CYP2C9*61, a rare missense variant identified in a Puerto Rican patient with low warfarin dose requirements

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Warfarin continues to be the mainstay therapy for preventing thrombus formation. Although pharmacogenetic algorithms have shown higher predictability of the optimal warfarin dose and lower occurrence of bleeding episodes, they often do not include ethno-specific genetic variants relevant to non-Europeans. This case report describes a rare missense variant at exon 9 of *CYP2C9* (rs202201137; c.1370A>G transition; p.Asn457Ser) found in a Puerto Rican patient with low warfarin dose requirements (3 mg/day). The haplotype characterized by two amino acid changes, Asn457Ser and Arg144Cys (rs1799853; c.430C>T), has been designated *CYP2C9**61 by the Pharmacogene Variation Consortium. According to prediction scores assessed with the Combined Annotation Dependent Depletion tool, *CYP2C9**61 (p.Asn457Ser) was classified as nondeleterious, therefore its impact on *CYP2C9* enzymatic activity cannot be postulated.

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Despite the advent of the new direct oral anticoagulants (e.g., Xarelto[®] and Eliquis[®]), warfarin continues to be a mainstay treatment for preventing thrombus formation and risk associated with cardiovascular conditions such as atrial fibrillation and deep vein thrombosis not only in the Commonwealth of Puerto Rico but in many other countries around the world. However, warfarin is also one of the most common prescription drugs implicated in emergency room visits for adverse drug events [1]. The occurrence of major bleeding episodes during warfarin initiation therapy is a leading cause of death in Puerto Rico, and related adverse events account for one in ten hospital admissions. The ARISTOTLE trial (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) found that the rate of major hemorrhages caused by warfarin was 3.09% per year [2].

Individual warfarin dosing estimation is multifactorial, depending on clinical and demographic determinants such as age, gender, self-reported race or ethnicity, comedications (e.g., amiodarone), smoking status, International Normalized Ratio (INR) – among others. Over the last decade, multiple reports describing clinical pharmacogenetic studies in warfarin patients have been published [3–6]. Overall, there is strong evidence that genetics accounts for a significant portion of the observed interpatient variability in warfarin dose requirements. Currently, there

are numerous population-derived pharmacogenetics-guided warfarin-dosing algorithms that explain 35–70% of the observed variability in dose requirements [7–12]. Since these algorithms are derived from cohorts of mostly European patients, only the common *CYP2C9**2, *CYP2C9**3 and *VKORC1*-1639G>A variants are typically included. Consequently, certain rare or relevant ethno-specific allelic variants such as *CYP2C9**8, *CYP2C9**11, *CYP2C9* rs12777823 and rs2860905, which occur in underrepresented minority populations, or *CYP4F2**3, are usually not part of genotyping panels or included in dosing algorithms. Furthermore, the performance of existing pharmacogenetic dosing algorithms has only been partially assessed in individuals of Hispanic heritage, a minority population that is often excluded from clinical studies. Consequently, there is a lack of knowledge regarding the impact of pharmacogenomics on warfarin dose requirements for Hispanic populations at large.

Puerto Rican Hispanics are a highly heterogeneous, admixed population with a complex, rich repertoire of combinatorial allelic variants shaped by more than 500 years history of mixture between Europeans, west Africans and Native Americans Taínos (Arawaks ethnicity). Here, we report a nonsynonymous missense variant rs202201137 (10:96748682; c.1370A>G transition; p.Asn457Ser) in exon 9 of the *CYP2C9* gene that has been identified in a single Hispanic individual from Puerto Rico who was found to be sensitive to warfarin. This patient required only 3 mg of warfarin daily compared with the standard dose of 5 mg daily to achieve an INR of 2–3. To the best of our knowledge, this SNP has never been described in previous studies as a genetic marker found to be associated with warfarin dose requirements. It is important to mention that this patient is also a carrier of the *CYP2C9**2 allele, which has previously been shown to be an independent predictor of low warfarin dose requirements in other populations worldwide [3,13,14]. To what extent this rare missense variant contributes to lower effective warfarin dose requirements in Hispanic Puerto Ricans remains to be determined on a larger population basis.

Case

A 78-year-old, self-identified Black Puerto Rican male (height: 167 cm; weight: 92 kg) with a past medical history of status-post aortobifemoral bypass surgery, dyslipidemia and major depression was recruited to participate in a clinical study on warfarin pharmacogenetics (participant ID: WPRA006). The patient had a history of nonsmoking and nonalcohol consumption. Hepatic enzymes as well as hematocrit, hemoglobin, serum creatinine (mg/dl) and blood urea nitrogen (BUN) were all within normal range. At the time of enrollment, the patient was taking warfarin, amitriptyline and gemfibrozil, with evidence of compliance on these treatments. The patient denied any report of adverse episodes at the time of recruitment. The cardiac examination was unrevealing. His laboratory tests revealed an INR of 2.73 on reported therapeutic dose (i.e., prescribed warfarin at a dose of 3 mg/day, *per os*, for prevention of thrombus formation). It took 111 days, however, to reach a target INR of 2–3.

Based on the diagnosis of status-post aortobifemoral bypass surgery, the INR therapeutic range is between 2.0 and 3.0, however, the baseline INR value for this patient was lower at 1.86. Warfarin dosage was changed three-times for this patient during the course of the 111-day adjustment period. A total of 17 INR tests were ordered before the patient reached his INR target. Seven INR measurements exceeded target level peaking at 7.4 suggesting a supratherapeutic level of warfarin dosage, and exposing him to a higher risk of bleeding, and three measurements were below the target. No major life-threatening bleeding episodes were reported and dosage was eventually stabilized at 3 mg/day, the lowest dose prescribed, at the time of achievement of the target INR. In this patient, the initial warfarin dose of 5 mg/day resulted in INRs that exceeded the target range. At the initiation of therapy, the dose was the highest dose given to the patient.

After obtaining written informed consent, this patient underwent genotype testing as a participant in the research study conducted at the VA Caribbean Healthcare System anticoagulation clinic to investigate the association of common minor alleles in *CYP2C9*, *CYP4F2*, *NQO1* and *VKORC1* pharmacogenes with warfarin-dosing requirements in the Puerto Rican population. The study was approved by the Institutional Review Board at the University of Puerto Rico, Medical Sciences Campus (IRB protocol: A4070109) and the VA Caribbean Healthcare System (IRB protocol: 00558). DNA testing to infer metabolic capacity and sensitivity to warfarin revealed that this patient is a carrier of three major variant alleles that have previously been associated with warfarin response, in other words, *CYP2C9**2/*5 and *CYP4F2**1/*3. In addition, we discovered the *CYP2C9* exon 9 variant rs202201137. Noteworthy, this patient is not a carrier of *VKORC1*-1639 G>A, a major genetic predictor of warfarin dosing, or the *NQO1**2 (4559C>T) allele that has also been implicated to contribute to warfarin dose requirements.

The variant *CYP2C9* rs202201137 (10:96748682; c.1370A>G; p.Asn457Ser) was first identified by performing targeted resequencing of the *CYP2C9* gene using the Ion PGM sequencing system (Thermo Fisher Scientific, MA, USA) as previously published by our group [15] and confirmed with Sanger sequencing (Figure 1). Variants

Figure 1. The presence of *CYP2C9* rs202201137 (10:96748682; c.1370 A>G; p. Asn457Ser) in exon 9 was confirmed by Sanger sequencing (highlighted in blue). The sequence shows two peaks at the position of interest representing A (green peak) and G (black peak) demonstrating that the individual is heterozygous. For color figures see online at: <https://www.futuremedicine.com/doi/full/10.2217/pgs-2018-0143>

Figure 2. Graphical display of the predicted *CYP2C9**61 haplotype (10:96748682, c.1370A>G p. Asn457Ser, rs202201137). This novel haplotype carries a series of SNPs including the SNP defining the *CYP2C9**2 allele, in other words, c.430C>T (rs1799853; Arg144Cys) as well as a number of 5' upstream and intronic SNPs, in other words, c.-3597A>G (rs61886768), c.-3360T>C (rs61886769), c.-1188T>C (rs4918758), c.-1096A>G (rs4917636) and c.481+197G>A (rs2860905). Positions are provided according to the NM_000771.3 cDNA reference sequence.

identified with the Ion PGM sequencing were phased using Beagle v4.1 [16] to estimate the haplotypic arrangements of *CYP2C9*. According to Beagle estimates, this rare variant was in linkage disequilibrium with *CYP2C9**2 and rs2860905, both previously found associated with low warfarin dose requirements in Puerto Ricans [15] but not with *CYP2C9**5. Moreover, these results were verified with data from 1000 Genomes Project and accordingly, none of the Puerto Rican individuals with the variant *CYP2C9* rs202201137 (samples HG01190 and HG01325) were carriers of *CYP2C9**5 (sample HG01170) confirming their independent inheritance predicted by Beagle. The novel haplotype containing rs202201137 (Figure 2) was submitted to the Pharmacogene Variation Consortium (www.PharmVar.org) [17] and designated as *CYP2C9**61.

Functional prediction of p.As457Ser defining the *CYP2C9**61 haplotype

*CYP2C9**61 was evaluated using the Combined Annotation Dependent Depletion tool (CADD v1.3 at <http://cadd.gs.washington.edu/info>). Predicted scores for potential deleteriousness are summarized in Table 1. Grantham, SIFT and PolyP scores provide information regarding the effects of amino acids substitutions and predict deleteriousness based on protein structure and function. These scores suggest that *CYP2C9**61 is tolerated and benign. On the other hand, GERP, PhastCons and PhyloP provide information regarding the conservation of the locus where this variant is mapped to. Based on the neutral evolution hypothesis, these conservation scores indicate that this variant site is not under evolutionary constraint and, therefore, is not highly conserved [18].

Table 1. Combined annotation depletion scores for predicted functionality and conservation of *CYP2C9*61*.

Variant	C-score (PHRED-like) [†]	Amino acid substitution scores			Conservation scores		
		Grantham [‡]	SIFT [§]	Polyphen [¶]	GERP [#]	PriPhastCons ^{††}	PriPhyloP ^{‡‡}
<i>CYP2C9*61</i> (Asn457Ser)	0.001	46	0.830 (tolerated)	0.009 (benign)	2.30	0.014	0.368
<i>CYP2C9*8</i> (Arg150His)	7.882	29	0.330 (tolerated)	0.005 (benign)	3.54	0.553	-0.450

[†]C-score is a PHRED-like CADD ranking system that estimates the probability that the variant is deleterious. This score allows prioritizing variants with the highest predicted deleteriousness. This value depends on the dataset.

[‡]Grantham score categorizes amino acids substitutions based on chemical dissimilarity where higher scores reflect higher evolutionary distance and are expected to be more deleterious.

[§]SIFT predicts deleteriousness based on the protein structure and function (scores range from 0 to 1 where 0 predicts a higher chance of being damaging).

[¶]PolyPhen is the probability that the substitution is damaging (scores from 0 to 1 where values closer to 1 predicts deleteriousness).

[#]GERP observes the number of substitutions expected under neutrality minus the number of observed substitutions (in a multiple sequence alignment); therefore, positives scores (fewer than expected) indicate that a site is under evolutionary constraint; values range from -12.3 to 6.17, where values closer to 6.17 indicate conservation.

^{††}PhastCons values in primates (Pri-PhastCons) are a ratio that represents the average rate of substitutions in conserved regions relative to the average rate in nonconserved regions; scores in closer to 0 indicate less conservation.

^{‡‡}PhyloP values in primates (Pri-PhyloP); PhyloP compares the probability of observed substitutions under the hypothesis of neutral evolutionary rate; positive values indicate conservation and negatives indicate selection.

However, these scores do not necessarily rule out an impact on activity. As a way of example, rs7900194 (c.449G>A, p.Arg150His) defining the *CYP2C9*8* allele in exon 3 was also predicted as tolerated and benign (see Table 1). In addition, it is not located in a highly conserved region (according to conservation scores), but this functional variant has been found to be significantly associated with low warfarin dose requirements in individuals of African ancestry (e.g., African-Americans and Puerto Ricans) [12,19]. In fact, this missense variant is listed by ClinVar as a drug-response allele based on sustained evidence linking this SNP with warfarin response (<https://preview.ncbi.nlm.nih.gov/clinvar/variation/226024/>). Clinical annotations of *CYP2C9*8* can be found on PharmGKB at www.pharmgkb.org/clinicalAnnotation/637879801. Furthermore, although Asn457Ser may not obliterate or severely reduce function on its own, it may, however, further reduce the activity of *CYP2C9*2* by way of a concerted effect caused by Arg144Cys and Asn457Ser as discussed below.

Using the published pharmacogenetic-guided algorithm to predict warfarin dosing in Puerto Ricans, the estimated effective dose in this individual (4.06 mg/day) deviates from the actual stable dose of 3 mg/daily (Δ dose = 1.06 mg/day) suggesting that *CYP2C9*61* (carrying Arg144Cys and Asn457Ser) further reduce the activity of the protein encoded by this haplotype. The presence of *CYP2C9*2* was associated with a 7% dose reduction per allele in Puerto Ricans [12] and therefore does not intrinsically explain this dose reduction by the presence of Arg144Cys. However, this patient is also carrier of *CYP2C9*5* (c.1080C>G; Asp360Glu), found to significantly decrease CYP2C9 activity (8–12% compared with *CYP2C9*1*) [20].

Heterozygosity of *CYP4F2*3* is unlikely to cause the degree of sensitivity observed in our patient despite an association with dose resistance in Hispanics [21]. Furthermore, the patient was genotyped as *VKORC1*-1639 G/G, which is known as a 'high-dose' haplotype. We cannot, however, discard with certainty that other factors are responsible for the low dose requirement. Factors like the advanced age of the patient (78 years), liver function, co-morbidities, drug-to-drug interactions and the presence of other polymorphisms (*CYP2C9*2* and *CYP2C9*5*) may be underlying causes of the low warfarin dose requirements in the reported case. Clearly, *in vitro* experiments are needed to further determine the impact of this variation on intrinsic clearance of warfarin and other CYP2C9-mediated drugs and ideally more patients to better characterize the functional consequences of this allele *in vivo*.

Allele frequency & designation

According to the reported minor allele frequencies in the 1000 Genome Project database (N = 2504 individuals from 26 populations), the prevalence of *CYP2C9* rs202201137 (Asn457Ser) is zero in all reference populations worldwide, except in Puerto Rican Hispanics. Among the 104 Puerto Rican subjects in the 1000 Genomes Project, two heterozygous individuals (HG01190 and HG01325) were identified (MAF = 0.010). Also, three individuals participating in eMERGE (two out of 14,788 Europeans and one out of 2837 Africans) were reported to have the *CYP2C9*61*. This variant was, however, not found in any individual in the Children's Mercy Hospital data warehouse (<https://warehouse.cmh.edu/>) with 4700 participants. Clearly, this genetic variant is more frequent in Puerto Ricans than any other ethnic group, but is not necessarily specific to this population.

Conclusion

In this case report, we describe the discovery of a novel haplotype, *CYP2C9*61*, in a Puerto Rican patient who required significantly lower weekly dosing of warfarin. Although *in-silico* analysis did not suggest that the *CYP2C9*61* signature SNP c.1370A>G (p.Asn457Ser) impacts enzymatic activity, the functional consequence of this amino acid change remains to be functionally validated.

Summary points

- The occurrence of major bleeding episodes during warfarin initiation therapy is a leading cause of death in Puerto Rico, and related adverse events account for one in ten hospital admissions.
- Population-derived pharmacogenetics-guided warfarin dosing algorithms explain 35–70% of the observed variability in dose requirements but they typically include variants derived from European cohorts (specifically *CYP2C9*2*, *CYP2C9*3* and *VKORC1* -1639 G>A).
- This case report presents the discovery of a rare missense variant in *CYP2C9*, rs202201137 (10:96748682; c.1370A>G; p.Asn457Ser) that was identified in a Puerto Rican patient requiring a low warfarin dose (3 mg/day), whose impact on the metabolic clearance of the anticoagulant needs further evaluation and proper validation.
- The *CYP2C9* rs202201137 (10:96748682; c.1370A>G; p.Asn457Ser) is the signature SNP of the haplotype *CYP2C9*61* recently accepted by the Pharmacogene Variation Consortium.
- *CYP2C9*61* is a rare variant with higher frequency in Puerto Ricans (MAF = 0.010) being found only in two participants of the 1000 Genomes Project (also Puerto Ricans) and in three individuals from eMERGE.
- According to a CADD evaluation of the *CYP2C9*61* defining SNP, this missense is tolerated and benign. Conservation scores suggest that this SNP is not under evolutionary constraint.
- Although we hypothesize that *CYP2C9*61* may have an impact on warfarin dose requirements in the present case report, factors like the advanced age of the patient, liver function, co-morbidities, drug-to-drug interactions and the presence of other polymorphisms (*CYP2C9*2* and *CYP2C9*5*) may be underlying causes of the low warfarin dose requirements observed in the reported case. Therefore, the impact of the *CYP2C9*61* signature SNP c.1370A>G (p.Asn457Ser) on the enzyme activity of *CYP2C9* needs to be evaluated by *in vitro* assays.

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