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Gamma-glutamyl carboxylase and its influence on warfarin dose

Cristi R. King¹, Elena Deych¹, Paul Milligan¹, Charles Eby^{1,2}, Petra Lenzini¹, Gloria Grice³, Rhonda M. Porche-Sorbet², Paul M. Ridker⁴, and Brian F. Gage¹

¹ Department of Internal Medicine, Washington University School of Medicine, Saint Louis, Missouri

² Department of Pathology & Immunology, Washington University School of Medicine, Saint Louis, Missouri

³ St. Louis College of Pharmacy, Saint Louis, Missouri

⁴ Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Abstract

Background—Via generation of vitamin K-dependent proteins, gamma-glutamyl carboxylase (*GGCX*) plays a critical role in the vitamin K cycle. Single nucleotide polymorphisms (SNPs) in *GGCX*, therefore, may affect dosing of the vitamin K antagonist, warfarin.

Methods—In a multi-centered, cross-sectional study of 985 patients prescribed warfarin therapy, we genotyped for two *GGCX* SNPs (rs11676382 and rs12714145) and quantified their relationship to therapeutic dose.

Results—*GGCX* rs11676382 was a significant ($p=0.03$) predictor of residual dosing error and was associated with a 6.1% reduction in warfarin dose (95% CI: 0.6%-11.4%) per G allele. The prevalence was 14.1% in our predominantly (78%) Caucasian cohort, but the overall contribution to dosing accuracy was modest (partial $R^2 = 0.2\%$). *GGCX* rs12714145 was not a significant predictor of therapeutic dose ($p = 0.26$).

Conclusions—*GGCX* rs11676382 is a statistically significant predictor of warfarin dose, but the clinical relevance is modest. Given the potentially low marginal cost of adding this SNP to existing genotyping platforms, we have modified our non-profit website (www.WarfarinDosing.org) to accommodate knowledge of this variant.

Keywords

gamma-glutamyl carboxylase; warfarin; pharmacogenetics; algorithm

INTRODUCTION

Warfarin (Coumadin™ and others) is an anticoagulant with a narrow therapeutic window and wide inter-individual response. In 2007, and again in January 2010, the United States Food and Drug Administration approved label changes for warfarin to recommend lower initial doses for patients known to have single nucleotide polymorphisms (SNPs) in two genes: one affecting warfarin metabolism (*CYP2C9*), and the other affecting sensitivity (*VKORC1*) (1-3). Several pharmacogenetic algorithms incorporate SNPs in both genes (e.g. *CYP2C9**2, *3 and *VKORC1* -1639 G>A), but these SNPs alone explain only one-third of the variability in warfarin dose (4-8). The inclusion of non-genetic factors can explain an additional one-fifth

of warfarin dosing variability (8,9). Incorporating other SNPs may further improve the accuracy of warfarin dosing (10-13).

Because of the critical pharmacodynamic role gamma-glutamyl carboxylase (*GGCX*) plays in the generation of vitamin K-dependent proteins, SNPs in the *GGCX* gene may affect the dose of warfarin and other vitamin K antagonists. *GGCX* resides in the endoplasmic reticulum membrane, oxidizing reduced vitamin K to vitamin K-2,3-epoxide while adding a carboxyl residue to the gamma carbon on selected glutamic acids to produce functional clotting factors II, VII, IX, and X and other vitamin K dependent proteins (6,14-16). Patients with defects in *GGCX* have elevated International Normalized Ratios (INRs), even in the absence of a vitamin K antagonist (17), and *GGCX*-knockout mice die at birth from massive hemorrhage (18).

Although previous investigators have examined *GGCX* polymorphisms in patients taking warfarin, initial reports of associations with warfarin dose have either been unsubstantiated or await validation. A SNP located in intron 2, rs12714145 G>A, was associated with a 3.3% increase in warfarin dose requirements per A allele in a Swedish study of 201 patients (6). This observation was not confirmed in two subsequent studies which genotyped only 186 European Americans and 318 Chinese patients, respectively (19,20). However, if validated, the association would be important because this SNP explained 1.4-3.3% of the dose variability in the Swedish population (6).

More recently, *GGCX* rs11676382 C>G, located in intron 14, correlated with lower warfarin doses in a single-center study of 186 European Americans, explaining 2% of the total variance of the warfarin maintenance dose in a dominant genetic model (19). However, the putative association was not significant in an additive model or after adjusting for multiple comparisons. Furthermore, Lubitz et al. did not confirm the significance of this SNP in 145 multiethnic patients (21). The goal of the present study, therefore, was to further investigate the putative associations between warfarin therapeutic dose and *GGCX* rs12714145 and rs11676382 in a large, multiracial, multi-centered cohort.

MATERIALS AND METHODS

Study Design

This was a cross-sectional study of 985 patients who have been well characterized (8,11,22, 23). Patients provided written, informed consent for use of their DNA, and the participating institutions approved the protocol. We genotyped adult patients (Table 1) who had their warfarin therapy managed at one of three sites: the anticoagulation clinics affiliated with Barnes-Jewish Hospital at Washington University Medical Center in St. Louis, the Anticoagulation Clinics of North America (San Antonio), and subjects who participated in the PREVENT (PREvention of VENous Thromboembolism) study (23). A stable therapeutic warfarin dose was defined as a dose that was unchanged for at least six days and resulted in two or more consecutive therapeutic INR values at least 3 days apart.

Genotyping

DNA was extracted from patients' whole blood or buffy coat samples using Puregene Purification Kits (Gentra, now Qiagen, Hilden, Germany). Genotyping was performed for *GGCX* rs11676382 and rs12714145 using PCR and Pyrosequencing® (24,25). PCR primers were designed using the UCSC Golden Path Human Genome Browser March 2006 Build (<http://genome.ucsc.edu/cgi-bin/hgGateway>) and *GGCX* Refseq NM_000821 (Appendix 1).

Statistical analysis

To test an additive effect of *GGCX* SNPs on warfarin dose requirements, we coded each *GGCX* SNP as 0, 1, or 2 for the number of variant alleles present. We then regressed the number of *GGCX* SNPs onto the residual of the prediction according to a validated pharmacogenetic algorithm (8,21): $\text{residual} = \ln(\text{observed therapeutic dose}) - \ln(\text{predicted dose}) = \beta_0 + \beta_1 * (\text{number of alleles})$, where $\ln$ is the natural logarithm. By exponentiating the residual error, we obtain the ratio of the observed dose/predicted dose, which can be used to correct the validated algorithm for *GGCX*, when genotype is known (Appendix 2).

To reduce the effect of extreme therapeutic dose values on regression coefficients, we Winsorized the 6 doses that fell outside of the usual (1-18 mg/day) range. We tested the model fit using standard regression diagnostic methods. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Participants had an average age of 60 years and half were female (Table 1). One hundred seventeen (12%) inherited one or two copies of the *GGCX* rs11676382 G allele (Table 2). The geometric means (SD) of the therapeutic dose (mg/day), stratified by rs11676382, were: C/C 4.9 (1.6); G/C 4.1 (1.6); and G/G 3.8 (2.2). *GGCX* rs11676382 was a significant predictor of residual dosing error ($p=0.03$), and was associated with a 6.1% (95% CI: 0.6%-11.4%) reduction in warfarin dose, per G allele (Table 3). Adding rs1167382 to the validated pharmacogenetic algorithm (8) increased the R^2 by 0.2% in the total population and by 0.3% in Caucasians.

Half of the subjects ($N = 492$) carried either one or both copies of the *GGCX* rs12714145 A allele (Table 2), but it was not a significant predictor of dose residual ($p=0.39$). The geometric mean (SD) therapeutic dose was 4.7 (1.6) for subjects with either the *GGCX* rs12714145 A/A or A/G genotype, and was 5.1 (1.6) for those with G/G genotype.

DISCUSSION

Although *GGCX* rs12714145 was not confirmed as a predictor of therapeutic warfarin dose requirements, *GGCX* rs11676382 C>G was significantly associated with decreased warfarin requirements: each G allele was associated with a 6.1% reduction (e.g. 93.9% of prediction) in dose. The effect of *GGCX* rs11676382 C>G on R^2 was modest, however, and could explain why Lubitz et. al were unable to confirm this association in their smaller population of 145 diverse patients (21). Given the allele frequency of rs11676382 C>G, we estimate about 14% of Caucasians would carry this SNP, and without genotyping for it, their initial pharmacogenetic-based therapeutic warfarin dose estimates would be 6%–12% too high. Because of warfarin's narrow therapeutic index, even this modest over-estimation of warfarin dose could contribute to supratherapeutic INR values or bleeding.

With *CYP2C9* (*2 and *3) and *VKORC1* -1639 G>A SNPs explaining about one-third of the variability in therapeutic warfarin dose (4-6,8,26), and emerging data from recent high throughput genotyping studies of Swedish and Chinese populations (27-29), few other genes are likely to have major effects on warfarin dose. However, by genotyping for *GGCX* rs11676382 and other SNPs that raise R^2 modestly (10-13), one could increase the accuracy of existing pharmacogenetic algorithms significantly.

In their sample of 201 predominantly Swedish patients, Wadelius et al. 2005, found a 3.3% increase in dose per *GGCX* rs12714145 A allele (6,30). While their univariate analysis was significant for this SNP ($p=0.036$), their multivariate analysis was not (6,30). Reider et al., on

the other hand, did not find any association with warfarin dose and rs10691423, a SNP that is in high linkage disequilibrium with rs12714145 (19). Similarly, our analysis of *GGCX* rs12714145 A SNP in a larger (n= 985), more diverse population found no association between this SNP and warfarin dose.

The scope of the present study was limited to validating two *GGCX* SNPs in a large and diverse cohort of therapeutic patients. We did not evaluate *GGCX* rs699664 (8016G>A; Q325R), which was associated with increased warfarin dosage in Japanese patients (31), even though prior studies in Caucasian populations did not confirm this putative association (19,32,33). A related limitation is that few Asian patients were included in the present study. However rs11676382 C>G has yet to be observed in Asian populations according to public databases such as HapMap Phase 3. Lastly, as with any warfarin related study, definitions of stable warfarin dose and selection of covariates vary among studies (34-38), and obfuscate attempts at repeatability.

In August, 2009 the United States Center for Medicare and Medicaid Services (CMS) announced it would reimburse for warfarin pharmacogenetic testing only in the setting of approved randomized clinical trials. We hope that CMS will remunerate laboratories involved in warfarin pharmacogenetic research not only for *VKORC1* and *CYP2C9* testing, but also for *GGCX* and other SNPs (10-13) that modestly improve accuracy. Although delaying warfarin therapy while waiting for a \$400 genotype of 2 genes may not be cost effective now,(39) in the future, genotyping of multiple SNPs will become faster and less expensive, so the cost effectiveness of pharmacogenetic dosing will improve. Using all available pharmacogenetic information during warfarin initiation would allow for optimal dosing accuracy. With this vision in mind, we have updated our non-profit website, www.WarfarinDosing.org, to accommodate, but not require, *GGCX* rs11676382. We will use this website in our upcoming trial (ClinicalTrials.gov Identifier NCT01006733).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1Patient Demographics for *GGCX* analysis.

Variables	
Demographic variables	N = 985
Age, mean (SD), y	60 (15)
Gender:	
Women, N (%)	486 (49.3%)
Men, N (%)	499 (50.7%)
Race:	
Caucasian, N (%)	772 (78.4%)
African-American, N (%)	180(18.3%)
Asian or East Indian, N (%)	3(0.3%)
Other or Unknown, N (%)	30 (3.0%)
Clinical variables	
Therapeutic warfarin dose, geometric mean (SD), mg/d	4.8 (1.6)
Target INR, mean (SD), units	2.3 (0.4)
Body surface area, mean (SD), m ²	2.0 (0.3)
Current smoker, N (%)	140 (14%)
Takes amiodarone, N (%)	33 (3%)

SD = standard deviation; INR = international normalized ratio

Table 2

Genotype Frequencies.

GGCX Alleles	Caucasians	African-Americans	Asian or East Indians	Other or Unknown Race	Association (95% CI) with dose residual, per allele
rs11676382					
C/C	662 (85.8%)	175 (97.2%)	3	28 (93.3%)	
G/C	103 (13.3%)	5 (2.8%)	0	2 (6.7%)	6.1% (0.6%-11.4%)
G/G	7 (0.9%)	0 (0.0%)	0	0 (0%)	
rs12714145					
G/G	394 (51.0%)	56 (31.1%)	3	14(46.7%)	
G/A	298 (38.6%)	91 (50.6%)	0	14 (46.7%)	NS
A/A	80 (10.4%)	33 (18.3%)	0	2 (6.7%)	

NS = not significant; CI = confidence interval.

Table 3Adjustment Factors for Existing Pharmacogenetic Dosing Algorithms, Depending on *GGCX* Genotype.

GGCX rs11676382 genotype	Formula for Correction Factor [*]	Adjustment in African-Americans and Asians [*]	Adjustment in Caucasians [*]
CC	$1 / (P_C^2 + 2P_C P_G \times 0.939 + P_G^2 \times 0.939^2)$	1.00	1.01
GC	$0.939 / (P_C^2 + 2P_C P_G \times 0.939 + P_G^2 \times 0.939^2)$	0.94	0.95
GG	$0.939^2 / (P_C^2 + 2P_C P_G \times 0.939 + P_G^2 \times 0.939^2)$	0.88	0.89

[†]P_C = allele frequency of the wildtype C allele.[‡]P_G = allele frequency of the G allele.^{*} multiply predicted dose by this correction factor.

Table 4**Warfarin Pharmacogenetics Snapshot: past, present and future.**

1. What is known about this topic?

- Warfarin is an anticoagulant with a narrow therapeutic window and wide intra-individual response.
- In 2007 and again in 2010, the US FDA approved labeling changes for warfarin to recommend lower initial doses for patients known to have SNPs in genes affecting warfarin metabolism (*CYP2C9*) and sensitivity (*VKORC1*).
- SNPs in *CYP2C9* and *VKORC1* explain one-third of the variability in warfarin dose.

2. What does this paper add?

- *GGCX* rs11676382 was associated with a 6.1% reduction in warfarin dose (95% CI: 0.6%-11.4%) per G allele.
 - Knowledge of *GGCX* rs11676382 genotype has been incorporated in www.WarfarinDosing.org for pharmacogenetic dose initiation.
 - *GGCX* rs12714145 was not a significant predictor of warfarin dose
-