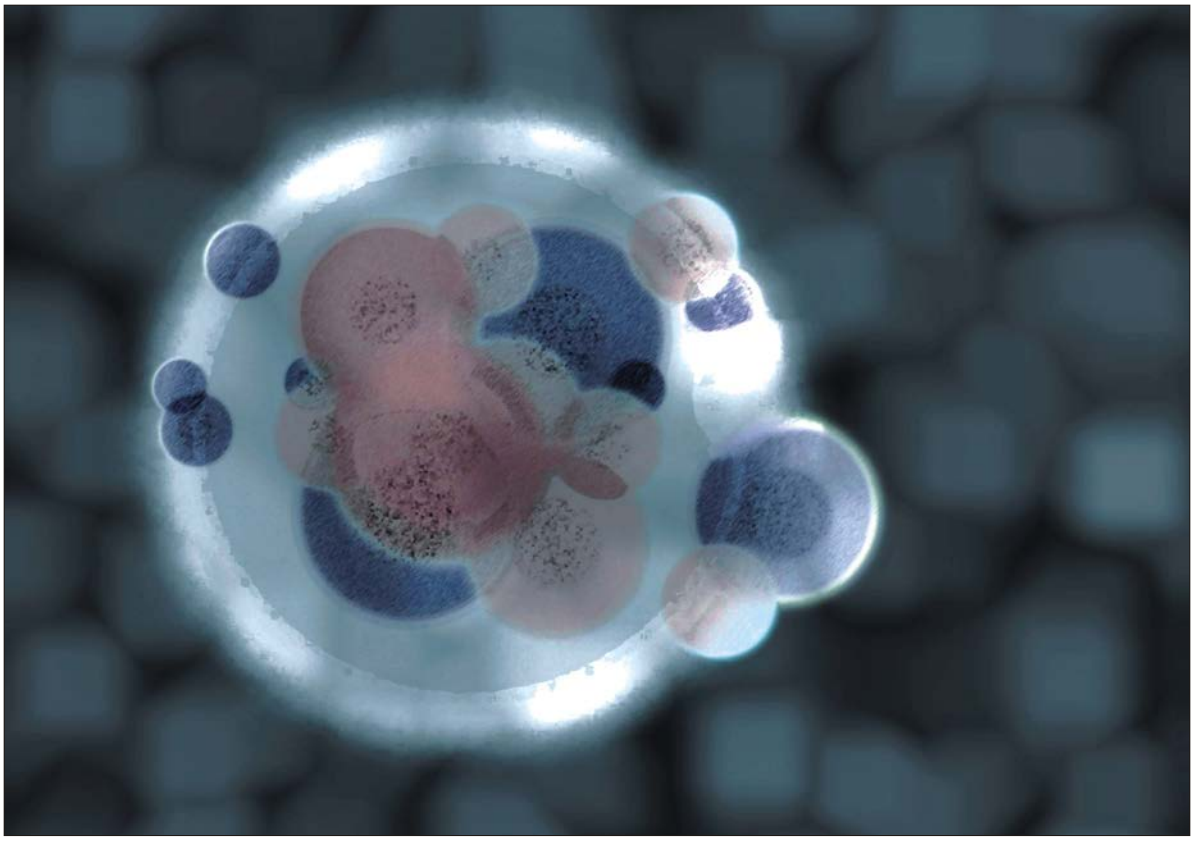

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# *VKORC1* Gene Analysis in an Iranian Warfarin Resistant Patient

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**Abstract:** The vitamin K epoxide reductase subunit 1 (VKORC1) has been identified recently. It is a component of the enzyme vitamin K epoxide reductase that is the therapeutic target site of warfarin. In order to investigate the relationship between *VKORC1* gene and warfarin dose response, we studied this gene in an Iranian warfarin resistant patient who receive more than 100 mg warfarin per day. The results showed that although warfarin concentration in his plasma was extremely higher than therapeutic level (22.8 mg $L^{-1}$) but no mutation(s) found in the exons of *VKORC1* gene. Other genes may be contributed in resistance to warfarin in this patient.

**Key words:** Warfarin, warfarin resistance, *VKORC1*

## INTRODUCTION

Warfarin is the most commonly used oral anticoagulant drug for the prevention and treatment of thromboembolic events (Landefeld and Beyth, 1993). The required dose of this drug is variable, in particular between individuals but also in any patient and depends on several factors.

The therapeutic response to warfarin is influenced by pharmacokinetic, pharmacodynamic, genetic variability (Loebstein *et al*., 2001; Meyer, 2000) so prediction of warfarin dose response in patients is difficult and the bleeding risk could be significantly high. Physicians are very interested in developing strategies in order to predict warfarin dose before prescription.

Anticoagulant effect of warfarin is exerted by inhibiting the vitamin k epoxide reductase enzyme complex (VKOR) which exists in vitamin K cycle (Stafford, 2005). Before the year 2004, glutathione S-transfrase has been identified as a component of VKOR that is responsible for resistance to warfarin in animal models (Cain *et al*., 1998). However, this proposal remains valid but requires additional experimental verification. Another component of VKOR termed VKORC1 has now been identified and 6 heterozygous missense mutations found in patients suffering from warfarin resistance, shown that it is the most probable target site of warfarin both in rat and human (Rost *et al*., 2004; Li *et al*., 2004; Harrington *et al*., 2005; Bodin *et al*., 2005).

The objective of the present study was to evaluate the relationship between *VKORC1* genotype and warfarin dose requirement in an Iranian patient who is resistant to warfarin.

## MATERIALS AND METHODS

A 56 years old man with rheumatic heart disease and three prosthetic valves was referred to us from the anticoagulation clinic of Imam Hospital (Tehran, Iran). The patient was firstly operated on 1974 for replacement of mitral and tricuspid valves. In the year 1992, he had re-do operation because of tricuspid prosthetic valve malfunction and aortic valve disease and these two valves replaced by prosthetic valves. The mitral prosthetic valve had no problem and remained as before.

He had a borderline blood glucose level and positive HBs antigen but otherwise nearly normal lab tests. He was taking the usual cardiac drugs and no any special drug with significant effect on warfarin metabolism.

This patient has been receiving more than 100 mg warfarin per day for several years (normal amount is 5-10 mg warfarin per day). The INR of this patient was just hardly in the therapeutic range with this amount of drug.

DNA was extracted from peripheral blood leukocytes using DNG™-plus solution (fermentase). DNA sample was amplified by polymerase chain reaction (PCR) in a final volume of 100 μL, consists of 0.5 μM from each primer, 0.2 mM deoxynucleoside triphosphate (dNTP), 1 μg

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Table 1: Primers used for amplification and sequencing of the *VKORC1* gene

| Exons | Primer sequences | |
|---|---|---|
| Exon 1 | F | 5′ GGCGGAACCTGGAGATAATG 3′ |
| | R | 5′ CCGATCCCAGACTCCAGAAT 3′ |
| Exon 2 | F | 5′ CCAAGGCACTGGGTTGAC 3′ |
| | R | 5′ GAGTGGGGCTGAGCTGAC 3′ |
| Exon 3 | F | 5′ TCTTCTGCCTTGCAGGTTG 3′ |
| | R | 5′ ACCCAGATATGCCCCCTTAG 3′ |

genomic DNA, 2.5 U Taq polymerase (fermentas) in 1x PCR buffer. The PCR were done in 35 cycles that in each one denaturation occurred 45 sec in 93°C, annealing occurred 30 sec in 58°C for exon one and two and 56°C for exon three and elongation occurred 90 sec in 72°C. Primer sequences are shown in Table 1. Then amplified DNA fragments of all three exons were sequenced to find any mutation(s).

In the parallel study assaying of warfarin concentration using HPLC method in his plasma was performed (Sadrai *et al*., 2008) and showed a very high concentration (22.8 mg $L^{-1}$) of plasma warfarin level, which is very higher than therapeutic warfarin level in Iranian patients ($1.19 \pm 0.3$ mg $L^{-1}$) that is determined by Andalibi *et al*. (1998).

## RESULTS AND DISCUSSION

The coagulation assay of this patient showed the following results:

Pt = 24 sec (normal 11-13), Aptt = 36 sec (normal 33-37), Factor II = 36%, Factor VII = 35%, Factor IX = 50%, Factor V and VIII = 100%, INR = three, that are consistent with moderate decrease in function of vitamin K dependent coagulation factors, excluding the possibility of non cooperation in drug ingestion. Also very high concentration of warfarin plasma level could exclude a significant impairment of bioavailability and intestinal absorption of the drug. Therefore, this patient was warfarin resistant.

However, the results of DNA extraction and sequencing showed a predicted normal sequence of all three exons and exclude these exons as the site of mutation and warfarin resistance in our patient. Our result is in contradict of the results of the some previous studies (Rost *et al*., 2004; Li *et al*., 2004; Harrington *et al*., 2005; Bodin *et al*., 2005) that reported VKORC1 as the site of mutation for warfarin resistance. So other genes than VKORC1 may be responsible for warfarin resistance in such patient that should be surveyed later.

## CONCLUSION

*VKORC1* gene is not the only gene that is responsible for resistance to warfarin in individuals, because in some warfarin resistant patients this gene is normal. More studies should be done about the other genes that are important for pharmacodynamics and pharmacokinetics of warfarin.

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