PPARA gene and phenprocoumon: a new predictor of response variability

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Phenprocoumon is an anticoagulant used for thromboembolic disorder prophylaxis metabolized mainly by CYP3A4. However, polymorphisms in this gene did not explain the observed variability. PPARA (peroxisome proliferator-activated receptor-α) is a nuclear receptor that, among others, influences *CYP3A4* gene expression. The aim of this study was to determine whether *PPARA* gene polymorphisms and the *CYP3A4*22* allele are associated with phenprocoumon dose variability. A total of 198 patients on a stable dose of phenprocoumon were included in the study. Genotyping was performed by allele discrimination using standardized TaqMan assays. Differences between the average phenprocoumon dose and genotypes/haplotypes were assessed by analysis of variance and multiple linear regression analyses. Patients with the *PPARA* rs4253728A allele needed higher phenprocoumon doses. However, the effect size (3%) of this association was small. The *CYP3A4*22* allele was not associated with the dose of phenprocoumon. As this is the first report of an association between *PPARA* gene polymorphisms and phenprocoumon dose, future studies are warranted to confirm these results. *Pharmacogenetics and Genomics* **25:93–95**

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Warfarin, phenprocoumon, and acenocoumarol are oral anticoagulants from the coumarin class. Oral anticoagulants are used for several thromboembolic disorder prophylaxis and treatment. Phenprocoumon is used less, but in some European countries and Brazilian centers, it is used widely. Similar to other coumarins, its mechanism of action is through inhibition of vitamin K reduction, which leads to a decrease in clotting factors II, VII, IX, and X.

The CYP2C9 enzyme plays an important role in the metabolism of coumarin. However, phenprocoumon biotransformation occurs mainly through the CYP3A4 enzyme. *CYP3A4* has a highly variable gene expression intraindividually and interindividually. This variability is because of several factors such as inflammatory disease, drug interactions, and genetic factors [1,2]. A recently reported *CYP3A4* allele, *CYP3A4*22*, has been described to be associated with simvastatin and tacrolimus response [3,4]. Nevertheless, absent or weak associations between *CYP3A4* gene polymorphisms and phenprocoumon doses were reported [1,5]. However, *CYP3A4*22* has not been investigated in phenprocoumon dose variability studies.

Recently, the possibility of transacting genes playing a role in *CYP3A4* regulation has been considered. The nuclear receptor *PPARA* (peroxisome proliferator-activated receptor-α) gene is located at the 22q13.31 chromosome and is composed by nine exons. Intron 4 rs4253728 and rs4823613 polymorphisms were associated with decreased activity and expression of *CYP3A4* [6]. However, *PPARA* variants have not been investigated in association with phenprocoumon dose so far. Therefore, the aim of this study was to investigate the possible association between *PPARA* gene rs4253728 and rs4823613 polymorphisms and the *CYP3A4*22* allele with phenprocoumon dose variation.

A total of 198 patients on phenprocoumon treatment were recruited at Hospital de Clínicas de Porto Alegre and at Instituto de Cardiologia – Fundação Universitária de Cardiologia. Most patients were of European ancestry (81%). Ethnicity was assessed by grandparents' ancestry and/or was self-reported. All patients included in the study were under a stable phenprocoumon dose. The weekly stable dose was defined as two consecutive international normalized ratio (INR) measurements in the therapeutic target (no variability was allowed) with the same phenprocoumon dose.

Clinical and demographic data were obtained by an interview with the patients, by reviewing their medical records and their phenprocoumon identification cards. The approval of the Ethics Committee of the Hospital de Clínicas de Porto Alegre and Instituto de Cardiologia – Fundação Universitária de Cardiologia was obtained for the study and all patients provided written informed consent to participate.

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Blood collection was performed in vacuum tubes containing citrate. The PureLink Genomic DNA Purification Kit (Invitrogen, Carlsbad, California, USA) was used to isolate genomic DNA from whole blood. *PPARA* gene rs4253728 and rs4823613 genotypes and *CYP3A4*22* were determined by allelic discrimination using TaqMan 5′-nuclease assays according to the manufacturer's recommended protocol. Prothrombin time for INR determination was assessed at the Clinical Laboratories of the hospitals.

Allele frequencies were estimated by counting the number of alleles and dividing them by the total number of chromosomes investigated. Agreement of genotype frequencies with Hardy–Weinberg expectations was tested using a goodness-of-fit χ^2-test. Linkage disequilibrium between polymorphisms was estimated with Mlocus 3.0 and haplotypes were derived with PHASE 2.1. Differences between the average phenprocoumon dose and haplotypes were assessed using analysis of variance or Student's *t*-test, as appropriate. Multiple linear regression analysis was used to assess the effect of polymorphisms on phenprocoumon dose controlling for nongenetic factors known to influence phenprocoumon dose variability. Assuming that the phenprocoumon dose does not have a normal distribution, $\log_{10}$ of the weekly dose was used to perform these tests. All analyses were carried out using SPSS software, version 18.0 (SPSS Inc., Chicago, Illinois, USA). Statistical significance was defined as a two-tailed *P*-value less than 0.05.

The mean age of the patients was 60.5 ± 12.4 and ranged between 28 and 97 years. This sample set has been fully characterized previously [5]. Briefly, 51.1% of the patients were women; the average dose of phenprocoumon was 15.27 ± 7.38 mg/week and ranged between 3.75 and 54 mg/week. The main indications for oral anticoagulation were atrial fibrillation and heart valve prosthesis. The INR target of most patients was between 2.0 and 3.0, the exception being patients with mechanical mitral valve prosthesis (the target was between 2.5 and 3.5).

All polymorphisms were in Hardy–Weinberg equilibrium ($P > 0.05$). A strong linkage disequilibrium was observed between *PPARA* rs4253728 and rs4823613 ($P < 0.001$). The D' and ρ^2 values were 0.967 and 0.685, respectively. Four haplotypes were derived and three of them (rs4253728G/rs4823613A – 72.2%, rs4253728A/rs4823613G – 21.7%, and rs4253728G/rs4823613G – 5.6%) accounted for 99.5% of the chromosomes investigated. Carriers of the rs4253728A/rs4823613G haplotype were on a higher phenprocoumon dose (17.05 mg) than rs4253728G/rs4823613G haplotype carriers (11.93 mg; $P = 0.001$; Table 1). Carriers of the rs4253728G/rs4823613A haplotype needed intermediate doses (14.83 mg), whereas the two individuals who were carriers of the rare rs4253728A/rs4823613A haplotype were on the highest dose; *CYP3A4*22* was not associated with the outcome (Table 1). As strong linkage disequilibrium was observed between *PPARA* gene polymorphisms, rs4253728 was used as a tag single nucleotide polymorphism in the multivariate analysis. Multiple linear regression analysis showed that *PPARA* was an independent predictor of phenprocoumon dose ($P = 0.02$). However, *PPARA* showed a small effect, explaining only 3% of phenprocoumon variability dose (Table 2). Even after controlling for confounders, *CYP3A4*22* single nucleotide polymorphism was not associated with the anticoagulant dose (Table 2). Because this is the first study to investigate *PPARA* gene association, and because of the small effect size of the *PPARA* polymorphisms on phenprocoumon dose, the clinical applicability of genetic testing of these variants needs to be further investigated.

Recently, nuclear receptors have been highlighted as important pharmacological players because of the control they exert on the expression of drug-metabolizing enzymes and membrane transporters. Peroxisome proliferator-activated receptors are known as master regulators of liver-specific gene expression [7]. Specific pharmacological effects of nuclear receptors deal with drug–drug interactions, in which one drug alters the systemic drug levels of a second coadministered medication by inducing activation of nuclear receptors [7–9]. The effect of several drugs on phenprocoumon dose has already been shown [5]; whether these interactions are modulated by *PPARA* variability is an open question for future studies.

De Mattia *et al.* [8] proposed that mutual influences between miRNAs and nuclear receptors would control downstream expression of proteins, such as CYP3A4 or CYP2D6. The interplay between these two classes of molecules could be key to the cellular integration of environmental stimuli in the cellular response phenotype with pharmacological treatment.

Two recent studies reported the combined effect of *CYP3A* and *PPARA* gene polymorphisms on tacrolimus and simvastatin pharmacokinetics [3,4]. However, in relation to phenprocoumon, no association was observed between dose and *CYP3A4*1B* [5] and/or *CYP3A4*22* investigated here. The *PPARA* genetic polymorphisms investigated are promising pharmacogenetic predictors of CYP3A4-dependent pharmacokinetics and drug-response phenotypes with respect to many clinically used drug substrates of this enzyme [3,4,6]. Thomas *et al.* [10] elucidated the mechanistic basis for constitutive and inducible transcriptional regulation of *CYP3A4* by *PPARA*, and provided evidence for a broader range of similarly regulated drug-metabolizing P450s. However, as *PPARA* is important in lipid pathophysiology and in inflammatory diseases, the target of the association observed between *PPARA* and phenprocoumon is also an open question.

Table 1 Frequencies of *PPARA* haplotypes and *CYP3A4*22* genotypes with their respective average phenprocoumon dose

PPARA haplotypes	rs4253728G/rs4823613G	rs4253728G/rs4823613A	rs4253728A/rs4823613G	rs4253728A/rs4823613A
Frequencies [*n* (%)]	22 (5.6)	286 (72.2)	86 (21.7)	2 (0.5)
Weekly dose (mg)	11.93	14.83	17.05	39.00
P (ANOVA)		0.001*		

CYP3A4 genotypes	CYP3A4*1/*1	CYP3A4*1/*22	CYP3A4*22/*22
Frequencies [*n* (%)]	182 (91.9)	15 (7.6)	1 (0.5)
Weekly dose (mg)	14.96	19.40	10.50
P (ANOVA)		0.222**	

ANOVA, analysis of variance.
*The analysis was carried out without the rs4253728A/rs4823613A haplotype because of the low number of chromosomes in this group.
**The analysis was carried out considering CYP3A4*1/*1 versus CYP3A4*1/*22 + *22/*22.

Table 2 Linear regression analysis for dose prediction controlling for nongenetic factors

	β	*P*	Partial coefficient
Age	−0.381	5.9×10^{-8}	0.1521
Sex	−0.173	0.007	0.0396
Ethnicity	0.050	0.460	0.0030
β-Blockers	−0.148	0.024	0.0279
PPARA rs4253728 GA + AA	0.155	0.020	0.0299
CYP3A4*1/*22 + CYP3A4*22/*22	0.010	0.876	0.0001

R^2 =0.255.

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Conflicts of interest

There are no conflicts of interest.

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