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a systematic review and meta-analysis of randomized controlled trials

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Genotype-guided warfarin dosing versus conventional dosing strategies: a systematic review and meta-analysis of randomized controlled trials

Short title: Meta-analysis of genotype-guided warfarin dosing

Gary Tse * MPH PhD FESC FACC FHRS FRCP [1, 2], Mengqi Gong MS * [3], Guangping Li MD, PhD [3], Sunny Hei Wong DPhil FRCPath FRCP [1, 2], William KK Wu PhD FRCPath [2, 4], Wing Tak Wong PhD [5], Leonardo Roever MHS [6], Alex Pui Wai Lee MD FACC FRCP [1], Gregory YH Lip MD FESC FACC FRCP [7, 8], Martin CS Wong MPH MBA MD FRACGP FFPH [9, 10], Tong Liu MD PhD [3]; **International Health Informatics Study (IHIS) Network**

[1] Department of Medicine and Therapeutics, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong, SAR, P.R. China;

[2] Li Ka Shing Institute of Health Sciences, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong, SAR, P.R. China

[3] Tianjin Key Laboratory of Ionic-Molecular Function of Cardiovascular disease, Department of Cardiology, Tianjin Institute of Cardiology, Second Hospital of Tianjin Medical University, Tianjin 300211, People's Republic of China

[4] Department of Anaesthesia and Intensive Care, State Key Laboratory of Digestive Disease, The Chinese University of Hong Kong, Hong Kong, China

[5] School of Life Sciences, Chinese University of Hong Kong, Hong Kong, SAR, People's Republic of China

[6] Department of Clinical Research, Federal University of Uberlândia, Uberlândia, MG, Brazil

[7] Institute of Cardiovascular Sciences, University of Birmingham, United Kingdom

[8] Aalborg Thrombosis Research Unit, Department of Clinical Medicine, Aalborg University, Denmark

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[9] JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong, China

[10] State Key Laboratory of Digestive Disease, Chinese University of Hong Kong, Hong Kong, China

**** equal contributors***

Correspondence to:

Assistant Prof. Gary Tse, MPH, PhD, FESC, FACC, FHRS, FRCP

Department of Medicine and Therapeutics,

Li Ka Shing Institute of Health Sciences,

Faculty of Medicine, The Chinese University of Hong Kong,

Hong Kong, SAR, P.R. China

Email: tseg@cuhk.edu.hk

Prof. Tong Liu, MD, PhD

Tianjin Key Laboratory of Ionic-Molecular Function of Cardiovascular disease,

Department of Cardiology, Tianjin Institute of Cardiology,

Second Hospital of Tianjin Medical University,

No. 23, Pingjiang Road, Hexi District, Tianjin 300211, People's Republic of China.

Tel.: +86-22-88328648

Fax: +86-22-28261158

E-mail:liutongdoc@126.com

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Abstract

Background: Previous trials on the effectiveness of genotype-guided warfarin dosing versus conventional dosing have been inconclusive. We conducted a systematic review and meta-analysis of randomized trials comparing genotype-guided to conventional dosing strategies.

Methods: PubMed and Cochrane Library were searched until 23rd October 2017.

Results: A total of 76 and 94 entries were retrieved. A total of 2626 subjects in the genotype-guided dosing (mean age: 63.3 ± 5.8 years; 46% male) and 2604 subjects in the conventional dosing (mean age: 64.7 ± 6.1 years; 46% male) groups (mean follow-up duration: 64 days) from 18 trials were included. Compared to conventional dosing, genotype-guided dosing significantly shortened the time-to-first therapeutic INR (mean difference: 2.6 days, standard error: 0.3 days, P<0.0001; I^2: 0%) and time-to-first stable INR (mean difference: 5.9 days, standard error: 2.0 days, P<0.01; I^2: 94%). Genotype-guided dosing also increased the Time in Therapeutic Range (TTR) (mean difference: 3.1%, standard error: 1.2%, P<0.01; I^2: 80%), and reduced the risks of both excessive anticoagulation defined as INR ≥ 4 (risk ratio [RR]: 0.87; 95% CI: 0.78-0.98; P<0.05; I^2: 0%) and bleeding (RR: 0.82; 95% CI: 0.69-0.98; P<0.05; I^2: 31%). No difference in thromboembolism (RR: 0.84; 95% CI: 0.56-1.26; P=0.40; I^2: 0%) or mortality (RR: 1.16; 95% CI: 0.46-2.91; P=0.76; I^2: 0%) was observed between the two groups.

Conclusions: Genotype-guided warfarin dosing offers better safety with less bleeding compared to conventional dosing strategies. No significant benefit on thromboembolism or mortality was evident.

Key words: warfarin; dosing; genotype; CYP2C9; VKORC1; CYP4F2

Introduction

Warfarin is one of the commonest prescribed drug, accounting for more than 35 million prescriptions in the United States alone (1). However, it is also responsible for more iatrogenic accident and emergency department visits in elder patients compared to other medications (2, 3). This may be related to over- or under-dosing because of wide inter-individual variability in dosing requirements. To better optimize anticoagulation control, the use of genetic-based algorithms, collectively termed genotype-guided dosing, has been devised. However, previously published randomized controlled trials (RCTs) comparing the effects of genotype-guided dosing against conventional dosing (either fixed dosing or clinically-guided dosing) strategies (4-14), and even their subsequent meta-analyses have yielded conflicting results (15-21). A meta-analysis published in 2015, which pooled the evidence from 11 RCTs with trial sequential analysis (21), reported shorter time taken to reach first therapeutic or stable International Normalised Ratio (INR), improvements in markers of anticoagulation control such as the time in therapeutic range (TTR) and the number of patients with out-of-range INR, but this did not translate into better clinical outcomes of reducing bleeding, thromboembolism or mortality.

Since the publication of this study, six additional trials have been published on this issue (22-27), with the most recent three showing conflicting results. For example, a RCT conducted in 2015 on non-valvular atrial fibrillation patients reported no significant difference in TTR or in the number of patients with out-of-range INR (22). Similarly, in a group of Han Chinese, TTR, excessive anticoagulation or adverse events did not differ between the genotype-guided and optimal clinical care arms (27). By contrast, the recently published Genetic Informatics Trial (GIFT) of Warfarin to Prevent Deep Vein Thrombosis in patients receiving warfarin at the time of elective hip or knee arthroplasty reported significant benefits with genotype-guided dosing when compared to clinically-guided dosing (25). In

GIFT, genotype-guided warfarin dosing increased the TTR, and reduced the combined risk of major bleeding, INR of 4 or greater, venous thromboembolism, or death. Given these new findings, we conducted a systematic review and meta-analysis of all randomized trials to evaluate the benefits and complication rates in genotype-guided dosing versus conventional dosing strategies.

Methods

Search strategy, inclusion and exclusion criteria

The systematic review and meta-analysis was performed according to the PRISMA statement (28). PubMed and Cochrane Library were searched for randomized trials that compared the efficacy in genotype-guided warfarin dosing compared to conventional dosing strategies. The following search terms were used for PubMed: [genotype AND warfarin AND randomized trial]. For Cochrane Library, the following terms were used: ["genotype " AND "warfarin"]. The search period was from 1966 for Pubmed and 1996f for Cochrane Library through to 23rd October 2017, with no language restrictions. The following inclusion criteria were applied: i) the design was a randomized trial in humans, ii) the study compared outcomes for genotype-guided versus conventional warfarin dosing strategies. Reference lists of included studies, and of previous meta-analyses identified, were searched. No additional studies were found. Given the recently published PRISMA-compliant systematic review and meta-analysis studies, a more robust search strategy used. Previously, the Tang 2015 meta-analysis was performed using the [(genotype OR polymorphism OR gene OR allele OR variant OR mutation OR single-nucleotide polymorphism) AND (algorithms OR regimen OR model OR strategy)] AND (warfarin OR coumarin OR anticoagula*). The same terms were searched in PubMed between 1st February 2017 and 31st March 2018, yielding an additional

128 studies. This failed to identify any further relevant studies (**Supplementary Figure 1**). Quality assessment of randomized controlled trials was performed using the Cochrane Risk Assessment Tool (**Supplementary Figures 2 and 3**).

Data extraction

Data from the different studies were entered in Microsoft Excel. All publications extracted from the search strategy were assessed for compliance with the inclusion criteria. In this meta-analysis, the extracted data elements consisted of: i) last name of first author and year of publication; ii) target INR, iii) follow-up duration; iv) characteristics of the genotype-guided and control groups including sample size, sex and age, v) genes tested and dosing algorithm for the genotype-guided group, vi) dosing algorithm and whether fixed dose or clinical information-guided was used for the control group. The search of the two databases was conducted by GT. The search results were then retrieved and independently screened by GT and MG. Any disagreements would be brought to the attention of a third reviewer (TL). However, this was not required as both reviewers arrived at the same list of RCTs for inclusion in the meta-analysis.

Endpoints and statistical analysis

The *a priori* pre-defined endpoints for this meta-analysis were: 1) time-to-first therapeutic INR, 2) time-to-first stable INR, 3) time in therapeutic range (TTR), number of patients with 4) excessive anticoagulation defined as INR $\geqslant$ 4, 5) bleeding, 6) thromboembolism and 7) mortality. For time-to-first therapeutic INR, time-to-first stable INR and TTR, mean difference between the genotype-guided dosing and conventional dosing strategies was extracted or calculated. For INR $\geqslant$ 4, bleeding, thromboembolism and

mortality, risk ratios were calculated. Where the data concerning a particular endpoint were not available, they were obtained from previously published meta-analyses.

Heterogeneity across studies was assessed using the I^2 statistic from the standard chi-square test, which describes the percentage of the variability in the effect estimates resulting from heterogeneity. $I^2 > 50\%$ was considered to reflect significant statistical heterogeneity and in such cases the random-effects model using the inverse-variance approach was used. Otherwise, the fixed-effects model was used. To explore the potential sources of the heterogeneity, subgroup analysis based on the type of warfarin dosing for the control group (fixed dose and clinical information-guided) was performed. Funnel plots showing standard errors against the mean difference or against the logarithms of the risk ratios were constructed. The Egger's test was used to detect publication bias.

Results

A QUORUM diagram detailing the above search terms with inclusion and exclusion criteria is depicted in **Figure 1**. A total of 76 and 94 studies were retrieved from PubMed and Cochrane Library, respectively. However, 152 studies did not meet the inclusion criteria. Therefore, a total of 18 trials were included in this meta-analysis (4-14, 22-27, 29). The baseline characteristics of these studies are listed in **Table 1**. This meta-analysis included 2626 patients in the genotype-guided dosing arm (mean age: 63.3 ± 5.8 years; 46% male) and 2604 patients in the conventional dosing arm (mean age: 64.7 ± 6.1 years; 46% male). The mean follow-up duration was 64 days. For the control group, two conventional dosing strategies have been used. The first is fixed dosing, where the patients received a fixed dose for a fixed number of days. This varied from 2.5 mg to 6 mg for 3-7 days, 10 mg-5 mg-5 mg regimens were also used. The lower doses were used in Chinese populations where warfarin

requirements are lower. For clinical information-guided dosing, the different definitions are illustrated in **Table 1**. This involved the use of regression models based on different clinical parameters such as age, gender, body surface area and valve status. For the genotype-guided group, 12 different algorithms were described in the 18 trials, incorporating the polymorphisms for CYP2C9, VKORC1, CYP4F2 to determine the warfarin dose (**Supplementary Table 1**).

Quality assessment of randomized controlled trials was performed using the Cochrane Risk Assessment Tool (**Supplementary Figures 2 and 3**). Overall, risk of bias assessment could be conducted for 16 of the 18 trials conducted, whereas the remaining two studies were conference abstracts (24, 29), which could not be judged on their quality due to the lack of information reported. One study (6) was deemed to be of low quality and whereas the remaining 15 studies generally showed high quality study designs for reducing risk of bias. Specifically, for random sequence generation, nine of the 18 trials included a low risk of bias. Similarly, for allocation concealment, only eight trials had an appropriate design to reduce selection bias. Nevertheless, to reduce performance bias, 12 trials had described proper blinding of participants and research personnel. For blinding of outcome assessment, most studies did not clearly illustrate an appropriate method except for two trials, which had low risk of attrition bias. On selective reporting, 15 of the 18 trials had appropriately described their data on their different endpoints, which therefore had a low risk of reporting bias. Funnel plots showing standard errors against the mean difference or against the logarithms of the risk ratios are shown in **Supplementary Figures 4 and 10**.

Genotype-guided warfarin dosing leads to shorter time to first therapeutic INR and time to stable INR

Seven studies provided information on the time taken to reach the first therapeutic INR (6, 9, 23, 25-27, 29), but only three of these provided sufficient information for the calculation of mean difference values (6, 9, 26) (**Figure 2**, *top left panel*). It was defined by Borgman 2012 study as "the time interval in days from the first warfarin dosage to the first time interval where the INR remains within the predefined acceptable range (INR 1.8 to 3.2) for a minimum of 4 consecutive days". By contrast, the Coraco 2008 study, defined stable anticoagulation as "two consecutive INR values, 7 days apart, were within the therapeutic range, without any intervening dose alteration". The Jin 2017 study defined it "as INR values maintained in the range of 2-3 for at least 3 times (⩾ 7 days) continuously". Our meta-analysis shows a significant shorter time to reach the first therapeutic INR in the genotype-guided dosing group when compared to controls, all of which used fixed dosing (mean difference: 2.6 days, standard error: 0.3 days, P < 0.0001; I^2: 0%; **Figure 2**, *top left panel*). Egger's test demonstrated no significant asymmetry (intercept 0.9, t-value 1.9; $P > 0.05$; **Supplementary Figure 4**). Subgroup analysis based on ethnicity showed that the mean differences remained statistically significant for each ethnicity (**Figure 2**, *top right panel*).

For the time taken to reach a stable INR, four studies provided the median (8, 10, 14, 23), and six studies reported the mean (6, 10, 11, 22, 26, 27). Of the latter six studies, one was excluded because standard deviation or another measure of dispersion was not available (27) (**Figure 2**, *bottom left panel*). Our meta-analysis of these five studies showed a shorter time to reach a stable INR with the genotype-guided dosing group (mean difference: 5.9 days, standard error: 2.0 days, P < 0.01; I^2: 94%). Egger's test demonstrated no significant asymmetry (intercept 0.6, t-value 0.2; $P > 0.05$; **Supplementary Figure 5**). Of the five studies, four used a fixed dosing regimen for the control group and the mean difference

remained statistically significant on subgroup analysis (**Figure 2**, *bottom left panel*). Subgroup analysis for ethnicity showed that the mean difference remained significant for Caucasians and Chinese subjects, but not in the study with both Caucasians and African subjects (**Figure 2**, *bottom right panel*).

Genotype-guided warfarin dosing leads to higher time in therapeutic range and lower number of patients with excessive anticoagulation when compared to conventional dosing strategies

Fourteen of the 18 trials reported TTR values (4-9, 11, 12, 14, 22, 23, 25, 27) but one study was excluded as no standard error, standard deviation or confidence interval was reported (24). Of the thirteen studies, five reported significantly higher TTRs in genotype-guided therapy compared to conventional dosing strategies, whereas the remaining studies reported no difference in the two groups (**Figure 3**, *top left panel*). Nevertheless, our meta-analysis shows that genotype-guided warfarin dosing significantly increased TTR compared to conventional dosing strategies (mean difference: 3.1%, standard error: 1.2%, $P < 0.05$; I^2: 80%). Egger's test demonstrated no significant asymmetry (intercept 0.2, t-value 0.2; $P > 0.05$; **Supplementary Figure 6**). Subgroup analysis showed that genotype-guided dosing produced greater TTR than fixed-dose regimens (mean difference: 7.4%, standard error: 2.0%, $P < 0.0001$; I^2: 71%) (**Figure 3**, *top left panel*). By contrast, no significant difference in TTRs was observed between genotype-guided dosing and clinical information-guided regimens (mean difference: 0.5%, standard error: 1.5%, $P = 0.73$; I^2: 55%). Subgroup analysis based on ethnicity showed that TTRs remained significantly different between both groups for Caucasians, Caucasians and Africans, and Chinese, with I^2 taking values of 84%, 54%, 0%, respectively (**Figure 3**, *top right panel*).

Moreover, 13 of the 18 trials (4-9, 11, 12, 14, 22, 24, 25, 27) reported the number of individuals with excessive anticoagulation defined as INR $\geqslant$ 4 and the total of individuals in each group. Of these, two reported a reduction in the risk ratio for excessive anticoagulation in genotype-guided therapy compared to conventional dosing strategies, whereas eleven studies reporting no significant difference (**Figure 3**, *bottom left panel*). Our overall meta-analysis demonstrated that genotype-guided warfarin dosing was associated with a lower risk of excessive anticoagulation (risk ratio [RR]: 0.87; 95% CI: 0.78-0.98; P < 0.05; I^2: 0%). Egger's test demonstrated no significant asymmetry (intercept 0.3, t-value 0.6; $P > 0.05$; **Supplementary Figure 7**). Subgroup analysis remained statistically significant when comparison was made to fixed-dose regimen (RR: 0.82; 95% CI: 0.68-0.99; P < 0.05; I^2: 0%) but not to clinical information-guided regimen (RR: 0.91; 95% CI: 0.78-1.06; P = 0.22; I^2: 0%) (**Figure 3**, *bottom left panel*). Subgroup analyses based on ethnicity resulted in RRs that were no longer statistically significant for Caucasians, Caucasians with Africans and Chinese (**Figure 3**, *bottom right panel*).

Genotype-guided warfarin dosing reduces bleeding without affecting thromboembolism or mortality compared to conventional dosing strategies

Fourteen of the 18 trials reported bleeding events (4, 6-8, 11, 12, 14, 23-27, 29), but one was excluded from the analysis due to zero events in both groups (22). Two trials reported significantly reduction in bleeding using genotype-guided dosing, whereas the other trials did not report significant differences (**Figure 4**, *top left panel*). Our overall meta-analysis shows that genotype-guided dosing was associated with a lower risk of bleeding (RR: 0.82; 95% CI: 0.69-0.98; P < 0.05; I^2: 31%). Egger's test demonstrated significant asymmetry (intercept -1.4, t-value 4.1; $P < 0.05$; **Supplementary Figure 8**). Subgroup analyses based on control group dosing regimen led to loss of statistical significance for the RRs (fixed-dose

regimen: 0.86, 95% CI: 0.70-1.06; P = 0.16; I^2: 22%; clinical information-guided regimen: 0.76; 95% CI: 0.57-1.01; P = 0.06; I^2: 45%) (**Figure 4**, *top left panel*). Subgroup analyses based on ethnicity showed that the risk of bleeding remained significantly lower for Chinese (RR: 0.46, 95% CI: 0.23-0.92; P < 0.05; I^2: 0%), but not for Caucasians alone or Caucasians with Africans (**Figure 4**, *top right panel*).

Thromboembolism was assessed by ten trials (4, 6, 8, 11, 12, 14, 22, 24, 25, 27), but three trials were excluded because zero events were reported for both genotype-guided dosing and conventional dosing groups. None of the remaining studies reported significant difference in thromboembolism events (**Figure 4**, *bottom left panel*), which is confirmed by our meta-analysis (RR: 0.84; 95% CI: 0.56-1.26; P = 0.40; I^2: 0%). Egger's test demonstrated no significant asymmetry (intercept -0.4, t-value 0.9; $P > 0.05$; **Supplementary Figure 9**). Subgroup analyses comparing against fixed-dose regimen (RR: 0.27; 95% CI: 0.03-2.38; P = 0.24; I^2: 0%) or clinical information-guided regimen (RR: 0.87; 95% CI: 0.58-1.32; P = 0.53; I^2: 0%) did not significantly alter the findings (**Figure 4**, *bottom left panel*). Subgroup analyses based on ethnicity also did not alter our results (**Figure 4**, *bottom right panel*). It was possible to calculate the number of patients needed to be genotyped in order to reduce one adverse event based on the absolute risk difference. This was estimated to be 40 patients for major bleeding but 238 for thromboembolism.

Mortality was reported in seven trials (8, 11, 12, 14, 23-25), but one was excluded from further analysis because of zero events in both groups (25). Of the remaining studies, none reported significant difference in mortality between both genotype-guided dosing and conventional dosing groups (**Figure 5**, *left panel*), which was confirmed by our meta-analysis (RR: 1.16; 95% CI: 0.46-2.90; P = 0.76; I^2: 0%). Egger's test demonstrated no significant asymmetry (intercept -1.1, t-value 0.8; $P > 0.05$; **Supplementary Figure 10**). Subgroup analyses comparing against fixed-dose regimen (RR: 2.63; 95% CI: 0.62-11.23; P = 0.19; I^2:

0%) or clinical information-guided regimen (RR: 0.66; 95% CI: 0.20-2.19; P = 0.50; I^2: 0%) did not significantly alter the findings. Similarly, subgroup analyses based on ethnicity did not alter the findings (**Figure 5**, *right panel*).

Meta-regression analysis was conducted to explore potential influences of continuous moderator variables. Thus, meta-regression of TTR mean difference on the logarithm of risk ratios for INR $\geqslant$ 4 (**Supplementary Figure 11**), bleeding (**Supplementary Figure 12**), thromboembolism (**Supplementary Figure 13**) or mortality (**Supplementary Figure 14**) did not reveal slopes or intercepts that were significantly different from zero (P > 0.05).

Discussion

The main findings of this meta-analysis are that, compared to conventional dosing strategies, genotype-guided warfarin dosing significantly 1) shortened the time to first therapeutic INR by 2.6 days, 2) shortened the time to first stable INR by 5.9 days, 3) improved TTRs by 3.1%, 4) reduced the number of patients with excessive anticoagulation (INR > 4) with an RR of 0.87, 5) reduced bleeding events with an RR of 0.82. No significant difference in the risk of 6) thromboembolism or 7) mortality was observed when comparing the two groups.

Warfarin has been one of the commonest prescribed anticoagulant medications since its approval in 1954, although non-vitamin K oral anticoagulants may have been overtaken since 2012 (30). Inactivation of warfarin occurs when it is metabolized to the 7-hydroxy metabolite by *CYP2C9* (31). Polymorphisms in *CYP2C9* are known to reduce the activity of the enzyme, thereby less effective warfarin inactivation (32). Moreover, polymorphisms in both the *VKORC1* and *CYP2C9* genes contribute to the inter-individual variability in dosing requirements (33) and patients' response to warfarin (34). Therefore, there has been

significant interests in whether genotype-guided dosing therapy will improve INR control and clinical outcomes for patients on warfarin.

Previous randomized controlled trials comparing the effectiveness of genotype-guided dosing and conventional dosing strategies, and even meta-analyses of these trials, have been inconclusive (15-18). Nevertheless, a subsequent meta-analysis of 11 RCTs with trial sequential analysis has demonstrated improvements in biochemical parameters of INR control and TTRs, but limited clinical utility with genotype-guided dosing (21). However, since its publication, an additional seven trials have been published. Of these newer trials, the Genetic Informatics Trial (GIFT) of Warfarin to Prevent Deep Vein Thrombosis was the largest trial to date with the largest number of 1597 subjects. This trial contributed approximately one third of included cohort, reporting that genotype-guided dosing prevented more adverse outcomes than clinically-guided dosing in patients undergoing hip and knee surgery. In orthopedic surgery, surgeons often have more time to obtain genotype data and use this information to plan for the surgery. By contrast, physicians who encounter patients with atrial fibrillation or venous thromboembolism often have little time to obtain genotype data before prescribing anticoagulants.

In our updated meta-analysis, our significant findings are that both biochemical measures of warfarin therapy were improved and bleeding complications were reduced. The endpoints were chosen as these were parameters that were critical for guiding the decision-making process in clinical practice. For example, both time-to-first therapeutic INR and time-to-stable INR can guide clinicians for deciding an appropriate follow-up duration and frequency. By contrast, TTR, INR ≥ 4 and risks of complications are important for resource allocation at the population level. The effect of genotype-guided warfarin dosing compared with conventional dosing on TTR is convincing and is clinically important. The significant

difference in TTR when a clinical information-guided warfarin dosing regimen is used in the conventional arm is striking.

Two of the studies included in the meta-analysis only genotyped for CYP2C9 variants (4, 6). The COAG trial also determined the CYP2C9*2 and *3 only, but not for other CYP2C9 variants (12). This is important because other CYP2C9 variants are more frequently found than CYP2C9*2 and *3 in African Americans, who constituted nearly one third of the study population. Therefore, the advantages of genotype-guided warfarin dosing could be diminished in populations with African ancestry. Nevertheless, in the COAG trial, TTR was improved, excessive anticoagulation was reduced, and the number of adverse events were significantly reduced. Consequently, in our meta-analysis this had little impact on the overall pooled effect estimates for these endpoints.

There are several considerations on the practicality of utilizing genotype-guided warfarin dosing. All of the included studies had applied complex proprietary algorithms for genotype testing to determine the suitable warfarin dose. Currently, it is unclear which algorithm is the best because no direct comparisons have been made. Currently, at least for physicians it is difficult to take the time for genotype-guided dosing to guide warfarin treatment during their busy workday, especially when a patient presents with acute venous thromboembolism or atrial fibrillation, anticoagulation needs to be started immediately. It may not be the case for orthopedic surgery, for which more time is available for planning of surgery and anticoagulation. It may well be that there are adequate time and resources for acquiring the genotype of patients in clinical trials or in university hospitals. The situation is different for doctors who are working in the average clinic or hospital without significant resources that can be used for such testing. Nevertheless, a feasibility study examined the procedural feasibility of a pharmacist-led interdisciplinary service for providing genotype-guided warfarin dosing for hospitalized patients newly starting warfarin (35). When these

tools were embedded into electronic health records, the majority of genotypes were available before the second warfarin dose and good adherence to genotype-guided dose recommendations by the medical staff was observed.

We estimated that genotyping is needed for 40 individuals to reduce one major bleeding event. By contrast, it is needed for 238 individuals to reduce one thromboembolic event. These findings suggest that genotype-guided warfarin dosing could be worthwhile for individuals who are at high risk of bleeding. A related key issue is whether the benefits of genotype-guided dosing are cost-effective. The widespread and increasing use of non-VKA anticoagulants is likely to strongly diminish the impact of genotype-driven dosing for VKA. For patients who are prescribed warfarin, the cost of genotyping is relatively modest and likely to be much less costly than the costs for hospital admissions, length of stay and medical or interventional treatment due to bleeding. Since cost effectiveness may also vary with the baseline risk of bleeding of the patients concerned, depending on the comorbidities, this issue requires formal health economic analyses in future studies to determine the subset of patients on warfarin for whom genotype-guided dosing is cost-effective (36). Indeed, cost-effective analyses have been conducted using pharmacogenetic information in warfarin dosing for patients with nonvalvular atrial fibrillation (37). Based on a Markov state transition decision model with effectiveness measured by quality-adjusted life-years (QALYs), it was shown that warfarin-related genotyping is unlikely to be cost-effective for typical patients, but may be cost-effective in those at high risk for hemorrhage who will be started on warfarin therapy. Recent work has demonstrated its cost-effectiveness in other conditions such as mechanical heart valve replacement (38).

In 11 of the 18 trials, genotype-guided therapy arm was compared with a fixed dosing strategy in the standard care arm. In these studies, it is difficult to attribute the beneficial effects entirely to genotyping because patients in this group also benefited from the

algorithms or regression models using clinical information, which also contributed to the accuracy of warfarin dosing. Therefore, the benefits of genotype-guided therapy alone are better estimated by comparing to the remaining seven studies using clinical information guided approach in the standard care arm. There was no apparent improvement in TTR, excessive anticoagulation, risk of thromboembolism or mortality between the genotype-guided and the clinical information-guided groups, although there may be a benefit in reducing bleeding events. From the previous meta-analyses (15-21), only three had performed subgroup analyses based on the dosing regimen in the control group (18, 20, 21). All of these three meta-analyses similarly demonstrated no significant improvement in either biochemical parameters of INR control or the clinical endpoints of bleeding and thromboembolism events in the genotype-guided warfarin dosing group when compared to the clinical information-guided group using an equation-based approach.

Strengths and limitations

There are many strengths of this study. It is the largest meta-analysis of randomized trials to date, including 5230 participants from 18 trials. No heterogeneity or a low level of heterogeneity was observed for our meta-analyses on excessive anticoagulation, bleeding, thromboembolism or mortality. Heterogeneity remained low even when different types of control groups (fixed dosing and clinical information-guided dosing) were analyzed together, indicating the appropriateness to do pool these studies.

However, several limitations inherent in the present meta-analysis should be noted. Firstly, significant heterogeneity was observed for time-to-stable INR analysis. Similarly, the meta-analysis of TTR showed high level of heterogeneity, which was only partially accounted for when fixed dosing and clinical information-guided dosing were analyzed

separately. Some of the heterogeneity may be clinical as a result of different patient populations studied, such as different indications for anticoagulation. As described above, any small benefits in lowering the risk of bleeding may be magnified in orthopedic surgery because of the presence of open surgical wounds. Secondly, our meta-analysis only focused on one coumarin anticoagulant, warfarin, but not others. For example, acenocoumarol and phenprocoumon, which may be more commonly prescribed in some countries, were not included. Whether genotype-guided dosing similarly is better than conventional dosing strategies await further analyses. Moreover, the mean follow-up duration was 64 days. Whilst this is sufficient for evaluating time-to-first therapeutic INR and time-to-stable INR, it cannot provide the full picture in terms of clinical outcomes. Furthermore, there appears to be considerable heterogeneity among the genotype testing regimens. Finally, although differences in bleeding rates could be detected in our meta-analysis, our study may not be powered sufficiently to detect differences in thromboembolism or mortality. Future work can also analyze whether the effectiveness similar in the perioperative period as compared with other clinical indications.

Conclusions

Genotype-guided warfarin dosing offers better safety with less bleeding for patients requiring anticoagulation compared to conventional dosing strategies. No significant benefit on thromboembolism or mortality was evident.

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GT and MG: article screening, data extraction, data analysis, drafting and critical revision of manuscript

GL, SW, WKKW, WTW, LR, APWL: data interpretation, critical revision of manuscript

GYHL, MCSW, TL: conception of study, study supervision, data interpretation, critical revision of manuscript

Conflicts of interests

None declared.

Disclosures

None declared.

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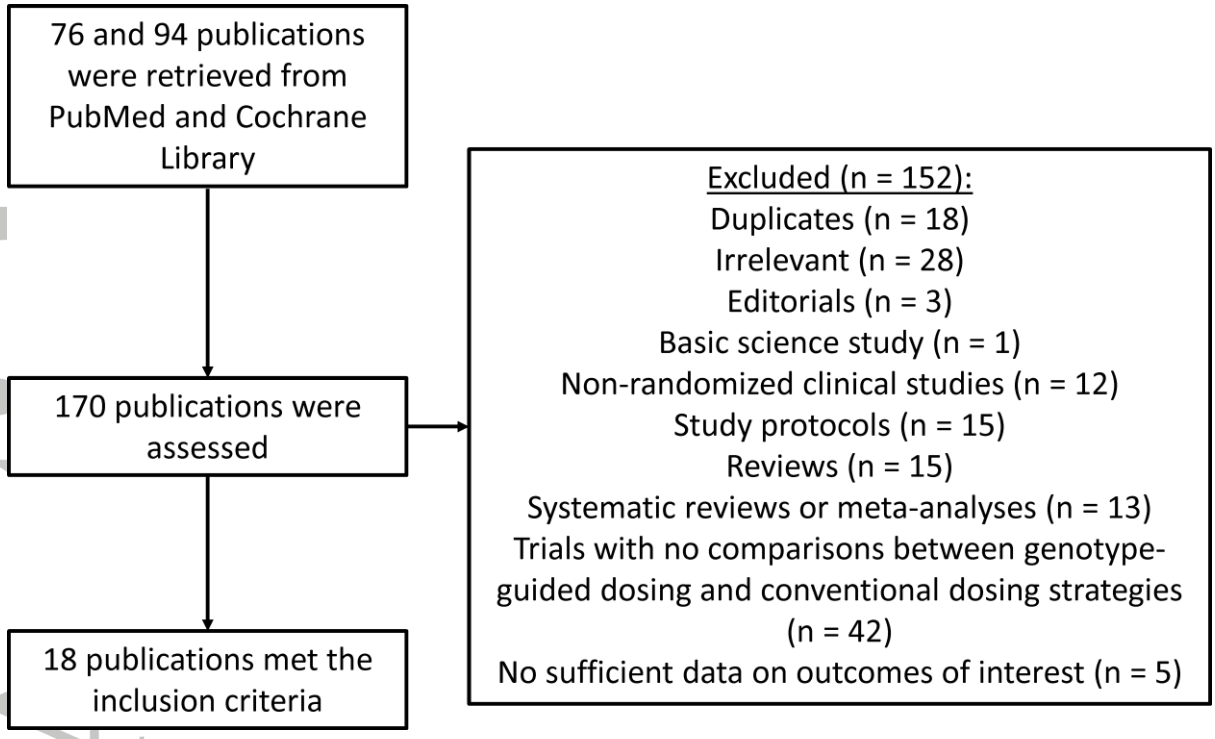

Figure 1. Flowchart of the database search and study selection process.

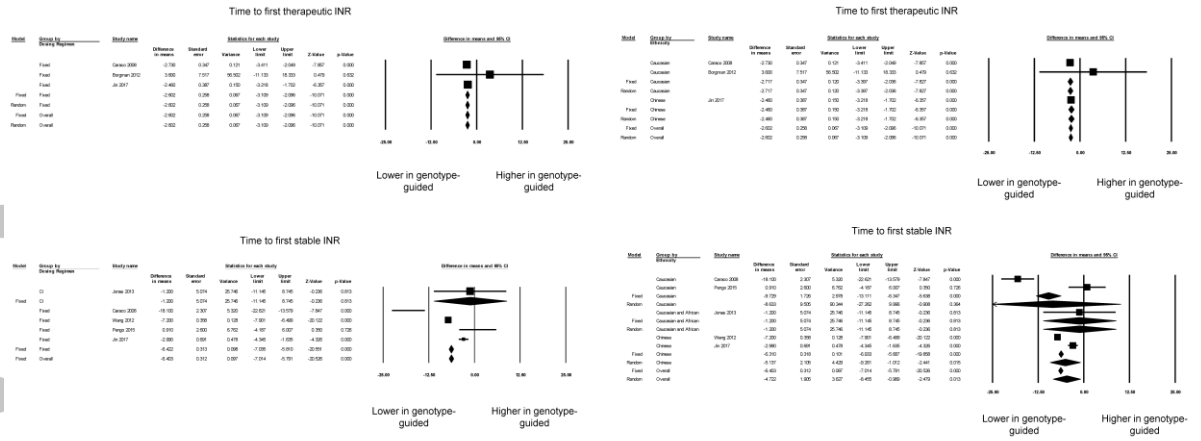

Figure 2. Mean difference in time-to-first therapeutic INR based on control group dosing regimen (*top left panel*) or ethnicity (*top right panel*). Mean difference in time-to-first stable INR based on control group dosing regimen (*bottom left panel*) or ethnicity (*bottom right panel*).

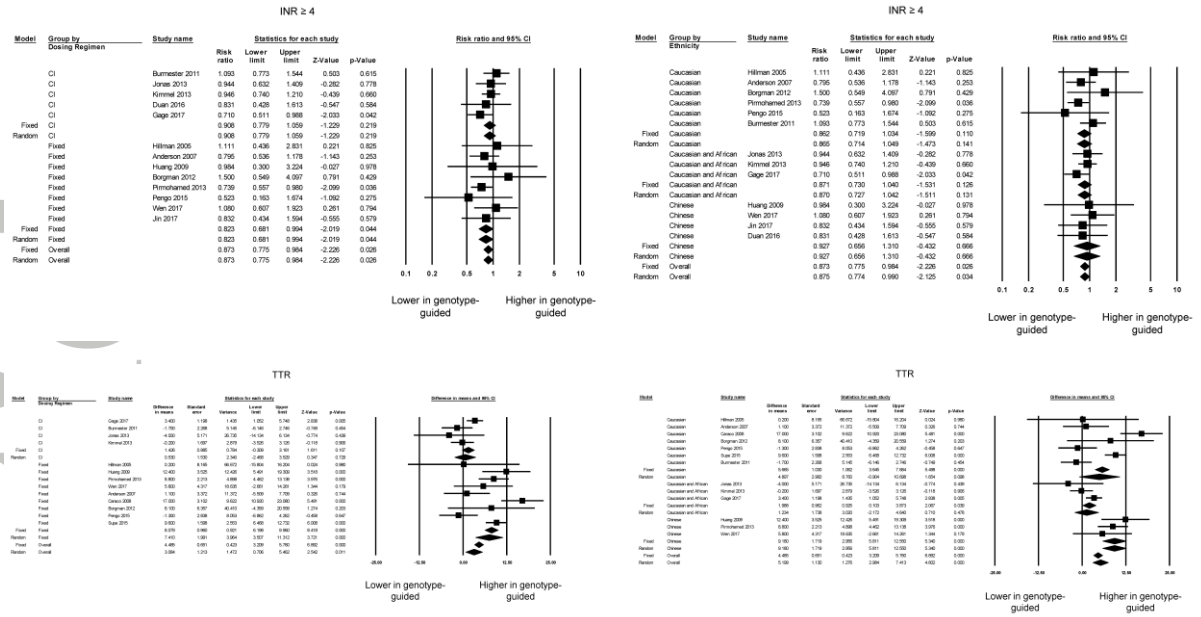

Figure 3. Mean difference in TTR based on control group dosing regimen (*top left panel*) or ethnicity (*top right panel*). Risk ratios comparing the number of individuals with excessive anticoagulation, defined as INR ≥ 4 between genotype-guided warfarin dosing and conventional dosing groups based on control group dosing regimen (*bottom left panel*) or ethnicity (*bottom right panel*).

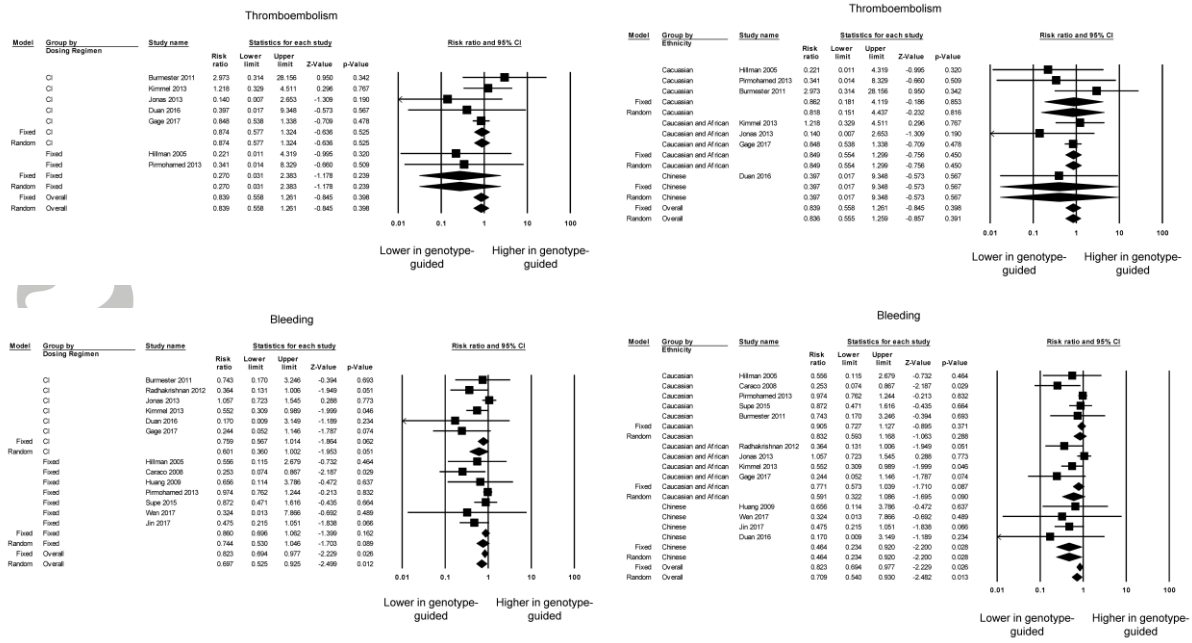

Figure 4. Risk ratios for comparing the number of individuals with bleeding symptoms between the genotype-guided warfarin dosing and conventional dosing groups based on control group dosing regimen (*top left panel*) or ethnicity (*top right panel*) with thromboembolism based on control group dosing regimen (*bottom left panel*) or ethnicity (*bottom right panel*).

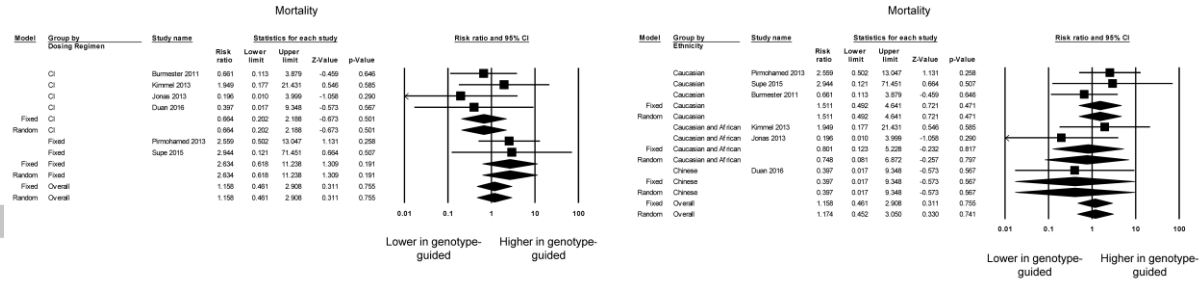

Figure 5. Risk ratios for comparing the mortality between the genotype-guided warfarin dosing and conventional dosing groups based on control group dosing regimen (*left panel*) or ethnicity (*right panel*).

Table 1. Characteristics of the trials included in this meta-analysis.

First author surname and year of publication	Target INR	Follow-up (days)	Ethnicity	Indication for warfarin	Genotype-guided group genes tested	Genotype-guided group dosing algorithm	Genotype-guided group total no.	Genotype-guided group no. of males	Genotype-guided group age, SD (yrs)	Control group dosing algorithm	Control group total no.	Control group no. of males	Control group age, SD (yrs)	Ref
Hillman 2005	1.9-3.2	28 ± 0	Caucasian (100%)	AF, DVT/PE, elective valvuloplasty or arthroplasty	*CYP2C9*	Hillman equation	18	8	68.8 ± 11.3	Fixed (5 mg for 7 days)	20	9	70.5 ± 13.3	(4)
Anderson 2007	2.0-3.0	46 ± 32	Caucasian (95%)	AF, DVT/PE, orthopedic surgery, others	*CYP2C9*, *VKORC1*	Carlquist equation	101	50	63.2 ± 15.3	Fixed (10 mg, 10 mg, 5 mg)	99	56	58.9 ± 16.0	(5)
Caraco 2008	2.0-3.0	31 ± 22	Not reported (Israeli patients)	AF, DVT/PE	*CYP2C9*	Algorithm designed by the authors	95	46	57.6 ± 19.6	Fixed (5 mg for an average of 6.5 days)	96	42	59.7 ± 18.5	(6)
Huang 2009	1.8-3.0	50 ± 0	Han Chinese (100%)	AF, DVT, valve replacement	*CYP2C9*, *VKORC1*	Sheng-WenHuang equation	61	20	41.6 ± 9.6	Fixed (2.5 mg; did not describe how many days)	60	18	43.0 ± 10.8	(7)
Borgman 2012	1.8-3.2	90 ± 0	Caucasian (100% in genotype group; 85% in conventional dosing)	AF, DVT, stroke, others	*CYP2C9*, *VKORC1*	5 mg + PerMIT software	13	7	59.0 ± 12.3	Fixed (5 mg for 7 days, but clinicians allowed to deviate)	13	7	45.0 ± 17.3	(9)

Wang 2012	1.8-3.0	50 ± 0	Han Chinese (100%)	Valve replacement for rheumatic heart disease	*CYP2C9*, *VKORC1*	Sheng-WenHuang equation	53	15	41.9 ± 6.3	Fixed (2.5 mg for 3 days)	53	16	42.8 ± 8.5	(10)
Pirmohamed 2013	2.0-3.0	90 ± 0	Caucasian (98.5%), African (1.1%), Asian (0.4%)	AF, DVT/PE	*CYP2C9*, *VKORC1*	Modified IWPC algorithm	227	145	67.8 ± 14.5	Fixed (10/5 mg, 5 mg, 5 mg)	228	132	66.9 ± 12.9	(14)
Pengo 2015	2.0-3.0	30 ± 0	Italian Caucasian (100%)	AF	*CYP2C9*, *VKORC1*, *CYP4F2*	Hamberg equation	88	58	71.0 ± 11.3	Fixed (5 mg for 4 days)	92	60	75.0 ± 10.0	(22)
Supe 2015	2.0-3.0	21 ± 0	Croatian Caucasian (100%)	Acute stroke	*CYP2C9*, *VKORC1*	IWPC algorithm	106	46	67.6 ± 13.5	Fixed (6 mg for days 2 to 5)	104	42	69.1 ± 12.2	(23)
Wen 2017	2.0-3.0	90 ± 0	Han Chinese (100%)	AF, DVT, PE, stroke, others	*CYP2C9*, *VKORC1*	Wen et al. algorithm	107	59	67.0 ± 15.5	Fixed (5 mg for 3 days)	104	63	66.0 ± 14.0	(27)
Jin 2017	2.0-3.0	84 ± 0	Han Chinese (100%)	PE	*CYP2C9*, *VKORC1*	IWPC algorithm	115	57	69.0 ± 12.0	Fixed (3 mg)	123	63	68.0 ± 12.0	(26)
Burmester 2011	2.0-3.5	60 ± 0	Caucasian including Hispanics (100%)	AF, DVT/PE, valve surgery	*CYP2C9*, *VKORC1*, *CYP4F2*	Burmester equation	115	66	67.4 ± 12.3	CI (Burmester equation, regression model based on age, gender, BSA, heart valve status)	115	70	69.2 ± 12.7	(8)

Radhakrishnan 2012	N/A	90 ± 0	Not mentioned (U.S. study based in Pittsburgh)	Any indication (not elaborated further)	*CYP2C9*, *VKORC1*	N/A	28	-	-	CI (N/A)	28	-	-	(29)
Li 2013	2.0-3.0	50 ± 0	Han Chinese (100%)	PE	*CYP2C9*, *VKORC1*	Li et al. algorithm	97	38	61.6 ± 13.6	CI (empirically by clinician for first 3 doses)	95	38	60.1 ± 14.2	(13)
Jonas 2013	2.0-3.5	90 ± 0	Caucasian (72.5%), African-American (27.5%)	AF, DVT, PE, heart valve, others	*CYP2C9*, *VKORC1*	Gage equation	55	24	59.0 ± 19.3	CI (Gage equation)	54	27	55.3 ± 19.1	(11)
Kimmel 2013	2.0-3.0	30 ± 0	Caucasian (66.5%), African (27.1%), Hispanic (6.4%)	AF, DVT/PE, multiple indications, other indications, no indication given	*CYP2C9*, *VKORC1*	Gage equation	514	272	59.0 ± 16.3	CI (Gage equation, based on age, BSA, African American race, amiodarone use, target INR, smoking status, and warfarin indication)	501	246	57.0 ± 16.3	(12)

Duan 2016	N/A	28 ± 0	Han Chinese (100%)	PE with or without DVT	*CYP2C9*, *VKORC1*	N/A	25	10	54.5 ± 14.9	CI (traditional model)	30	13	54.5 ± 14.9	(24)
Gage 2017	1.5-2.1 (50%), 2.0-3.0 (50%)	90 ± 0	Caucasian (91.0%), African (6.4%), Asian or Indian subcontinent (1.8%), American Indian or Alaskan Native (0.1%), others	Arthroplasty	*CYP2C9*, *VKORC1*, *CYP4F2*	IWPC algorithm	808	286	72.2 ± 5.3	CI (Gage equation, based on age, BSA, African American race, amiodarone use, target INR, smoking status, and warfarin indication)	789	293	72.0 ± 5.5	(25)

Abbreviations: AF: atrial fibrillation; BSA: body surface area; DVT: deep vein thrombosis; PE: pulmonary embolism; CI: clinical information