

Influence of CYP2C9, VKORC1 and environmental factors on patient response to warfarin: a HuGENet systematic review and meta-analysis

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Background

Warfarin is the anticoagulant of choice in the UK. A coumarin anticoagulant, it is prescribed to patients with venous thrombosis, pulmonary embolism, chronic atrial fibrillation and prosthetic heart valves. Several randomised controlled trials and meta-analyses have demonstrated the efficacy of warfarin. However, this efficacy is dependent on maintaining a patient's anticoagulation within a clinically acceptable therapeutic range, measured in terms of the International Normalised Ratio (INR).

Maintaining anticoagulation within this clinically acceptable therapeutic range, however, is not always an easy task. Firstly, warfarin has a very narrow therapeutic range i.e. the dose required to ensure therapeutic anticoagulation is very close to the dose that leads to over-anticoagulation. Secondly, there is very large inter-individual variability in the maintenance dose required to achieve therapeutic range: whereas some patients may require only 0.5mg a day others may require more than 10mg a day.

The most feared side effect of warfarin and anticoagulation treatments in general is bleeding. According to a meta-analysis of 33 studies¹, major and fatal bleeding events occur at a rate of 7.2 and 1.3 per 100 patient years in warfarin treated patients. In addition to the obvious mortality and morbidity implications of warfarin-related bleeds, cost is also an important aspect: a recent analysis showed the average cost per patient of a bleeding event to be approximately \$15,988².

Several previous studies have examined the influence both of environmental factors such as age, gender, interacting medications, diet and medical history and genetic factors on anticoagulation control, dose requirements and bleeding events in warfarin treated patients. All these factors have been shown to have a role to play in determining how an individual patient will respond to warfarin therapy and in determining their optimal course of treatment.

In terms of genetic factors, it has been suggested that at least 30 genes may be involved in the biological pathway of warfarin and these are discussed in detail in a recent review undertaken by Wadelius and Pirmohamed³. Of these many genes, the most central to the pharmacokinetics of warfarin is CYP2C9 whilst the most central to the drug's pharmacodynamic effects is VKORC1³.

Several variants have been identified within CYP2C9 but most warfarin studies to date have focused on just two variants: CYP2C9*2 and CYP2C9*3. Patients homozygous for the wild-type CYP2C9*1 allele have been shown to be extensive metabolisers of warfarin. Being homozygous for the *2 allele has been shown to reduce enzyme activity to 12% compared to extensive metabolisers whilst being homozygous for the *3 allele has been shown to reduce activity to 5%⁴⁻⁶. As would be expected, it follows that patients with the *2 and *3 variant alleles require a lower mean daily dose of warfarin. Indeed, a systematic review and meta-analysis

investigating the effect of the CYP2C9 variants on mean daily dose requirement⁷ showed that patients carrying at least one copy of the *2 alleles needed a 17% reduction in mean daily dose compared to those homozygous for *1. Patients carrying at least one copy of the *3 allele needed a 37% reduction. This same meta-analysis showed that a patient with at least one copy of the *2 allele had a 91% increased risk of experiencing a bleeding event whilst on warfarin and a patient carrying at least one copy of the *3 allele had a 77% increase in risk.

Warfarin exerts its effect by interfering with the vitamin K cycle in patients. Genes involved in the Vitamin K cycle are therefore prime candidates for influencing treatment response. VKORC1 is one of these genes and several studies have shown association between common single nucleotide polymorphisms in VKORC1 and a reduced warfarin mean daily dose requirement⁸⁻¹⁰. Some further studies^{11,12} have shown rare mutations within this gene to be associated with warfarin resistance. All the VKORC1 single nucleotide polymorphisms found to be associated with warfarin are however within a region of strong linkage disequilibrium which means that a combination of several SNPs does not contribute any greater information than one individual SNP^{9,10,13}.

Although some studies into pharmacogenetic influences on warfarin treatment response have focused only on variants in just one of the aforementioned genes, others have assessed the combined effect of variants in both genes together with various environmental factors. Five such studies^{9, 13, 14, 15, 16} undertaken have been able to explain approximately 50% of inter-individual variation in dose requirement by way of a combination of CYP2C9 and VKORC1 variants and environmental factors, all modelled as main effects. These studies varied in terms of the ethnic population within which they were undertaken and also in terms of the environmental factors investigated and found to be statistically significant.

The purpose of this systematic review and meta-analysis is to evaluate the current evidence on the effect of genetic variants in CYP2C9 and VKORC1 on response to warfarin treatment. Studies will be evaluated in terms of their quality and strength of evidence and, if appropriate, their results synthesised to provide the best evidence and estimates of association available so far. A previous meta-analysis⁷ undertaken in 2003 reviewed the role of CYP2C9 variants on clinical outcomes in warfarin-treated patients. Our work will extend this meta-analysis to studies on CYP2C9 variants undertaken since 2003 as well as those investigating the role of VKORC1. Some further meta-analyses investigating the role of variants in both CYP2C9 and VKORC1 on response to warfarin were undertaken as part of an ACCE review of allele testing to inform warfarin dosing²². However, although the review is highly informative and addresses several key clinical questions, no information is provided regarding the search strategy employed in identifying studies to include in the meta-analyses. Further, heterogeneity in effect estimates between studies is not evaluated or investigated. We feel it appropriate to undertake a systematic review incorporating a structured search strategy, to ensure that all relevant studies so far are identified and that the meta-analyses conducted reflect information from all accumulated evidence to date. We will investigate the role the genes have to play both in isolation and in combination in how patients respond to warfarin treatment.

Methods

Selection Criteria

Types of studies

It is anticipated that the design of the majority of studies will be either retrospective or prospective cohort, however, we may also identify some case-control studies and randomised controlled trials and these types of studies will also be included in our analysis. For example, case-control studies may involve a comparison of warfarin patients who have experienced a bleeding event (cases) with those who have not (controls). If randomised controlled trials are identified which compare genotype-based prescribing of warfarin to standard prescribing without genotyping, data on patients from the genotyped arm will also be included in our review.

Types of participants

The review will include all studies that recruited patients either already established on or commencing warfarin treatment and in which patients were genotyped for CYP2C9 and/or VKORC1 variants. The studies should have investigated the effect of these variants on response to warfarin treatment.

Outcomes

In terms of defining response, the co- primary outcomes will be:

- stable maintenance dose (mgs/day);
- time to stable dose;
- bleeding events.

The secondary outcomes will be:

- INR>4 in week 1 of treatment;
- Time to achieving therapeutic INR range;
- Proportion of time spent within therapeutic INR range during the course of treatment;
- Warfarin sensitivity (defined as a dose of 1.5 mg or less on 3 successive clinic visits);
- Warfarin resistance (dose of more than 10mg/day on 3 successive clinic visits).

In terms of classifying bleeding events, the methods used will vary between studies. A decision will be made as to how bleeding events will be classified for the purpose of our meta-analysis after studies have been identified for inclusion but before any analyses are undertaken.

Identification of Studies

The search strategy will involve searching PUBMED, EMBASE, BIOSIS, The Web of Science and the HuGENet database of genetic association studies up to and including March 2007 and will be undertaken as set out in section 4.1.1 of the HuGENet HUGE Review Handbook¹⁸. Reference lists of all primary studies

identified will also be scrutinised for further papers of potential interest. Study authors and experts in the field will be contacted in an attempt to identify unpublished studies.

Once potentially relevant papers have been identified by way of the search strategy all lists of references will be merged and any duplicates removed. This combined list of titles and their corresponding abstracts will then be scanned and any obviously irrelevant reports removed. For the remaining reports, the full text will be retrieved and each assessed individually for compliance with the inclusion criteria. This process of elimination and selection will be undertaken by two people independently with any differences being resolved by way of discussion.

Reports will be carefully appraised in terms of geographic location, author names and period of study in order to ensure that two reports do not relate to the same or overlapping datasets. If indeed this is found to be the case, and both reports include relevant results, the larger will be used for analysis purposes.

Data Collection

Data will be extracted in accordance with the methods set out in the Cochrane Handbook for Systematic Reviews of Interventions¹⁷ as recommended in The HuGENet HuGE Review Handbook¹⁸. A data collection form will be prepared and piloted in a subset of randomly selected papers. This form will include information pertinent to assessing that inclusion criteria have been met, assessing study quality, study design, study methods, participant characteristics and also collection of outcome data. Outcome data will be collected in the format that it was reported with any necessary transformations made at a later stage. A section will also be provided at the start of the form for important notes of interest to be written relating to the study being abstracted. If it is not possible to extract key information from the published report the original investigators will be contacted to see if this information can be supplied.

Assessment of Study Quality

It is likely that our review will include studies of more than one type of design e.g. case control, cohort etc. and each of these designs will need to be quality-assessed according to specific criteria. Tools aimed at assessing quality for each of the different study designs will be developed prior to undertaking the review. For the purpose of developing these tools the HuGENet handbook¹⁸ and tools developed in a previous meta-analysis²¹ of observational studies will be referred to for guidance.

In addition to assessing the quality of design for each study, consideration will also be given to the following issues which are specific to the quality assessment of genetic association studies:

- a) Genotyping quality
 - was the primer sequence used for genotyping reported and indeed was there confirmation that the primer-annealing site was absent of any polymorphisms that could lead to biased genotyping?

- were any quality control procedures undertaken e.g. DNA re-sequencing or re-genotyping of a random subset of samples. If so, what was the degree of agreement quoted?
- was the genotyping failure rate quoted and if so what was the extent of the failure?
- if the study is case-control were the two groups genotyped together on the same assay or separately ?
- do authors comment on whether genotype frequencies are consistent with other reports from the same population and if so, are they?
- were genotyping personnel blinded to pertinent characteristics and to the hypothesis being tested?
- did the genotyping involve visual inspection and interpretation of electrophoresis gels? If so, was scoring and data entry done double blind followed by electronic comparison of the blind entries with discrepancies flagged and adjusted by a third person ?

b) Population Stratification

- does the study population consist of more than one ethnic origin? If so, how was this dealt with in the analysis?
- did the study undertake a test for population stratification or adjust for population stratification in any way? If so, what methods were used?

c) Hardy Weinberg Equilibrium (HWE)

- was deviation from HWE tested for ?
- if a SNP was found to deviate from HWE was it excluded from the analysis and were the reasons for the deviation explored further ?

d) Missing genotype data

- Is the extent of any missing genotype data reported/clear from the paper? If so, what is the extent of this missingness ?
- Have the reasons for missing data been explained/explored?
- Are the analyses adjusted in any way for missing data e.g. data imputation. If so, what methods are used?
- If missing data is imputed, have analyses been undertaken to check whether extent of missingness is independent of both phenotype and genotype?

e) Compliance

- Was compliance with treatment measured in the study? If so, how?
- If compliance was measured, to what extent were the patients non-compliant?
- Was compliance adjusted for in the analysis? If so, how?

A table will be designed with questions geared at addressing these issues and completed for each included study.

Statistical Analysis

- For each genetic variant a pooled analysis will be undertaken of the effect of genotype on each outcome.

Separate effect estimates will be calculated for both heterozygotes and homozygotes. Forest plots will be prepared and an assessment for heterogeneity will be made both by visually inspecting the plots and by calculating the I^2 statistic¹⁹ which measures the proportion of variation across studies that is due to genuine differences rather than due to random error.

Where studies differ in terms of the ethnicity of included patients, separate effect estimates will be calculated for each ethnic group. If the separate estimates appear similar, they will subsequently be pooled to provide a single effect estimate. This is in view of the controversy surrounding possible confounding from population stratification and is the approach suggested in The HuGENet HuGE Review Handbook.¹⁸ The impact of ethnicity on effect estimates will also be explored by way of meta-regression, with studies categorised in terms of the ethnicity of participants.

Since the underlying genetic model is not known for the variants being investigated, a 'genetic model-free'²⁰ approach to pooling the effect estimates will be taken. Using this method the underlying genetic model, which is assumed unknown yet constant across all studies, is estimated from the data. If it is evident on inspecting the data that the genetic model varies across the studies then joint pairwise comparisons will be undertaken instead using a general bivariate meta-analysis model. Whilst this model does not assume a common genetic model across studies it still takes into account the correlation which exists between the odds ratio for homozygous-mutant versus homozygous wildtype genotypes and the odds ratio for heterozygous versus homozygous wildtype genotypes.

If heterogeneity of effect is detected, we will examine whether the results of the studies change in a consistent direction over time, since it is common to see early studies providing exaggerated estimates of effect. We will also consider the use of meta-regression to investigate heterogeneity of effect across studies further.

Even though it is not necessarily the case that these factors will interact with genetic effects they are already known to influence response to warfarin in their own right and so are considered to be worthwhile exploring further in the presence of heterogeneity.

In an attempt to minimise the risk of publication bias, study authors and experts in the field will be contacted in an attempt to identify unpublished studies, as detailed in the search strategy. Given that some unpublished studies may not have been peer reviewed such unpublished studies will only be included in the review provided that it is possible to assess their quality adequately. Funnel plots will also be used to assess for the likelihood of publication bias being present.

Consideration will also be given to the issue of within-study selective reporting, in particular whether it is likely to be an issue in the included studies and, if so, the potential consequences.

Finally, as recommended in the HuGENet HuGE Review Handbook¹⁸, sensitivity analysis will be undertaken to assess the impact of any deviation from HWE.

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