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Hypersensitivity to Acenocoumarol Revealing a Homozygous Mutation for *VKORC1* - 1639 G > A and *VKORC1* 1173 C > T and Heterozygous for *CYP2C9* * 2 and *CYP2C9* * 3

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ABSTRACT

*The initiation of treatment with acenocoumarol is a critical phase that can lead to an iatrogenic event in some patients carrying polymorphisms of the genes involved in the response to treatment, notably VKORC1 and CYP2C9. We report the first case in Morocco and Africa of hypersensitivity to acenocoumarol at the initiation dose in a 70-year-old patient who required an extremely low maintenance dose (0.25 mg / day) to achieve INR target. Genotyping results showed that the patient was homozygous mutated for (VKORC1) -1639 G> A and (VKORC1) -11173 C> T and heterozygous for (CYP2C9) * 2 and (CYP2C9) * 3 demonstrating extreme sensitivity to acenocoumarol due to the cumulative effect of these genetic polymorphisms on the maintenance dose of the anticoagulant. Our study shows the major benefit of prospective genotyping of CYP2C9 and VKORC1 prior to initiation of acenocoumarol treatment, as a good predictor of extreme susceptibility to VKA.*

Keywords: Hypersensitivity, Acenocoumarol, *VKORC1*, *CYP2C9*

INTRODUCTION

Vitamin K antagonists (VKA) are the most prescribed oral anticoagulants for the prevention and treatment of thromboembolic diseases and acenocoumarol is the only coumarin derivative available in Morocco. These drugs are responsible for severe hemorrhages, firstly because of their narrow therapeutic margin, and secondly because of the large inter-individual and intra-individual variability of the response to treatment [1,2].

This variability can be explained by demographic, clinico-biological, therapeutic, and genetic factors identified over the past ten years. The polymorphisms of two genes related to the metabolism of VKA, cytochrome P450 2C9 "*CYP2C9*" and their pharmacological target, vitamin K epoxide reductase "*VKORC1*", are associated with hypersensitivity to VKA. Indeed, the combination of *CYP2C9* and *VKORC1* polymorphisms allows identifying approximately 50% of the individual variability in acenocoumarol dose requirements and response to treatment [3]. Predictive models of acenocoumarol dose requirements based on these parameters have been proposed.

We report the first case in Morocco and Africa of hypersensitivity to acenocoumarol revealing homozygous mutation of the two variants of *VKORC1* (*VKORC1*-1639 G > A and *VKORC1* 1173 C > T) and heterozygous *CYP2C9* mutation (*CYP2C9* *2/*3).

CASE DESCRIPTION

A 70 year-old-male patient, followed for a dystrophic aortic insufficiency requiring a valve replacement and complicated by atrioventricular block leading to the replacement of a single-chamber pace maker. He was treated with acenocoumarol (Sintrom® 4 mg) at a dose of 1 mg/day. The patient had mild bleeding disorders in the form of bruises, one week after the start of treatment. The hemostasis assessment showed that the International Normalized Ratio (INR) was 10, requiring stopping acenocoumarol treatment and vitamin K until normalization of the INR.

The relay was taken by the mini Sintrom® 1 mg at a dose of 0.25 mg/day with satisfactory controls of the INR (Figure 1).

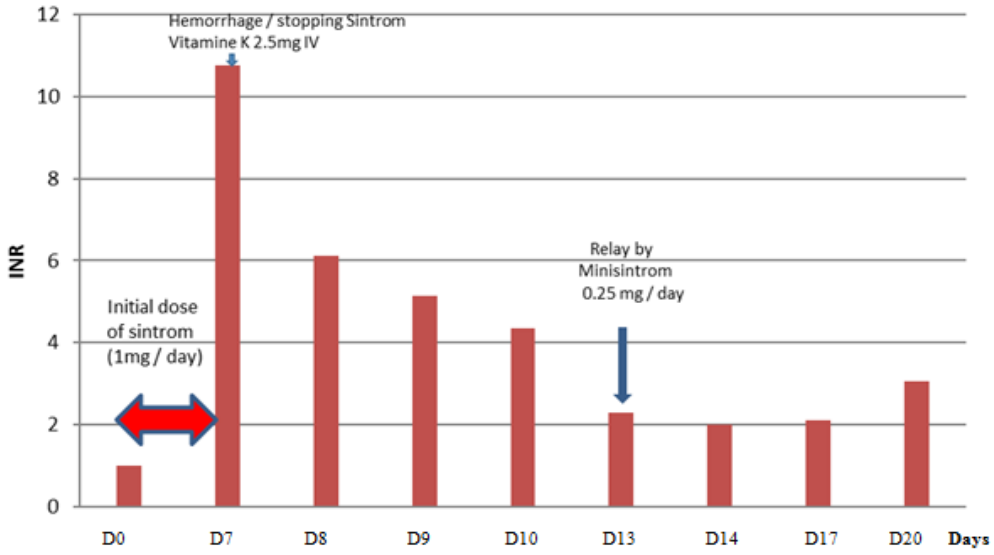

Figure 1 INR variability and clinically significant events in our patient

Genotyping was performed for detecting the polymorphisms of the genes involved in the metabolism of acenocoumarol. This genetic analysis was carried out at the Medical Biotechnology Laboratory of the Faculty of Medicine and Pharmacy of Rabat on 4 ml of blood sample collected in EDTA tube for DNA extraction. DNA was extracted from the blood sample by the Qiagen DNA extraction kit according to the manufacturer's protocol and then frozen at -20°C until genotyping. The DNA sample was genotyped for *CYP2C9* * 2 (rs1799853), * 3 (rs1057910), *VKORC1*-1639 G > A (rs9923231) and *VKORC1* 1174 C > T by PCR RFLP (Tables 1 and 2).

Table 1 Primers used for amplification of *VKORC1* (1639G > A), *VKORC1* (1173C > T, *CYP2C9* * 2 (430C > T) and *CYP2C9* * 3 (1075A > C)

Genetic polymorphism	Primer sequences
VKORC1 (1639 G>A)	F5'GAGCCAGCAGGAGAGGGAAATAT 3'
	R-5'GTTTGGACTACAGGTGCCTGCC 3'
VKORC1 (1173 C>T)	F-5'CTAAGATGAAAAGCAGGGCCTAC3'
	R-5'CTGCCCGAGAAAGGTGATTTCC3'
*CYP2C9*2 (430 C>T)	F-5'TCCTAGTTTCGTTTCTCTTCCTGT3'
	R-5'ATAGTAGTCCAGTAAGGTCAGTGA3
*CYP2C9*3 (1075 A>C)	F-5'CACGAGGTCCAGAGATGCATTG3'
	R-5'CTTCGAAAACATGGAGTTGCAGT3'

Table 2 PCR RFLP conditions for genotyping of *VKORC1* (1639G> A), *VKORC1* (1173C> T, *CYP2C9* * 2 (430C> T) and *CYP2C9* * 3 (1075A> C)

Genetic polymorphism	Condition PCR		PCR Products size	Restriction enzyme (RE)	RE digestion product size
VKORC1 (1639 G>A)	95°C/5 min		291 bp	Msp I	WT-167 + 124 bp Htz-291 + 167 + 124 bp Mut-291 bp
VKORC1 (1173 C>T)	95°C/30 sec 68°C/30 sec 72°C/30 sec	X32	201 bp	Sty I	WT-127 + 74 bp Htz-201 + 127 + 74 bp Mut-201 bp
*CYP2C9*2* (430 C>T)	72°C/5 min		221 bp	Ava II	WT-122 + 99 bp Htz-221 + 122 + 99 bp Mut-221 bp
*CYP2C9*3* (1075 A>C)			135 bp	Nsi I	WT-116 + 19 bp Htz-135 + 116 + 19 bp Mut-135 bp

RESULTS

Genotyping revealed heterozygosity for the *CYP2C9* * 2 (* 1/* 2) and *CYP2C9* * 3 (* 1/* 3) genes and a double mutation homozygous for the *VKORC1* 1639 (AA) and *VKORC1* 1173 (*TT*) genes (Figure 2).

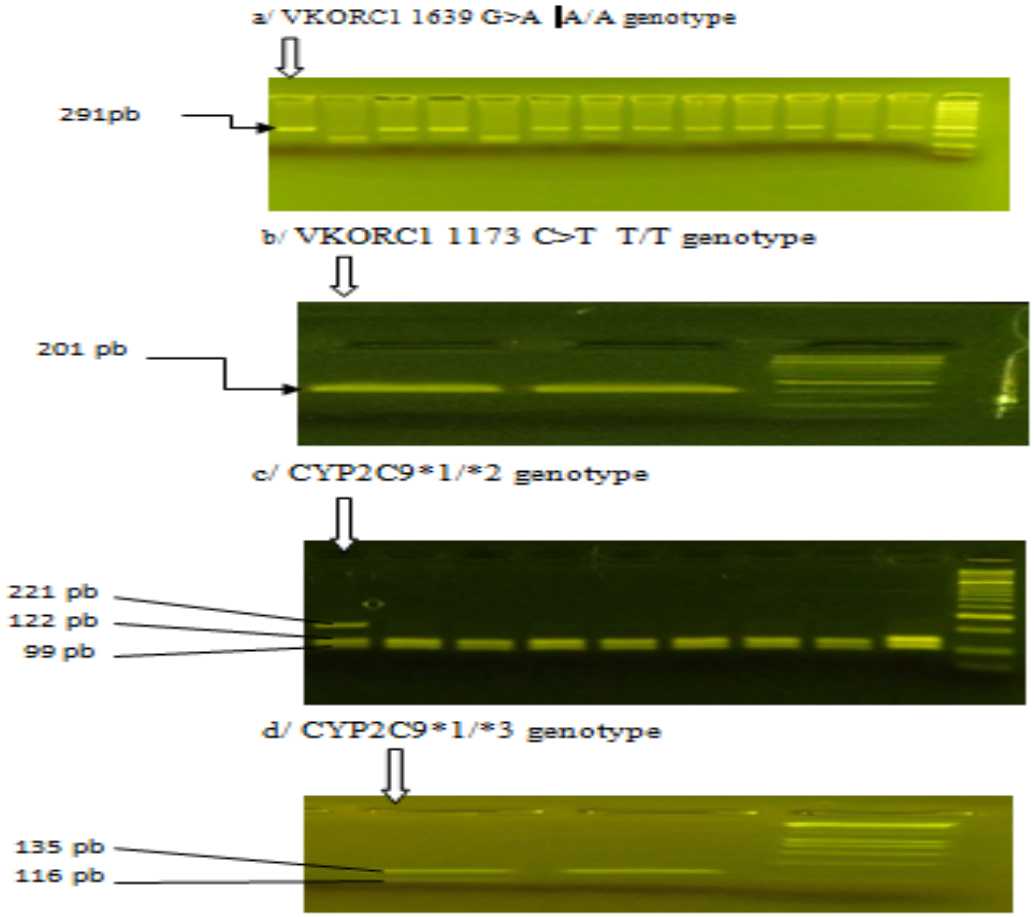

Figure 2 Representative gel picture and chromatogram showing *VKORC1*1173 C>T, *VKORC1* 1639 G>A, *CYP2C92 and *CYP2C9**3 genotypes**

a/Lane 1 – Patient genotype: Homozygous mutant for *VKORC1*1639 (291pb)

b/Lane 1 – Patient genotype: Homozygous mutant for *VKORC1* 1173 (201pb)

c/Lane 1 – Patient genotype: Heterozygous for *CYP2C9* * 2;(221 + 122 + 99 pb)

d/Lane 1 – Patient genotype: Heterozygous for *CYP2C9* * 3(135 + 116 + 19pb)

DISCUSSION

VKA are characterized by an important inter-individual and intra-individual variability in response to treatment. This variability is largely explained by the polymorphism of two genes: the one coding for vitamin K epoxide reductase: *VKORC1*, which is the pharmacological target of VKA, and the other coding for cytochrome P450, 2C9: *CYP2C9*, responsible for the metabolism of coumarin derivatives [4,5].

The polymorphism within the *VKORC1* gene located in the promoter at position 1639G> A (in complete linkage disequilibrium with 1173C> T polymorphism in intron 1) is associated with a significant decrease in the expression of the *VKORC1* enzyme and an increased risk of bleeding in patients treated with VKA [5,6].

The gene coding for cytochrome *CYP2C9* has polymorphisms that affect its activity. The *CYP2C9* * 1 allele corresponds to the wild form and constitutes the reference sequence, the two main allelic variants are *CYP2C9* * 2 (Arg144Cys) and *CYP2C9* * 3 (Ile359Leu). These polymorphisms of *CYP2C9* are associated with a decrease in enzyme activity, hence the need to use lower doses of VKA to reach the INR target [7,8] (Figure 3).

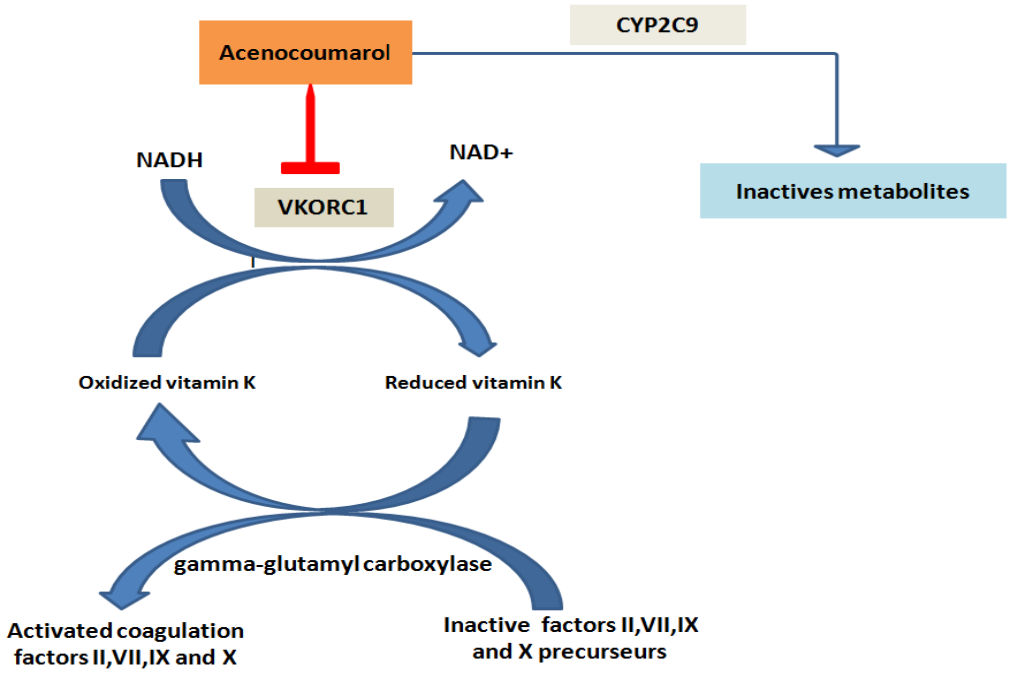

Figure 3 Vitamin K cycle and mode of action of acenocoumarol adopted by Fabien Lamoureux and Thomas Duflotab [9].

The combination of *VKORC1* and *CYP2C9* polymorphisms has a cumulative effect. In fact, individuals with at least two polymorphisms are exposed to the risk of overdose and therefore to an increased risk of bleeding, requiring lower doses of VKA compared to wild subjects or carriers of a single polymorphism, or an average reduction of 50% in the initial dose [10-12].

The combination of *VKORC1* 1639 A/A, *VKORC1* 1173 T/T and *CYP2C9* * 2/* 3 genotypes is extremely rare. Antoaneta Dimitrova-Karamfilova reported two similar cases in two Bulgarian patients, the first case was that of a 46-year-old woman with an optimal dosage of 0.25 mg/day, and the second was that of a 60-year-old man with optimal dosage of 1 mg/day [13]. In our case, the initial dosing of acenocoumarol was reduced to 75%, or 0.25 mg/day to reach the steady-state acenocoumarol dosing. The optimal dose of our patient is same to that of the Bulgarian patient.

Bodin showed that the patient's weight and the presence of a *CYP2C9* polymorphism account for 19% of the variability of the acenocoumarol response, and that the polymorphism of the *VKORC1* gene represents 37% of the variability

[3]. Markatos has shown that age, polymorphism of *VKORC1* and *CYP2C9* are responsible for 55% of the variability of the acenocoumarol dosage, and that the presence of the *CYP2C9* * 2/* 3 haplotype requires a 56% decrease in the dose, with increased risk of hemorrhagic stroke.

He also reported that patients with *VKORC1* 1639 A/A homozygous require lower doses less than 63% compared to patients with *VKORC1* 1639 G/G [14]. Similar percentages were found in an Austro-German (52%) [15], Serbianne (62%) [15] and Lebanese (50%) population [16,17]. Reitsma showed that Dutch patients with the *VKORC1* 1173 T/T polymorphism required a dose of less than 47% compared to wild-type (1173 C/C) patients [18]. Algorithms for predicting an appropriate acenocoumarol dosage based on pharmacogenetic, demographic, physiopathological and therapeutic factors have been proposed.

We have collected in the literature ten dose prediction algorithms for acenocoumarol: the first published is that of Markatos in 2008 [14], followed by van Schie in 2011 [19], and Borobia in 2012 [20], Cerezo-Manchado in 2013 [21], Krishna Kumar in 2013 [22], Pop in 2013 [23], Rathore in 2012 [24], Wolkanin-Bartnik in 2013 [25], Enrique Jinénez-varo in 2014 [26] and finally that of Hoi Y. Tong published in 2016 [2]. We selected five models that perfectly match to our data in order to calculate the optimal dose of our patient. However, these algorithms are specific to each population which explains the difference in dose found in Table 3.

Table 3 Selected algorithms and calculated doses for our patient

Algorithm references	Predicted daily maintenance dose (mg/day)	Actual daily maintenance dose (mg/day)
Markatos, et al. [13]	0.748	0.25
van Schie, et al. [18]	0.897	
Krishna Kumar, et al. [21]	0.538	
POP, et al. [22]	0.772	

Among these five algorithms, that of Krishna Kumar provides the closest value to the actual dose of our patient (0.538 mg/day).

CONCLUSION

The current debate thus remains open on the interest of systematic genotyping of patients before initiation of VKA treatment in order to identify patients at high risk of bleeding demonstrating hypersensitivity [27,28]. By genotyping *VKORC1*, *CYP2C9* and CYP4F2, and based on demographic and clinical factors, the acenocoumarol stable dosage could be determined a priori, which would avoid hemorrhagic stroke.

Several individual dosing algorithms for acenocoumarol maintenance dosage have been published. These mathematical models can be implemented in practice to predict the appropriate dose of VKA. However, the effectiveness of these models remains uncertain and lacks arguments [29].

DECLARATIONS

Acknowledgement

The presented study has been carried with interdisciplinary assistance of all authors.

Conflict of Interest

The authors have disclosed no potential conflicts of interest, financial or otherwise.

Ethical Considerations

This genetic study was carried out after ethical approval from the ethics committee of the Faculty of Medicine and Pharmacy of Rabat and after informed and signed consent of the patient.

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