

Warfarin Pharmacogenetics: *CYP2C9* and *VKORC1* Genotypes Predict Different Sensitivity and Resistance Frequencies in the Ashkenazi and Sephardi Jewish Populations

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Warfarin is a widely used anticoagulant that has a narrow therapeutic range because of both genetic and environmental factors. *CYP2C9**2 (p.R144C), *CYP2C9**3 (p.I359L), and the *VKORC1* promoter (g.-1639G→A) polymorphisms occur frequently in patients who are warfarin “sensitive” and require lower doses, whereas patients with *VKORC1* missense mutations are warfarin “resistant” and require higher doses. To compare the *CYP2C9* and *VKORC1* allele and genotype frequencies among 260 Ashkenazi (AJ) and 80 Sephardi Jewish (SJ) individuals, we genotyped six *CYP2C9* and eight *VKORC1* alleles by using the Tag-It Mutation Detection Kit and PCR-RFLP assays. The “sensitive” *CYP2C9**2 and *3 alleles had significantly higher frequencies in SJ than in AJ individuals, 0.194 and 0.144 versus 0.127 and 0.081, respectively ($p \leq 0.001$). In contrast, the *VKORC1* p.D36Y mutation, which predicts warfarin “resistance,” had a significantly higher frequency in AJ than in SJ individuals, 0.043 versus 0.006, respectively ($p \leq 0.025$). Of note, 11.3% of AJ individuals predicted to be *CYP2C9* extensive metabolizers and 8.7% of those predicted to be intermediate and poor metabolizers were *VKORC1* p.D36Y carriers who require markedly higher warfarin doses. Thus, ~10% of all AJ individuals would be misclassified when only genotyping *CYP2C9**2, *3, and *VKORC1* g.-1639G→A, underscoring the importance of screening for p.D36Y prior to initiating warfarin anticoagulation in AJ individuals. Taken together, our findings show that ~85% of AJ and ~90% of SJ individuals have at least one “sensitive” (*CYP2C9**2, *3, *VKORC1* g.-1639G→A) or “resistant” (*VKORC1* p.D36Y) allele, indicating that each group has different warfarin pharmacogenetics and would benefit from genotype-based dose predictions.

Warfarin is one of the most widely used anticoagulants, yet interindividual differences in drug response, a narrow therapeutic range, and a high risk of bleeding or stroke complicate its clinical use.^{1–3} The metabolism and anticoagulant action of warfarin presumably are moderated by many genes; however, the polymorphic cytochrome P450-2C9 (*CYP2C9* [MIM 601130]) and vitamin K epoxide reductase complex subunit 1 (*VKORC1* [MIM 608547]) genes alter warfarin pharmacokinetics and pharmacodynamics, respectively. In addition, environmental (e.g., vitamin K intake, comedications, age, and body-surface area) and other factors contribute to individual dose requirements, and together the currently identified genetic and environmental components account for approximately half of the interindividual warfarin dose variation.^{2,4–7} Importantly, dosing algorithms that incorporate several of these factors and the variant *CYP2C9* and *VKORC1* genotypes recently have been described.^{8,9}

The variant *CYP2C9**2 (p.R114C) and *3 (p.I359L) alleles and the *VKORC1* g.-1639G→A promoter polymorphism occur in patients who are drug “sensitive” and require a lower average warfarin dose,^{10,11} whereas *VKORC1* missense mutations have been described in patients who are “resistant” and require higher warfarin doses.^{12–17} To facilitate the use of *CYP2C9* and *VKORC1* genotyping in routine anticoagulation practice, it is important to characterize the allele frequencies in various populations. Recently, we reported the *CYP2C9*, *CYP2C19*, and *CYP2D6* allele and genotype frequencies in 250 healthy Ashkenazi Jewish (AJ) individuals.¹⁸ Here, we extend these

studies to include the *VKORC1* g.-1639G→A promoter polymorphism (rs9923231), previously reported missense mutations, p.V29L, p.A41S, p.V45A, p.R58G, p.V66M, and p.L128R, and the recently described warfarin-resistant p.D36Y missense mutation in a separate cohort of 260 AJ and 80 Sephardic Jewish (SJ) individuals.

Peripheral blood samples were obtained with informed consent from 592 unrelated healthy AJ and 80 SJ individuals from the greater New York metropolitan area. All personal identifiers were removed, the samples were tested anonymously, and genomic DNA was isolated with the Puregene DNA Purification kit (Gentra). 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]; Figure 1) was performed on 260 AJ and all 80 SJ individuals with the Tag-It Mutation Detection Kit (Luminex Molecular Diagnostics) according to the manufacturer's instructions. The *CYP2C9* and *VKORC1* genotypes for each sample were determined with 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.⁷ The *CYP2C9* allele designations refer to those defined by the Cytochrome P450 Allele Nomenclature Committee,¹⁹ and representative heterozygous samples for all identified alleles were confirmed by direct sequencing.

The variant *CYP2C9**2, *3, *4, *5, and *6 alleles have been identified in individuals with impaired *CYP2C9*-mediated metabolism and lower warfarin dose requirements,^{4,20}

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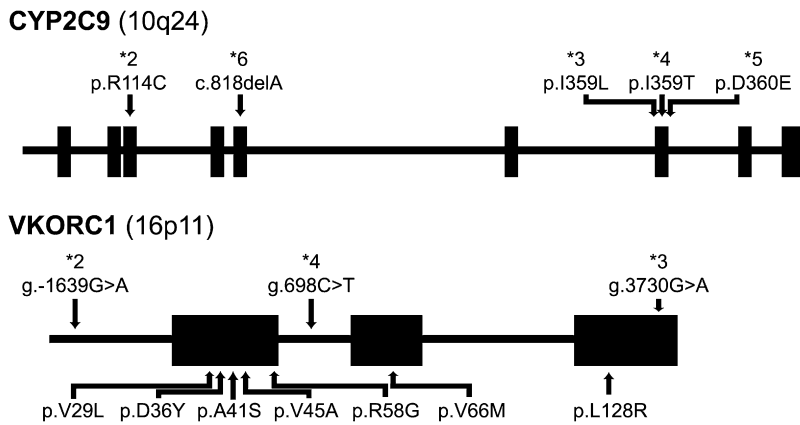


Figure 1. Schematic of the *CYP2C9* and *VKORC1* Genes

The locations of the nucleotide variations and missense mutations analyzed in this study are noted and are based on accession numbers M61857 and AY587020. The g.-1639G→A (*rs9923231*), g.3730G→A (*rs7294*), and g.698C→T (*rs17708472*) tag-SNPs for the *VKORC1**2,*3, and *4 haplotypes correspond to 3673G→A, 9041G→A, and 6009C→T (accession number AY587020), respectively. Illustrations are not to scale.

yet only the *2 and *3 alleles were identified in our healthy AJ and SJ cohorts. When the *CYP2C9* data from the 250 AJ individuals reported previously¹⁸ were combined with the results from the 260 AJ individuals studied here, the combined AJ allele frequencies (n = 510; Table 1) were distinct from those in Asian and African American populations and similar to those in other populations with European ancestry.²¹ In contrast, the SJ cohort had significantly higher ($p \leq 0.001$) *CYP2C9**2 and *3 allele frequencies when compared to the AJ population, 0.194 and 0.144 versus 0.127 and 0.081, respectively (Table 1). On the basis of their genotypes, the assigned *CYP2C9* metabolic phenotypes¹¹ among the AJ and SJ were distributed as extensive, intermediate, and poor metabolizers (Table 2). Given the increased frequency of the deficient *CYP2C9**2 and *3 alleles in the SJ cohort, this population had significantly higher ($p \leq 0.002$) frequencies of intermediate and poor metabolizers when compared to the AJ, 42.6% and 12.6% versus 32.4% and 4.7%, respectively (Table 2). Furthermore, the *CYP2C9**2 and *3 allele frequencies observed in our SJ cohort were considerably higher than those in other populations with European ancestry.^{20,22} Given that *CYP2C9**2 and *3 are associated with an increased risk of excessive anticoagula-

tion and bleeding events among warfarin-treated patients,^{23,24} our results suggest that the SJ population is predisposed to warfarin sensitivity when compared to other populations with European ancestry.

Warfarin exerts its anticoagulant effect by inhibiting vitamin K epoxide reductase, the catalytic subunit of which is encoded by *VKORC1*, thereby preventing the regeneration of vitamin K from vitamin K epoxide. After the identification of *VKORC1* in 2004,^{12,13} common *VKORC1* polymorphisms and haplotypes that are strongly associated with warfarin response were reported.^{4,25,26} Although the molecular mechanisms by which *VKORC1* polymorphisms modulate warfarin response remain unclear, recent evidence suggests that the g.-1639G→A promoter polymorphism reduces hepatic *VKORC1* expression and, therefore, decreases warfarin dose requirements.^{10,26} The g.-1639G promoter allele is present in the *VKORC1**1, *3, and *4 haplotypes and is typically associated with a “normal” warfarin dose.^{27,28} In contrast, the g.-1639A promoter allele, which is in strong linkage disequilibrium with 6484C→T (*rs9934438*), 6853G→C (*rs8050894*), and 7566C→T (*rs2359612*; accession number AY587020),²⁷ is present in the *VKORC1**2 haplotype^{17,27–29} and is consistently

Table 1. *CYP2C9* and *VKORC1* Allele Frequencies

Allele	AJ ^a			SJ		
	Alleles	Frequency	95% CI	Alleles	Frequency	95% CI
<i>CYP2C9</i>						
*1	806	0.790	0.765–0.815	106	0.663 ^c	0.589–0.763
*2	130	0.127	0.107–0.148	31	0.194 ^c	0.133–0.255
*3	83	0.081	0.065–0.098	23	0.144 ^c	0.089–0.198
*5	1	0.001	0.000–0.003	0	-	-
<i>VKORC1</i> ^b						
<i>g.-1639G</i>	277	0.533	0.490–0.576	80	0.500	0.423–0.577
<i>g.-1639A</i>	243	0.467	0.424–0.510	80	0.500	0.423–0.577
<i>g.106G</i>	1133	0.957	0.945–0.969	159	0.994 ^d	0.982–1.000
<i>g.106T (p.D36Y)</i>	51	0.043	0.031–0.055	1	0.006 ^d	0.000–0.018

^a Combined AJ *CYP2C9* data with Scott et al.¹⁸

^b Based on accession number AY587020 (*g.-1639G*→*A* and *g.106G*→*T* correspond to 3673G→A and 5417G→T, respectively).

^c $p \leq 0.001$.

^d $p \leq 0.025$.

Table 2. CYP2C9 Genotype Frequencies

Predicted Metabolizer Phenotype/Genotype	AJ ^a		SJ	
	n ^b	Observed (Expected ^c) Frequency (%)	n ^b	Observed (Expected ^c) Frequency (%)
Extensive (EM)				
*1/*1	321	62.8 (62.5)	36	45.1 ^d (43.9)
Intermediate (IM)				
*1/*2	105	20.5 (20.1)	23	28.8 (25.7)
*1/*3	60	11.7 (12.8)	11	13.8 (19.0)
*1/*5	1	0.2 (0.2)	0	0 (0.0)
Total	166	32.4 (33.1)	34	42.6 ^d (44.7)
Poor (PM)				
*2/*2	6	1.2 (1.6)	1	1.3 (3.8)
*2/*3	13	2.5 (2.1)	6	7.5 (5.6)
*3/*3	5	1.0 (0.7)	3	3.8 (2.1)
Total	24	4.7 (4.4)	10	12.6 ^d (11.5)

^a Combined AJ *CYP2C9* data with Scott et al.¹⁸

^b Number of subjects.

^c Predicted Hardy-Weinberg frequencies.

^d $p \leq 0.002$.

associated with warfarin-“sensitive” individuals who require lower warfarin doses.^{6,7,9,30,31} Unlike African Americans and similar to other individuals of European descent,²⁷ the g.-1639A allele was present at high frequencies in both our AJ (0.467) and SJ (0.500) cohorts (Table 1). Similar G/G, G/A, and A/A genotype frequencies were observed in the two Jewish subpopulations (Table 3), and they were comparable to those reported in other individuals of European descent.^{26,27} Interestingly, haplotype analysis based on the PERLEGEN SNP public database indicates that the g.-1639A allele is predominant in the Chinese population (0.958),²⁷ and such a finding is consistent with the lower warfarin dose requirements typically observed in this group.³²

Heterozygous *VKORC1* missense mutations have been identified in individuals who are resistant to warfarin and homozygous mutations have been reported in families with combined deficiency of vitamin-K-dependent clotting factors type 2 (MIM 607473).^{13,14} Although other nongenetic factors can lead to increased warfarin doses (e.g., excessive vitamin K intake, drug interactions, etc.), heterozygous *VKORC1* missense mutations alter vitamin K epoxide reductase activity and, therefore, play a consider-

able role in the warfarin-“resistant” phenotype.^{13,14,16} However, these mutations presumably are rare given that p.V29L, p.V45A, p.R58G, and p.L128R were not identified in 384 healthy non-Jewish control chromosomes.¹³ Moreover, none of these and other (p.A41S and p.V66M) missense mutations were identified among the healthy AJ and SJ individuals in our study.

The p.D36Y *VKORC1* mutation was recently identified in individuals who required an average warfarin dose greater than 10 mg/day.¹⁷ This missense mutation is located in the conserved luminal loop (L1) *VKORC1* region that also contains p.V29L, p.A41S, p.V45A, p.R58G, and p.V66M (Figure 1).^{13,33} For an analysis of *VKORC1* p.D36Y in our AJ and SJ cohorts, a PCR-RFLP assay was employed¹⁷ with sense (5'-AACCTGGAGATAATGGGCAGC A-3') and antisense (5'-ACACCGATCCCAGACTCCAGAA TA-3') PCR primers and RsaI (New England BioLabs). The frequency of p.D36Y in 100 AJ and 100 East African individuals was 0.040 and 0.150, respectively,¹⁷ and it had a comparable allele frequency of 0.043 in our larger AJ cohort (n = 592), and this was significantly higher than that observed among the SJ individuals (0.006; Table 1). The high carrier frequency of p.D36Y in the AJ (1 in 12) indicates that this population may be predisposed to warfarin resistance.

Table 4 summarizes the combined *CYP2C9* and *VKORC1* genotype frequencies for the 260 AJ and 80 SJ cohorts, which were in Hardy-Weinberg equilibrium. Predicted *CYP2C9* metabolizer phenotypes¹¹ for both the AJ and SJ were subdivided on the basis of *VKORC1* g.-1639G→A status and the presence of the p.D36Y warfarin-resistant mutation. Although the *VKORC1* phase could not be determined in our study, it is notable that no p.D36Y carriers were identified in g.-1639A/A (*VKORC1* *2/*2) individuals, suggesting that p.D36Y occurs on a g.-1639G background

Table 3. VKORC1 g.-1639G→A Genotype Frequencies

Genotype	AJ		SJ	
	n ^a	Observed (Expected ^b) Frequency (%)	n ^a	Observed (Expected ^b) Frequency (%)
g.-1639G→A				
G/G	76	29.2 (28.4)	20	24.1 (26.2)
G/A	125	48.1 (49.8)	45	54.2 (50.0)
A/A	59	22.7 (21.8)	18	21.7 (23.8)

^a Number of subjects.

^b Predicted Hardy-Weinberg frequencies.

Table 4. CYP2C9 and VKORC1 Combined Genotype Frequencies

CYP2C9 Genotype (Predicted Metabolizer Phenotype ^a)	VKORC1 g.-1639G→A	AJ			SJ		
		n ^b	Frequency (%)	No. of p.D36Y Carriers	n ^b	Frequency (%)	No. of p.D36Y Carriers
*1/*1 (EM)							
	G/G	49	18.8	9 ^c	9	11.3	1
	G/A	81	31.2	10	14	17.5	0
	A/A	38	14.6	0	13	16.3	0
*1/*2 or *1/*3 (IM)							
	G/G	26	10.0	2	11	13.8	0
	G/A	38	14.6	3	17	21.3	0
	A/A	20	7.7	0	6	7.5	0
*2/*2, *2/*3, or *3/*3 (PM)							
	G/G	1	0.4	1	2	2.5	0
	G/A	6	2.3	2	5	6.3	0
	A/A	1	0.4	0	3	3.8	0

^a EM, extensive metabolizer; IM, intermediate metabolizer; and PM, poor metabolizer.

^b Number of subjects.

^c One p.D36Y homozygote was found in this group.

(Table 4). Given that the *VKORC1**3 and *4 haplotypes, which are associated with “normal” warfarin dose requirements,²⁸ also occur on a g.-1639G background,²⁷ additional PCR-RFLP genotyping to identify these alleles was performed on all p.D36Y carriers. Tag-SNPs 9041G→A (rs7294; g.3730G→A) and 6009C→T (rs17708472; g.698C→T; accession number AY587020; Figure 1)²⁷ were interrogated for identification of *VKORC1**3 and *4 with sense (*VKORC1**3: 5'-TTTGCTTTGGCATGTGAGCCTTGC-3'; *VKORC1**4: 5'-GCATAATGACGGAATACAGAGGAGGC-3') and antisense (*VKORC1**3: 5'-ACAGTCCATGGCAGACACATGGTT-3'; *VKORC1**4: 5'-GGTAGAGACAGGC TTTCACCATGT-3') PCR primers and *Aci*I and *Bfa*I (New England BioLabs), respectively. The *VKORC1**1 haplotype was assigned in the absence of other detectable alleles, and representative p.D36Y, *VKORC1**3, and *4 positive samples were confirmed by direct sequencing. Interestingly, all the p.D36Y heterozygous individuals evaluated in our AJ and SJ cohorts were heterozygotes for *VKORC1**1 and *2, *3, or *4, and importantly, one AJ individual was identified who was homozygous for both p.D36Y and *VKORC1**1 (data not shown). Thus, our findings strongly support the hypothesis that p.D36Y tags a unique haplotype found on a *VKORC1**1 background.¹⁷

Notably, patients with p.D36Y who have dose-reducing variant *CYP2C9**2, *3, or *VKORC1**2 alleles still require higher warfarin doses (>10 mg/day), indicating that this missense mutation is “dominant” when present in individuals with warfarin-“sensitive” *CYP2C9* and *VKORC1* alleles.¹⁷ Importantly, 11.3% of AJ individuals predicted to be *CYP2C9* extensive metabolizers and 8.7% of those predicted to be intermediate and poor metabolizers were *VKORC1* p.D36Y carriers who would require markedly higher warfarin doses (Table 4). Thus, ~10% of all AJ individuals would be misclassified when only genotyping

*CYP2C9**2, *3 and *VKORC1* g.-1639G→A, thereby underscoring the importance of screening for p.D36Y prior to initiating warfarin anticoagulation in AJ individuals.

The recently reported warfarin dosing algorithms that incorporate genotype information only include *CYP2C9**2, *3, and *VKORC1* g.-1639G→A.^{8,9} On the basis of these dosing guidelines and given that 211 AJ and 71 SJ individuals had at least one variant *CYP2C9* (*2 and *3) or *VKORC1* (g.-1639G→A) allele, 81.2% of AJ and 88.8% of SJ individuals would be candidates for genotype-based dose adjustments (Table 4). The high frequency of variant *CYP2C9* and *VKORC1* alleles in AJ and SJ individuals indicates that clinical testing of these genes is warranted among individuals initiating warfarin therapy, as recently recommended by a Food and Drug Administration Advisory Committee (see Web Resources). Moreover, warfarin-“resistant” *VKORC1* missense mutations are currently not incorporated into published dosing algorithms, yet their identification prior to warfarin administration may reduce complications because of ineffective anticoagulation. By including p.D36Y in our analysis, 84.6% of AJ and 90.0% of SJ individuals would benefit from *CYP2C9* and *VKORC1* genotyping prior to initiating warfarin-based anticoagulation. Further studies are necessary to correlate warfarin dose requirements in patients with the p.D36Y allele and various other *CYP2C9* and *VKORC1* alleles. Such information is required to accurately model the contribution of p.D36Y to warfarin dose for inclusion in warfarin dosing algorithms for the AJ population.

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Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://ncbi.nlm.nih.gov/Omim/> (for *CYP2C9*, *VKORC1*, and combined deficiency of vitamin-K-dependent clotting factors type 2)

U.S. Food and Drug Administration, <http://www.fda.gov/cder/drug/infopage/warfarin/>

WarfarinDosing.org, <http://warfarindosing.org>

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