. Also, there was a statistically significant difference in warfarin dose (P < 0.001) and R-warfarin plasma concentrations (P < 0.01) between *VKORC1*2* and *VKORC1*3* or **4* haplotypes. Patients with *VKORC1*2* haplotype required much lower warfarin doses than other patients. The combination of genotype and clinical factors explains approximately 50% to 60% of the variance in warfarin dose requirements in Caucasians and Asians, but only 25% to 40% in African Americans. Racial differences in the association between genotype and patient response to warfarin treatment may be caused by racial differences in the frequencies of the variant *CYP2C9* and *VKORC1* alleles and/or by the influence of non-genetic factors.

Warfarin resistance

Warfarin resistance is a relatively uncommon phenomenon in humans and has been related to several missense mutations in the *VKORC1* gene that have been discovered so far. Results obtained by Rost group corroborate the

cije koje dovode do otpornosti na varfarin (25,48). Drugi su potvrdili ove nalaze (26,37,49,50). *Missense* mutacije p.D36Y, p.V29L, p.A41S, p.V45A, p.R58G i p.V66M koje su odgovorne za otpornost na varfarin smještene su u očuvanoj luminalnoj petlji (L1) regije *VKORC1* (51). Za *VKORC1* kodirajući polimorfizam Asp36Tyr je utvrđeno da nositelji Asp36Tyr zahtijevaju značajno više doze varfarina od 80,9 ± 10,1 mg/tjedan u usporedbi s 42,7 ± 7,5 mg/tjedan kod osoba koje nisu nositelji Asp36Tyr (F = 9,79; P = 0,002) (52). Druga je skupina istraživača dokumentirala jedan irski slučaj prave otpornosti na varfarin kao rezultat mutacije u *VKORC1* (383 T>G tranzicija u eksonu 2) (53). No, mehanizam(me) kojim mutacije u genu *VKORC1* posreduju neosjetljivost na kumarine *in vivo* tek treba rasvijetliti.

CYP4F2 je oksidaza vitamina K(1) (VK1) i nositelji alela CYP4F2 V433M (*rs2108622*) imaju smanjenu sposobnost metaboliziranja VK1 kao posljedicu o *rs2108622* ovisnog smanjenja stabilne jetrene koncentracije ovoga enzima. Stoga će bolesnici s polimorfizmom *rs2108622* vjerojatno imati povišene razine VK1 u jetri, što zahtijeva više doze varfarina kako bi se postigao isti antikoagulacijski odgovor (54). Drugi istraživači također su potvrdili ulogu polimorfizma CYP4F2 V433M u farmakogenetici kumarinskih antikoagulansa (55,56).

Utjecaj genotipa *CYP2C9* i *VKORC1* na rizik od krvarenja

U Sjedinjenim Državama se godišnji rizik od većeg krvarenja u bolesnika na oralnim antikoagulansima procjenjuje na 1-5% (57,58). Kombinacija za kumarinske lijekove osjetljivih alela gena *VKORC1* i *CYP2C9* mogla bi snažno ukazivati na visok rizik od teške prekomjerne antikoagulacije (43). U bolesnika s alelima *CYP2C9*2* i **3* se prvo krvarenje pojavilo ranije i s višom učestalosti nego u bolesnika s izvornim genotipom (divljeg tipa) (59-61). U istraživanju provedenom među Amerikancima afričkog i europskog podrijetla (38) varijantni genotip *CYP2C9* doveo je do povećanog rizika za veća, ali ne i za manja krvarenja. Rizik za veće krvarenje bio je 5,3 puta viši prije stabiliziranja terapije, 2,2 puta viši nakon stabiliziranja, te 2,4 puta viši tijekom svih razdoblja kad antikoagulacija nije bila stabilna. Varijantni genotip *VKORC1* 1173C/T nije izazvao značajan porast rizika niti za veće niti za manje krvarenje. Kod nositelja bilo koje varijante *CYP2C9* ili *VKORC1* rizik od gastrointestinalnog krvarenja za vrijeme terapije acenokumarolom jako se povećao s izlaganjem tjednim dozama acenokumarola višim od 15 mg ili s primjenom amjodarona ili aspirina (62).

Iako se unošenjem podataka o genotipu poboljšava točnost u predviđanju terapije, dosad nije dokazano uvjerljivo poboljšanje u smanjenju komplikacija krvarenja.

VKORC1 gene as the main target for spontaneous mutations conferring warfarin resistance (25,48). Other researchers confirmed these findings (26,37,49,50). The missense mutations p.D36Y, p.V29L, p.A41S, p.V45A, p.R58G and p.V66M, responsible for warfarin resistance, are located in the conserved luminal loop (L1) *VKORC1* region (51). It was found for a coding *VKORC1* Asp36Tyr polymorphism that carriers of Asp36Tyr required significantly higher warfarin doses of 80.9 ± 10.1 mg/wk as compared with 42.7 ± 7.5 mg/wk in non-carriers (F = 9.79; P = 0.002) (52). Another group documented an Irish case of true warfarin resistance as the result of a mutation in *VKORC1* (383 T>G transition in exon 2) (53). However, the mechanism(s) of how mutations in the *VKORC1* gene mediate insensitivity to coumarins *in vivo* remains to be elucidated.

CYP4F2 is a vitamin K(1) (VK1) oxidase and carriers of the CYP4F2 V433M allele *(rs2108622*) have a reduced capacity to metabolize VK1, secondary to an *rs2108622*-dependent decrease in steady-state hepatic concentrations of the enzyme. Therefore, patients with the *rs2108622* polymorphism are likely to have elevated hepatic levels of VK1, necessitating a higher warfarin dose to elicit the same anticoagulant response (54). Other researchers have also confirmed the relevant role of CYP4F2 V433M polymorphism in the pharmacogenetics of coumarin anticoagulants (55,56).

Effect of *CYP2C9* and *VKORC1* genotype on hemorrhage risk

In the United States, the annual major bleeding risk in patients on oral anticoagulants is estimated to 1%-5% (57,58). A combination of the coumarin-sensitive alleles of both the *VKORC1* and *CYP2C9* genes may strongly indicate a high risk of severe overanticoagulation (43). Patients with the *CYP2C9*2* and **3* allele experienced the first bleeding event sooner and showed a higher bleeding rate than patients with the wild-type genotype (59-61). In a study conducted among African and European Americans (38), the variant *CYP2C9* genotype conferred an increased risk of major but not minor hemorrhage. The risk of major hemorrhage was 5.3-fold before therapy stabilization, 2.2-fold after therapy stabilization, and 2.4-fold during all periods when anticoagulation was not stable. The variant *VKORC1* 1173C/T genotype did not confer a significant increase in the risk of major or minor hemorrhage. The risk of gastrointestinal bleeding during acenocoumarol therapy in carriers of any of the *CYP2C9* or *VKORC1* variants was severely increased with exposure to weekly doses of acenocoumarol higher than 15 mg or the use of amiodarone or aspirin (62).

Although the incorporation of genotype information improves the accuracy of dose prediction, no convincing improvement has been demonstrated in the reduction of hemorrhagic complications.

Genomske studije udruženosti

Genomska studija udruženosti (engl. *genome-wide association study*, GWAS) za česte genetičke varijante s većim utjecajem na dozu održavanja varfarina, što su je proveli Cooper i suradnici na otprilike 550.000 ispitanih polimorfizama, utvrdila je da je najznačajniji neovisni učinak udružen s polimorfizmima *VKORC1* ($P = 6,2 \times 10^{-13}$) (63). *CYP2C9* (*CYP2C9*3* i *CYP2C9*2*) je bio udružen s dozom na razini umjerene značajnosti ($P = 10^{-4}$). Autori zaključuju kako je malo vjerojatno da će se česti SNP sa znatnim učinkom na dozu varfarina otkriti izvan gena *CYP2C9* i *VKORC1*. Takeuchi i sur. (64) su proveli prvu GWAS dovoljne snage za otkrivanje varijanata DNA sa skromnim utjecajem na dozu varfarina potrebnu za postizanje terapijske antikoagulacije. Univarijatnom analizom GWAS SNP oni su također identificirali iznimno jaku povezanost ($P = 10^{-78}$ do 10^{-13}) sa SNP u i blizu *VKORC1* i *CYP2C9*. Primjenom multivarijatne regresije prilagođene za poznate genetičke i negenetičke prediktore doze oni su također otkrili genomsku značajnost na razini $P < 8,3 \times 10^{-10}$ za *CYP4F2* (rs2108622), koja je činila otprilike 1,5% razlike u dozi. I drugi autori opisuju udruženost *CYP4F2* s razlikom u dozi (65).

Izgleda da je važnost genskog polimorfizma u drugim enzimima i strukturama uključenim u učinak antikoagulansa, kao što su gama-glutamilkarboksilaza, glutation-S-transferaza A1, mikrosomna epoksid hidrolaza i apolipoprotein E, zanemariva.

Algoritmi zasnovani na farmakogenetici

Algoritmi zasnovani na farmakogenetičkim podatcima predlažu se za doziranje oralnih antikoagulansa. Većina modela uzima u obzir dob, spol, površinu tijela, istodobno primijenjene lijekove, istodobne bolesti i kliničke indikacije. Jedna od najčešće navođenih jednadžba kojima se procjenjuje doza varfarina (mg/dan) je algoritam za doziranje utemeljen na regresijskom modelu:

$$[0,628 - 0,0135 \times \text{dob (god)} - 0,24 \times \text{CY2C9*2} - 0,37 \times \text{CYP2C9*3} - 0,241 \times \text{VKORC1} + 0,062 \times \text{visina (cm)}]^2,$$

gdje je iznos vrijednosti za genotip CYP2C9 0, 1 ili 2 za broj alela *1, *2 i *3 u bolesnikovom genotipu, a genotip *VKORC1* je 1 za GG, 2 za GA i 3 za AA.

Autori su našli R^2 od 54% (jednadžba je objasnila 54% varijabilnosti doze varfarina u izvedenoj skupini) (5). Nekoliko objavljenih algoritama za doziranje varfarina (Washington University, UCSF, Louisville i Newcastle) uspoređeno je prema točnosti predviđanja doze varfarina u retrospektivnoj analizi mjesne bolesničke populacije na dugotrajnoj stabilnoj terapiji varfarinom. Vrednovanje farmakogenetičkog ispitivanja odgovora na varfarin obuhvatilo je dokazivanje analitičke valjanosti ispitnih

Genome-wide association study

A genome-wide association study (GWAS) of common genetic variants with major effect on warfarin maintenance dose, performed by Cooper *et al*. from approximately 550,000 polymorphisms tested, found the most significant independent effect to be associated with *VKORC1* polymorphisms ($P = 6.2 \times 10^{-13}$) (63). *CYP2C9* (*CYP2C9*3* and *CYP2C9*2*) was associated with the dose at moderate significance levels ($P = 10^{-4}$). The authors conclude that common SNPs with large effects on warfarin dose are unlikely to be discovered outside the *CYP2C9* and *VKORC1* genes. Takeuchi *et al*. (64) conducted the first GWAS sufficiently powered to detect DNA variants with a modest influence on the warfarin dose needed to achieve therapeutic anticoagulation. On univariate analysis of GWAS SNPs they also identified extremely strong association signals ($P = 10^{-78}$ to 10^{-13}) at SNPs in and near *VKORC1* and *CYP2C9*. By applying multivariate regression adjusting for known genetic and nongenetic dose predictors, they also detected genome-wide significance of $P < 8.3 \times 10^{-10}$ at *CYP4F2* (rs2108622) that accounted for approximately 1.5% of the dose variance. *CYP4F2* association with dose variance has also been reported by other authors (65).

Genetic polymorphism in further enzymes and structures involved in the effect of anticoagulants such as gamma-glutamylcarboxylase, glutathione S-transferase A1, microsomal epoxide hydrolase and apolipoprotein E appear to be of negligible importance.

Pharmacogenetics-based algorithms

Algorithms based on pharmacogenetic data have been proposed for oral anticoagulant dosing. The majority of models accounted for age, sex, body surface area, concomitant medications, comorbidities and clinical indications. One of the most frequently cited equations that estimate warfarin dose (mg/day) was the dosing algorithm based on a regression model:

$$[0.628 - 0.0135 \times \text{age (years)} - 0.24 \times \text{CY2C9*2} - 0.37 \times \text{CYP2C9*3} - 0.241 \times \text{VKORC1} + 0.062 \times \text{height (cm)}]^2,$$

where CYP2C9 genotype is 0, 1, or 2 for the number of *1, *2 and *3 alleles within the patient's genotype and *VKORC1* genotype is 1 for GG, 2 for GA, and 3 for AA.

The authors found a R^2 of 54% (the equation explained 54% of the variability in the warfarin dose in the derivation cohort) (5). Several published warfarin-dosing algorithms (Washington University, UCSF, Louisville, and Newcastle) were compared for accuracy in predicting warfarin dose in a retrospective analysis of a local patient population on long-term, stable warfarin therapy. Validation of pharmacogenetic testing for warfarin responses

platforma i kliničke valjanosti ispitivanja (66). Gage i sur. su razvili i vrednovali farmakogenetički algoritam u izvedenoj skupini od 1.015 sudionika (67). Neovisni prediktori terapijske doze bili su sljedeći: polimorfizam *VKORC1* -1639/3673 G>A (-28% po alelu), tjelesna površina (engl. *body surface area*, BSA) (+11% po 0,25 m^2, *CYP2C9*3* (-33% po alelu), *CYP2C9*2* (-19% po alelu), dob (-7% po desetljeću), ciljni INR (+11% po 0,5 jediničnog porasta), uzimanje amjodarona (-22%), pušenje (+10%), rasa (-9%) i postojeća tromboza (+7%). Predložena farmakogenetička jednadžba objasnila je 53-54% varijabilnosti doze varfarina u izvedenoj i vrednovanoj (N = 292) skupini. Za usporedbu, klinička jednadžba objasnila je samo 17-22% varijabilnosti doze (P < 0,001). Kako bi se olakšala uporaba ovih farmakogenetičkih i kliničkih algoritama izrađena je neprofitna mrežna stranica: http://www.WarfarinDosing.org. Polimorfizmi *VKORC1* i/ili *CYP2C9* uvedeni su i u neke druge kliničke algoritme za doziranje te u prospektivne kliničke studije (68-72). U istraživanju što ga je proveo Međunarodni konzorcij za farmakogenetiku varfarina (73) upotrebljeni su podatci o 4,043 bolesnika u izradi algoritma za doziranje zasnovanog isključivo na kliničkim varijablama i drugog algoritma u kojem su uz kliničke varijable dodani i genetički podatci. Objavljeni su najnoviji podatci, a glavni zaključci su slijedeći: primjena farmakogenetičkog algoritma za procjenu odgovarajuće početne doze varfarina rezultira preporukama koje su značajno bliže potrebnoj stabilnoj terapijskoj dozi od onih izvedenih iz kliničkog algoritma ili metodom fiksne doze. Najveće koristi su zabilježene u 46,2% populacije koji su zahtijevali 21 mg ili manje varfarina na tjedan ili 49 mg ili više na tjedan za terapijsku antikoagulaciju.

Isplativost

Iako Radna skupina za farmakogenetičko testiranje alela *CYP2C9* i *VKORC1* za varfarin Američkog kolegija medicinske genetike zasad ne podupire rutinsku primjenu genotipiziranja za varfarin, ističu kako u nekim situacijama ispitivanje na *CYP2C9* i *VKORC1* može biti korisno i potrebno kako bi se utvrdio uzrok neuobičajenih terapijskih odgovora na liječenje varfarinom (74). Eckman i sur. (75) pak zaključuju kako je malo vjerojatno da bi genotipiziranje za varfarin bilo isplativo u bolesnika s nevalvularnom atrijskom fibrilacijom, ali bi moglo biti isplativo u bolesnika s visokim rizikom od krvarenja a koji započinju liječenje varfarinom. Lippi i sur. (76) ističu kako ograničenja genetičkog ispitivanja uključuju zasad uvelike nepoznat optimalan sastav ispitnih panela, podatke o interindividualnim razlikama, nedostatak analitičkih i kvalitativnih specifikacija, nedostatak sveobuhvatnih analiza ishoda koje bi omogućile procjenu isplativosti, nedostatak općeg suglasja o pouzdanim algoritmima doziranja, te druga etička i društvena pitanja. Rezultati što su ih Wadelius

included demonstration of analytical validity of testing platforms and of the clinical validity of testing (66). Gage *et al*. developed and validated a pharmacogenetic algorithm in the derivation cohort of 1015 participants (67). The independent predictors of therapeutic dose were: *VKORC1* polymorphism -1639/3673 G>A (-28% *per* allele), body surface area (BSA) (+11% *per* 0.25 m^2, *CYP2C9*3* (-33% *per* allele), *CYP2C9*2* (-19% *per* allele), age (-7% *per* decade), target INR (+11% *per* 0.5 unit increase), amiodarone use (-22%), smoker status (+10%), race (-9%), and current thrombosis (+7%). The pharmacogenetic equation proposed explained 53%-54% of the variability in the warfarin dose in the derivation and validation (N = 292) cohorts. For comparison, a clinical equation explained only 17%-22% of the dose variability (P < 0.001). To facilitate the use of these pharmacogenetic and clinical algorithms, a non-profit website has been developed (http://www.WarfarinDosing.org). *VKORC1* and/or *CYP2C9* polymorphisms have been introduced in several other clinical dosing algorithms and prospective clinical studies (68-72). In a study conducted by the International Warfarin Pharmacogenetics Consortium (73), data from 4.043 patients were used to create a dose algorithm that was based on clinical variables only and an algorithm in which genetic information was added to the clinical variables. Most recent data are published. The main conclusions were as follows: the use of a pharmacogenetic algorithm to estimate the appropriate initial dose of warfarin produces recommendations that are significantly closer to the required stable therapeutic dose than those derived from a clinical algorithm or a fixed-dose approach. The greatest benefits were observed in 46.2% of the population that required 21 mg or less of warfarin *per* week or 49 mg or more *per* week for therapeutic anticoagulation.

Cost-effectiveness

Although the American College of Medical Genetics Working Group on Pharmacogenetic Testing of *CYP2C9* and *VKORC1* alleles for warfarin use have not endorsed the routine use of warfarin genotyping at this time, they pointed that in certain situations, *CYP2C9* and *VKORC1* testing may be useful, and warranted, on determining the cause of unusual therapeutic responses to warfarin therapy (74). Eckman *et al*. (75) conclude that warfarin-related genotyping is unlikely to be cost-effective for typical patients with non-valvular atrial fibrillation, but may be cost-effective in patients at a high risk of hemorrhage that are starting warfarin therapy. Lippi *et al*. (76) point out that limitations of genetic testing include still largely unknown optimal composition of test panels, information concerning interindividual variability, lack of analytical and quality specifications, lack of comprehensive outcome analyses to enable assessment of cost-ef-

i sur. (77) dobili genotipiziranjem 183 polimorfizma u 29 gena kandidata kod 1,496 bolesnika iz Švedske koji su započinjali liječenje varfarinom i ispitali njihovu udruženost s ishodom govore snažno u prilog uvođenja terapije varfarinom na osnovu farmakogenetičke analize. Ostaje nejasno je li genotipski kontrolirano doziranje klinički korisno, međutim, u tijeku su studije koje će pomoći razjasniti ovo pitanje (78).

Zaključak

Na učinak oralnih antikoagulansa utječu genetički i okolišni čimbenici. Česte mutacije gena koji kodiraju citokrom P450 (CYP2C9) i vitamin K epoksid reduktazu s jednom ili više kombinacija njihovih polimorfizama odgovorne su za smanjene potrebne doze varfarina ili za otpornost na varfarin. Zapažene su rasne razlike u vezi između genotipa i bolesnikova odgovora na liječenje varfarinom. Genotipovi utječu na vrijeme potrebno da bi se postigla terapijska antikoagulacija, kao i na rizik od prekomjerne antikoagulacije i krvarenja. Iako uvođenje podataka o genotipu poboljšava točnost predviđanja doze, poboljšanje u kontroli antikoagulacije ili smanjenje komplikacija krvarenja tek treba dokazati u smislu učinkovitosti i isplativosti (79). Korist će vjerojatno imati osobe koje trebaju krajnje - granične doze varfarina, no sveukupnu kliničku korist od antikoagulacijskog liječenja prilagođenog genotipu u čitavoj bolesničkoj populaciji tek treba utvrditi u budućim prospektivnim kliničkim studijama.

fectiveness, lack of universal agreement related to reliable dosing algorithms, and other ethical and social issues. Results obtained by Wadelius group, which genotyped 183 polymorphisms in 29 candidate genes in 1.496 Swedish patients starting warfarin treatment, and tested for association with response, strongly support that warfarin initiation be guided by pharmacogenetics (77). Whether genotype-guided dosing is clinically beneficial remains unclear, but studies are currently underway that will help elucidate this issue (78).

Conclusions

The effect of oral anticoagulants is influenced by genetic and environmental factors. Common mutations in the genes coding for the cytochrome P450 (CYP2C9) and vitamin K epoxide reductase with one or more combinations of its polymorphisms are responsible for the reduced warfarin requirements or warfarin resistance. Racial differences in the association between genotype and patient response to warfarin treatment have been observed. Genotypes influence the time required to attain therapeutic anticoagulation and the risk of overanticoagulation and hemorrhage. Although the incorporation of genotype information improves the accuracy of dose prediction, an improvement in anticoagulation control or a reduction in hemorrhagic complications has yet to be demonstrated in terms of efficacy and cost-benefit (79). While individuals at the extremes of dose requirements are likely to benefit, the overall clinical merits of a genotype-adapted anticoagulant treatment regimen in the entire patient population remain to be determined in future prospective clinical studies.

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