**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-\(\alpha\)) 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-\(\alpha\)) 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.
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 number of alleles and dividing them by the total number of chromosomes investigated. Carriers of the rare rs4253728A/rs4823613A haplotype were on a higher phenprocoumon dose (17.05 mg) than rs4253728G/rs4823613G carriers of the rare rs4253728A/rs4823613A haplotype were on a higher phenprocoumon dose (17.05 mg) than rs4253728G/rs4823613G haplotype carriers (11.93 mg; 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.
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Conflicts of interest
There are no conflicts of interest.

References
8 De Mattia E, Dreussi E, Cecchin E, Toffoli G. Pharmacogenetics of the nuclear hormone receptors: the missing link between environment and drug effects? Pharmacogenomics 2013; 14:2035–2054.

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

<table>
<thead>
<tr>
<th>PPARA haplotypes</th>
<th>rs4253728G/rs4823613G</th>
<th>rs4253728G/rs4823613A</th>
<th>rs4253728A/rs4823613G</th>
<th>rs4253728A/rs4823613A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequencies [n (%)]</td>
<td>22 (5.6)</td>
<td>286 (72.2)</td>
<td>86 (21.7)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>Weekly dose (mg)</td>
<td>11.93</td>
<td>14.83</td>
<td>17.05</td>
<td>39.00</td>
</tr>
</tbody>
</table>

P (ANOVA) 0.001*

CYP3A4* genotypes

<table>
<thead>
<tr>
<th>CYP3A4* genotypes</th>
<th>CYP3A4*1/*1</th>
<th>CYP3A4*1/*22</th>
<th>CYP3A4*22/*22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequencies [n (%)]</td>
<td>182 (91.9)</td>
<td>15 (7.6)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Weekly dose (mg)</td>
<td>14.98</td>
<td>19.40</td>
<td>10.50</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Partial coefficient</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.381</td>
<td>5.9 × 10^-8</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.173</td>
<td>0.007</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.050</td>
<td>0.460</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>-0.148</td>
<td>0.024</td>
</tr>
<tr>
<td>PPARA rs4253728 GA + AA</td>
<td>0.155</td>
<td>0.020</td>
</tr>
<tr>
<td>CYP3A4*1/<em>22 + CYP3A4</em>22/*22</td>
<td>0.010</td>
<td>0.876</td>
</tr>
</tbody>
</table>

R² = 0.255.