

# Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications

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## Summary

**Background** The cytochrome P450 CYP2C9 is responsible for the metabolism of S-warfarin. Two known allelic variants *CYP2C9\*2* and *CYP2C9\*3* differ from the wild type *CYP2C9\*1* by a single aminoacid substitution in each case. The allelic variants are associated with impaired hydroxylation of S-warfarin in in-vitro expression systems. We have studied the effect of *CYP2C9* polymorphism on the in-vivo warfarin dose requirement.

**Methods** Patients with a daily warfarin dose requirement of 1.5 mg or less (low-dose group, n=36), randomly selected patients with a wide range of dose requirements from an anticoagulant clinic in north-east England (clinic control group, n=52), and 100 healthy controls from the community in the same region were studied. Genotyping for the *CYP2C9\*2* and *CYP2C9\*3* alleles was done by PCR analysis. Case notes were reviewed to assess the difficulties encountered during the induction of warfarin therapy and bleeding complications in the low-dose and clinic control groups.

**Findings** The odds ratio for individuals with a low warfarin dose requirement having one or more *CYP2C9* variant alleles compared with the normal population was 6.21 (95% CI 2.48–15.6). Patients in the low-dose group were more likely to have difficulties at the time of induction of warfarin therapy (5.97 [2.26–15.82]) and have increased risk of major bleeding complications (rate ratio 3.68 [1.43–9.50]) when compared with randomly selected clinic controls.

**Interpretation** We have shown that there is a strong association between *CYP2C9* variant alleles and low warfarin dose requirement. *CYP2C9* genotyping may identify a subgroup of patients who have difficulty at induction of warfarin therapy and are potentially at a higher risk of bleeding complications.

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## Introduction

Warfarin is the oral anticoagulant of choice in the UK and many other countries and is being prescribed to an increasing number of patients. The risk of serious haemorrhage during warfarin therapy ranges from 1.3 to 4.2 per 100 patient years of exposure.<sup>1,2</sup> Haemorrhage incidence is associated with the intensity of anticoagulation, with the deviation in prothrombin time

ratio shown to be the strongest risk factor for bleeding complications.<sup>3</sup>

Widespread interindividual variation in the response to a given dose of warfarin makes the prediction of an accurate maintenance dose difficult, with an effective daily dose ranging from 0.5 mg to 60 mg.<sup>4,5</sup> Standardised induction regimens, with monitoring of International Normalised Ratio (INR) over the first 4 days, have only a 69% success rate in predicting the correct maintenance dose,<sup>6</sup> and, although complex normograms and computer programs requiring protein C and S estimations have been shown to improve the prediction they are not suitable for routine use.<sup>7</sup> An understanding of the genetics of warfarin metabolism and the identification of genetic factors that contribute to the individuality of the warfarin dose response may help to ameliorate clinical difficulties associated with warfarin therapy.

The asymmetric carbon of warfarin (C9) gives rise to two enantiomeric forms, R-warfarin and S-warfarin, which are differentially metabolised. When administered as a racemate, S-warfarin is about three times as potent as R-warfarin.<sup>8</sup> The cytochrome P450 CYP2C9 is the principal enzyme that catalyses the conversion of S-warfarin to inactive 6-hydroxy and 7-hydroxy metabolites whereas the oxidative metabolism of R-warfarin is mainly catalysed by CYP1A2 and CYP3A4.<sup>9</sup> The existence of genetic polymorphisms in *CYP2C9* giving rise to functionally significant effects on enzyme activity is now well established. In addition to the wild-type (*CYP2C9\*1*) allele, point mutations in the *CYP2C9* gene result in two allelic variants—*CYP2C9\*2*, where cysteine substitutes for arginine at aminoacid 144, and *CYP2C9\*3*, where leucine substitutes for isoleucine at residue 359.<sup>10</sup> Both allelic variants have impaired hydroxylation of S-warfarin when expressed in vitro.<sup>11,12</sup> The *CYP2C9\*3* variant is less than 5% as efficient as the wild-type enzyme, while *CYP2C9\*2* shows about 12% of wild-type activity, apparently as a result of the aminoacid substitution altering the interaction of the enzyme with cytochrome P450 oxidoreductase.<sup>11–13</sup> Because these in-vitro data and some preliminary in-vivo data<sup>7,14</sup> have suggested that the known polymorphisms in *CYP2C9* significantly impair S-warfarin metabolism, we have now investigated whether either *CYP2C9* allelic variant was more common in patients with a lower than average warfarin dose requirement and have also examined whether the incidence of bleeding complications is associated with *CYP2C9* genotype.

## Methods

The investigation was approved by the Newcastle upon Tyne Joint Ethics Committee and all the participants gave informed consent.

### Participants

Patients whose warfarin dose requirement was 1.5 mg per day or less, had a stable warfarin dose requirement for at least three

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consecutive clinic visits with a target INR of 2.0 to 3.0, and no apparent cause for low-dose requirement (eg, drug interactions or liver disease) were recruited from an anticoagulation clinic in north-east England. Of a total of 828 patients registered with the clinic at the time of recruitment, 76 fulfilled the requirements for the study. Of these, 57 patients had prescheduled clinic visits during the study period (July 1, 1997–July 31, 1997) and were invited to take part in the study. The other 19 patients either had clinic visits prescheduled for a later date (n=14) or failed to attend the scheduled visit during the study period (n=5) and were not invited to take part in the study. Of 57 patients, 36 (17 male, 19 female) aged 55–88 (median 73) years, who agreed to take part in the study, formed the low-dose group. Reasons for refusing consent in the other 21 patients were fear of needles, lack of time, and lack of interest in research.

From a group of 124 consecutive attendees to the anticoagulant clinic invited to take part in the study, 52 individuals (26 men, 26 women) aged 33–94 (median 70.5) years, with a wide range of daily warfarin dose requirement, were recruited to the clinic control group during the month starting June 5, 1998. One patient previously included in the low-dose group was excluded. The community control group consisted of 100 individuals (58 men, 42 women) aged 38–91 (69) years recruited from the registers of general practitioner's practices in the same area of north-east England. The recruitment rate for the community controls was 50%.

### Genotyping

10 mL of blood was taken from each individual, DNA was extracted<sup>15</sup> and analysed by PCR. Genotyping for the *CYP2C9*\*2 and *CYP2C9*\*3 alleles was done by *Ava*II digestion to detect the *CYP2C9*\*2 allele and *Nsi*I digestion for *CYP2C9*\*3.<sup>16</sup>

### Assessment of complications of anticoagulation

All records from inpatient admissions and visits to the anticoagulation clinic for the patients in the low-dose group and the clinic control group were reviewed. To determine the presence of difficulties during the induction of anticoagulation, the details of dosage regimen used, peak INR during the first week, and the medical consequences (delayed discharges, outpatient visits, and referrals) of poor control of anticoagulation during the induction were noted.

Bleeding complications associated with raised INR above the therapeutic range of greater than 4.0, were classified as minor (requiring no additional testing, referral, or outpatient visits), serious (requiring medical evaluation, blood transfusion of two units or less), or life threatening (requiring surgical or angiographic intervention, transfusion of three or more units of blood, leading to irreversible sequelae).<sup>3</sup> Bleeding episodes that occurred within the first 4 weeks of anticoagulation were classified as early, the rest as late.

### Statistical analysis

Comparisons of genotype frequency and frequency of complications of anticoagulation between the groups were made using Fisher's exact test. Odds or rate ratios with 95% CIs were calculated where appropriate. Age and duration of follow-up in the low-dose group and random clinic group were compared with the Mann-Whitney U test.

## Results

*CYP2C9* genotype distributions in the low-dose warfarin group compared with the clinic controls and the community controls are summarised in table 1. In the low-dose warfarin group, 29 (81%) of the 36 patients had one or more of the variant alleles present compared with 40 (40%) of 100 in the control group. The odds ratio for individuals with a low warfarin dose requirement having one or more *CYP2C9* variant alleles compared with the normal population was 6.21 (95% CI 2.48–15.6). When possession of either one or two variant alleles was

Genotype	Low-dose group (n=36)	Random clinic control group (n=52)	Community control group (n=100)
<i>CYP2C9</i> *1/*1	7 (0.19)	32 (0.62)	60 (0.60)
<i>CYP2C9</i> *1/*2	12 (0.33)	9 (0.17)	20 (0.20)
<i>CYP2C9</i> *1/*3	10 (0.28)	10 (0.19)	17 (0.17)
<i>CYP2C9</i> *2/*3	5 (0.14)	0 (0.00)	2 (0.02)
<i>CYP2C9</i> *2/*2	2 (0.06)	1 (0.02)	0 (0.00)
<i>CYP2C9</i> *3/*3	0 (0.00)	0 (0.00)	1 (0.01)

Table 1: *CYP2C9* genotype distribution

considered separately, the odds ratio for a patient on low-dose warfarin with one variant allele only compared with the general population was 2.68 (95% CI 1.22–5.86) and with two variant alleles was 7.8 (95% CI 1.90–32.1).

Genotype and allele frequencies were measured in the clinic controls to test whether particular *CYP2C9* genotypes were associated with an increased risk of requiring anticoagulant treatment. There was no significant difference in *CYP2C9* genotype frequencies between the clinic controls and the community control group. Age and sex were not confounders between the low-dose group and clinic controls because the groups were not significantly different with respect to these factors. A further control group of 37 patients randomly selected from patients attending another anticoagulant clinic in the same region had the same *CYP2C9* genotype frequencies as the community control group. Allele frequencies of 0.785 for *CYP2C9*\*1, 0.110 for *CYP2C9*\*2, and 0.105 for *CYP2C9*\*3 in our control groups are comparable to those of 0.79, 0.125, and 0.085 for *CYP2C9*\*1, \*2 and \*3 alleles, respectively, reported in a study of a white British population.<sup>10</sup>

All the patients in the study group were started on warfarin as inpatients. Each patient received an initial dose of 10 mg warfarin, with further dose requirements determined by a modified Fennerty formula<sup>17</sup> with regular estimations of INR. Peak INR during the first week of warfarin treatment in the low-dose group ranged from 2.0 to 10.0 (median 4.4).

20 of 36 patients (two patients with \*1/\*1 genotype and 18 with one or more of the mutant alleles) had a peak INR above the therapeutic range of greater than 4.0 during induction of warfarin therapy when compared with nine of 52 patients in the clinic control group (odds ratio 5.97 [2.26–15.82]). Nine of 20 patients in the low-dose group with supra-therapeutic INR during induction had their inpatient stay prolonged for 1–9 days (median 2 days) while optimum anticoagulation was achieved. In seven additional patients, raised INR resulted in frequent visits to the anticoagulant clinic or additional investigations, or both. No patient in the clinic control group had their hospital stay prolonged because of poor control of anticoagulation.

Duration of warfarin treatment was 0.2–17 (median 2) years for patients in the low-dose group and 0.1–30 (3.1) years for patients in the clinic control group. In the low-dose group, during 132.8 patient years of warfarin treatment, seven minor, five serious, and six life-threatening bleeding episodes occurred in 11 patients, including one patient where bleeding contributed to death. This compares with six minor, five serious, and two life-threatening bleeding episodes in 11 patients during 311.1 patient years of warfarin therapy in the clinic control group. In the low-dose group, six minor and one major bleeding episode occurred in five patients within the first 4 weeks of induction of warfarin therapy. Each of these patients had one or more variant *CYP2C9* alleles.

	Low-dose group (n=36)	Random clinic control group (n=52)	p
Male: female	17:19	26:26	
Age (years)	55-88 (median 73)	39-95 (median 70.5)	0.11
Follow up (years)	0.2-17 (median 2)	0.1-30 (median 3.1)	0.35
Number with INR >4.0 at induction	20	9	0.0002
Minor bleeding episodes (% per person years)	5.27	1.93	0.07
Major bleeding episodes (% per person years)	8.28	2.25	0.007

Table 2: Comparison of low-dose group with random clinic controls

The incidence of minor bleeding episodes was higher in the low-dose group (seven in 132.8 patient years) when compared with that in random clinic controls (six in 311.1 patient years) though this did not reach significance (rate ratio 2.73 [95% CI 0.92-8.1],  $p=0.07$ ). Significantly higher numbers of major (serious and life-threatening) bleeding episodes occurred in the low-dose group (11 in 132.8 patient years) when compared with the random clinic controls (seven in 311.1 patient years; rate ratio 3.68 [1.43-9.50],  $p=0.007$ ). Comparison between the low-dose group and the random clinic controls is shown in table 2.

## Discussion

In this study we show that there is a strong association between *CYP2C9* genotype and warfarin sensitivity. An individual requiring a low warfarin dose is six times more likely to be positive for one or more of the variant alleles associated with impaired S-warfarin metabolism (*CYP2C9\*2* and *CYP2C9\*3*) compared with the general population. *CYP2C9* genotyping appears to have the potential to identify a subgroup of individuals who are poor metabolisers of warfarin, hence they require a very low dose of drug. Our findings also indicate that a significant proportion of these individuals have difficulties at the time of induction of warfarin therapy and have an increased risk of bleeding complications when compared with clinic controls.

With the exception of homozygosity for *CYP2C9\*3*, all the other combinations of variant alleles occurred more frequently in the low-dose group compared with the controls. One of 100 controls was homozygous for *CYP2C9\*3* while none in the low-dose group had this genotype. It is predicted from the control genotype frequencies that eight individuals attending the anticoagulation clinic would be expected to be *CYP2C9\*3* homozygotes, making the absence of *CYP2C9\*3* homozygotes in the low-warfarin-dose group unexpected. However, because in-vitro studies have shown extremely slow hydroxylation of S-warfarin when *CYP2C9\*3* is expressed, it is possible that individuals homozygous for this allele have such a low warfarin dose requirement that stabilisation is not successful and treatment with warfarin is abandoned. Interestingly, diminished clearance of S-warfarin with exacerbated response to normal doses of warfarin has been recently reported in a patient who was homozygous for *CYP2C9\*3*.<sup>14</sup>

Impaired metabolism of a low therapeutic index drug such as warfarin has important clinical consequences. Difficulty in establishing optimum anticoagulation was experienced in 20 (56%) of 36 patients in the low-dose group with their peak INR rising above the target range after a fixed-dose regimen. 18 of these patients carried one or more variant *CYP2C9* alleles. Difficult induction resulted in delayed discharges, multiple visits to the

clinics, and additional investigations in an attempt to seek an explanation for warfarin sensitivity. A smaller warfarin dose requirement may be associated with greater variability of INR<sup>3</sup> and this, coupled with warfarin's low therapeutic index, may lead to bleeding complications. These are likely to occur later during the course of warfarin therapy, presumably when monitoring is relaxed. Consistent with this, the incidence of major bleeding complications in the low-dose group was four times higher than that in the clinic controls.

We suggest that knowledge of *CYP2C9* genotype when deciding on treatment with warfarin may help to reduce problems during induction. In addition, the likely increased risk of bleeding in patients with mutations may influence the decision to start warfarin therapy, particularly in patients in whom the benefit is perceived to be small.

## Contributors

Guruprasad Aithal was involved in genotyping and wrote the paper. Christopher Day and Ann Daly jointly supervised the study and revised the paper. Patrick Kesteven contributed to the study design and data analysis.

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