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*CYP2C9*8* is prevalent among African–Americans: implications for pharmacogenetic dosing

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Abstract

Aims—Although the frequencies of pharmacogenetic variants differ among racial groups, most pharmacogenetic algorithms for genotype-guided warfarin dosing only include two *CYP2C9* alleles (**2* and **3*) and a single *VKORC1* allele (g.-1639G>A or g.1173C>T) commonly found among Caucasians. Therefore, this study sought to identify other *CYP2C9* and *VKORC1* alleles important in warfarin dose variability and to determine their frequencies in different racial and ethnic groups.

Materials & methods—The *CYP2C9* and *VKORC1* genes were sequenced in selected sensitive (<21 mg/week) and resistant (>49 mg/week) individuals with discrepant therapeutic and algorithm-predicted warfarin doses based on prior *CYP2C9* and *VKORC1* genotyping. The *CYP2C9* and *VKORC1* allele frequencies were determined in healthy, racially self-identified blood donors.

Results—Sequencing identified an African–American male with a lower than predicted therapeutic warfarin dose (14.4 mg/week), previously genotyped as *CYP2C9*1/*1*, who was homozygous for *CYP2C9*8* (c.449G>A; p.R150H). Genotyping 600 African–American alleles identified *CYP2C9*8* as their most frequent variant *CYP2C9* allele (0.047), and the combined allele frequency of *CYP2C9*2*, **3*, **5*, **6*, **8* and **11* was 0.133. Given most warfarin pharmacogenetic dosing algorithms only include *CYP2C9*2* and **3*, the inclusion of *CYP2C9*8* alone could reclassify the predicted metabolic phenotypes of almost 10% of African–Americans, or when combined with *CYP2C9*5*, **6* and **11*, more than 15%. In addition, the African–American *VKORC1* g.-1639A allele frequency was 0.108 and three g.1331G>A (p.V66M) carriers were identified.

Conclusions—*CYP2C9*8* is prevalent among African–Americans (~1 in 11 individuals). Thus, in this racial group, the incorporation of *CYP2C9*8* into genotyping panels may improve dose prediction of CYP2C9-metabolized drugs, including warfarin.

Keywords

African-American; allele frequencies; *CYP2C9*8*; pharmacogenetics; *VKORC1*; warfarin

The highly polymorphic cytochrome P450 2C9 (*CYP2C9*) gene has over 50 known variant alleles, many of which result in decreased enzyme activity [1,101]. *CYP2C9* encodes a microsomal monooxygenase involved in the oxidative metabolism of many endogenous and xenobiotic compounds including anticoagulant, anticonvulsant, nonsteroidal anti-inflammatory and antihypertensive drugs. Prominent among these is warfarin, a commonly

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

used anticoagulant that is metabolized, in part, by CYP2C9, and variant *CYP2C9* alleles are associated with reduced therapeutic warfarin doses [2–6]. In addition, common polymorphisms and rare sequence variants in the vitamin K epoxide reductase complex 1 (*VKORC1*) gene alter warfarin pharmacodynamics and also affect warfarin dose requirements [3–9]. Consequently, genetic variation in *CYP2C9* and *VKORC1* combined with clinical and environmental factors (e.g., age, gender, body weight, concomitant medications and diet), account for approximately half of interindividual warfarin dose variation [10–13].

The identification of variant *CYP2C9* and *VKORC1* alleles led to their inclusion in warfarin-dosing algorithms (for review, see [14]). However, a current limitation of pharmacogenetic-predicted warfarin dosing is its application to multiracial and multi-ethnic patient populations. In part, this is owing to differences in *CYP2C9* and *VKORC1* allele frequencies between racial groups as *CYP2C9*2* (c.430C>T; p.R114C) and **3* (c.1075A>C; p.I359L) are common in Caucasians, but less frequent in African–Americans and Asians [15]. Since these variant alleles are used in the majority of pharmacogenetic-based dosing algorithms, their lower frequencies in African–Americans may account for why recent algorithms derived from multi-ethnic cohorts better explain dose variation in Caucasians than in African–Americans [16,17]. For example, the multi-ethnic algorithm recently reported by Gage *et al*. accounted for 57% of the dose variability in Caucasians, but only 31% in African–Americans [16].

To determine if other *CYP2C9* and *VKORC1* alleles are important in warfarin dose variability in African–Americans and other non-Caucasian populations, the *CYP2C9* and *VKORC1* genes were sequenced in selected patients from a multi-ethnic cohort with sensitive (<21 mg/week) and resistant (>49 mg/week) warfarin doses. An African–American male was identified who required a very low warfarin dose (14.4 mg/week) to achieve stable anticoagulation intensity (International Normalized Ratio [INR] 2–3) and who was homozygous for the *CYP2C9*8* variant allele. Consequently, the frequencies of variant *CYP2C9* (**2*, **3*, **4*, **5*, **6*, **8*, **11* and **13*) and *VKORC1* (g.-1639G>A, g.1331G>A) alleles were determined in a cohort of 300 healthy African–American individuals. Of note, the *CYP2C9*8* allele had the highest allele frequency (0.047) among African–Americans and the combined allele frequency of *CYP2C9*5*, **6*, **8* and **11* was 0.085. These studies underscore the importance of incorporating at least *CYP2C9*8* into pharmacogenetic-based dosing algorithms for CYP2C9-metabolized drugs, including warfarin.

Materials & methods

Human subjects

Since July 2007, adult (>18 years) blood samples have been collected with Institutional Review Board approval and informed consent from patients with arrhythmias or venous thrombosis treated with warfarin in a large anticoagulation program at an urban academic medical center. The anticoagulation clinic serves a population of over 500 patients, of which approximately 55% are Caucasian (~30% Ashkenazi Jewish) 25% Hispanic, 15% are African–American and 5% are Asian. A total of 114 patients were enrolled in this study and 65 were either sensitive (<21 mg/week) or resistant (>49 mg/week) with a target INR of 2–3. Of these, six patients had discrepant therapeutic and algorithm-predicted warfarin doses based on prior *CYP2C9* and *VKORC1* genotyping; therefore, their *CYP2C9* and/or *VKORC1* exonic regions were sequenced. To determine the frequency of *CYP2C9* and *VKORC1* alleles, blood samples from healthy donors who indicated their racial background and gave informed consent for the use of their DNA for research were obtained from the New York Blood Center with Institutional Review Board approval.

Genotyping

The *CYP2C9* allele designations refer to those defined by the Cytochrome P450 Allele Nomenclature Committee [1,101]. All patients enrolled in the study were initially genotyped for eight *CYP2C9* alleles (**1, *2, *3, *4, *5, *6, *11* and **13*) and 12 *VKORC1* variants (g.-4931T>C, g.-1639G>A, g.1173C>T, g.2255C>T, g.3730G>A, g.85G>T [p.V29L], g. 106G>T [p.D36Y], g.121G>T [p.A41S], g.134T>C [p.V45A], g.172A>G [p.R58G], g. 1331G>A [p.V66M] and g.3487T>G [p.L128R]). The coagulation factor VII (*F7*) and apolipoprotein E (*APOE*) alleles were also genotyped in selected patients by direct sequencing, and additional *CYP2C9* and *VKORC1* genotyping was performed to determine allele and genotype frequencies.

Genotyping of six *CYP2C9* alleles (**1, *2, *3, *4, *5* and **6*), and seven *VKORC1* nucleotide variants (g.-1639G>A, g.85G>T [p.V29L], g.121G>T [p.A41S], g.134T>C [p.V45A], g. 172A>G [p.R58G], g.1331G>A [p.V66M] and g.3487T>G [p.L128R]) was performed using the Tag-It™ Mutation Detection Kit (Luminex Molecular Diagnostics, ON, Canada) as previously described [18]. The *CYP2C9* and *VKORC1* genotypes were determined using Tag-It Data Analysis Software (Luminex Molecular Diagnostics) and the wild-type *CYP2C9*1* allele was assigned in the absence of other detectable variant alleles. Genotyping of the *VKORC1* g.106G>T (p.D36Y), g.698C>T (6009C>T, Accession Number AY587020) and g. 3730G>A (9041G>A, Accession Number AY587020) alleles was performed as previously described [18].

The *CYP2C9*8, *11* and **13* alleles were amplified by PCR and interrogated using RFLP assays. *CYP2C9*8* and **13* were amplified using *CYP2C9* exon 2/3 primers and **11* was amplified using exon 7 primers (Table 1). Reactions were performed in 25 μl containing approximately 100 ng of DNA, 1X PCR buffer (Invitrogen, CA, USA), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 mM of each primer and 1.0 unit of Platinum® Taq DNA Polymerase (Invitrogen). Amplification consisted of an initial denaturation step at 94°C for 5 min followed by 35 amplification cycles (94°C for 30 s, 58°C for 30 s, and 72°C for 45 s) and a final incubation at 72°C for 5 min. Genotyping of *CYP2C9*8, *11* and **13* was performed using *Aci*I, *Bst*NI and *Msp*I (New England BioLabs, MA, USA), respectively. The *VKORC1* g.-4931T>C, g.1173C>T, and g.2255C>T alleles were genotyped by PCR-RFLP using the primers, annealing temperatures and $MgCl_2$ concentrations listed in Table 1 and *Aci*I, *Mly*I and *Nco*I (New England BioLabs), respectively. Digested PCR products were visualized by agarose gel electrophoresis and positive control samples were confirmed by sequencing.

The *F7* g.-401G>T allele was PCR-amplified as described above using primers, annealing temperatures and $MgCl_2$ concentrations listed in Table 1. The common *APOE* alleles (ε2, ε3 and ε4) were PCR-amplified in 25 μl containing approximately 100 ng of DNA, 1X GC-RICH Reaction buffer (includes 1.5 mM $MgCl_2$), 0.2 mM of each dNTP, 0.4 mM of each primer (Table 1), 0.5 M GC-RICH Resolution Solution, and 0.4 units of GC-RICH Enzyme mix (Roche Applied Science, Basel, Switzerland). Amplification of *APOE* consisted of an initial denaturation step at 95°C for 5 min followed by 40 amplification cycles (95°C for 30 s, 62°C for 30 s and 72°C for 45 s) and a final incubation at 72°C for 5 min. Individual *F7* and *APOE* PCR products were purified using QIAquick columns (Qiagen, Hilden, Germany) and bidirectionally sequenced using amplification primers.

CYP2C9 & *VKORC1* exonic sequencing

Primers for *CYP2C9* sequencing were aligned against other *CYP2C* family members to ensure specific amplification of the *CYP2C9* gene and are listed with the *VKORC1* exon primers in Table 1. Amplification reactions were performed as described above using the indicated $MgCl_2$ concentrations and annealing temperatures (Table 1). Individual PCR products were

purified using QIAquick columns and bidirectionally sequenced using appropriate amplification primers.

Results

Analysis of *CYP2C9* & *VKORC1*

Of the 65 patients who were designated sensitive (<21 mg/week) or resistant (>49 mg/week), six were identified whose therapeutic dose was discrepant from their algorithm-predicted dose based on the alleles tested below. Of these, one patient was identified with a nonsynonymous variant following *CYP2C9* and *VKORC1* exonic sequencing. This patient was a 61.1 year old HIV-positive African–American male (height: 188 cm; weight: 86.2 kg) with a non-ischemic cardiomyopathy (New York Heart Association class III heart failure) and stage three chronic kidney disease who was treated with anticoagulation since 2002 for paroxysmal atrial fibrillation with a target INR of 2–3 and a mean therapeutic warfarin dose of 14.4 mg/week. Other medications included aspirin, amiodarone, 200 mg daily, carvedilol, 6.25 mg twice daily, lisinopril, 5 mg daily, spironolactone, 25 mg daily, furosemide, 120 mg twice daily, lamivudine, 150 mg twice daily, zidovudine, 300 mg twice daily, potassium chloride and docusate. The patient had not developed bleeding complications during anticoagulation, nor had he required a blood transfusion. His blood chemistry values including liver function tests were normal and hepatitis serologies revealed no evidence of infection.

Patients enrolled in the study were genotyped for eight *CYP2C9* and 12 *VKORC1* variant alleles (see Materials & methods) and the index patient described above had the following genotypes used for algorithm-based dose prediction: *CYP2C9*1/*1; VKORC1* g.-4931T/C, g.-1639G/G, g.1173C/C, g.2255C/T, g.3730G/G; *F7* g.-401G/G; *APOE* ε2/ε3. None of the seven tested warfarin resistant *VKORC1* mutations were identified in this patient. The predicted doses from the 15 tested algorithms ranged from 24.9 to 52.2 mg/week and are summarized in Table 2. Based on the clinical features and identified genotypes, all algorithms predicted a significantly higher maintenance warfarin dose (24.9 to 52.2 mg/week) than this patient actually required, even when adjusted for renal clearance [19]. In addition, although amiodarone inhibits CYP2C9 and decreases warfarin requirements, the eight algorithms [6,16,17,20–22], which included an adjustment for amiodarone or concomitant drug interaction predicted doses ranging from 24.9 to 50.1 mg/week, all greater than the observed 14.4 mg/week therapeutic dose.

Sequencing identified *CYP2C9*8*

The sensitivity to warfarin and the discrepancy between the therapeutic (14.4 mg/week) and algorithm-predicted (24.9 to 52.2 mg/week) warfarin doses in this patient prompted *CYP2C9* and *VKORC1* sequencing. In the *CYP2C9* gene, one homozygous base substitution was identified in exon 3 (rs7900194, c.449A/A) and three heterozygous nucleotide changes were identified in exon 9 (rs9332240, c.1540C/T; rs9332241, c.1561C/T; rs9332243, c.1628C/T). The exon 3 variant, a known arginine to histidine substitution (p.R150H; Figure 1), has been classified by the Cytochrome P450 Allele Nomenclature Committee as *CYP2C9*8*. All three exon 9 transition polymorphisms were in the 3′;-UTR and have not been reported in known *CYP2C9* haplotypes according to the Cytochrome P450 Allele Nomenclature Committee. No additional *VKORC1* coding region or intron–exon boundary changes were identified.

African–American *CYP2C9* allele frequencies

The *CYP2C9*2*, **3*, **4*, **5*, **6*, **8*, **11* and **13* allele frequencies were determined in 300 anonymous healthy African–American individuals from the greater New York, USA, metropolitan area and were compared with previous African–American *CYP2C9* population data (Table 3). Representative PCR-RFLP genotyping for *CYP2C9*8*, **11* and **13* is illustrated in Figure 2. No *CYP2C9*4* or **13* alleles were identified and **8* was the most

prevalent variant allele (0.047) in this cohort with a combined heterozygote and homozygote frequency of approximately 1 in 11 individuals. The wild-type (**1*) allele frequency was 0.867 and the identified variant alleles, **2*, **3*, **5*, **6*, **8* and **11*, had a combined frequency of 0.133 (~1 in 4 individuals). To determine if *CYP2C9*8* is specific to the African–American population, genomic DNAs (100 each) from Caucasian, Ashkenazi Jewish, Hispanic and Asian individuals were interrogated and the allele was only detected in Hispanics and Asians with frequencies of 0.015 and 0.010, respectively. The Hispanic frequency may represent an African contribution to the Caribbean Hispanic community of the New York metropolitan area [23].

All African–American *CYP2C9* allele frequencies were in Hardy–Weinberg equilibrium and the genotype frequencies are summarized in Table 4. Based on their genotypes, the assigned metabolic phenotypes [24–27] among the African–American individuals in our cohort were distributed as extensive (75.7%), intermediate (22.7%) and poor (1.7%) metabolizers. Notably, the high *CYP2C9*8* frequency (0.047) made **1*/**8* the most common intermediate metabolizer genotype in this population (8.7%). In addition, the inclusion of *CYP2C9*5*, **6*, **8* and **11* in the genotyping panel reclassified approximately 16% of African–Americans compared with the predicted phenotypes using a panel consisting of only *CYP2C9*2* and **3*.

African–American *VKORC1* allele frequencies

The *VKORC1* allele frequencies were determined in the African–American cohort and were compared with previous African–American population data (Table 5). The *VKORC1* g.-1639A sensitive allele, which is in strong linkage disequilibrium (LD) with g.1173C>T, g.1542G>C and g.2255C>T in Caucasians [7,8], had a frequency of 0.108, consistent with other reports of the g.-1639G>A and g.1173C>T tag-SNPs [5,28,29], but lower than the reported g.1542G>C frequency in African–Americans (0.255) [30]. No g.85G>T (p.V29L), g.121G>T (p.A41S), g.134T>C (p.V45A), g.172A>G (p.R58G), or g.3487T>G (p.L128R) warfarin resistant alleles were detected; however, three African–American g.1331G>A (p.V66M) carriers were identified. These three samples were interrogated for additional *VKORC1* alleles and the genotypes are listed in Table 6. In addition, the *VKORC1* g.106G>T (p.D36Y) warfarin resistant allele found at a high frequency in Ashkenazi Jewish [18,31] and Ethiopian [32] individuals was analyzed and no African–American carriers were identified.

All African–American *VKORC1* allele frequencies were in Hardy–Weinberg equilibrium and the g.-1639G>A genotype frequencies are summarized in Table 7. By contrast to the Caucasian and Asian populations, the low g.-1639A frequency (0.108) resulted in only approximately 20% of African–American individuals carrying either the G/A or A/A genotype.

Discussion

By sequencing the *CYP2C9* and *VKORC1* genes in patients with discrepant therapeutic and algorithm-predicted warfarin doses, an African–American male was identified who required a very low warfarin dose (14.4 mg/week) to achieve stable anticoagulation (INR of 2–3) and who was homozygous for the *CYP2C9*8* variant allele. To date, the frequency of the *CYP2C9*8* allele has not been systematically determined in distinct racial and ethnic populations. Therefore, we investigated the frequency of *CYP2C9*8* in healthy cohorts from various racial and ethnic groups and detected the allele only in African–American (0.047), Hispanic (0.015) and Asian (0.010) individuals, with frequencies of approximately 1 in 11, 1 in 34 and 1 in 50, respectively.

The *CYP2C9*8* c.449G>A transition polymorphism results in a nonsynonymous amino acid substitution (p.R150H) and was originally identified by resequencing *CYP2C9* in lymphoblastoid lines derived from African–American and African–Pygmy individuals [33]. In addition, Blaisdell *et al.* determined the *CYP2C9*8* allele frequency to be 0.036 in a small

African–American cohort (n = 14), and measured its *in vitro* metabolizing activity towards tolbutamide [33]. Kinetic studies revealed a slight reduction in K_m and an increased intrinsic clearance compared with *CYP2C9*1*, suggesting that **8* may have increased, not decreased, catalytic activity towards tolbutamide. In addition, an *in vivo* pharmacokinetic study of *CYP2C9*8* using the urinary losartan:E-3174 ratio as a marker of CYP2C9 oxidation in Beninese subjects indicated that *CYP2C9*8* activity was not significantly different than wild-type; however, the number of heterozygous **1/*8* human subjects in the study was small (n = 2), which may have confounded the results [34].

By contrast, when using phenytoin as a substrate for *in vivo* CYP2C9 activity, heterozygous *CYP2C9*8* carriers displayed significantly impaired metabolic activity [26], suggesting that variant *CYP2C9* alleles may have differing catalytic activities when measured *in vitro* or *in vivo*, and/or with different substrates. Similar contradictory phenotypes have been reported for *CYP2C9*11* with tolbutamide [33] or losartan [34] as substrates. However, *CYP2C9*11* recently has been associated with reduced therapeutic warfarin [25] and acenocoumarol [35] dose requirements. Importantly, heterozygosity for the *CYP2C9*8* allele was also recently found to significantly reduce warfarin requirements in a multi-ethnic cohort, indicating that this allele has impaired *in vivo* metabolism of warfarin [27].

In addition to oral anticoagulants, CYP2C9 is involved in the metabolism of hypoglycemic, anticonvulsant, nonsteroidal anti-inflammatory and antihypertensive drugs. For example, the hydroxylation and elimination of phenytoin, a commonly used anti-epileptic, is impaired by the *CYP2C9*2* and **3* variants both *in vitro* [36] and *in vivo* [37]. Moreover, the *CYP2C9*6* variant, originally identified in an African–American with phenytoin toxicity, results in defective phenytoin clearance [38] and similar phenotypic responses to phenytoin have been reported for *CYP2C9*5* and **11* [26]. Given the combined allele frequency of 0.133 for *CYP2C9*2, *3, *5, *6, *8* and **11* in the African–American population, our data suggest that approximately 1 in 4 African–Americans may be at risk for phenytoin or other important CYP2C9-mediated drug toxicity.

Although markedly more common in Caucasians and Asians [5,7], the *VKORC1* g.-1639G>A promoter polymorphism allele frequency in our African–American cohort (0.108) was consistent with other studies involving African–American populations. The g.-1639A allele reduces hepatic *VKORC1* expression [8,9,39] and is frequently identified in warfarin-sensitive individuals [3,5,7,8,11,21,22,28,40,41]. Importantly, this allele is in strong LD with g.1173C>T, g.1542G>C and g.2255C>T among Caucasians [7,8]. However, the haplotype structure of *VKORC1* among African–Americans is much more complex [8,42,43]. This may, in part, be why the commonly tested *VKORC1* alleles (g.-1639G>A and g.1173C>T) contribute less to warfarin dose variability in African–Americans than Caucasians [16,28]. Furthermore, the weaker LD between g.-1639G>A and g.1542G>C among African–Americans [29,43] could explain the discrepancy between the g.-1639G>A frequency in our study and the previously reported g.1542G>C frequency [30]. Recently, a comprehensive analysis of *VKORC1* polymorphisms identified 12 common African–American haplotypes and concluded that g.-1639G>A or g.1173C>T were still the best predictors of warfarin dose variability in this racial group [42], supporting their use in multi-ethnic dosing algorithms.

Interestingly, three *VKORC1* g.1331G>A (p.V66M) carriers were detected in our African–American cohort. This variant was originally identified shortly after the discovery of *VKORC1* in an African–Caribbean individual who required more than 25 mg/day of warfarin for therapeutic anticoagulation [44] and subsequently was reported in other warfarin-resistant individuals requiring at least 20 mg/day [45,46]. Like other *VKORC1* resistant mutations, p.V66M occurs within the cytoplasmic loop of the VKORC1 protein, and it is hypothesized that this domain may serve as an important accessory binding site for coumarin anticoagulants

[46]. To investigate if p.V66M occurs on a common haplotype among the African–American p.V66M carriers, additional *VKORC1* genotyping was performed. Based on these studies (Table 6), in this racial group the p.V66M allele likely occurs on a CGCCGTG haplotype background which corresponds to the H4 or BHT4 haplotype using the nomenclature of Rieder [8] or Limdi *et al*. [42], respectively. Given the diverse *VKORC1* African–American haplotype structure [8,42,43], further testing on additional p.V66M carriers is required to confirm this hypothesis.

Although the frequency of *VKORC1* g.1331G>A (p.V66M) is low among African–Americans (~1 in 100), the extreme resistance to warfarin consistently associated with the allele suggests that its inclusion into genotyping panels, if cost-effective, may also be considered. In addition, the *CYP4F2*3* (p.V433M) variant recently shown to be responsible for approximately 1–2% of the variation in warfarin dosing among Caucasians [47,48] may also warrant inclusion into genotyping panels following evaluation in multi-ethnic cohorts.

Conclusion

The frequency of the *CYP2C9*8* allele in the African–American population (~1 in 11 individuals) and the finding that homozygosity and heterozygosity [27] are associated with reduced warfarin-dose requirements underscores the importance of its incorporation into future pharmacogenetic-based dosing algorithms. Furthermore, as the majority of warfarin algorithms only include *CYP2C9*2* and **3* (with *VKORC1* g.-1639G>A or g.1173C>T), our data indicate that the inclusion of *CYP2C9*8* alone could reclassify the predicted metabolic phenotypes of almost 10% of African–Americans, and when combined with *CYP2C9*5, *6* and **11*, more than 15%. Thus, in the African–American population, the incorporation of *CYP2C9*8* into genotyping panels may improve dose prediction of CYP2C9-metabolized drugs, including warfarin.

Future perspective

Dosing algorithms that include common *CYP2C9* and *VKORC1* polymorphisms have been developed and currently are being prospectively evaluated to determine if they predict the initial warfarin dose more accurately than standard clinical algorithms. Given that the majority of pharmacogenetic algorithms perform better in Caucasians than other racial and ethnic groups, it is likely that other genetic variants and nongenetic factors will be identified that significantly influence warfarin dosing and drug response in specific subpopulations. The identification of novel and racial-specific CYP450 alleles and other pharmacogenetic variants that influence drug metabolism will permit improved phenotypic prediction and drug selection. Inclusion of known and novel variants into future clinical testing panels must be accompanied by careful interpretation of genotype–phenotype relationships, physician awareness and cost-effective testing.

Executive summary

- Although the frequencies of pharmacogenetic variants differ among racial groups, most pharmacogenetic algorithms for genotype-guided warfarin dosing only include two *CYP2C9* alleles (**2* and **3*) and a single *VKORC1* allele (g.-1639G>A or g.1173C>T) commonly found among Caucasians.
- To identify other *CYP2C9* and *VKORC1* alleles important in warfarin dose variability, these genes were sequenced in selected sensitive (<21 mg/week) and resistant (>49 mg/week) individuals with a target International Normalized Ratio of 2–3.

Identification of *CYP2C9*8* & African–American allele frequencies

- Sequencing identified an African–American male with a lower than predicted therapeutic warfarin dose (14.4 mg/week), previously genotyped as *CYP2C9*1*/**1*, who was homozygous for *CYP2C9*8* (c.449G>A; p.R150H).
- Genotyping 600 alleles from healthy African–American blood donors identified *CYP2C9*8* as their most frequent variant *CYP2C9* allele (0.047; ~1 in 11 individuals) and the combined allele frequency of *CYP2C9*2, *3, *5, *6, *8* and **11* was 0.133 (~1 in 4 individuals).

VKORC1 African–American allele frequencies

- The *VKORC1* g.-1639A allele frequency was 0.108 and three warfarin resistant g. 1331G>A (p.V66M) carriers were identified (1 in ~100 individuals).

Conclusion

- Inclusion of *CYP2C9*8* alone reclassified the predicted metabolic phenotypes of almost 10% of African–Americans, and when combined with *CYP2C9*5, *6* and **11*, more than 15%.
- For the African–American population, the incorporation of *CYP2C9*8* into genotyping panels may improve dose prediction of CYP2C9-metabolized drugs, including warfarin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial & competing interests disclosure

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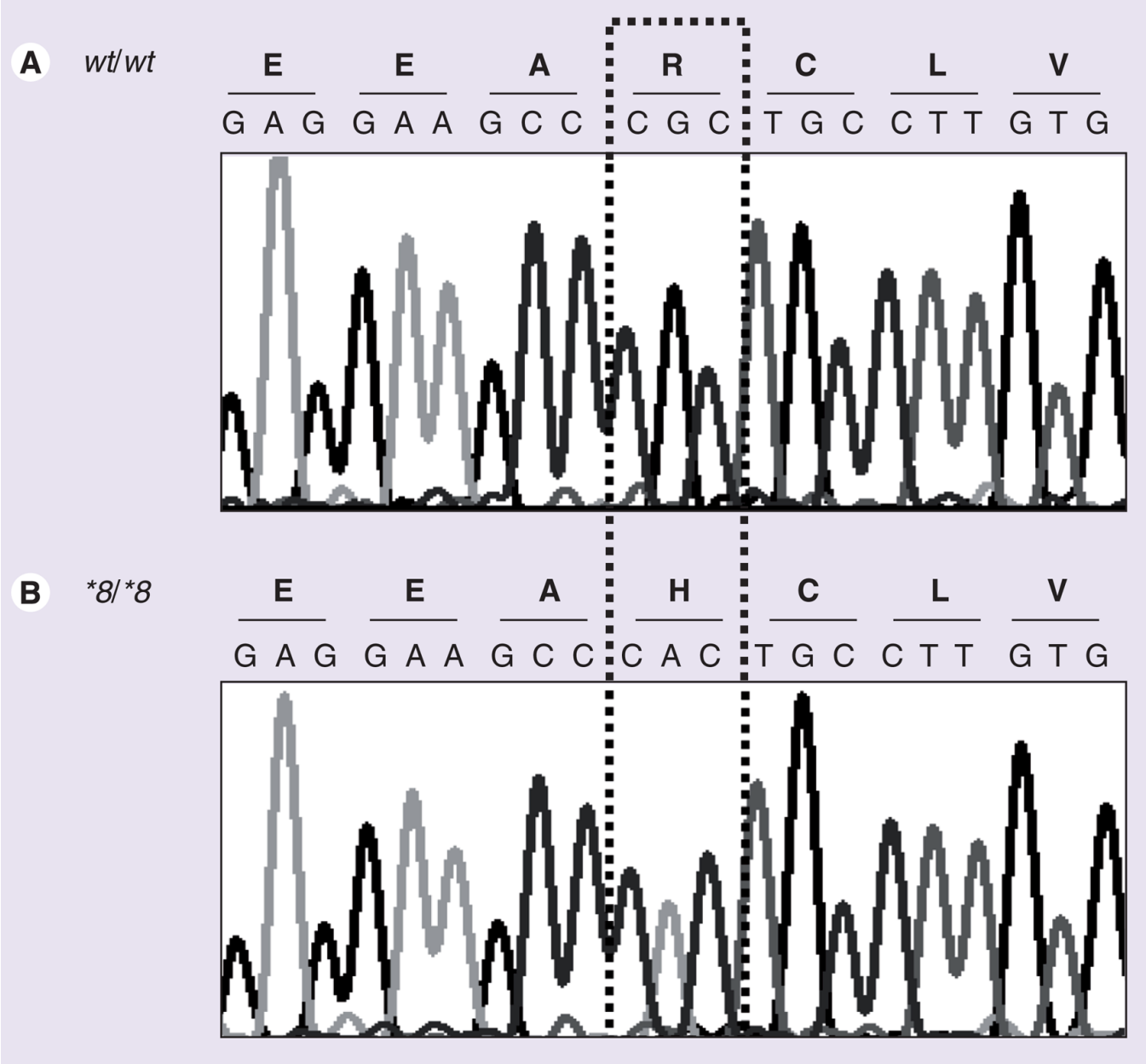

Figure 1. Sequence analysis of *CYP2C9* exon 3 in the African–American index patient compared with that from a homozygous wild-type individual

(A) Electropherogram illustrates the wild-type genomic DNA and amino acid sequences. **(B)** Electropherogram illustrates the *CYP2C9*8/*8* genomic DNA (c.449G>A) and amino acid (p.R150H) sequences identified in the African–American index patient.

wt: Wild-type.

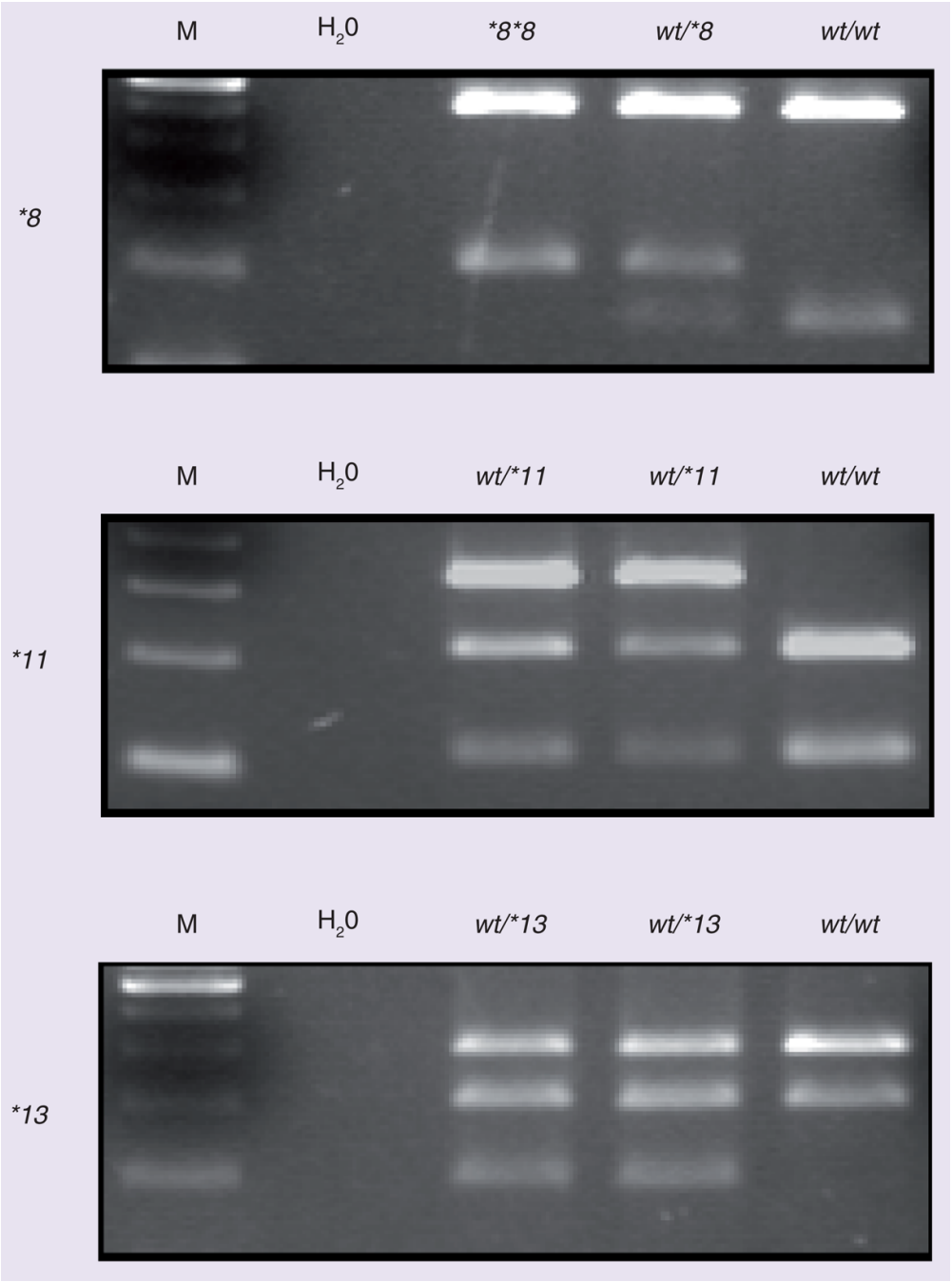

Figure 2. Representative PCR-RFLP genotyping of *CYP2C9 *8, *11* and *13*

Digested PCR products were visualized by agarose gel electrophoresis and compared with 100 bp size markers. Genotypes of all samples are noted above appropriate gel lanes.
M: Size marker; H_2O: Reagent control; wt: Wild-type.

Table 1
Primer sequences for PCR amplification.

Primer	Sequence (5′–3′)	T_a (°C)	$MgCl_2$ (mM)	Product (nt)
CYP2C9				
Exon 1-FWD*	GCCTTCAGGAATTTTTTTTA	53	2.5	929
Exon 1-REV*	TTTTACTTTACCATTACCTCTTG			
Exon 2/3-FWD‡	TACAAATACAATGAAAATATCATG	58	2.5	690
Exon 2/3-REV‡	CTAACAACCAGGACTCATAATG			
Exon 4-FWD	TGTTAAGGGAATTTGTAGGTAAGA	58	2.5	366
Exon 4-REV	TGCACTTCAGAGCTTGATCC			
Exon 5-FWD§	CAGAGCTTGGTATATGGTATG	58	2.5	321
Exon 5-REV§	GTAAACACAGAACTAGTCAAC			
Exon 6-FWD*	GTTTGGGCAAGTTGGTCTA	58	2.5	395
Exon 6-REV*	AGAAACAGGAAGGAGGACAC			
Exon 7-FWD	CTGAATTGCTACAACAAATGTG	58	2.5	315
Exon 7-REV	GATACTATGAATTTGGGGACTTC			
Exon 8-FWD	ACCACTGTTTCTTCAACCTTCATGG	58	2.5	370
Exon 8-REV	TTAGGATGTATCATGAGCAGGGTG			
Exon 9-FWD*	TATTGCATATTCTGTTTGTGC	58	2.5	803
Exon 9-REV*	CAAGTAACTCTAACACTCACCC			
VKORC1				
g.-4931T>C-FWD	TCCAGCTTTAGCCACCATCT	60	2.5	560
g.-4931T>C-REV	TTTTGACCATATTTTCTTCTAGCATTT			
g.1173C>T-FWD	AGGGTCAGTGACATGGAATCCTGA	60	1.5	178
g.1173C>T-REV	GGGTGGAACCAGGTTAGGACTGT			
g.2255C>T-FWD	ATGCCATATGCCGGAGATGAGACT	60	1.5	222
g.2255C>T-REV	ACTGGTCTCTGAAGCTCTTGCCAT			
Exon 1-FWD	AAGTGCTGGGATTACAAGCGTGAG	60	1.5	720
Exon 1-REV	GTCCCTTGCCTCGCACTCTTATTT			
Exon 2-FWD	AGGGTCAGTGACATGGAATCCTGA	60	1.5	535

Primer	Sequence (5′–3′)	T_a (°C)	$MgCl_2$ (mM)	Product (nt)
Exon 2-REV	GGGACCTAGGATGTCTTTAAGGG			
Exon 3-FWD	TTTAGAGACCCTTCCCAGCAGCTC	60	1.5	700
Exon 3-REV	CACTCTCCCTCTGACTCACCCTT			
F7				
g.-401G>T-FWD[¶]	TAAGAAACCAGCCTCCCTTG	58	1.5	229
g.-401G>T-REV[¶]	CGTGCAGGTGTTAAGGTGTG			
APOE				
APOE-FWD	TGAAGGCCTACAAATCGGAACTGG	62	1.5	459
APOE-REV	GGCTGCCCATCTCCTCCAT			

[*] Primer sequence from [49].

[‡] Primer sequence from [50].

[§] Primer sequence from [38].

[¶] Primer sequence from [17].

FWD: Forward (sense); nt: Nucleotides; REV: Reverse (antisense); T_a: Annealing temperature.

Table 2

Mean therapeutic and algorithm-predicted warfarin doses in the index patient.

Warfarin dose	mg/week	Ref.
Mean therapeutic dose	14.4	–
Algorithm-predicted dose ‡		
Sconce *et al.* (2005)	47.6	[41]
Wadelius *et al.* (2005)	49.7	[21]
Herman *et al.* (2006)	42.5	[51]
Takahashi *et al.* (2006)	50.3	[5]
Tham *et al.* (2006)	32.9	[52]
Anderson *et al.* (2007)	45.8	[53]
Miao *et al.* (2007)	49.1	[54]
Zhu *et al.* (2007)	52.2	[40]
Gage *et al.* (2008)	34.6	[16]
Schelleman *et al.* (2008) (C)	41.9	[17]
Schelleman *et al.* (2008) (AA)	28.4	[17]
Schelleman *et al.* (2008) (C + AA)	41.5	[17]
Wu *et al.* (2008)	24.9	[20]
Klein *et al.* (2009)	35.4	[6]
Wadelius *et al.* (2009)	50.1	[22]

‡Based on the following genotypes: *CYP2C9*: **1/*1; VKORC1* (Accession Number AY587020) g.-4931T>C (381T>C): T/C, g.-1639G>A (3673G>A): G/G, g.1173C>T (6484C>T): C/C, g.2255C>T (7566C>T): C/T, g.3730G>A (9041G>A): G/G; F7 g.-401G>T: G/G; APOE: ε2/ε3.

AA: African–American; C: Caucasian.

Table 3

African–American *CYP2C9* allele frequencies.

Allele	This study (n = 600)		Blaisdell (2004) [33](n = 28)‡	Takahashi (2006) [5] (n = 128)	Momary (2007) [30] (n = 202)	Kealey (2007) [55] (n = 336)	Limdi (2008) [29] (n = 536)
	Frequency	*95% CI*					
*CYP2C9*1*	0.867	0.839–0.894	0.831‡	0.953	0.946	0.961	0.937
*CYP2C9*2*	0.028	0.015–0.042	0.000	0.000	0.030	0.036	0.013
*CYP2C9*3*	0.020	0.009–0.031	0.000	0.008	0.015	0.003	0.019
*CYP2C9*4*	0.000	0.000–0.000	0.000	0.000	ND	ND	ND
*CYP2C9*5*	0.015	0.005–0.025	0.000	0.008	0.010	ND	0.009
*CYP2C9*6*	0.010	0.002–0.018	0.000	0.008	ND	ND	0.007
*CYP2C9*8*	0.047	0.030–0.064	0.036	ND	ND	ND	ND
*CYP2C9*11*	0.013	0.004–0.023	0.000	0.023	ND	ND	0.015
*CYP2C9*13*	0.000	0.000–0.000	0.000	ND	ND	ND	ND

‡This study performed *CYP2C9* sequencing on 14 or 15 African–American samples. Of these, 1 of 14 had *CYP2C9*8*, 4 of 15 (0.133) had *CYP2C9*9* (p.H251R) and several noncoding or synonymous variants were identified. The frequency of *CYP2C9*1* was therefore calculated as 1 – the sum of *CYP2C9*8* and **9*.

n: Number of alleles; ND: Not determined.

Table 4

African–American *CYP2C9* genotype frequencies (n = 300).

Predicted metabolizer phenotype/genotype	Number of subjects	Observed (expected‡) frequency (%)
Extensive metabolizer		
**1/*1*	227	75.7 (75.1)
Intermediate metabolizer		
**1/*2*	13	4.3 (4.9)
**1/*3*	10	3.3 (3.5)
**1/*5*	8	2.7 (2.6)
**1/*6*	5	1.7 (1.7)
**1/*8*	26	8.7 (8.1)
**1/*11*	6	2.0 (2.3)
Total	68	22.7 (23.1)
Poor metabolizer		
**2/*2*	1	0.3 (0.1)
**2/*3*	1	0.3 (0.1)
**3/*11*	1	0.3 (0.1)
**5/*6*	1	0.3 (0.0)
**8/*11*	1	0.3 (0.1)
Total	5	1.7 (0.4)

‡Predicted Hardy–Weinberg frequencies.

Table 5

African–American *VKORC1* allele frequencies.

Allele	This study (*n* = 600)		Takahashi (2006) [5] (n = 128)*	Momary (2007) [30] (n = 216)‡	Schelleman (2007) [28] (n = 318)	Limdi (2008) [29] (n = 518)*
	Frequency	*95% CI*				
VKORC1 g.-1639G	0.892	0.867–0.917	0.914	0.745	0.896	0.894
VKORC1 g.-1639A	0.108	0.083–0.133	0.086	0.255	0.104	0.106
VKORC1 g.1331G	0.995	0.989–1.000	0.984	ND	ND	ND
VKORC1 g.1331A (p.V66M)	0.005	0.000–0.011	0.016	ND	ND	ND

*Based on tag-SNP g.1173C>T (6484C>T).

‡Based on tag-SNP g.1542G>C (6853G>C).

n: Number of alleles; ND: Not determined.

Table 6

VKORC1 Tag-SNP genotypes among g.1331G>A (p.V66M) carriers.

Subject	g.-4931T>C (381*)	g.-1639G>A (3673*)	g.698C>T (6009*)	g.1173C>T (6484*)	g.1542G>C (6853*)	g.2255C>T (7566*)	g.3730G>A (9041*)
1	C/C	G/G	C/C	C/C	G/G	C/T	G/G
2	C/C	G/G	C/C	C/C	G/C	C/T	G/G
3	C/T	G/G	C/C	C/C	G/G	C/T	G/A

*Position based on Accession Number AY587020.

Table 7

African–American *VKORC1* g.-1639G>A genotype frequencies (n = 300).

Genotype	Number of subjects	Observed (expected*) frequency (%)
G/G	241	80.3 (79.5)
G/A	53	17.7 (19.3)
A/A	6	2.0 (1.2)

* Predicted Hardy–Weinberg frequencies.