Case Report

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Pharmacogenomics

Warfarin dose requirements in a patient with the *CYP2C9**14 allele

We describe a 64-year-old male of Indian descent with a history of atrial fibrillation who was started on warfarin after hospital admission for acute stroke. He received genotype-guided warfarin dosing as per the standard-of-care at our hospital, with daily dose recommendations provided by the pharmacogenetics service. Genotyping revealed the rare *CYP2C9**1/*14 genotype and warfarin insensitive *VKORC1* -1639GG and *CYP4F2* 433Met/Met genotypes. The patient received an initial warfarin loading dose of 4 mg for 2 days, followed by 2–3 mg/day for the following 11 days. He reached a therapeutic international normalized ratio on day 5, which was maintained over the following week. This report adds to the limited data of the effects of the *CYP2C9**14 allele on warfarin dose requirements.

Keywords: anticoagulation • *CYP2C9**14 • pharmacogenetics • *VKORC1* • warfarin

Background

Warfarin is commonly prescribed for the prevention and treatment of thromboembolism, but its dosing is complicated by a narrow therapeutic index and wide interindividual variability in anticoagulation response. Specifically, warfarin is dosed to achieve an international normalized ratio (INR) of 2–3 for most indications [1]. An INR >4 increases the risk of bleeding, while an INR <2 increases the risk for thromboembolism [2,3]. The dose required to achieve an INR of 2–3 varies considerably among patients [4].

The genes for *CYP2C9* and *VKORC1* influence warfarin pharmacokinetics and pharmacodynamics, respectively, and significantly contribute to warfarin dose requirements. Specifically, the *CYP2C9**2 (p.Arg144Cys) and *3 (p.Ile359Leu) alleles are the most commonly described *CYP2C9* variants and are associated with reduced enzyme activity, *S*-warfarin clearance and warfarin dose requirements [5,6]. The *VKORC1* c.-1639G>A polymorphism affects *VKORC1* expression and sensitivity to warfarin, with reduced expression, increased warfarin sensitivity and decreased warfarin

dose requirements in carriers with the AA genotype [7]. The gene for *CYP4F2*, which metabolizes vitamin K, provides lesser contribution to dose requirements. In particular, the *CYP4F2* p.Val433Met polymorphism leads to reduced *CYP4F2* activity, reduced vitamin K metabolism and higher warfarin dose requirements [8,9].

Dosing recommendations based on *CYP2C9**2, *CYP2C9**3 and *VKORC1* -1639G>A genotypes are now included in US FDA-approved warfarin labeling. The Clinical Pharmacogenetics Implementation Consortium (CPIC) published guidelines in 2011 strongly recommending genotype-guided warfarin dosing when genotype is available [10]. Starting in August 2012, genotyping became available at the University of Illinois (UI, IL, USA) Hospital to assist with warfarin dosing [11]. Genotyping is carried out at the UI Hospital clinical laboratory, using a commercially available platform (Genmark Dx platform with eSensor® technology, GenMark Diagnostics, Inc., CA, USA) that detects the *CYP2C9**2, *3, *5 (p.Asp360Glu), *6 (null allele), *11 (p.Arg335Trp), *14 (p.Arg125His), *15 (Ser-

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162null) and *16 (p.Thr299Ala); as well as *VKORC1* -1639G>A and *CYP4F2* p.Val433Met variants. A pharmacogenetics service provides genotype interpretation and daily warfarin dose recommendations until therapeutic anticoagulation is achieved. While the commercially available genotyping panel used includes a number of rare *CYP2C9* alleles, there are limited data on the effects of rare alleles on warfarin metabolism or dosing. Herein, we describe a patient with the *CYP2C9**14 allele and his INR response to initial warfarin doses. The patient described provided written, informed consent for reporting of his data, and the study was approved by the institution review board at the University of Illinois at Chicago.

Case report

A 64-year-old male originally from India suffered a left middle cerebral artery stroke and was transferred from an outside medical center to the UI Hospital for management. His past medical history was significant for hypertension, diabetes, cardiomyopathy (left ventricular ejection fraction of 15% by echocardiography) and nonvalvular atrial fibrillation. The patient was taking dabigatran for stroke prevention until 3 days prior to the stroke when dabigatran was held for a lithotripsy procedure with ureteral stent placement. Dabigatran had not been resumed at the time of the stroke.

On admission, the patient was taking digoxin, furosemide and propranolol. He weighed 55 kg (BMI of 21 kg/m²), with normal renal and liver function tests, and a baseline INR of 1.2. The patient was admitted to the neurosurgery service and started on intravenous heparin titrated to an activated partial thromboplastin time of 45–60 s while the source of the stroke was interrogated. The stroke was determined to be cardioembolic in origin, with no evidence of hemorrhagic conversion. A percutaneous endoscopy gastrostomy tube was placed on day 5, and then warfarin was started with an INR goal of 2–3, as recommended for stroke prevention in atrial fibrillation [1]. Warfarin was chosen over dabigatran because of the ease of reversing anticoagulant effects should hemorrhagic transformation occur.

Blood for genotyping was drawn on the morning of day 4 in anticipation of warfarin initiation after percutaneous endoscopy gastrostomy tube placement. Genotype was available later that day and revealed the rare *CYP2C9**1/*14 genotype in addition to the warfarin insensitive *VKORC1* -1639 GG and *CYP4F2* 433Met/Met genotypes. The pharmacogenetics service provided an initial warfarin dose recommendation of 4 mg for 2 days, with subsequent daily dose recommendations refined based on INR response. On day 5 of hospitalization, a CT scan ordered in response

to patient complaints of new right-sided weakness, revealed a new right-sided temporoparietal infarct, and heparin and warfarin were continued. His other medications during the warfarin dose titration period included atorvastatin, digoxin, prednisone at a dose of 40 mg daily, and ampicillin from days 3–12 for a urinary tract infection. All warfarin doses recommended by the pharmacogenetics service were ordered by the primary service and are shown in Figure 1, with daily INR values.

The patient achieved an INR in the target range on day 5 of warfarin dosing and was maintained in the target INR range for the following week with doses of 2–3 mg/day. The pharmacogenetics service signed off on day 7, recommending continued dosing at 2.5 mg/day with INR monitoring. The patient continued on warfarin during his hospitalization requiring an average daily warfarin dose of 2.9 ± 0.62 mg/day and a weight adjusted dose of 0.052 ± 0.011 mg/kg/day over the initial 13 days. His remaining hospital course was complicated by urosepsis secondary to the ureteral stent placement during his recent lithotripsy, requiring broad spectrum intravenous antibiotic therapy (day 16–33 of hospitalization). His INR rose to 3.6 on day 14, likely secondary to his infectious course, and warfarin was held. Warfarin was restarted on day 16, with his INR fluctuating from 1.4 to 3.3 thereafter, as shown in Figure 1. Warfarin was discontinued on day 23 prior to planned ureteral stent removal. Following the procedure, the patient was started on rivaroxaban for chronic secondary stroke prevention, with follow-up by the home-health service after discharge.

Discussion

The *CYP2C9**14 (rs72558189) allele results from a nonsynonymous SNP (c.374G>A, p.Arg125His) in exon 3 [12]. Its reported frequency is 0.003 in Europeans [13] and 0.002 to 0.004 in Asians [14,15]. *In vitro* studies show that the *CYP2C9**14 allele is associated with 80–90% lower catalytic activity toward tolbutamide and 8% of the wild-type activity against *S*-warfarin [6,16]. As such, it is anticipated that *CYP2C9**14 carriers will require lower warfarin doses, as described for the patient in this report.

Even though there are limited data on the clinical effects of the *CYP2C9**14 variant, it is included on commercially available genotyping platforms, such as the one used at our institution. Therefore, it is important to elucidate its effects on warfarin response in order to inform appropriate dosing when the allele is detected. In addition, there is evidence that accounting for both common and rare variants improves the accuracy of genotype-guided warfarin therapy [17]. This may reduce the risk for overdosing warfarin in patients

Figure 1. Warfarin doses and international normalized ratio values during the warfarin initiation period for a 64-year-old male with the *CYP2C9*1/*14* genotype. The patient reached the target INR range of 2–3 (shaded area) by day 5 and was maintained within range until the Warfarin Genetics Service signed off on his care on day 7.

INR: International normalized ratio.

with rare *CYP2C9* genotypes associated with reduced enzyme activity and dose requirements. The risk may be particularly high with variants such as *CYP2C9*14* that have substantial effects on enzyme activity toward warfarin [6].

The available data on the *CYP2C9*14* allele are from cohort studies where a single individual had the heterozygous genotype. Specifically, in a study of 125 warfarin-treated patients of Asian descent, the *CYP2C9*14* allele was identified in a single Indian male who required a weight-adjusted warfarin dose of 0.053 mg/kg/day [14]. However, neither *VKORC1* nor *CYP4F2* genotype information was provided to determine the effect of the *CYP2C9*14* allele in combination with other genotypes. A second cohort study of 265 warfarin-treated Koreans, revealed a single patient with the *CYP2C9*1/*14* genotype who required a warfarin dose of 2 mg/day to maintain an INR in the target range of 1.7–2.8 [15]. The patient

also had the *VKORC1* 1173TT genotype, which is in linkage disequilibrium with the -1639AA genotype and associated with high warfarin sensitivity and low warfarin dose requirements [18]. It is difficult to interpret the effects of the *CYP2C9*14* genotype on warfarin dosing in the context of the highly warfarin sensitive *VKORC1* genotype.

By contrast, the patient described herein had the *CYP2C9*1/*14* genotype together with the *VKORC1* -1639GG genotype, associated with reduced sensitivity and higher warfarin dose requirements. In addition, the patient had the *CYP4F2* 433Met/Met genotype associated with reduced metabolism of vitamin K [8]. Specifically, the 433Met/Met genotype is associated with approximately 1 mg/day higher dose requirements compared with the Val/Val genotype [9]. Despite this, after receiving warfarin 4 mg for the initial 2 days, he required doses between 2 and 3 mg/day to maintain therapeutic anticoagulation during the

initial week of therapy. His weight-adjusted average dose was similar to that reported by Zhao *et al.* [14]. Even these low doses led to supratherapeutic anticoagulation after the patient developed urosepsis. While we cannot comment on the effects of the *CYP2C9*14* allele on chronic maintenance dosing, as the patient discontinued warfarin before discharge, our report does provide evidence of low dose requirements with this allele during the warfarin initiation period. Hence, we believe that this case adds to the accumulating data on the implications of the *CYP2C9*14* allele for warfarin dosing. Genotyping was limited to the variants detected by the commercial platform used, and thus, we cannot rule out the presence of other *CYP2C9* variants, such as *4, *8, *13 or *35, which have been associated with reduced enzyme activity against warfarin [6,19–20]. Additionally, no plasma or urine samples were collected from the patient to confirm that the low dose requirements were secondary to reduced warfarin clearance. However, the lower dose requirements observed were consistent with previous *in vitro* pharmacokinetic data with the *CYP2C9*14* allele [6].

Several clinical factors, may have contributed to low warfarin dose requirements in this patient, including small body size and prednisone use. However, based on recommended warfarin pharmacogenetic dosing algorithms, which take into account both clinical factors and genotype, in the absence of the *CYP2C9*14* genotype, the patient's estimated warfarin maintenance dose would be 6 mg/day, a dose more than 50% higher than required by the patient in this case [21]. The pharmacist on the pharmacogenetics service empirically reduced the recommended warfarin dose by 35% to account for potential effects of the *CYP2C9*14* allele on *S*-warfarin metabolism. This proved to be appropriate in this particular patient case and serves as a useful guide for clinicians managing warfarin dosing for future patients with the *CYP2C9*14* allele.

Conclusion

In summary, this case report provides further evidence that the *CYP2C9*14* allele leads to lower warfarin dose requirements. Importantly, low doses were required despite the presence of the low sensitivity *VKORC1* and warfarin resistant *CYP4F2* genotypes. We believe that these data support the initiation of low warfarin doses to avoid overanticoagulation in patients found to carry the *CYP2C9*14* allele.

Future perspective

There is a paucity of data on the effects of rare *CYP2C9* variants on warfarin dose requirements. This is par-

ticularly true for variants occurring predominately in non-European populations. Current guidelines recommend genotype-guided warfarin dosing when genotype is available [10]. Results from a recent clinical trial in a homogeneous European population support this approach [22]. Another trial in a diverse patient population showed no benefit with genotype-guided dosing versus dosing based on clinical factors alone [23]. African-Americans, who comprised one-third of the population, actually did worse with genotype-guided versus clinically guided dosing. Genotyping for both trials was limited to the *CYP2C9*2* and *3 alleles and *VKORC1* -1639G>A genotype. These are the most important variants contributing to warfarin dose requirements in Europeans [24,25]. However, additional variants provide important effects in non-European populations. For example, over 15% of African-Americans have a *CYP2C9*5*, *6, *8 or *11 allele, which significantly reduce *S*-warfarin clearance and dose requirements [26–30]. By contrast, only about 6% of African-Americans have a *CYP2C9*2* or *3 allele. The *CYP2C9*5*, *6, *8 and *11 alleles were not detected in clinical trials, potentially leading to overdosing of warfarin in patients with one of these alleles and to the worse performance of genotype-guided versus clinical dosing observed in African-Americans. The clinical effectiveness of genotype-guided warfarin therapy in a diverse population remains unclear. However, appropriate evaluation of genotype-guided dosing in ethnically diverse populations is contingent on the use of well-informed genotyping that includes variants with significant effects on warfarin dosing across populations. This highlights the importance of uncovering the effects of variants that exist in populations beyond Europeans, which this report of warfarin dosing in a patient with the *CYP2C9*14* allele attempts to do. As institutions such as our own move toward genotype-guided warfarin dosing, it is now more important than ever to discern the effects of understudied genotypes on warfarin response to ensure optimally informed genotype-guided dosing. In the case of rare variants, such as *CYP2C9*14*, case reports or cases series will be the most likely source of data on associations with warfarin response.

Financial & competing interests disclosure

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the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained verbal and written informed consent from the patient described for the inclusion of his medical and treatment history within this case report.

Executive summary

Background

- Warfarin dosing is influenced by both genetic and clinical factors.
- The *CYP2C9* and *VKORC1* genes influence the pharmacokinetic and pharmacodynamics of warfarin, respectively, and serve as major genetic determinants of warfarin dose requirements.
- While the *CYP2C9**2 and *3 alleles are widely studied and well known to reduce warfarin dose requirements, much less is known about other *CYP2C9* alleles.

Case report

- We report a 64-year-old male of Indian descent with *CYP2C9**14 allele who achieved therapeutic anticoagulation with warfarin doses between 2 and 3 mg/day. He required a low warfarin dose despite also having the insensitive *VKORC1* -1639GG and *CYP4F2* 433Met/Met genotypes.

Discussion

- The *CYP2C9**14 (rs72558189) allele results from the p.Arg125His polymorphism in exon 3, with a reported frequency <0.01 in Europeans and Asians.
- *In vitro* studies show significant reductions in enzyme activity against S-warfarin with the *CYP2C9**14 allele.
- There are limited data on the effects of the *CYP2C9**14 allele on warfarin dosing, especially in patients with *VKORC1* and *CYP4F2* genotypes associated with higher warfarin dose requirements.

Conclusion

- These data support low warfarin dose requirements in patients with the *CYP2C9**14 allele.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

- Holbrook A, Schulman S, Witt DM *et al.* Evidence-based management of anticoagulant therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 141(2 Suppl.), e152S–e184S (2012).
- Hylek EM, Evans-Molina C, Shea C, Henault LE, Regan S. Major hemorrhage and tolerability of warfarin in the first year of therapy among elderly patients with atrial fibrillation. *Circulation* 115(21), 2689–2696 (2007).
- Hylek EM, Go AS, Chang Y *et al.* Effect of intensity of oral anticoagulation on stroke severity and mortality in atrial fibrillation. *N. Engl. J. Med.* 349(11), 1019–1026 (2003).
- Wadelius M, Chen LY, Lindh JD *et al.* The largest prospective warfarin-treated cohort supports genetic forecasting. *Blood* 113(4), 784–792 (2009).
- Scordo MG, Pengo V, Spina E, Dahl ML, Gusella M, Padriani R. Influence of *CYP2C9* and *CYP2C19* genetic polymorphisms on warfarin maintenance dose and metabolic clearance. *Clin. Pharmacol. Ther.* 72(6), 702–710 (2002).
- Niinuma Y, Saito T, Takahashi Met *et al.* Functional characterization of 32 *CYP2C9* allelic variants. *Pharmacogenomics J.* 14(2), 107–114 (2014).
- Examined the *in vitro* activity of *CYP2C9* variants towards warfarin and other drugs.
- Rieder MJ, Reiner AP, Gage BF *et al.* Effect of *VKORC1* haplotypes on transcriptional regulation and warfarin dose. *N. Engl. J. Med.* 352(22), 2285–2293 (2005).
- McDonald MG, Rieder MJ, Nakano M, Hsia CH, Rettie AE. *CYP4F2* is a vitamin K1 oxidase: an explanation for altered warfarin dose in carriers of the V433M variant. *Mol. Pharmacol.* 75(6), 1337–1346 (2009).
- Caldwell MD, Awad T, Johnson JA *et al.* *CYP4F2* genetic variant alters required warfarin dose. *Blood* 111(8), 4106–4112 (2008).
- Johnson JA, Gong L, Whirl-Carrillo M *et al.* Clinical pharmacogenetics implementation consortium guidelines for *CYP2C9* and *VKORC1* genotypes and warfarin dosing. *Clin. Pharmacol. Ther.* 90(4), 625–629 (2011).
- Provides recommendations for interpretation and application of genotype information for warfarin dosing.
- Nutescu EA, Drozda K, Bress AP *et al.* Feasibility of implementing a comprehensive warfarin pharmacogenetics service. *Pharmacotherapy* 33(11), 1156–1164 (2013).
- CYP2C9* allele nomenclature. www.cypalleles.ki.se/cyp2c9.htm
- Veenstra DL, Blough DK, Higashi MK *et al.* *CYP2C9* haplotype structure in European American warfarin patients and association with clinical outcomes. *Clin. Pharmacol. Ther.* 77(5), 353–364 (2005).
- Zhao F, Loke C, Rankin SC *et al.* Novel *CYP2C9* genetic variants in Asian subjects and their influence on maintenance warfarin dose. *Clin. Pharmacol. Ther.* 76(3), 210–219 (2004).
- Examined novel *CYP2C9* genetic variants among Asian patients and reported the effect of the *CYP2C9**14 allele on the maintenance dose of warfarin in an Indian patient.
- Kim HS, Lee SS, Oh M *et al.* Effect of *CYP2C9* and *VKORC1* genotypes on early-phase and steady-state warfarin

- dosing in Korean patients with mechanical heart valve replacement. *Pharmacogenet. Genomics* 19(2), 103–112 (2009).
- **Reported an association between the *CYP2C9*14* allele and warfarin dose requirements in a Korean patient in the context of the *CYP2C9* and *VKORC1* genotypes.**
- 16 DeLozier TC, Lee SC, Coulter SJ, Goh BC, Goldstein JA. Functional characterization of novel allelic variants of *CYP2C9* recently discovered in southeast Asians. *J. Pharmacol. Exp. Ther.* 315(3), 1085–1090 (2005).
- 17 Cavallari LH, Langaee TY, Momary KM *et al.* Genetic and clinical predictors of warfarin dose requirements in African Americans. *Clin. Pharmacol. Ther.* 87(4), 459–464 (2010).
- 18 Wang D, Chen H, Momary KM, Cavallari LH, Johnson JA, Sadee W. Regulatory polymorphism in vitamin K epoxide reductase complex subunit 1 (*VKORC1*) affects gene expression and warfarin dose requirement. *Blood* 112(4), 1013–1021 (2008).
- 19 Lee MY, Borgiani P, Johansson I *et al.* High warfarin sensitivity in carriers of *CYP2C9*35* is determined by the impaired interaction with P450 oxidoreductase. *Pharmacogenomics J.* doi:10.1038/tpj.2013.41 (2013) (Epub ahead of print).
- 20 Ciccacci C, Falconi M, Paolillo N *et al.* Characterization of a novel *CYP2C9* gene mutation and structural bioinformatic protein analysis in a warfarin hypersensitive patient. *Pharmacogenet. Genomics*. 21(6), 344–346 (2011).
- 21 Gage BF, Eby C, Johnson JA *et al.* Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. *Clin. Pharmacol. Ther.* 84(3), 326–331 (2008).
- 22 Pirmohamed M, Burnside G, Eriksson N *et al.* A randomized trial of genotype-guided dosing of warfarin. *N. Engl. J. Med.* 369(24), 2294–2303 (2013).
- **Prospective, multicenter trial conducted in the UK and Sweden that compared the impact of genotype-guided versus standard warfarin dosing on anticoagulation control over 12 weeks. The investigators found that genotype-guided dosing resulted in a greater proportion of time spent in the therapeutic range compared with standard dosing in a European population.**
- 23 Kimmel SE, French B, Kasner SE *et al.* A pharmacogenetic versus a clinical algorithm for warfarin dosing. *N. Engl. J. Med.* 369(24), 2283–2293 (2013).
- **Prospective, multicenter trial conducted in the USA comparing the effect of genotype-guided versus clinically guided dosing of warfarin on anticoagulation control over 4 weeks. The study showed no significant difference between dosing strategies in a diverse population in which 27% of participants were African–American.**
- 24 Cooper GM, Johnson JA, Langaee TY *et al.* A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. *Blood* 112(4), 1022–1027 (2008).
- 25 Teichert M, Eijgelsheim M, Rivadeneira F *et al.* A genome-wide association study of acenocoumarol maintenance dosage. *Hum. Mol. Genet.* 18(19), 3758–3768 (2009).
- 26 Perera MA, Gamazon E, Cavallari LH *et al.* The missing association: sequencing-based discovery of novel SNPs in *VKORC1* and *CYP2C9* that affect warfarin dose in African Americans. *Clin. Pharmacol. Ther.* 89(3), 408–415 (2011).
- 27 Dickmann LJ, Rettie AE, Kneller MB *et al.* Identification and functional characterization of a new *CYP2C9* variant (*CYP2C9*5*) expressed among African Americans. *Mol. Pharmacol.* 60(2), 382–387 (2001).
- 28 Redman AR, Dickmann LJ, Kidd RS, Goldstein JA, Ritchie DM, Hon YY. *CYP2C9* genetic polymorphisms and warfarin. *Clin. Appl. Thromb. Hemost.* 10(2), 149–154 (2004).
- 29 Liu Y, Jeong H, Takahashi H *et al.* Decreased warfarin clearance associated with the *CYP2C9* R150H (*8) polymorphism. *Clin. Pharmacol. Ther.* 91(4), 660–665 (2012).
- 30 Tai G, Farin F, Rieder MJ *et al.* *In-vitro* and *in-vivo* effects of the *CYP2C9*11* polymorphism on warfarin metabolism and dose. *Pharmacogenet. Genomics* 15(7), 475–481 (2005).