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Genetic polymorphism of *CYP2C9* and *VKORC1* in the Nigerian population: significance for warfarin therapy in the population

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ABSTRACT

Introduction: *CYP2C9* and *VKORC1* are important pharmacogenes for warfarin dosing. Variability in response to warfarin therapy, which may carry fatal consequences, have been explained by the presence of functionally relevant variants of these genes.

Aim: This study investigated the prevalence and highlights the clinical implications of some relevant variants of *CYP2C9* and *VKORC1* in the Nigerian population as they affect the safe administration of warfarin.

Material and methods: Genotype analysis via a Sequenom iPLEX platform and direct Sanger sequencing was carried out in 158 healthy, unrelated subjects, drawn from the main Nigerian ethnicities. The alleles screened for comprised *CYP2C9*2* (rs1799853), **3* (rs1057910) and **8* (rs7900194). Subjects were also genotyped for *VKORC1*3* (rs7294) allele.

Results and discussion: All the studied alleles were in Hardy-Weinberg equilibrium, and *CYP2C9*8* was the most prevalent *CYP2C9* allele with a frequency of 0.073 in the population. The other *CYP2C9* alleles, *CYP2C9*2* and **3*, occurred at frequencies of 0.006 and 0.003, respectively. *VKORC1*3*, however, was observed at a frequency of 0.437.

Conclusions: Although all alleles detected in the population are significantly related to warfarin dosing, *VKORC1*3* might be the most critical owing to its high prevalence (108 out of 158) across individuals from different Nigerian ethnicities. Plasma warfarin-level monitoring may also be desirable in instances where individuals carry both the *CYP2C9*8* and *VKORC1*3* alleles, an occurrence observed in 9% of the studied individuals.

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1. INTRODUCTION

Warfarin is a widely-prescribed anticoagulant characterised by a narrow therapeutic index and interindividual differences in response to its therapy. Its use spans the prevention and treatment of deep-vein thrombosis, pulmonary embolism, stroke and myocardial infarction.[1,2] The drug is often administered as a racemate with S-warfarin, one of its enantiomers almost exclusively metabolised by *CYP2C9*, predominating the exaction of warfarin's inhibitory effect on its target enzyme. This target enzyme, vitamin K_1 2,3 epoxide reductase (VKOR), is encoded by vitamin K_1 epoxide reductase complex subunit 1 (*VKORC1*).[3]

The activity of VKOR usually generates reduced vitamin K which is a cofactor for γ-glutamyl-carboxylase, a critical enzyme in the blood coagulation process that activates the precursor-forms of blood clotting factors.[4] Warfarin's inhibition of VKOR and by extension a disruption of the blood coagulation process underpins its delicate rational use as an anticoagulant. Unfortunately, wide variations in plasma levels of warfarin does carry a potential risk of major or fatal bleeding in humans, thus necessitating the monitoring of prothrombin time (PT) often expressed as an international normalised ratio (INR). The INR is an index derived from the measurement of the sum of activity of the vitamin K dependent coagulation factors.[1,5]

Although warfarin plasma levels is known to be affected by gender, dietary intake of vitamin K, age and disease such as liver dysfunction, genetic variability in genes encoding CYP2C9 and VKOR, warfarin's metabolic enzyme and target, respectively, impacts significantly on the safety of warfarin therapy.[1,6] Functional polymorphisms in the *CYP2C9* gene, especially the **2*, **3* and **8* alleles which have previously been shown to result in decreased metabolism of CYP2C9 substrates, are potential sources of alteration in initial warfarin dose sensitivity which often delays the attainment stable maintenance doses, and increases the risk of adverse events.[5,7,8] In the same vain, genetic variants of *VKORC1* have been noted to be predictive of warfarin phenotypes and such polymorphisms in this gene have been reported to account for about 25% of variance in stabilized warfarin dose.[9] These findings have reinforced the notion that the safe administration of warfarin may benefit hugely from pharmacogenetics.

2. AIM

A study of the prevalence of the main genetic contributors to warfarin therapy in Nigerians, a population for which genetic data for her many ethnicities is sparse, was hence carried out to evaluate the potential usefulness of pharmacogenetics in warfarin therapy in the population.

3. MATERIAL AND METHODS

Ethical approval for this study was provided by the Ethics Committee of Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria. Study subjects – 158 non-related Nigerians drawn from the main ethnicities (20 *Hausa*, 31 *Igbo*, 95 *Yoruba*, and 12 from some other minor ethnicities), who had provided written informed consent – participated in this study by providing 5 mL of blood which were collected in EDTA bottles. Genomic DNA was extracted from blood samples by a phenol-chloroform method and screened for *CYP2C9*2* (rs1799853), **3* (rs1057910) and **8* (rs7900194) through the Sequenom MassARRAY platform (kits and chemicals supplied by Agena Bioscience, San Diego, CA, USA). *VKORC1*3* was screened for by directly amplifying the corresponding VKORC1 locus and subsequently Sanger sequencing the amplicons. The population data was assessed for Hardy-Weinberg equilibrium with a Fisher's exact test, inferring statistical significance from $P < 0.05$.

4. RESULTS

Alleles detected in the population were all in Hardy-Weinberg equilibrium, and a detailed description of the population data is provided in Table 1. *CYP2C9*2* and **3* were not detected in the Igbo and Hausa ethnicities, whereas the *CYP2C9*8* allele was most frequent in Yoruba with a prevalence of 0.095. *VKORC1*3* was detected across all ethnicities with a cumulative allele frequency of 0.437 in the Nigerian population. In 158 studied individuals 14 were, however, carriers of both *CYP2C9*8* and *VKORC1*3*.

5. DISCUSSION

*CYP2C9*2* and **3*, alleles which had been predominantly reported in Caucasians,[10,11] occurred at frequencies below 1% in the Nigerian population. Although these alleles had previously been shown to necessitate a 20% reduction in warfarin dose for *CYP2C9*2*[12] and 75% in Caucasians who were homozygous carriers of *CYP2C9*3*,[13] their overall importance for warfarin dosing in Nigerians may be limited owing their low prevalence in the population. *CYP2C9*8*, an allele quite prevalent across Nigerian ethnicities, may be more critical for warfarin therapy instead. This allele is known to exhibit significantly-lower catalytic activity for S-warfarin compared with the wild-type from a study which used recombinant CYP2C9 protein.[8] The likely importance of this allele for warfarin dosing in the Nigerian population is further reinforced by previous studies in South African patients[14] which found correlation between *CYP2C9*8* and warfarin variability, and in African-Americans by Cavallari et al.[15] which observed a 19% reduction in median warfarin dose (34.4 *vs* 42.5 mg/week) for homozygous and heterozygous *CYP2C9*8* carriers. In addition, a similar study of the role of *CYP2C9*8* in warfarin therapy in another African-American population by Liu et al.[16] observed a 30% reduction in the unbound oral clearance of S-warfarin and 25% lower R- to S-warfarin plasma levels in carriers of *CYP2C9*8* compared to the wildtype allele carriers. Among

Table 1. Prevalence and distribution of allelic variants of *CYP2C9* and *VKORC1* in the Nigerian population.

	Nigerian ethnicities				
	Hausa $n = 20$	Igbo $n = 31$	Yoruba $n = 95$	Others* $n = 12$	Total $n = 158$
Allele prevalence					
*CYP2C9*2* (rs1799853)	1	–	1	–	2
*CYP2C9*3* (rs1057910)	–	–	1	–	1
*CYP2C9*8* (rs7900194)	1	3	12	1	17
*VKORC1*3* (rs7294)	13	19	70	6	108
CYP2C9*2/VKORC1*3	1	–	–	–	1
CYP2C9*3/VKORC1*3	–	–	1	–	1
CYP2C9*8/VKORC1*3	–	2	12	–	14
Allele frequencies					
*CYP2C9*2* (rs1799853)	0.025	–	0.005	–	0.006
*CYP2C9*3* (rs1057910)	–	–	0.005	–	0.003
*CYP2C9*8* (rs7900194)	0.025	0.048	0.095	0.042	0.073
*VKORC1*3* (rs7294)	0.425	0.387	0.474	0.292	0.437

Comments: * Isoko ($n = 1$), Bini ($n = 4$), Urhobo ($n = 2$), Ebira ($n = 3$), Ibibio ($n = 2$).

other findings from this group, as much as 30% lower intrinsic clearance of S-warfarin was also seen with the cDNA-expressed *CYP2C9*8* protein.

*VKORC1*3* (rs7294), another allele critical for warfarin dosing, was found to be quite prevalent across Nigerian ethnicities, occurring in about 68% of the subjects, comprising of 30 homozygous carriers and 78 heterozygous carriers. This variant has been reported to impact significantly on warfarin efficacy, especially at the early stages of therapy commencement, in a study of 460 Chinese patients.[17] The same study observed suboptimal INR values in about 90% of patients who were carriers of *VKORC1*3* after 7 days of daily 2.5 mg warfarin administration. Another study in Sudanese subjects who had been on warfarin for at least three months showed that the mean daily dose was about 14% higher in homozygous and heterozygous *VKORC1*3* carriers,[18] while variability in warfarin plasma levels and an expected adjustment of warfarin doses was also explainable by *VKORC1*3* in addition to *CYP2C9*8* in South Africans.[14] D'Andrea et al.,[19] however, reported a 24% increase in mean daily warfarin dose for homozygous *VKORC1*3* carriers in a Caucasian cohort whereas dose requirements were similar for heterozygotes and wild type carriers.

In the context of the Nigerian population, *VKORC1*3* (rs7294) would appear to be the most critical genetic factor for consideration in warfarin dosing owing to its prevalence. Consequently, upward adjustment of maintenance doses to achieve INR values indicative of optimal clinical response would seem logical when suboptimal responses are observed. However, the less prevalent *CYP2C9*8* relative to the *VKORC1*3* allele in the population calls for a cautious approach. Ideally, the presence of this allele, as shown from previous data, would necessitate a downward dose adjustment in therapy with warfarin. Incidentally, this is further complicated by the likelihood of carriers of both *CYP2C9*8* and *VKORC1*3* in the population as seen in about 9% of our study population. Such interesting occurrence of both alleles in Nigerians emphasizes the huge relevance of genetic screening prior to commencement of warfarin therapy. Since there is no data, to date, quantifying the effect of the co-presence *CYP2C9*8* and *VKORC1*3* on warfarin maintenance doses, it becomes imperative that drug levels be monitored until stability is achieved in individuals with these alleles.

It is, however, worth noting that the small sample sizes of the Igbo and Hausa samples analysed in the present study may limit statistical projections for these ethnicities. In any case, cohort studies which take into the consideration the polymorphic forms of these genes, especially their co-inheritance in individuals, while also monitoring response to warfarin therapy in the population appears necessary.

6. CONCLUSIONS

In conclusion, this study reports that *CYP2C9*8* and *VKORC1*3* are prevalent pharmacogenes of warfarin therapy in the Nigerian population. The distribution of these alleles in the population clearly makes a strong case for genetic screening prior to warfarin dosing, and drug levels monitoring afterwards until stability is achieved in patients.

Conflicts of interest

The authors declare no conflict of interest.

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