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Full Length Research Paper

VKORC1 haplotype diversity in the admixed Omani population: Significant presence of atypical haplotypes

Pathare A. V.[1]*, Alzadjali S.[1], Misquith R.[1], Alkindi S.[2], Sahaya P.[3], Paldi A.,[4] and Krishnamoorthy R.[3]

[1]Department of Hematology, Sultan Qaboos University Hospital, Muscat, Oman.
[2]College of Medicine and Health Sciences, Muscat, Oman.
[3]INSERM, UMR_S 665, Inserm/Université Paris Diderot, Paris, France.
[4]GENETHON, 1bis rue de l'Internationale, BP60, 91002 Evry, France.

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There are significant differences in the performances of dosing algorithms between Caucasians, African Americans and Oriental populations owing to differences in the prevalence of genetic polymorphisms in enzymes involved in the pharmacokinetic and pharmacodynamic pathway, although other non-genetic factors do also contribute. The purpose of this work was to assess the vitamin K-epoxide reductase complex unit 1 (*VKORC1*) haplotypes in the Omani population for predicting specificity of warfarin dose response. We studied the pattern of five single nucleotide polymorphisms (SNPs) (rs 9923231; rs 9934438; rs 2884737; rs 17708472 and rs 7294) that define the *VKORC1* haplotypes in healthy adult Omani subjects using a PCR-based targeted genomic DNA sequencing. The observed frequencies for *VKORC1*1, *2,*3,*4* haplotypes were 0.08, 0.28, 0.29, 0.14 respectively. Four different novel haplotypes were found, two of which were present at a frequency above 3% in the Omani subjects. This is the first study to establish the *VKORC1* haplotypes in Omanis. The predicted prevalence of warfarin sensitive *VKORC1*2* haplotype was 27.8%, whereas it was 29.4 and 14.4% respectively for the haplotypes *3 and *4. The significant presence of *VKORC*1*1 haplotype (8%) in Omanis, (otherwise quite rare in Caucasians and Asians) can be traced back to their ancestral African admixture.

Key words: Pharmacogenetics, *VKORC1*, Omani, allele, haplotype.

INTRODUCTION

There are significant differences in the performances of dosing algorithms between Caucasians, African Americans and Oriental population (Klein et al., 2009; Perera et al., 2011; Pathare et al., 2012a). These are believed to be due to significant differences in the prevalence of functional polymorphisms in the genes encoding key enzymes, namely, the vitamin K epoxide reductase complex subunit 1 (*VKORC1*) and cytochrome P450 2C9 and 4F2 (*CYP2C9, CYP4F*2). *VKORC1* is an enzyme essential for the post-translational gamma-carboxylation of vitamin K-dependent clotting factors (Rost et al., 2004; Berkner et al., 2004).

*Corresponding author. E-mail: avp16@hotmail.com.

The pharmacodynamic effect of warfarin is influenced by the genetic polymorphisms of the *VKORC1* locus (Takahashi and Echizen, 2003; Rieder et al., 2005). Several non-randomly linked polymorphic sequence changes in the coding and non-coding regions of the *VKORC1* gene define the *VKORC1* haplotype and are shown to be associated with variable response to warfarin treatment in different population groups (Rieder et al., 2005; D'Andrea et al., 2005; Geisen et al., 2005). On the basis of warfarin dose requirement for reaching steady state target international normalized ratio (INR) values, Reider et al. (2005) classified the patients into low warfarin dose-requiring, group A (haplotypes H1 and 2) and high dose-requiring Group B haplotypes (haplotypes H7 to 9). D'Andrea et al. (2005) reported that the mean therapeutic warfarin requirement in patients homozygous for the wild type allele dbSNP:

rs 9934438:g.1173CC (SNP in Group B) was significantly higher than in those with the TT variant (SNP in Group A). Geisen et al. (2005) described and characterized *VKORC1**2, *3 and *4 haplotypes which account for 99% of the genetic variability in the Caucasian population from Europe and have shown that the *VKORC1**2 haplotype was associated with warfarin sensitivity (which comprises the rs 9934438T variant) while the others with variable degree of warfarin resistance. Spreafico et al. (2008), highlighted the importance of testing not only the *VKORC*1*2 - defined rs9934438 T allele but also the *VKORC*1*3 and *VKORC*1*4 (defined respectively by rs7294 A allele and rs17708472 T allele) polymorphisms to identify patients with the extreme phenotypes especially in the initial phase of acenocoumarol (a warfarin derivative) therapy.

The purpose of this study is to determine, the *VKORC1* haplotype structure and its prevalence in the Omani population. The present day Omani population is a genetic admixture with gene flow from Caucasian, Asian and African population. Given the fact that there are significant differences in the performances of warfarin dosing algorithms between these three major population groups which correlate with the relative prevalence of polymorphisms/haplotypes in warfarin dose-influencing genes including the *VKORC1* locus, it is important to characterize the genetic structure of this locus in order to understand the specificity of this admixed population with respect to warfarin pharmacogenetics.

MATERIALS AND METHODS

Subjects and samples

The study was initiated after obtaining formal approval from the institutional ethics committee. Study subjects were unrelated healthy adult blood donors attending the blood bank at the Sultan Qaboos University Hospital, Muscat, Oman. A written informed consent was obtained from each participant as per our study protocol. A total of 157 subjects (67 males and 90 females) agreed to participate. Their mean age was 49.76 (± 17.65 SD) years.

A 5 ml blood sample was collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Genomic deoxyribonucleic acid (DNA) was isolated using the semi-automated ABI PRISM™ 6100 nucleic acid prep station, [Applied Biosystems, Foster city, CA, USA] and samples were stored at -20°C pending analysis. All the DNA polymorphisms were studied by direct sequencing of the relevant polymerase chain reaction (PCR)- amplified genome segment on ABI PRISM™ 3100 genetic analyzer (Applied Biosystems, Foster city, CA, USA) using home-designed primers.[available on request].

DNA segments encompassing the relevant *VKORC1* gene fragments were amplified by PCR and the PCR products were submitted for DNA sequencing to explore the following single nucleotide polymorphisms (SNPs): rs 9923231 (3673G>A or -1639G>A); rs 2884737 (5808T>G or +497T>G); rs 17708472 (6009 C>T or +698 C>T); rs 9934438 (6484 C>T or +1173 C>T) and rs 7294 (9041G>A or +3730G>A) using a sequencing protocol provided by the manufacturer of the commercial kit (Applied Biosystems, Foster city, CA, USA). Using the aforementioned SNPs, haplotypes were inferred by the PHASE program (version2.0) (Stephens and Donnelly, 2003).

Statistical analysis

Frequency distribution of the *VKORC1* polymorphisms was compared by Chi square and Fishers exact test. A p value of < 0.05 was considered as statistically significant. The observed allele frequencies were used in Hardy-Weinberg's equation for analyzing the degree of deviation, if any, between the observed and expected genotype frequencies by using weighted least square estimates of allele frequencies and chi-square goodness-of-fit tests. All statistical analyses were carried out using SPSS software (ver.15).

RESULTS

The prevalence of inferred *VKORC1* *1,*2,*3,*4, and atypical haplotypes and genotypes are described in Table 1. There was a complete linkage disequilibrium between the SNP, -1639 G>A in the *VKORC1* promoter and the SNP +1173 C>T in the intron 1 and hence only -1639G>A position was considered in further analysis. The frequency of *VKORC1*2 /*2* genotype (associated with warfarin sensitivity in various populations) was 8.9%. The results obtained in this study were compared with compiled data reported for Caucasian, African and Chinese populations (Geisen et al., 2005; Spreafico et al., 2008; Scott et al., 2010).

DISCUSSION

Although it is well known that single-nucleotide polymorphism (SNP's) in *CYP2C9, CYP4F2* and *VKORC*1 loci have impact on the warfarin dose response, polymorphisms of the *VKORC*1 seems to be the most important among them by the high prevalence of functionally relevant polymorphisms that influence the warfarin dose requirement (Rieder et al., 2005; D'Andrea et al., 2005; Geisen et al., 2005; Limdi et al., 2008; Spreafico et al., 2008; McDonald et al., 2009).

This study, documents the frequencies of *VKORC1* haplotypes in healthy adult Omani subjects with a

Table 1. Haplotype and genotype frequencies for *VKORC1* variants in present study and comparison with other population groups.

a) Haplotype frequencies for *VKORC1*					
SNPs*	**Present study Oman [n=157]**	**Europeans[$]**	**Italian study[@]**	**Africans[$]**	**Chinese[$]**
1 2 3 4	**(%)**	**(%)**	**(%)**	**(%)**	**(%)**
G, T, C, G [*1]	8.0	<0.1[#]	3.3[#]	31[#]	<0.1%[#]
A, (T/G), C, G [*2]	27.8	42[#]	42.6[#]	14[#]	95[#]
G, T, C, A [*3]	29.4	38[#]	36.2[#]	43[#]	4[#]
G, T, T, G [*4]	14.4	20[#]	17.9	12	<1[#]
Atypical haplotypes					
G, G, C, G [Aty. A]	16.6				
A, T, C, A [Aty. B]	3.2				
A, T, T, G [Aty. C]	0.3				
A, T, T, A [Aty. D]	0.3				

(b) Genotype frequencies	**Oman, present study**		**Italian study[@]**
Genotype	**No.**	**(%)**	**(%)**
VKORC1 *1/*1	4	2.6	0
VKORC1 *1/*2	7	4.5	1.4
VKORC1 *1/*3	8	5.1	3.6
VKORC1 *1/*4	2	1.3	1.8
VKORC1 *2/*2	14	8.9[+]	21.8[+]
VKORC1 *2/*3	34	21.7	27.3
VKORC1 *2/*4	11	7.0	13.6
VKORC1 *3/*3	10	6.4	13.2
VKORC1 *3/*4	6	3.8	14.5
VKORC1 *4/*4	6	3.8	2.7
Atypical genotypes			
VKORC1Aty. A/Aty. A	9	5.7	
VKORC1Aty. A/*2	5	3.2	
VKORC1Aty. A/*3	21	13.4	
VKORC1Aty. A/*4	8	5.1	
VKORC1Aty. B/*2	1	0.6[+]	
VKORC1Aty. B/*3	3	1.9	
VKORC1Aty. B/*4	6	3.8	
VKORC1Aty. C/*2	1	0.6[+]	
VKORC1Aty. D/*4	1	0.6	
Total	157	100	

Data compiled from Geisen et al. (2005[$]) and Spreafico et al. (2008[@]); *Genebank accession No: AY587020:-SNP Position No.1- 3673G>A; No. 2-5808T>C (haplotypes *2 can be divided into 2A and 2B based on 5808T>G polymorphism); No. 3-6009C>T; No.4- 9041G>A; [#]p<0.05 when compared with the frequency data on Omani Subjects; [+]Warfarin sensitive genotype.

native population of around 2 million. The present day Omani population is known to be genetically heterogeneous with Caucasian, African and Asian ancestries. Each of these three major population groups has previously been shown to require distinct average warfarin dose in direct correlation with the relative prevalence of *VKORC1* haplotypes (Rieder et al., 2005).

Some common SNPs of *VKORC*1 locus defining the *VKORC*1 haplotypes have been associated with clinically significant differences in warfarin maintenance dose (Spreafico, 2008). Indeed a single SNP, either -1639 G>A [3673G>A] in the *VKORC*1 promoter or 1173C>T in intron 1 behaved as a tagSNP in distinguishing the warfarin sensitive *VKORC*1 *2 haplotype from the other haplotypes (Rieder et al., 2005; Geisen et al., 2005; Spreafico et al., 2008). Nevertheless, other haplotypes especially *VKORC*1*3 and *VKORC*1*4 do also influence warfarin dose requirement by conferring different degrees of warfarin resistance. Indeed subjects bearing these haplotypes have the risk of being outside the International Normalized Ratio (INR) target range during the initial titration period of this anticoagulant (Spreafico et al., 2008; Ansell et al., 2008). Perera et al. (2011) have reported that *VKORC*1 -8191 SNP (rs 61162043) in African American population was associated with higher warfarin dose. Thus the determination of *VKORC*1 haplotypes/genotypes is of interest when a geographically distinct population (for example Omani

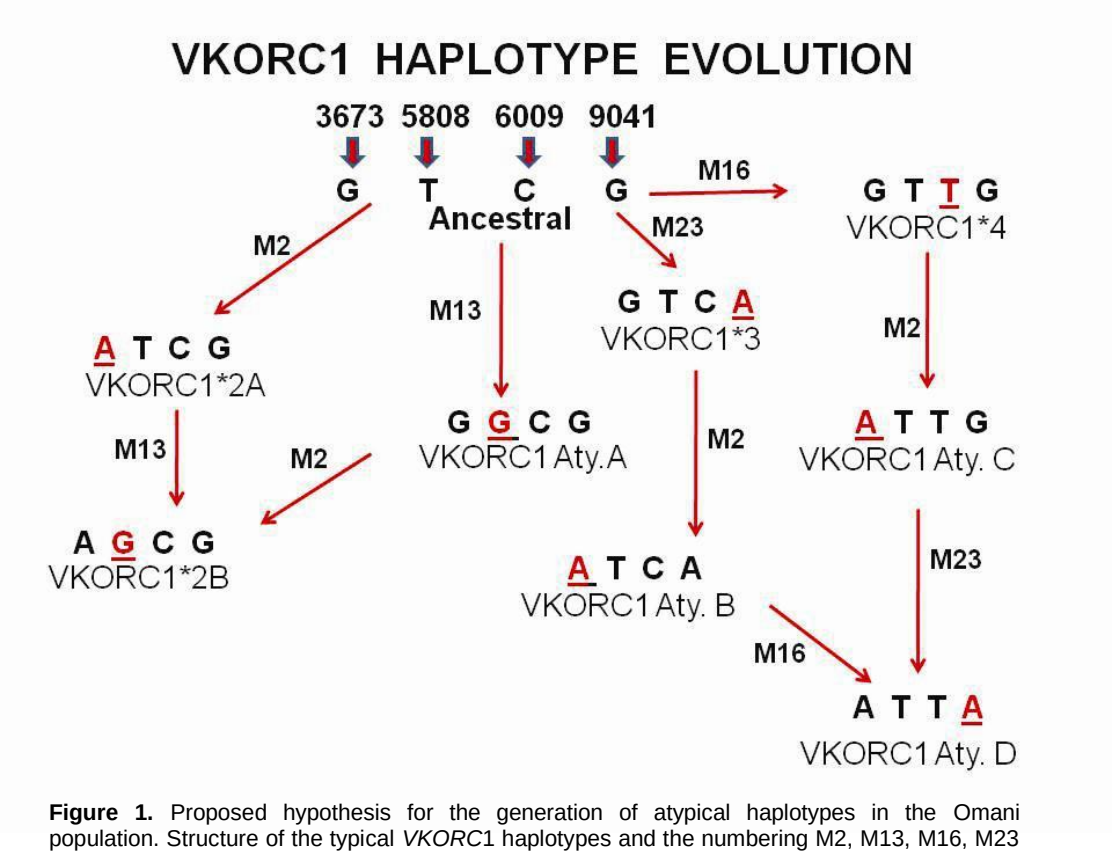

Figure 1. Proposed hypothesis for the generation of atypical haplotypes in the Omani population. Structure of the typical *VKORC*1 haplotypes and the numbering M2, M13, M16, M23 are adapted with nucleotide positions as per genebank accession number AY587020 (Geisen et al., 2005). *VKORC1**1 is the ancestral allele considered to be specific to Africans.

herein studied) is examined (Pathare et al., 2012b).

Our study revealed that warfarin sensitive tag-SNP or the *VKORC*1*2 haplotype was found in approximately 28% of the Omani population, with 8.9% in homozygous state. Strikingly, in this Omani population, the *VKORC1* locus was associated with nine different haplotypes including the four previously described common haplotypes namely *VKORC*1 *1,*2,*3 and*4 (seven of them had an allele frequency above 1%). Among them, five were already described in different population groups (including the sub-haplotypes *VKORC*1*2A and *2B) and four are novel, which we designated as atypical haplotypes A to D. The seven haplotypes observed above the frequency of 1% in Oman [GTCG (*1), ATCG (*2A), AGCG (*2B), GTCA (*3), GTTG (*4) and GGCG (atypical A) and ATCA (atypical B)] accounted for 99.4% of the haplotype diversity in our study population (Table 1). Furthermore we also observed that the prevalence of each of the major *VKORC*1 haplotypes (namely, *VKORC1* *1, *2,*3 and *4) in Omani population had values intermediate between those of Caucasians and African Americans, conforming further to the known admixture of Omanis (Table 1).

The presence of atypical haplotypes allowed us to revise the previously proposed *VKORC*1 haplotype evolutionary scheme as shown in Figure 1 (Geisen et al., 2005). It is likely that the atypical haplotype A, which was also found in homozygous state (5.7%) very likely arose from the putative ancestral African *VKORC*1*1 haplotype by a single substitution at position 5808T>G [an event assumed to have occurred in generating *VKORC*1*2B from *VKORC*1*2A] (Figure 1). Similarly, atypical haplotypes B and C probably arose each by a single substitution event at position 3673G>A involving *VKORC*1*3 and *4 haplotypes respectively. However, the minor haplotype atypical D requires two substitutional events to have generated from a major haplotype.

Presence of *VKORC*1 atypical haplotypes at significant proportions in the Omani population bears some relevance with the genetic admixture of present day Omanis with other major population groups by gene flow. This genetic profile is quite reminiscent of what had been observed for the sickle cell and thalassaemic genes in the Omanis (Daar, et al., 1998). Furthermore, the association of atypical *VKORC*1 haplotypes in this admixed Omani population is quite similar to the atypical haplotypes of sickle cell gene-linked haplotypes that were reported to have occurred due to recombination events in the admixed population from Brazil (Zago et al., 2001).

In conclusion, this study establishes the frequencies of the *VKORC1* haplotypes in the Omani population. The prevalence of homozygous warfarin sensitive

genotype (if assumed to be due either to 3673AA or 1173TT) is approximately 10%. Nevertheless the potential functional relevance of these variations linked to atypical haplotypes needs to be assessed. Overall the warfarin sensitive *VKORC1* alleles and genotypes in Omanis are significantly less than in Caucasians and Oriental populations with intermediate values between Africans and Caucasians conforming to the known population admixture of the present day Omanis.

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