Review

Pharmacogenomics of anticoagulants: steps toward personal dosage

Ann K Daly

Address: Institute of Cellular Medicine, Newcastle University Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK.
Email: A.K.Daly@ncl.ac.uk

Published: 21 January 2009

Genome Medicine 2009, **1**:10 (doi:10.1186/gm10)

The electronic version of this article is the complete one and can be found online at http://genomemedicine.com/content/1/1/10

Abstract

Warfarin and other coumarin anticoagulants are widely used clinically, but currently dosing is determined individually on the basis of patient response. There is increasing evidence that genetic factors, together with several non-genetic patient-specific factors, are important determinants of stable dose requirement for these compounds. Genotype for *CYP2C9*, which encodes the main cytochrome P450 enzyme that metabolizes warfarin, and *VKORC1*, the gene encoding the warfarin target vitamin K epoxide reductase, together account for approximately 30% of the variability in dose requirement. The past two years have seen several advances in the area of genetic factors affecting coumarin anticoagulant response. In particular, prospective studies have taken place to analyze whether earlier small retrospective studies can be confirmed, and the question of whether genes other than *CYP2C9* and *VKORC1* are important in determining dose requirement has been examined. So far, no strong evidence that other genes contribute to dose requirement has been found, apart from a minor but novel role for another cytochrome P450 gene, *CYP4F2*. A recently published whole genome association study confirms that the main genes important in warfarin response are *CYP2C9* and *VKORC1*. Clinical trials comparing genotype-guided and conventional warfarin initiation have suggested that genotyping may be of value, but larger studies are still needed to show clear clinical benefit. Current knowledge of genetic factors affecting other coumarin anticoagulants is more limited and this area requires further study, as does the impact of ethnic variation in genes relevant to coumarin responses. Here we review recent advances in the area of coumarin anticoagulant genetics and its potential clinical application.

Introduction

Coumarin anticoagulants, including warfarin, are among the most widely prescribed drugs in modern medicine. A difficulty with their use is that dosage needs to be individually determined for each patient, usually by following a standard initial dosing protocol, measuring the coagulation rate regularly (using the international normalized ratio, INR, which is a measure of prothrombin time. A high INR value indicates overcoagulation) and then adjusting the dose until the required rate of coagulation is obtained. Overcoagulation places the patient at risk of potentially fatal hemorrhage, so improving protocols for initiation of anticoagulant treatment remains an important issue. In particular, warfarin has been shown to be frequently implicated in emergency admissions relating to adverse drug reactions in a survey of two UK hospitals [1]. Approximately 10% of Europeans require an unusually low dose of warfarin (1.5 mg/day or less) and these patients could be at increased risk of developing serious bleeds and undesirably high levels of anticoagulation, especially during the initial weeks of treatment [2]. Although the current oral coumarin anticoagulants, such as warfarin, acenocoumarol and phenprocoumon, are likely to be replaced eventually by other drugs under development, such as the specific thrombin inhibitors, the current drugs will probably continue to be the main oral anticoagulants prescribed in the short to medium term.

The metabolism of warfarin and the other coumarin anticoagulants is well understood, with the cytochrome P450 enzyme CYP2C9 having a major role in their phase I metabolism (reviewed in [3]). *CYP2C9* is subject to a genetic polymorphism affecting its activity, and the fact that this polymorphism contributes to individual anticoagulant dose requirement is now well established, although its effect on phenprocoumon metabolism is less pronounced than that on either warfarin or acenocoumarol [2,4-7]. Coumarin anticoagulants exert their effect by inhibiting the enzyme vitamin K epoxide reductase, which regenerates vitamin K following its oxidation in the gamma glutamyl carboxylase reaction. This reaction takes place during the normal activation of clotting factors in the coagulation cascade [8]. *VKORC1*, the gene encoding the target enzyme vitamin K epoxide reductase, was identified relatively recently, in 2004 [9]. Polymorphisms in this gene's non-coding sequences have been shown to affect levels of gene expression, resulting in inter-individual variation in the amount of this protein present in hepatocytes [10], and recent studies have shown the basis for this: an allele with an A at position -1639 (upstream of the transcription start site) is associated with lower transcription than the G found more commonly in European populations [11,12]. VKOR protein levels seem to affect the required dose of anticoagulant. A clear association between the G-1639A polymorphism and warfarin dose requirement has been reported in many independent studies [10,13,14], and similar associations for acenocoumarol and phenprocoumon also occur [11,15].

In addition to the contribution of *CYP2C9* and *VKORC1* to the coumarin anticoagulant dose requirement, there is clear evidence for a contribution by other patient-specific factors, including age, weight, height, concurrent drug therapy and possibly diet [10,14,16,17]. Worldwide, warfarin is the most commonly used of the three coumarins and has also been the most widely studied in relation to factors affecting dose requirement and various aspects of patient response. The literature on pharmacogenetic aspects of coumarin anticoagulants continues to expand rapidly. An important recent development is the inclusion of reference to genetic factors affecting response (both *CYP2C9* and *VKORC1*) in the prescribing information for warfarin in the USA by the Food and Drug Administration (FDA) [18], which is likely to prompt more interest and further studies in the area. Detailed background information on coumarin anticoagulants is provided in several recent review articles [19-21].

Studies of the effect of genes other than *CYP2C9* and *VKORC1* on warfarin response

There is general agreement among published retrospective population studies that the combination of *VKORC1* and *CYP2C9* genotype, together with age, body mass index or height or weight, and concurrent medication, predicts approximately 50% of warfarin dose requirement. Identifying additional factors affecting dose requirement, particularly genetic factors, is of considerable interest. Some additional genes (for a detailed list see [20]) have been suggested to contribute to dose but, in all cases, the observed effects are smaller than those seen for *VKORC1* and *CYP2C9* and, generally, the findings have not been independently confirmed by additional studies.

One possible exception to this is the cytochrome P450 *CYP4F2* gene. This gene was shown to contribute to warfarin dose requirement in a study using the Affymetrix ADME gene chip, which includes a single nucleotide polymorphism (SNP) in *CYP4F2* that is associated with the amino acid change V433M [22]. The effect for warfarin was observed in three independent populations, and it was found that in each case there was an average 1 mg/day increase in dose requirement in those homozygous for the variant compared with those homozygous for the wild-type allele. Further support for a minor role for this polymorphism in warfarin dose requirement has come from a whole genome association study [23] and from a separate UK-based retrospective study (Hatch E, AKD and Kamali F, unpublished results).

A problem with this association is that the biological basis remains unclear. CYP4F2 has a well established role in eicosanoid metabolism, producing 20-hydroxyeicosatetraenoic acid, and the V433M polymorphism seems to be functionally significant, decreasing activity [24]. However, its relevance to warfarin action is still unclear, although a role for *CYP4F2* in vitamin K metabolism has been suggested [22] and it also remains possible that it contributes directly to warfarin metabolism. Interestingly, several independent studies suggest that the *CYP4F2* genotype affects systolic blood pressure [25,26], and an increased risk of ischemic stroke has been suggested among those positive for the variant form associated with the higher warfarin dose requirement [26]. The effect of *CYP4F2* on warfarin dose requirement is biologically interesting, but the overall 1-2% contribution to dose requirement confirmed so far [22] may be too small for genotyping for the relevant SNP to be clinically useful.

A recent whole genome association study on patients treated with warfarin [23] provides some new insights into the likelihood of additional genetic effects, and at least two other such studies are currently under way. In particular, a published study [23] confirmed the large effect of *VKORC1* on dose requirement. No other genome region showed *p*-values lower than the significance threshold set by Bonferroni correction in both the original population studied and a replication population, indicating that only this region was significantly associated with dose requirement. However, SNPs in the *CYP2C* locus gave a moderate *p*-value in the original population and showed stronger effects lower than the significance threshold in both the replication cohort and the original population combined with the replication cohort. Evidence for a minor role for *CYP4F2* was also

obtained, with the effect on dose requirement in the same direction as that reported previously [22].

Recent large-scale population studies and proposals for new dosing algorithms

Following the discovery of *VKORC1*, several studies proposed dosing algorithms on the basis of *VKORC1* and *CYP2C9* genotypes together with patient-specific non-genetic factors, such as age, body size and other prescribed drugs [14,27]. However, these studies were generally based on small sample numbers and retrospective study designs, which could limit their widespread usefulness. Some recent approaches aim to improve on these proposed algorithms by inclusion of larger numbers of patients and by using prospective study designs [27]. In addition, the International Warfarin Pharmacogenetics Consortium [28] has pooled genetic data relating to warfarin from researchers worldwide to develop a dosing algorithm that will be more applicable to a range of different ethnic groups. The current dataset includes data from more than 5,000 patients and quality control of genotyping has been performed to ensure that all the data are comparable. One caveat about this approach concerns the original study designs. Most were retrospective and somewhat selective in the patients they included, which could limit the applicability of the algorithm to patients for whom the stabilization of the drug is more difficult to achieve. Prospective studies in which patients are recruited at the start of their treatment are likely to eliminate this issue.

In the largest prospective study yet published [27], involving 1,496 Swedish patients and genotyping for a range of SNPs in 29 candidate genes, the only significant genetic predictors of dose were SNPs in *CYP2C9* and *VKORC1*. An algorithm for dose was developed and used the significant genotypes together with age, sex (as a surrogate for body mass) and additional drugs. This large study also demonstrated a particular risk for high INR (overcoagulation) soon after treatment in individuals homozygous for the variant *CYP2C9*3*. A second prospective study [29] involved a smaller sample and more limited genotyping but also showed a relationship between *CYP2C9* genotype and high INR while suggesting that individuals homozygous for A-1639 of *VKORC1* were particularly at risk of overcoagulation soon after the start of treatment.

Clinical trials incorporating genotyping

With the development of warfarin dosing algorithms that incorporate genotyping, clinical trials have been initiated to investigate their usefulness in warfarin treatment. The decision by the FDA to include reference to genetic testing in warfarin's label is an additional impetus to perform these studies. Of several factors that need to be taken into account in order to maximize the usefulness of any clinical trial on the value of genotype-guided dosing, the main one is the precise questions to be asked. Will genotyping increase patient safety, or is the benefit more likely to lie in the area of economics because fewer consultations or, in some cases, a shorter hospital stay is needed?

Three published clinical trials [30-32] have focused on the question of whether genotyping will lead to patients spending more time in the therapeutic range (the dose that gives the best effects without side-effects) and achieving a stable dose sooner, and all three have addressed this using relatively small numbers of patients. The first study [30] looked at *CYP2C9* genotype only and adjusted dose according to genotype on the second day of treatment. A total of 95 dose-adjusted patients and 96 control patients were studied. Patients with the *CYP2C9* genotype-adjusted dose achieved stable dose sooner, spent more time in the target INR range and experienced less minor bleeding. In a study using both *CYP2C9* and *VKORC1* genotyping [31], in which genotype was used to determine intervention on day 3 of dosing, no difference in the incidence of out-of-range INRs was seen between 100 patients on a genotype-guided dose and 100 on a traditional standard protocol. However, some benefits were shown both for patients homozygous for both wild-type alleles and for carriers of multiple variant alleles, which raises the possibility that the failure to see overall significance might be due to insufficient statistical power. A non-randomized prospective study of a similar size but using a different protocol, with dose adjustment on day 4 of treatment on the basis of either clinical factors only or clinical factors combined with genotype [32], reported more time spent in the therapeutic range in the genotype-guided group.

All three trials [30-32] have predominantly involved populations of European origin, but a prospective study based in Taiwan involving genotype-guided dosing without a non-genotyped control group has also been reported [33]. In this study, 83% of 108 patients reached a stable INR within two weeks without any bleeding incidents reported. The majority of patients in that study [33] were homozygous wild type for *CYP2C9* and also homozygous for the *VKORC1* variant genotype, which limited the ability to study patients who might be particularly at risk of high INR.

There is a need for larger prospective studies to provide adequate power to look at the incidence of bleeding events and also to collect information on the economic value of genotyping. In addition, the application of genotyping prior to acenocoumarol and phenprocoumon prescription needs to be assessed, although suitable dosing algorithms will need to be formulated. No algorithm that uses genotype to predict a suitable loading dose for use on day 1 has yet been developed, but such algorithms could be important in maximizing the benefit of using genotype to guide anticoagulant dose.

Conclusions

Progress on our understanding of the genetic factors affecting anticoagulant response has been rapid since the discovery of the *VKORC1* gene in 2004. The availability of genome-wide association data and large prospective studies in relation to warfarin have provided an excellent framework for further research. All indications suggest that incorporating genetic testing into warfarin use will probably be beneficial, but the extent of that benefit still needs to be determined in well-designed clinical trials if testing is to become routine in clinical practice.

The use of genetic tests in relation to drug prescription remains confined to some very specific examples, mainly in oncology [34]. Warfarin is prescribed more commonly than any of the drugs for which genetic testing is already widely used, and adoption of routine genetic testing during initiation of dosing would be an important advance clinically. Acenocoumarol and phenprocoumon continue to be the main oral anticoagulants used in several European countries, and further studies to bring understanding of genetic factors affecting response to these compounds up to the level currently available for warfarin is desirable. Finally, if a useful algorithm involving genetic testing that will benefit all users of coumarin anticoagulants is to be finalized, it will also be important to assess its validity in a range of different ethnic groups.

Abbreviations

CYP2C9, cytochrome P450 2C9; FDA, Food and Drug Administration; INR, International normalized ratio; SNP, single nucleotide polymorphism; VKORC1, vitamin K epoxide reductase complex 1.

Competing interests

The author declares that she has no competing interests.

References

1. Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ, Farrar K, Park BK, Breckenridge AM: **Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients.** *BMJ* 2004, **329:**15-19.
2. Aithal GP, Day CP, Kesteven PJL, Daly AK: **Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications.** *Lancet* 1999, **353:**717-719.
3. Daly AK, King BP: **Pharmacogenetics of oral anticoagulants.** *Pharmacogenetics* 2003, **13:**247-252.
4. Higashi MK, Veenstra DL, Kondo LML, Wittkowsky AK, Srinouanprachanh SL, Farin FM, Rettie AE: **Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy.** *JAMA* 2002, **287:**1690-1698.
5. Scordo MG, Pengo V, Spina E, Dahl ML, Gusella M, Padrini R: **Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance.** *Clin Pharmacol Ther* 2002, **72:**702-710.
6. Tassies D, Freire C, Puoan J, Maragall S, Monteagudo J, Ordinas A, Reverter JC: **Pharmacogenetics of acenocoumarol: cytochrome P450CYP2C9 polymorphisms influence dose requirements and stability of anticoagulation.** *Haematologica* 2002, **87:**1185-1191.
7. Kirchheiner J, i**t not CYP4A11 gene is associated with increased 20-HETE excretion and blood pressure.** *Hypertension* 2008, **51:**1393-1398.
26. Fava C, Montagnana M, Almgren P, Rosberg L, Lippi G, Hedblad B, Engstrom G, Berglund G, Minuz P, Melander O: **The V433M variant of the CYP4F2 is associated with ischemic stroke in male Swedes beyond its effect on blood pressure.** *Hypertension* 2008, **52:**373-380.
27. Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S, Holm L, McGinnis R, Rane A, Deloukas P: **The largest prospective warfarin-treated cohort supports genetic forecasting.** *Blood* 2008. doi: blood-2008-04-149070v1.
28. Owen RP, Altman RB, Klein TE: **PharmGKB and the International Warfarin Pharmacogenetics Consortium: the changing role for pharmacogenomic databases and single-drug pharmacogenetics.** *Hum Mutat* 2008, **29:**456-460.
29. Schwarz UI, Ritchie MD, Bradford Y, Li C, Dudek SM, Frye-Anderson A, Kim RB, Roden DM, Stein CM: **Genetic determinants of response to warfarin during initial anticoagulation.** *N Engl J Med* 2008, **358:**999-1008.
30. Caraco Y, Blotnick S, Muszkat M: **CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study.** *Clin Pharmacol Ther* 2008, **83:**460-470.
31. Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, Kahn SF, May HT, Samuelson KM, Muhlestein JB, Carlquist JF: **Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation.** *Circulation* 2007, **116:**2563-2570.
32. Lenzini PA, Grice GR, Milligan PE, Dowd MB, Subherwal S, Deych E, Eby CS, King CR, Porche-Sorbet RM, Murphy CV, Marchand R, Millican EA, Barrack RL, Clohisy JC, Kronquist K, Gatchel SK, Gage BF: **Laboratory and clinical outcomes of pharmacogenetic vs. clinical protocols for warfarin initiation in orthopedic patients.** *J Thromb Haemost* 2008. doi: 10.1111/j.1538-7836.2008.03095.x.
33. Wen MS, Lee M, Chen JJ, Chuang HP, Lu LS, Chen CH, Lee TH, Kuo CT, Sun FM, Chang YJ, Kuan PL, Chen YF, Charng MJ, Ray CY, Wu JY, Chen YT: **Prospective study of warfarin dosage requirements based on CYP2C9 and VKORC1 genotypes.** *Clin Pharmacol Ther* 2008, **84:**83-89.
34. Daly AK: **Individualized drug therapy.** *Curr Opin Drug Discov Devel* 2007, **10:**29-36.