

Warfarin pharmacogenetics: up close and personalized



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'The application of pharmacogenetic warfarin dosing may establish the necessary infrastructure for more expanded genotype-directed therapeutics.'

The term 'journey' as an allegorical symbol has been overused in a variety of narratives. However, in describing the pharmacogenetics of warfarin, it seems appropriate and has literal as well as metaphorical relevance. The journey began with the discovery in 1940 of the parent compound of warfarin, coumarin, a prothrombin inhibitor found to be the cause of mysterious hemorrhagic death of cattle ingesting sweet clover. Efforts to derive an increased-potency prothrombin inhibitor resulted in the development of warfarin (named after the Wisconsin Alumni Research Foundation). The chemical was initially patented as a rodent poison and later approved as a therapeutic anticoagulant in 1954. The drug is notoriously difficult to manage due its narrow therapeutic range, with elevated risk for bleeding and thromboembolic events for supra- and sub-therapeutic doses, respectively, coupled with marked interindividual dosing requirements.

Physiologically, *S*-warfarin, the more potent enantiomer of warfarin, is metabolized by the p450 enzyme, *CYP2C9*; genetic alterations in the *CYP2C9* gene (the *2 and *3 variants) have been shown to partially influence dose variability [1,2]. The molecular target of warfarin is the enzyme vitamin K epoxide reductase; variations in the gene encoding the enzyme (*VKORC1*) were also found to be associated with differences in warfarin dose [3]. In a retrospective study of genotype versus stable warfarin dose, our group found as much as an eightfold difference in mean warfarin dose between those individuals homozygous for the wild-type alleles for *CYP2C9* and *VKORC1* C1173T compared with those bearing variants at both loci [4]. This observation has been independently replicated.

As a result of the dramatic differences in stable dose arising from the allelic forms of these genes, in 2005, the Clinical Pharmacology Subcommittee of

the US FDA Advisory Committee for Pharmaceutical Science recommended that the FDA relabel warfarin with language indicating that *CYP2C9* and *VKORC1* genotyping can assist in optimizing warfarin dosing. Although a large prospective, randomized trial to test the efficacy of genotype-guided warfarin therapy is currently in the planning phase, there is presently little prospective information to define 'optimized warfarin dosing'. Despite the absence of data to guide dosing, the FDA, faced with overwhelming evidence that *CYP2C9* and *VKORC1* genotypes relate to therapeutic dose, and respecting the recommendation of the Pharmacology Subcommittee, relabeled warfarin with genomic information on August 16, 2007. The revised label for warfarin suggests that lower doses may be best for patients with variations in one or both of these genes. Thus, in a rather novel twist in the tortuous journey of the compound developed to poison rodents, warfarin had become pharmacogenetics' 'poster child'.

It was assumed by those working in the field and by the FDA that randomized trials would show that genotype-guided warfarin therapy shortens the time to stable therapeutic dosing and would increase time in therapeutic range, thereby reducing the risks associated with out-of-range prothrombin time, measured as the International Normalized Ratio (INR). We shared this view and set out to prospectively test this in a randomized trial. Optimistically, we powered the study to detect a reduction in out-of-range INR measurements by 50% or more (i.e., from 40 to 20%). This trial, the Couma-Gen study [5,6], was the first randomized trial using both the *CYP2C9* and *VKORC1* genotypes (plus clinical variables) to guide therapy in the treatment arm versus the control arm receiving standard therapy. Standard therapy was managed by an anticoagulation management service. The study enrolled 206 consenting patients; 200 were evaluable for the efficacy end points. Standard dosing used an empirical protocol, whereas the pharmacogenetic arm used a dosing regimen obtained from a regression equation that included genetic variants, age, weight and sex. In the pharmacogenetic arm, the algorithm more accurately predicted the stable maintenance dose ($R^2 = 0.47$; $p < 0.001$).

Moreover, there were fewer INR measurements and fewer dose adjustments, and the absolute amount of dose adjustment was significantly smaller in the pharmacogenetic group. Surprisingly, the primary end point, the percentage of out-of-range INRs, did not differ between groups (30.7% pharmacogenetic vs 33.1% standard therapy).

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Except for the primary end point, this study generally performed as predicted. Although the study was small and clearly powered for much larger differences, it did have an important efficacy signal for the future study and potential clinical uses of genotype-guided warfarin therapy. In a prespecified subset analysis, a nominally significant reduction ($p = 0.03$) in out-of-range INRs (from 39 to 29%; relative reduction: 26%; absolute reduction: 10%) was achieved by pharmacogenetic dosing for the combined groups of subjects possessing more than one genetic variant and wild-type subjects, that is, the dose-requirement extremes. For these two groups of subjects, the pharmacogenetic algorithm quite accurately predicted the stable dose, whereas large adjustments to the empirical (standard dose) algorithm were required. Individuals with a single variant comprised the largest subgroup of the population under study (43%). Thus, this group heavily influenced the development of empirical dosing algorithms and also contributed importantly to the overall neutral results in the pharmacogenetics-guided arm. In contrast, the wild-type subjects (30% of the study population) and carriers of multiple variants (27% of the study sample), representing important extremes of the dosing range, were found to require 14 mg more and 10 mg less per week (both $p < 0.001$), respectively, than the standard empirical dose of 35 mg/week (5 mg/day). Thus, they represent the subgroups most poorly served and were most likely to be off target (and require adjustment) with an empirical algorithm based on the population mean dose requirement of 35 mg/week (a concurrent randomized study has also reported that wild-type subjects are underdosed by an empirical algorithm) [7]. In retrospect, these observations are quite predictable: wild-type patients were underdosed and carriers of multiple

variants were overdosed. A future study, powered to account for these subgroup differences, appears to be highly appealing.

Although not addressed by Couma-Gen, another unresolved issue is the relative contributions of pharmacokinetic and pharmacodynamic interactions dictating response to warfarin. Whereas the *CYP2C9* gene product influences warfarin metabolism and elimination (pharmacokinetic effect), *VKORC1* encodes the molecular target for warfarin (pharmacodynamic effect). A model has been proposed by Hamberg *et al.* [8] that attributes variability in warfarin clearance to *CYP2C9* genotype and warfarin sensitivity to variants in *VKORC1*. These investigators describe a two-compartment transit model to explain the delay between treatment and anticoagulant effect. The delay is most pronounced for *CYP2C9* variant carriers. Carriers of two *CYP2C9* variant alleles are particularly at risk for excessively high INRs, but this effect arises slowly, requiring three or more days to become evident even for double variant carriers [9]. Conversely, the effects of the *VKORC1* variants are already detectable with the first three warfarin doses [10]. It is probable that the most appropriate dosing algorithm will not simply incorporate the presence and number of variants, but will also make temporal adjustments based on the variant gene. One might envision that early dosing would be dictated by the presence and number of *VKORC1* variants and be incorporated into an initial dosing algorithm (first few days), whereas *CYP2C9* genotype would affect later maintenance dosing and be incorporated (together with *VKORC1*) in a second stage, maintenance dosing algorithm, beginning after the first few doses. Here, the concept of a journey becomes literal rather than symbolic. It seems the pathway followed to arrive at a stable warfarin dose may have as much clinical relevance as the actual quantity of the final stable dose.

Couma-Gen clearly demonstrated that genotype-guided dosing can be effective, but it does not clearly demonstrate a clinical improvement over an experienced anticoagulation management service making daily INR measurements and appropriate dose adjustments on an inpatient cohort. It is likely that the successful management of the comparator control group in Couma-Gen was an important contributor to the failure to achieve the primary end point. Indeed, the percentage of out-of-range INRs in the control arm was 7–8% lower than the overall system average at Intermountain Healthcare

(UT, USA). However, it should be borne in mind that warfarin recipients being closely monitored by an experienced and dedicated anticoagulant management service represent a very small minority of the total number of patients undergoing anticoagulation. Most out-patients are managed by their physicians and some by warfarin clinics. Although these clinics concentrate their expertise, they usually assume patient management only after stable dosing is achieved, the time at which the peak risk for adverse events has passed and after pharmacogenetics-guided dosing might have its greatest impact. Thus, the vast majority of warfarin initiators are likely to accrue some benefit from pharmacogenetic dosing. Potential benefits include increased time in therapeutic range (or fewer out-of-range INR measurements), fewer and smaller dose adjustments and fewer INR measurements. In addition to reduced bleeding and thromboembolic risks, this should translate into reduced personal burden, physician time, clinic visits, and laboratory and overall healthcare costs.

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It must be recalled that, despite the broad range of stable doses and the ability to accurately predict these doses, the effects on intermediate and clinical end points have been small. The Couma-Gen study, powered to detect a 50% effect-size reduction, did not meet the primary end point. In order to adequately test for a smaller clinical benefit, a more optimal dosing algorithm(s) must be fashioned and applied in such a way as to smooth the inherently unstable initiation period. It is this period where the greatest potential for benefit appears to reside. It is our belief that the optimal algorithm is likely to follow a dynamic rather than a static dosing model and respond to both the pharmacodynamic and pharmacokinetic effects of different genotypes through quantitatively and temporally adjusted personalized dosing.

As frequently observed, reality is more complex than what is envisioned, and, as in this case, the effects less dramatic. Nonetheless, through careful refinements in this approach, warfarin

therapy could, and should, be improved for the up to 2 million individuals per year who will begin warfarin therapy in the upcoming years.

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Future perspective

To return to the metaphor of the journey, warfarin therapy has followed an interesting and twisting path from its unlikely beginning as rodent poison to a pharmacogenetics case study. The journey is not over, but the destination may soon come into sight. The potential to improve patient care is clearly within grasp. The application of pharmacogenetic warfarin dosing may establish the necessary infrastructure for more expanded genotype-directed therapeutics – that is, given the widespread use of warfarin, pharmacogenetic guidance for its dosing should hasten familiarity with the concept of personalized medicine, easing the transition into more widely practised pharmacogenetics.

Specifically with respect to warfarin anticoagulation, the advantages are likely to be more pronounced when viewed from a system rather than an individual perspective. Current accounting practices are silo-based and may disguise generalized benefit. For example, reducing the number of INR measurements and clinic visits may reduce revenue to the laboratory and the clinic. However, this lost revenue may be more than compensated for by the reduction in hospitalizations that often accompany frequent dose adjustments. Avoidable hospitalizations excessively burden the healthcare system and divert resources from others in serious need of medical attention. In this way, personalized medicine provides hope of more broadly distributing healthcare resources.

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