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Impact of gamma-glutamyl carboxylase gene polymorphisms on warfarin dose requirement: A systematic review and meta-analysis

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ABSTRACT

Background: The Gamma-glutamyl carboxylase (GGCX) gene, as with Vitamin K Epoxide Reductase Complex Subunit 1(VKORC1), CytochromeP450 Complex Subunit 14 F2 (CYP4F2) and CytochromeP450 Complex Subunit2C9 (CYP2C9), is a candidate predictor for appropriate maintenance warfarin dose. However, the association between GGCX gene polymorphisms and warfarin dose requirement is still controversial. To quantify the influence of GGCX polymorphisms on warfarin dose requirements, we performed a systematic review and meta-analysis.
Methods: According to PRISRM statement (Preferred reporting items for systematic reviews and meta-analyses), a comprehensive literature search was undertaken through August 2014 looking for eligible studies in Embase, Pubmed,Web of Science and the Cochrane Library. The impact of GGCX polymorphisms on mean daily warfarin dose (MDWD) was counted by means of Z test. RevMan 5.2.7 software (developed by the Cochrane Collaboration) was applied to analyze the relationship between GGCX gene polymorphisms and warfarin dose requirements.
Results: Nineteen articles including 21 studies with a total of 6957 patients were included in the meta-analysis. Among three investigated single nucleotide polymorphisms (SNPs), rs11676382 showed higher CC genotype frequencies in Asian than those in Caucasian(97.7% vs. 86.9%); patients who were "G carriers" (that is, carried the GGCX rs11676382 CG or GG genotypes) required 27% lower warfarin dose than CC genotype[95%Confidence Interval(CI) = 17%-37%, $P = 0.000$, $I^2\% = 82.0$ and $P_Q = 0.000$], moreover, stratified analysis by ethnicity showed similar results in Caucasian(23% lower, 95%CI = 12%-33%), but not in Asian. With respect to genetic variation of rs699664 and rs121714145 SNPs, no significant impact on warfarin dose requirements were demonstrated.
Conclusions: This meta-analysis suggested that *GGCX* rs11676382 polymorphism may be one of factors affecting the dose of warfarin requirement, and the effects are different in different ethnicities. Further studies about this topic in different ethnicities with larger samples are expected to be conducted to validate our results.

Introduction

Warfarin is commonly used in clinical treatment of various disorders, especially thromboembolic disease. The narrow therapeutic range of warfarin dosage may increase the risk of recurrence of thromboembolism and bleeding [1], so it is difficult to establish the appropriate dose of warfarin to achieve anticoagulation. Over time, many factors have proven to be associated with therapeutic warfarin dose requirements, including basic elements such as gender, age, weight, and body surface area [2–4]; however, these elements do not explain all of the differences that currently exist among warfarin dose requirements among different individuals and different ethnicities.

In recent years, it has been shown that the genetic variability of the pharmacokinetics and pharmacodynamics of warfarin may be important in determining the individual and interethnic differences in appropriate warfarin dosage [5–9]. VKORC1, CYP4F2, and CYP2C9 polymorphisms contribute to inter-population difference in warfarin doses among diverse geographic regions [5,7,8]. GGCX, as a key cofactor for the activation of clotting factors (VII, IX, X) to reduce Vitamin K [10], is also a possible candidate, given that recent studies have found a significant relationship between *GGCX* genotype and warfarin dose [11–18].

The association between *GGCX* SNPs polymorphisms and warfarin dose requirements is biologically plausible. The *GGCX* gene, located on chromosome 2p12 in humans and consisting of 15 exons, plays the critical pharmacodynamic role in the generation of vitamin K-dependent

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proteins. GGCX can active the clotting factors VII, IX, X, and prothrombin by reducing vitamin K to vitamin K-2, 3-epoxide [19]. In addition, GGCX-knockout mice die at birth from massive hemorrhage [20]. Crosier et al. [21] found that *GGCX* SNPs showed significant associations with percent undercarbosylated osteocalcin, a measure of vitamin K-dependent carboxylation in extra-hepatic proteins, which would influence warfarin dose requirements in order to achieve stable oral anticoagulation.

For the past decades, a number of studies on the relationship between *GGCX* gene and warfarin dose requirement had been reported [11–18, 22–30]. These studies provide certain evidences that the genotype-based dose predictions may in future enable personalised drug treatment from the start of warfarin therapy. In those studies, the rs699664 SNP in exon 8, rs12714145 SNP in intron 2, and rs11676382 SNP in intron 14 were the most researched SNPs. However, variant *GGCX* alleles have been associated with warfarin dosing in some studies with different ethnicities [12,23,31], but have not been consistently replicated in other studies [32,33]. Therefore, it is necessary to substantiate or validate the impact of *GGCX* polymorphisms in patients taking warfarin. The aim of this study was to quantify the effects of individual *GGCX* genotype on warfarin dose requirements, by means of a systematic literature review and meta-analysis.

Materials and Methods

This meta-analysis was conducted according to PRISMA statement (Preferred reporting items for systematic reviews and meta-analyses), including search strategy, selection criteria,data extraction and data analysis [34].

Identification of Eligible Studies

We used the following search terms: “Gamma-glutamyl carboxylase” or “GGCX” in combination with “polymorphism,” “mutation,” or “variant” in combination with “warfarin,” in Embase, PubMed, Web of Science and the Cochrane Library up to Aug, 2014. Two investigators (YFS and LS) conducted an extensive literature search independently for all publications. There was no language restriction. Articles in reference lists were also hand-searched. Only human studies were searched.

Inclusion and Exclusion Criteria

The following criteria were used to choose suitable studies:

(1) The following *GGCX* gene SNPs was studied: rs699664 (G8016A, c.974G>A), rs12714145 (c.214+597G>A, 3261G>A), rs11676382 (12970C>G);
(2) Studies offering information regarding the number of genotypes, the maintenance dose of warfarin (mean with either standard deviation or 95% confidence interval) separately for *GGCX* genotype groups.

Publications were excluded from the meta-analysis if:

(1) Review articles, letters, case reports, editorials, and conference abstracts.
(2) The articles did not include genotype frequencies or warfarin dose, and this information was not available from the authors contacted.

Data Extraction

Two investigators (YFS and LS) independently extracted data from the included studies. The recorded data mainly included genotype frequencies of *GGCX* and warfarin dose (mean, SD) for each genotype. Also extracted from the eligible studies was basic information including the first author’s name, publication date, country, ethnicity, indication of warfarin, total sample size (male, female), mean age of sample, target value of international normalized ratio (INR), and genotyping method. Accuracy of the extracted information was verified by ensuring that the data recorded by the 2 investigators matches; if it did not, the investigators rechecked the data extracted. If the two investigators could not reach an agreement, the dispute was submitted to a third reviewer (QX) to decide.

Quality Score Assessment

The quality of the selected studies was independently assessed by two investigators (CL and YFS) independently following the criteria predefined by Little et al. [35]. These quality criteria were based on: (1) analytic validity of genotyping (genotyping method, quality control measures, timing of sample collection and analysis, and types of samples used); (2) selection of study subjects (geographic area recruitment period, exclusion criteria for cases and controls, mean age and standard deviation or age range of study subjects, and distribution by sex); (3) confounding factors, including population stratification (potential correlation of the genotype identified and taken into consideration in design or analysis); and (4) statistical issues (method of analysis used in reference, and software used to perform the analysis). If most of the above criteria were satisfied, a study was graded as “++”. A study that fulfilled some of the criteria would be graded as “+”; few or no criteria fulfilled resulted in a grade of “-” [35].

Statistical Analysis

To remove any heterogeneity caused by deviation in warfarin pharmacokinetics and pharmacodynamics sensitivity among different study populations, in our meta-analysis, the maintenance of warfarin dose for each *GGCX* genotype group was normalized using the homozygous wild-type group as a reference. The normalization procedure was performed by dividing the mean dose and associated standard deviations in each group by the mean maintenance dose in the GGCX reference group [36].

For the *GGCX* rs699664 and rs12714145, we defined carriers of AG or AA genotype as “A carriers”, A carriers (AG+AA), AA and AG were compared with GG genotype (reference group).With respect to *GGCX* rs11676382, CG or GG genotype were defined as “G carriers”, because GG frequencies were too small, only G carriers (CG+GG) were compared with CC genotype (reference group). The calculated mean differences represent relative rather than absolute differences in maintenance dose; in other words, a mean difference of 0.5 would indicate a 50% increase in warfarin dose requirement [36].

The weight is the inverse of its standard differences of warfarin dose in each study, and the effect of each *GGCX* genotype on warfarin dose was defined as mean difference (MD), which was calculated by subtracting the normalized mean warfarin dose for the respective genotypes from the reference. The Weight Mean Difference (WMD) was used as indicator of effect, which was calculated by multiplying the MD and related weight of each study. Each study’s WMD was summed to arrive at the total WMD [31]. The impact of *GGCX* SNPs on mean daily warfarin dose (MDWD) was counted by means of Z test; a P value <0.05 was considered statistically significant. Heterogeneity was assessed by a chi-squared Q test and I-squared statistics, as has been previously described in the literature [37, 38]. If $P_Q < 0.1$ or $I^2 > 50\%$, we considered the heterogeneity significant, and a random-effects model was conducted using the DerSimonian and Laird method. Otherwise, a fixed-effects model (the Mantel-Haenszel method) was used. Stratified analysis was carried out by ethnicity and the mean target INR. We defined the mean target INR < 2.5 and INR $\geqslant 2.5$ as two separated subgroups.

Sensitivity analyses were carried out by excluding studies one by one, especially excluded study with low quality or small sample size. Begg’s funnel plot and Egger’s test were used to investigate the publication