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

Ayorinde Adehin¹, Oluseye Oladotun Bolaji¹, Simran Maggo², Martin A. Kennedy²

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo, University, Ile-Ife, Osun State, Nigeria
²Department of Pathology and Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand

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.
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. The drug is often administered as a racemate with S-warfarin, one of its enantiomers almost exclusively metabolised by CYP2C9, predominating the exactation of warfarin's inhibitory effect on its target enzyme. This target enzyme, vitamin K, 2,3 epoxide reductase (VKOR), is encoded by vitamin K, epoxide reductase complex subunit 1 (VKORC1).

The activity of VKOR usually generated 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. 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.

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. 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 of stable maintenance doses, and increases the risk of adverse events. 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. 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 Ibo, 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, 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 and 75% in Caucasians who were homozygous carriers of CYP2C9*3, 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. The likely importance of this allele for warfarin dosing in the Nigerian population is further reinforced by previous studies in South African patients which found correlation between CYP2C9*8 and warfarin variability, and in African-Americans by Cavalli et al. 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. 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
higher in homozygous and heterozygous months showed that the mean daily dose was about 14% of patients who were carriers of the polymorphic forms of these genes, especially their co-inheritance in individuals, while also monitoring response to warfarin therapy in the population appears necessary.

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 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.

References

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

<table>
<thead>
<tr>
<th>Allele prevalence</th>
<th>Hausa (n = 20)</th>
<th>Igbo (n = 31)</th>
<th>Yoruba (n = 95)</th>
<th>Others* (n = 12)</th>
<th>Total (n = 158)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2 (rs1799853)</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>CYP2C9*3 (rs1057910)</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>CYP2C9*8 (rs7900194)</td>
<td>1</td>
<td>3</td>
<td>12</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>VKORC1*3 (rs7294)</td>
<td>13</td>
<td>19</td>
<td>70</td>
<td>6</td>
<td>108</td>
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<tr>
<td>CYP2C9<em>2/VKORC1</em>3</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>CYP2C9<em>3/VKORC1</em>3</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>CYP2C9<em>8/VKORC1</em>3</td>
<td>–</td>
<td>2</td>
<td>12</td>
<td>–</td>
<td>14</td>
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</tbody>
</table>

Allele frequencies

<table>
<thead>
<tr>
<th>Allele</th>
<th>Hausa (n = 20)</th>
<th>Igbo (n = 31)</th>
<th>Yoruba (n = 95)</th>
<th>Others* (n = 12)</th>
<th>Total (n = 158)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2 (rs1799853)</td>
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<td>–</td>
<td>0.005</td>
<td>–</td>
<td>0.006</td>
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<tr>
<td>CYP2C9*3 (rs1057910)</td>
<td>–</td>
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<td>0.005</td>
<td>–</td>
<td>0.003</td>
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<tr>
<td>CYP2C9*8 (rs7900194)</td>
<td>0.025</td>
<td>0.048</td>
<td>0.095</td>
<td>0.042</td>
<td>0.073</td>
</tr>
<tr>
<td>VKORC1*3 (rs7294)</td>
<td>0.425</td>
<td>0.387</td>
<td>0.474</td>
<td>0.292</td>
<td>0.437</td>
</tr>
</tbody>
</table>

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


