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## **Long-term anticoagulant effects of *CYP2C9* and *VKORC1* genotypes in phenprocoumon users**

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**Running head:** Long-term pharmacogenetic effects in phenprocoumon

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Anticoagulant treatment with phenprocoumon is challenging because of the narrow therapeutic range and the wide inter- and intra-patient variability in dose response. Frequent monitoring of the international normalized ratio (INR) is therefore required. Polymorphisms in two genes, *CYP2C9* and *VKORC1* explain approximately one third of the variation in dose requirements [1-3]. *CYP2C9* encodes the main metabolizing enzyme of coumarins, the cytochrome P450 2C9 enzyme (*CYP2C9*), while *VKORC1* encodes the pharmacodynamic target enzyme for coumarins, vitamin K epoxide reductase multiprotein complex 1 (*VKORC1*).

Earlier this year, we found that in the first month of acenocoumarol therapy, the risk of underdosing is highest in patients with a *VKORC1* wild-type [4]. This increased risk of a subtherapeutic

INR was also seen in months 2 and 3, but not after the third month of coumarin treatment. In addition, the risk of overdosing was highest in patients with a *VKORC1* TT genotype in the first 6 months. The effect of *CYP2C9* genotype on under- or overdosing of acenocoumarol was smaller than the effect of *VKORC1* and this effect was only found in the first month of therapy and not after the initiation period [4]. This has not been investigated for phenprocoumon yet. The aim of this study was therefore to examine the association of *CYP2C9* and *VKORC1* polymorphisms with the risk of over- and underanticoagulation after the initiation period of phenprocoumon.

To investigate this, we looked at data from two different studies, the pre-EU-PACT study [5] and the study of Schalekamp *et al.* [6]. The study protocols of both studies were approved by a Medical Ethics Committee (Leiden University Medical Center, Leiden for pre-EU-PACT, Utrecht Medical Centre, Utrecht for the study of Schalekamp) and patients provided informed consent before study inclusion. All procedures were conducted in accordance with the Helsinki Declaration. More details about the design and data collection in both studies can be found elsewhere [4].

We examined the occurrence of at least one INR <2, >3.5 or >6.0 in several time periods up to 1.5 years after treatment initiation and tested for differences among the genotypes with chi-square analysis. The time periods we used were: 0-1 month (day 1-30), 1-3 months (day 31-90), 3-6 months (day 91-180), 6-9 months (day 181-270), 9-12 months (day 271-360), 12-15 months (day 361-450) and 15-18 months (day 451-540) after treatment initiation. We also looked at the time within, below and above the therapeutic range, since this method is more robust when the frequency of INR measurements differs between patients. All analyses were performed using SPSS 18.0.

In total, 794 phenprocoumon users from the two studies [5,6] were eligible for analyses in this study. Patient characteristics and genotypes of all 794 patients are shown in the Supplement Table 1. Data on height and weight were only available in the Pre-EU-PACT study (n=486). The most frequent indication for phenprocoumon treatment was atrial fibrillation. The average number of INR measurements per time period ranged from 3.4 to 5.5. Only data of patients using phenprocoumon during

the entire time period were included in the analysis of that period. The maximum follow-up in the Schalekamp study was six months (n=308).

Significant differences in out-of-range INR values between the genotypes were only found during the first month of phenprocoumon therapy. In the first month, 89% of the patients with a *VKORC1* wild-type had at least one subtherapeutic INR. This frequency was significantly lower among patients with CT (76%,  $p<0.001$ ) and TT (50%,  $p<0.001$ ). Supratherapeutic INR values occurred in 33% of the *VKORC1* wild-type patients, versus 48% ( $p<0.001$ ) and 66% ( $p<0.001$ ) in patients with a CT or TT genotype, respectively. Of the wild-type patients, 3% had at least one  $\text{INR}>6$ . This percentage was increased in patients with a TT genotype (17%,  $p<0.001$ ), but there was no statistically significant difference for patients with a CT genotype (6%,  $p=0.12$ ).

Occurrences of subtherapeutic INR values or INR values  $>6$  were not significantly different among the *CYP2C9* genotypes. However, INR values  $>3.5$  occurred more often in carriers of a *CYP2C9*\*3 allele (62%,  $p<0.001$ ) or a *CYP2C9*\*2 allele (52%,  $p=0.01$ ) than in wild-type patients (40%). For both *VKORC1* and *CYP2C9* genotypes, no significant differences in out-of-range INRs were found after the first month. The risk of out-of-range INRs for the different periods and genotypes are shown in the Supplement (Supplement Figures 1-4).

Similar results were obtained in the analyses of time within, below and above therapeutic INR range (see Figure 1). In the first month, time below therapeutic INR range was longest in *VKORC1* and *CYP2C9* wild-type patients (up to 33%) and time above therapeutic INR range was longest in *VKORC1*-TT and *CYP2C9*\*3 carriers (up to 37%). The risk of having at least one  $\text{INR}<2$  did not vary significantly among the *CYP2C9* genotypes, but the time spent below therapeutic INR range was significantly shorter in \*2 carriers (19%) and \*3 carriers (14%) than in wild-type patients (26%,  $p<0.001$ ). No significant differences were found after month 1 of the treatment.

Our study demonstrated that in the first month of phenprocoumon therapy, the risk of underdosing is highest in patients with *VKORC1* and *CYP2C9* wild-types. In addition, the risk of overdosing was highest in patients with a *VKORC1* TT genotype or carriers of a *CYP2C9* variant allele.

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These results correspond with the results we have seen for acenocoumarol users, as described in a previous article [4]. However, the results beyond the first month of treatment are not similar. Specifically, while there were no differences in the risk of out-of-range INRs between the different genotypes after the first month of phenprocoumon therapy, there were differences in risk between the *VKORC1* genotypes up to the sixth month of acenocoumarol treatment.

A limitation of this study is the fact that the Pre-EU-PACT study contained retrospective data [5]. The data of Schalekamp *et al.*, however, was collected prospectively [6]. Data for a specific time period was only used in the analysis if the patient used phenprocoumon for this entire period. Because very unstable patients are expected to stop the therapy early, this patient group might be underrepresented in our study.

Information about the patient's genotype can be used to predict the right dose of phenprocoumon [5]. Carriers of a *VKORC1* or *CYP2C9* variant allele require a lower dose and have an increased risk of supratherapeutic INR values. If these patients are genotyped before treatment initiation, they could be treated with a lower dose, thereby decreasing the risk of overanticoagulation. In both this study and our previous study on acenocoumarol, we also found an increased risk of a subtherapeutic INR in *VKORC1* and *CYP2C9* wild-type patients during the first month. Information about the patient's genotype could therefore also be used to identify patients need a higher dose to decrease the risk of complications from underdosing. In this way, genetic information could be used to improve the safety and efficacy of anticoagulation treatment in both wild-type patients and variant carriers. The relevance of pharmacogenetic information for phenprocoumon users, however, seems to be limited to the first month of treatment.

Phenprocoumon has a longer elimination half life than acenocoumarol (110-130 hours versus 6-8 hours) [3,7]. Treatment with phenprocoumon is therefore somewhat more stable and patients on phenprocoumon spend more time within the therapeutic INR range than patients on acenocoumarol [8]. This might be a reason why only acenocoumarol users –and not phenprocoumon users- show differences between the genotypes in the risk of out-of-range INRs after the first month of treatment.

The results of this study suggest that pharmacogenetic information might help to prevent subtherapeutic or suprathreshold INRs in the first month of phenprocoumon therapy and thereby reduce the risk of adverse events. The value of this information after the first month of phenprocoumon treatment appears to be limited. Currently, clinical trials are underway to investigate the effectiveness and cost-effectiveness of a genotype-guided dosing regimen versus a standard dosing regimen [9,10].

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### **Conflict of Interest/Disclosure**

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#### Figure Legends

**Figure 1.** Percentage time in different INR ranges during the first month of phenprocoumon use. A: *VKORC1* genotypes B: *CYP2C9* genotypes

