Influence of 516G>T Polymorphisms at the Gene Encoding the CYP450-2B6 Isoenzyme on Efavirenz Plasma Concentrations in HIV-Infected Subjects

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We examined 516G>T polymorphisms at the gene encoding the cytochrome P450 in 100 human immunodeficiency virus–positive subjects who were receiving efavirenz (EFV). Elevated plasma EFV concentrations were found in 40% of subjects with the polymorphic homozygous genotype and 19% of subjects with the heterozygous genotype. Conversely, 20% of subjects with the wild-type genotype had subtherapeutic concentrations of EFV. CYP2B6-516 genotyping may help to identify subjects who have plasma EFV concentrations that are outside of the therapeutic range.

Plasma concentrations of efavirenz (EFV) have been correlated with the efficacy and safety of the drug [1]. HIV-infected subjects with plasma EFV concentrations of <1 μg/mL appear to have a greater risk for emergence of selective drug resistance and for treatment failure [2], and subjects with EFV concentrations of >4 μg/mL may experience neurological adverse effects more frequently [3]. The high interindividual variability in EFV pharmacokinetics complicates the achievement of a long-term benefit with EFV therapy. Differences in the hepatic metabolism of EFV seem to explain much of this discrepancy. Although several cytochrome P450 (CYP450) isoenzymes have been shown to be involved in the metabolism of EFV in the liver [4–7], CYP450-2B6 is the cytochrome responsible for the 8-hydroxylation of the drug and for clearance of ~90% of the circulating EFV [8]. Interestingly, several polymorphisms at the gene encoding CYP2B6 may influence the isoenzyme activity [9] and, therefore, the hepatic clearance of EFV. The most significant allelic variant is a G to T change at codon 516 (i.e., 516G>T polymorphism) [10]. In homozygosis, a 516G>T polymorphism may reduce CYP2B6 activity and, as a consequence, increase EFV plasma concentrations, leading to more frequent drug-related adverse effects [11]. Herein, we describe the prevalence of 516G>T polymorphism at the gene encoding CYP2B6 and examine its influence on EFV plasma concentrations and subsequent clinical outcome in a large group of HIV-infected subjects.

Patients and methods. All consecutive HIV–infected subjects who initiated a regimen that contained EFV during the first 6 months of 2003 at our institution (Hospital Carlos III, Madrid, Spain) were identified. Only subjects who were receiving EFV, 600 mg once daily at bedtime, along with 2 nucleoside analogues were analyzed. Subjects who were taking drugs that potentially might interact with EFV metabolism were excluded. Treatment adherence was assessed by a specific questionnaire and pharmacy records, and subjects with poor compliance were excluded from the study.

Plasma samples collected 3 months after the initiation of EFV therapy were obtained ~12 h after the last capsule of EFV had been taken. Plasma EFV concentrations were measured by a validated high-performance liquid chromatography method [12].

The genetic characterization of the CYP2B6 gene was performed using genomic DNA extracted from PBMCs. The complete amplification of exon 4 at the isoenzyme 2B6 gene was performed using 2 different primers: forward primer CYP2B6-4F (5’-GGTCTCCTCCCATCTATAAAC-3’) and reverse primer CYP2B6-4R (5’-CTGATTCTCTACATGTCTTGCG-3’) [13]. The amplicon was directly sequenced using the dRhodamine Terminator Cycle Sequencing kit (Applied Biosystems).

Statistical analyses were conducted with the SPSS package, version 11.0 (SPSS). Descriptive results of continuous variables are expressed as median and interquartile ranges (IQR). Mean values were compared by a parametric test (Student’s t test) or a nonparametric test (Wilcoxon test), as required. For the comparison of proportions, the χ² test was used, with Yates or Fisher corrections applied when needed.

Results. A total of 104 patients initiated therapy with EFV along with 2 nucleoside analogues and were compliant with their medication. Four patients discontinued treatment with EFV before completing 12 weeks of therapy because of adverse effects (neurological symptoms in 2 patients, liver toxicity in
Plasma concentrations of EFV that were considered to be subtherapeutic (i.e., <1 μg/mL) [2] were found in only 1 (2%) of 48 subjects with at least 1 allele with the 516G>T polymorphism but were found in 10 (19%) of 52 subjects with the wild-type genotype (P = .01). Conversely, 2 (40%) of 5 subjects with the TT variant in homozygosis, 8 (19%) of 43 subjects with the G516T polymorphism in heterozygosis, but only 3 (5%) of 52 subjects with wild-type homozygosis had EFV concentrations of >4 μg/mL (P < .01). The proportion of subjects with EFV concentrations below, within, and above the therapeutic range, according to their CYP450-2B6 genotype, is depicted in figure 2.

**Discussion.** Exposure to subtherapeutic amounts of antiretroviral drugs is one of the major causes of treatment failure in HIV-infected subjects [1]. When drugs do not attain optimal plasma concentrations, the efficacy of a given combination is compromised, and drug-resistant viruses are easily selected, limiting future therapeutic options [14]. Three main factors influence plasma concentrations of antiretroviral drugs: compliance, interactions, and differences in absorption and metabolism. Among subjects who had almost complete adherence to their medications and who were not taking other medications, most variability in plasma concentrations of a given antiretroviral agent are the result of differences in host factors, genetics being one of the main determinants [15]. In the present study, we confirmed that there was a high interindividual variability in EFV plasma concentrations and that these differences were

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**Table 1. Main characteristics of HIV-1–infected subjects with different CYP2B6-516 genotypes who initiated a regimen that contained efavirenz.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GG</th>
<th>GT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>52</td>
<td>43</td>
<td>5</td>
</tr>
<tr>
<td>Male sex</td>
<td>39</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td>Age, median years (IQR)</td>
<td>40 (37–48)</td>
<td>42 (37–45)</td>
<td>43 (30–44)</td>
</tr>
<tr>
<td>HCV coinfection</td>
<td>16</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>ALT level, median IU/L (IQR)</td>
<td>34 (23–76)</td>
<td>39 (24–67)</td>
<td>40 (28–81)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of subjects, unless otherwise indicated. ALT, alanine aminotransferase; GG, homozygous wild-type genotype; GT, heterozygous genotype; HCV, hepatitis C virus; IQR, interquartile range; TT, polymorphic homozygous genotype.

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**Figure 1.** Median efavirenz (EFV) plasma concentrations, by CYP2B6-516 genotype. Horizontal lines, median values; bars, interquartile ranges; whiskers, full range. GG, homozygous wild-type genotype; GT, heterozygous genotype; TT, polymorphic homozygous genotype.
largely dependent on genetic variation at the gene encoding the CYP450-2B6 isoenzyme, which is involved in the main catabolic pathway for EFV in the liver.

There is no doubt that, because of the intricacy of the CYP450 complex, it is more accurate to correlate EFV plasma concentrations with certain haplotypes—which may represent several groups of mutations—rather than with point mutations. A recent report [9] has highlighted that CYP2B6 haplotypes *6 and *7 are strongly associated with elevated EFV plasma concentrations and, accordingly, with a higher rate of side effects. We have confirmed those findings, but we have found, in addition, that the identification of a single mutation, the 516G>T variant, is the main determinant of CYP2B6 haplotypes *6 and *7. On the basis of our results, baseline CYP2B6-516 genotyping might give a good indication of how EFV would be metabolized in a given person. Because the full characterization of CYP450 haplotypes requires sophisticated and time-consuming laboratory work, our findings offer an easier approach to identify subjects who are likely to present with EFV concentrations outside of the therapeutic range.

Previous studies have demonstrated that the 516G>T mutation is highly prevalent among African Americans [11] and is less prevalent among Asians and whites [9]. The frequency of the 516G>T mutation in the white subjects in the present study was as high as 48%, which increases the clinical utility of this marker at least for testing populations of Mediterranean ancestry. Additional CYP450-2B6 genotypic studies are warranted to assess the rate of 516G>T polymorphisms in white subjects of Northern European origin, as well as of other ethnic origins.

As expected, we found that subjects with the 516G>T variant more frequently have EFV plasma concentrations within the toxic range. In our cohort, 40% of TT homozygotes and 19% of heterozygotes had EFV concentrations of >4 μg/mL. Conversely, only 5% of subjects with a wild-type genotype showed elevated EFV concentrations and were therefore at risk for developing EFV-related neurological toxicities [2, 3]. Furthermore, we found that the analysis of the 516G>T polymorphism led to the identification of subjects who were at risk for presenting with subtherapeutic EFV concentrations. Nearly one-fifth of subjects with a wild-type CYP2B6-516 genotype presented with EFV concentrations of <1 μg/mL. Conversely, only 2% of subjects with 516G>T polymorphisms had EFV concentrations below therapeutic levels.

Close follow-up of the population in the present study has been undertaken to assess the long-term clinical consequences of different EFV plasma concentrations. In the meantime, several clinical implications may be derived from our results. For example, the absence of 516G>T allelic variants in subjects initiating treatment with EFV may warrant close therapeutic drug monitoring to avoid insufficient antiviral exposure, especially in treatment-naive individuals who show high levels of virus replication. Conversely, in subjects carrying 516G>T polymorphisms, especially when present in homozygosis, complaints of neurological symptoms after starting treatment with EFV should prompt the measurement of plasma drug concentrations, and subjects with concentrations of >4 μg/mL may benefit from an adjustment of the EFV dose.

In conclusion, the results of the present study suggest that CYP2B6-516 genotyping at baseline may allow clinicians to optimize antiretroviral therapy in patients who initiate an EFV-based regimen. The information given by this single-codon genotypic analysis may help to easily identify patients who have a risk for treatment failure or drug toxicity.

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