Warfarin-induced life-threatening bleeding associated with a CYP3A4 loss-of-function mutation in an acute limb ischemia patient: Case report and review of the literature

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Abstract. Patients with acute limb ischemia, deep venous thrombosis and pulmonary artery embolism may be treated with warfarin. The dose-response interaction of warfarin is associated with numerous factors, depending on which an uncommon life-threatening bleeding may occur. The present case study reported on a patient with acute limb ischemia and a history of warfarin-induced bleeding ten years previously and who again developed life threatening bleeding associated with warfarin treatment and received vascular surgery. In this patient, a cytochrome P450 3A4 loss-of-function mutation decreased the effective dose of warfarin. Although this was a rare case, clinicians should be alert to the bleeding risk associated with such rare genetic mutations.

Introduction

Warfarin therapy effectively reduces ischemic stroke and mortality amongst patients with various types of thromboembolism, such as deep venous thrombosis, as well as heart valve prosthesis, atrial fibrillation and stroke (1).

It is the most frequently used oral anti-coagulant. Bleeding is an important adverse drug response (ADR) of anti-coagulants due to its narrow therapeutic index and the wide variability in drug responses among individuals.

To date, the warfarin dose response has been associated with race, environmental, clinical status, and particularly genetic factors (2). Warfarin exerts its anti-coagulant effect by antagonizing vitamin K epoxide reductase complex (VKORC1), thereby reducing the activation of vitamin K-dependent clotting factors II, VII, IX and X. A series of genetic variations related with the pharmacodynamics and pharmacokinetics of warfarin have been reported, such as VKORC1, cytochrome P450 (CYP) 2C9 and CYP4F2 (3,4).

Only few reports on warfarin-induced side effects associated with CYP3A4 mutations are currently available, and the present study reported on warfarin-induced major bleeding in a patient with acute limb ischemia (ALI) associated with a CYP3A4 loss-of-function mutation, which may promote the development of warfarin research.

Case report

The present study was approved by the institutional review board (CWO) of Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. Patients provided written informed consent. A 60-year-old man was admitted to the vascular surgery department due to ALI in the left lower extremity in May 2015. On reviewing his medical history, it was revealed that the patient's blood pressure, blood glucose, cholesterol, triglyceride, homocysteine, erythrocyte sedimentation rate, anti-thrombin-III as well as protein C and S were within normal ranges. His life style was healthy and he had never smoked. He had neither atrial fibrillation nor a ventricular aneurysm. According to the angiography results, his left profunda femoris was completely occluded, and his distal superficial femoral artery (SFA) to popliteal artery (PA) was filled with thrombus. A 4F/30 cm Uni-Fuse™ Thrombolytic catheter (AngioDynamics, Queensbury, NY, USA) was inserted and the patient was intra-arterially injected 1 million units urokinase by exact pump over the next 24 h. One day later, his SFA-PA-peroneal artery track was visualized and the thrombus was basically cleared. The occluded proximal anterior tibial artery (ATA) and strait distal superficial femoral artery were then re-canalized by implanting a XIENCE-PRIME 3.5x38 mm (Abbott Pharmaceutical Co. Ltd., Lake Bluff, IL, USA) and an Everflex 7x120 mm (Covidien/Medtronic, Dublin, Ireland). With the ATA and peroneal artery serving as below-the-knee (BTK) outflow tracks, his ischemic symptoms totally disappeared and the

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left ankle-brachial index was recovered from 0.43 to 1.14. The patient was discharged after 6 days and was prescribed warfarin (2.5 mg per day) and clopidogrel (75 mg per day).

Unexpectedly, at 9 days after discharge, the patient presented to the emergency room with a sudden onset of hemoptysis. Laboratory analysis revealed that the international normalized ratio (INR) of the prothrombin time was 5.02 R. After intravenous infusion of 40 mg vitamin K1, his INR was decreased to 1.06R (reference value: 0.8-1.5). The patient's prescription was therefore modified by eliminating warfarin and retaining clopidogrel when he was discharged 24 h later. The patient's clinical characteristics on second admission are listed in Table I.

Fifty days later, the patient presented with another left ALI with typical 6P symptoms. The angiogram revealed that a thrombus had formed in the upper part of the left SFA, which was more proximal to BTK arteries than the previous time. Mechanical thrombectomy using an Angioget system (Boston Scientific Corp., Marlborough, MA, USA) and traditional balloon angioplasty to re-establish the SFA-PA-ATA/peroneal artery tracks. The patient was prescribed aspirin (100 mg per day) and cilostazol (100 mg per day) to inhibit platelet aggregation, as well as rivaroxaban (10 mg per day) to neutralize coagulation factor X. To date, the patient has remained in remission. The treatment of the patient is illustrated in a flow diagram in Fig. 1.

The genetic information was also analyzed. DNA extraction was performed using a QIAamp DNA Blood Mini kit (Syngen, Inc., Sacramento, CA, USA) according to the manufacturer's protocol. The concentration of isolated DNA was measured using a NanoDrop spectrophotometer (NanoDrop; Thermo Fisher Scientific, Inc., Pittsburgh, PA, USA) according to the manufacturer's protocol. The associated genetic variation in the pharmacogenomics knowledge base (https://www.pharmgkb.org/) was obtained by whole-genome sequencing (Illumina Hiseq2500; Illumina Inc. San Diego, CA, USA; sequencing depth, x100). Rare genetic variations [minor allele frequency (MAF) <1%] were analyzed in this patient. There was no rare mutation in CYP2C9 or VKORC1, but a splicing mutation in CYP3A4 (rs55808838; MAF=0.04%) was identified by the Sanger method (Fig. 2).

Table I. Patient characteristics at the time-point of second admission (sudden onset of hemoptysis after warfarin + clopidogrel treatment for 9 days).

Variable	Result	Reference value
Ethnicity	Chinese-Han	
Sex	Male	
Height	175 cm	
Weight	64 kg	
Fever	No	
Smoking	No	
Alcohol consumption	No	
Diabetes mellitus	No	
Hypertension	No	
Hepatic disease	No	
Chronic kidney disease	No	
Protein C/S deficiency	No	
Age (years)	60	
Alanine aminotransferase (IU/l)	19.5	13-69
Aspartate aminotransferase (U/l)	26.0	15-46
Blood urea nitrogen (μmol/l)	2.80	2.5-7.1
Creatinine (μmol/l)	51.0	53-115
White blood cells (x10^9/l)	8.99	3.97-9.15
Platelets (x10^9/l)	176	85-303

Discussion

As is known, the dose-response association of warfarin is influenced by several factors, including ethnicity, environmental factors, drug interactions, clinical status and genetic factors. With regard to bleeding complications, as many aspects as possible were considered for the present case:

i) Clinical characteristics. It has been reported that abnormal kidney/liver function and several complications, such as fever, hypertension and diabetes mellitus affect the effective dose of warfarin (5-7). Recently, the white blood cell count was also found to affect inter-patient variations in the response to warfarin (8).

ii) Drug interactions. A multitude of drugs have been associated with the effective dose of warfarin, such as amiodarone, fluconazole and antibiotics, according to the warfarin product monograph. The patient of the present study was only prescribed clopidogrel (75 mg/day) besides warfarin. To the best of our knowledge, there is no evidence that clopidogrel affects the dose of warfarin and it was not a major factor associated with the bleeding.

iii) Vitamin K supplementation. Another common concern regarding the use of warfarin is the interaction with food rich in vitamin K (9). It has been reported that vitamin K intake was responsible for ~3% of dosage variations in Chinese patients (10), while certain studies found a negative correlation with vitamin K intake (11). The available evidence does not support the modification of dietary vitamin K intake when starting therapy with warfarin. The patient of the present study was on a regular Chinese diet and it was not the major factor associated with the bleeding.

iv) Genetic factors. Given that the clinicopathological findings were associated with genetic factors, written informed consent was obtained from the patient to perform genetic testing. Five genetic variations associated with a required increase in the warfarin dose, namely CYP4F2, γ glutamyl carboxylase, protease, serine 53 and NAD(P)H quinone dehydrogenase 1 were identified, besides the common mutation in VKORC1 (rs7294) in Chinese individuals (Table II) (12-17).

The patient's warfarin dose was adjusted using five built-in pharmacogenetics-based warfarin dosing algorithms for the Chinese population and one by the International Warfarin

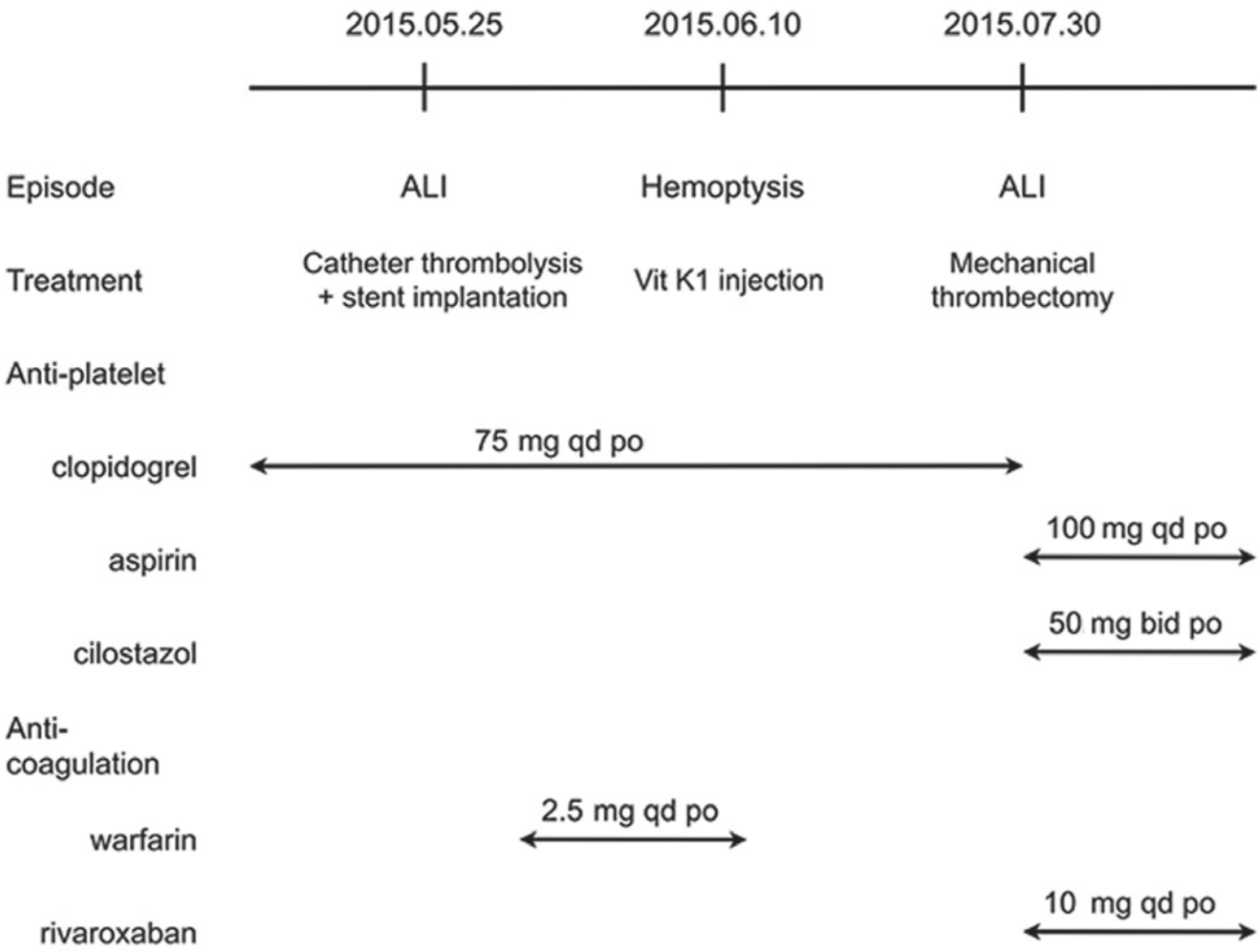

Figure 1. Schematic illustrating the treatment flow of the patient. ALI, acute limb ischemia; po, per os; bid, twice daily; qd, once a day; Vit, vitamin.

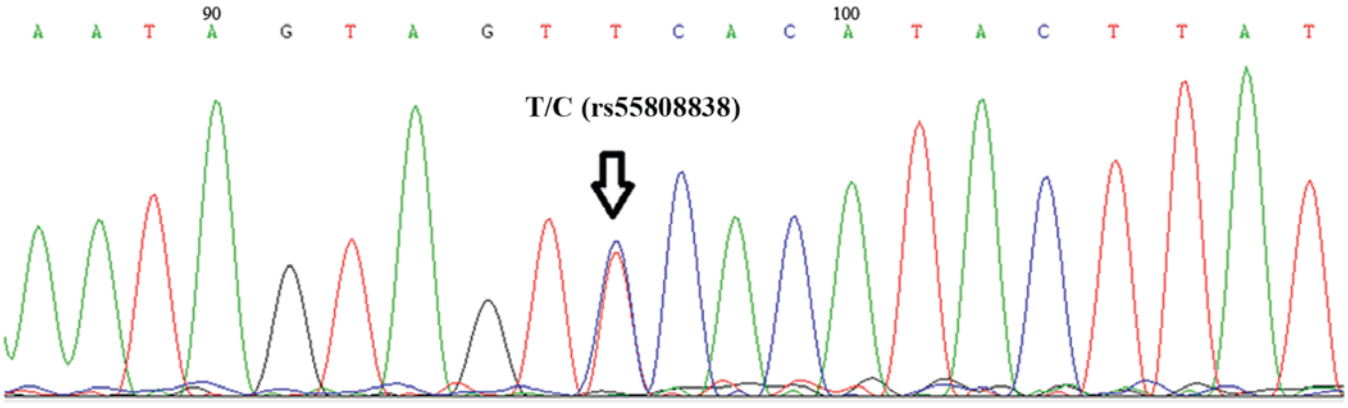

Figure 2. rs55808838 mutation of cytochrome P450 3A4 identified by the Sanger method.

Pharmacogenetics Consortium algorithm, as the equations based on Caucasian populations may not be suitable for predicting the dose of warfarin in Chinese patients (Table III).

Even if no other genetic variations were included in the dosing algorithms, not all of the dosing algorithms listed may be suitable for predicting the dose of warfarin in the present Chinese patient. In addition, the patient had a similar response to warfarin treatment ~10 years previously.

Compared to the normal Chinese population, the patient was unusually sensitive to warfarin. It was suggested that he had a rare genetic mutation leading to the high sensitivity to warfarin, as no other decrease-dose factors were found in this patient.

To the best of our knowledge, the present study was the first to report on warfarin-induced life threatening bleeding associated with a CYP3A4 loss-of-function mutation. S-warfarin

Table II. Genetic variations.

Gene	Single nucleotide polymorphism	Evidence level	Genotype	Effect on dose
CYP2C9	rs1799853	1A	CC	
CYP2C9	rs1057910	1A	AA	
VKORC1	rs9923231	1A	AA	Decrease
VKORC1	rs7294	1B	CC	
VKORC1	rs9934438	1B	GG	
CYP4F2	rs2108622	1B	CT	Increase
CYP2C9	rs7900194	2A	GG	
CYP2C9	rs4917639	2A	AA	
CYP2C9	rs56165452	2A	TT	
CYP2C9	rs28371686	2A	CC	
VKORC1	rs2359612	2A	AA	
VKORC1	rs8050894	2A	CC	
VKORC1	rs17708472	2A	GG	
VKORC1	rs2884737	2A	AA	
VKORC1	rs61742245	2A	CC	
CALU	rs339097	2B	AA	
CALU	rs12777823	2B	GG	
CALU	rs7196161	2B	GG	
NR1I3	rs2501873	3	CC	
EPHX1	rs1877724	3	CC	
GGCX	rs2592551	3	GA	Increase
GGCX	rs699664	3	CT	Increase
	rs12714145	3	CC	
CYP2C9	rs9332096	3	CC	
CYP2C9	rs7089580	3	AA	
CYP2C9	rs9332131	3	AA	
CYP2C9	rs10509680	3	GG	
CYP2C9	rs28371685	3	CC	
CYP2C9	rs1057910	3	AA	
STX4	rs10871454	3	CC	
PRSS53	rs11150606	4	CC	Increase
VKORC1	rs7200749	4	GG	
PRSS53, VKORC1	rs17886199	4	AA	
VKORC1	rs9934438	4	GG	
VKORC1	rs17880887	4	GG	
VKORC1	rs61162043	4	AA	
NQO1	rs10517	4	AA	
NQO1	rs1800566	4	GA	Increase
	rs2189784	4	GG	
THBD	rs1042580	4	TT	
HNF4A	rs3212198	4		
VKORC1	rs104894542	4	AA	
	rs104894541		TT	
VKORC1	rs104894540	4	AA	
VKORC1	rs104894539	4	CC	

CYP, cytochrome P450; VKORC1, vitamin K epoxide reductase complex; CALU, calumenin; NR1I3, nuclear receptor subfamily 1 group I member 3; EPHX1, epoxide hydrolase 1; GGCX, γ glutamyl carboxylase; STX4, syntaxin 4; PRSS53, protease, Serine 53; NQO1, NAD(P)H quinone dehydrogenase 1; THBD, thrombomodulin; HNF4a, hepatocyte nuclear factor 4α.

Table III. Six published pharmacogenetics-based warfarin dosing algorithms.

Author/(Ref.), year	Ethnicity	Pharmacogenetics-based warfarin algorithm	Calculated dose (mg/day)
IWPC; Klein *et al* (12), 2009	Mixed	√Weekly dose = 5.6044-0.2614 (age in decades) +0.0087 (height in cm) +0.0128 (weight in kg) -0.8677 (VKORC1 A/G) -1.6974 (VKORC1 A/A)-0.9357 (CYP2C9*1/*3) -0.1092 (Asian)[a]	3
Miao *et al* (13), 2007	Chinese	Daily dose = 6.22-0.011 (age in years) +0.017 (weight in kg) -0.775 (CYP*3)-3.397 (VKORC1 x 1) -4.803 (VKORC1 x 2)[a]	2.02
Huang *et al* (14), 2009	Chinese	Daily dose = exp [0.727-0.007 (age in years)+0.384 (BSA in square meters)+0.403 (VKORC1 6484TC) -0.482 (CYP2C9*1/*3)-1.583 (CYP2C9*3/*3)]	2.67
Zhong *et al* (15), 2012	Chinese	√Daily dose = 1.68143-0.0029 (age, years) +0.30784 (BSA, m^2) -0.2633 (VKORC1 g.3588G.A) -0.19114 (CYP2C9*3) +0.14735(CYP4F2 c.1297G>A) -0.1797 (amiodarone) -0.4138 (fluconazole) -(0.1888) diltiazem	2.79
Tan *et al* (16), 2012	Chinese	√Daily dose = 2.140-0.370 (VKORC1-1639 G>A)-0.332 (CYP2C9*3)+0.324 (BSA, m^2)-0.004 (age, years) -0.231 (no. of INR-increasing drugs) +0.105 (smoking habit) -0.135 (pre-operative stroke history) -0.108 (hypertension)	2.99
Chen *et al* (17), 2014	Chinese	Daily dose = 0.135+1.7816 (rs7294) -1.2146 (rs1057910) +1.2886 (BSA, m^2) -0.0196 (age, years) +0.7086 (target INR) +0.1596 (rs2108622) +0.3736 (diabetes mellitus) -0.5816 (amiodarone) -0.2526 (digoxin)	2.84

[a]Genotyped for VKORC1-1639G>A (rs9923231) and CYP2C9*3 (rs1057910). IWPC, international Warfarin Pharmacogenetics Consortium; INR, international normalized ratio; BSA, body surface area.

is metabolized via CYP3A4 and the splicing mutation of CYP3A4 may therefore affect the effective dose of warfarin.

Only few studies have previously assessed CYP3A4 polymorphisms (18,19), and to the best of our knowledge, the rs55808838 polymorphism has not been previously reported. These previous studies on pharmacogenetic polymorphisms reported that other CYP3A4 polymorphisms may be associated with drug-induced thrombosis. CYP3A4*1G is the most frequent mutant allele of CYP3A4 in Asians and may be associated with a lower rate of clopidogrel resistance, which was, however, not the case in the patient of the present study.

The major limitation of the present study was that the effect of this rare mutation on the effect of warfarin in a single case was hard to verify. Therefore, as many aspects of this case as possible were reviewed, such as the patient's clinicopathological characteristics, drug interactions and vitamin K supplementation. All known genetic variations were considered and included into the pharmacogenetics-based warfarin dosing algorithms. However, all not all factors reviewed explain for the sensitivity to the warfarin in this patient. However, it is likely that this rare genetic mutation of CYP3A4 was associated with the recurrent warfarin-induced bleeding.

The present case report illustrated the importance of considering genetic mutations for assessing unusual warfarin-induced bleeding and presented the methods used for this. Although this was a rare case, clinicians should be alert to the bleeding risk associated with such rare genetic mutations.

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