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Slow Efavirenz Metabolism Genotype Is Common in Botswana

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To the Editors

The nonnucleoside reverse transcriptase inhibitor efavirenz is metabolized by the hepatic cytochrome P450 isoenzyme 2B6.¹ Homozygotes for a single polymorphism at position 516 in the CYP2B6 enzyme (G > T) have an approximately 3-fold higher area under the curve of efavirenz plasma concentrations.² Central nervous system toxicity is associated with elevated efavirenz plasma concentrations, and lack of response is associated with low efavirenz plasma concentrations.³

The 516T polymorphism represents alleles *6, *7, and *9.⁴ The prevalence seems to differ by race with the highest rates found to date in populations of African origin.⁵ Efavirenz is commonly used in southern Africa. Although slow metabolizing genotypes might have an important impact on human immunodeficiency virus (HIV) treatment, the prevalence in this setting is currently unknown.

We conducted a cross-sectional study of a convenience sample of HIV-infected Botswana citizens (Batswana) under care at the Infectious Diseases Care Clinic of the Princess Marina Hospital in Gaborone, Botswana. All individuals of African origin attending clinical visits over 1 week in July 2007 were invited to participate. Buccal mucosa was obtained using sterile brushes and stored at –80°C. DNA was extracted using the Qiagen protocol (Qiagen, Valencia, CA). CYP2B6 genotypes were determined using ABI Taqman assays (Applied Biosystems, Foster City, CA).

Tests for violations of Hardy–Weinberg equilibrium were performed. Haplotypes were inferred using the Bayesian PHASE approach⁶ and implemented using Phase 2.1 software.

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The frequency distribution of the alleles and observed frequency of the genotypes were tabulated. Haplotype frequency estimates were confirmed using the expectation–maximization approach,⁷ implemented with the `haplo.em()` function⁸ of the `haplo.stats` package in R version 2.6.0. Variance estimates were derived based on Louis' formula for the inverse information.⁹ The population frequency estimate of *6 homozygotes in the population was calculated as (frequency of the *6 allele)², and the corresponding 95% confidence interval (CI) was calculated using the delta method.

The study was approved by research ethics committees at the Princess Marina Hospital, the Botswana Ministry of Health, and the University of Pennsylvania. Informed consent was obtained from all participants.

We enrolled 101 Batswana of African origin over 5 days in July 2007, during which approximately 800 patients attended clinic. The population included 52 (52%) females, a median age of 39 years (interquartile range 34–47 years), a median current CD4 count of 352 cells/mm³ (interquartile range 266–541 cells/mm³), a median nadir CD4 count of 122 cells/mm³ (52–180 cells/mm³), and baseline HIV RNA of 5.2 log₁₀ copies/mL (interquartile range 4.6–5.8 log₁₀ copies/mL).

A median of 29 ng/mL of DNA was extracted from the buccal cells. CYP2B6 genotype results were obtained successfully in all samples. Observed allele frequencies were in Hardy–Weinberg equilibrium for CYP2B6*4 ($P = 0.09$), CYP2B6*9 ($P = 0.14$), and CYP2B6*18 ($P = 0.14$). Because all CYP2B6*5 genotypes were homozygous CC, testing for Hardy–Weinberg equilibrium was not performed. After assigning haplotypes based on the highest estimated posterior probability, the CYP2B6*6 allele was present in 37 (36.6%) individuals and the homozygous haplotype was present in 18 (17.8%) of the cohort. The population estimate of the frequency of CYP2B6*6 homozygosity was 13.4% (95% CI 8.5% to 18.3%). No other slow efavirenz metabolism genotypes were identified.

The CYP2B6*6 allele associated with slow efavirenz metabolizer phenotype is common in Batswana with a prevalence over 30%. The highest prevalence of this allele has been observed in another African population, in Ghana, with a frequency of 49%⁵ and the lowest in a Japanese population with a frequency of 18%.¹⁰ African Americans have been found to have higher frequencies than whites,^{2,11} with both higher than Asians.⁵

This finding has potentially important implications for the treatment of HIV within this setting. The homozygous genotype has been associated with higher efavirenz concentrations irrespective of racial group.^{2,10–14} These higher concentrations have been associated with increased incidence of central nervous system toxicity. A recent study demonstrated proof of concept by adjusting the dose of efavirenz down for slow metabolizers.¹⁵ They found that central nervous system side effects were decreased, yet viral loads remained suppressed at the lower doses. This tailored approach to dosing would need to be tested in larger studies before being adopted more broadly.

Studies to date in clinical trials populations have not demonstrated an improved or worsened clinical response in individuals with higher concentrations.^{11,16} It should be noted that these trials were relatively small and therefore may have been underpowered to find an effect.

Further, in clinical populations, where adherence may be lower than in clinical trials, the effects of slow efavirenz metabolism may be different than reported to date. A potential beneficial effect might be that the higher efavirenz concentrations offset the adverse impact of the relatively lower efavirenz concentrations in suboptimal adherers. Alternatively, suboptimal adherence to all the drugs in the regimen might result in functional efavirenz monotherapy in slow metabolizers due to the prolonged half-life.¹⁴ In this scenario, the slow metabolizers might be at increased risk of developing efavirenz resistance.

This study has several potential limitations including the relatively small sample size of HIV patients from a single clinic with a participation rate on the order of 10%–15%. However, the Infectious Diseases Care Clinic (IDCC) population is thought to be representative of the population of Botswana in general. If CYP2B6*6 homozygosity confers either an advantage or disadvantage for survival in HIV patients on efavirenz, then it is possible that our estimate of CYP2B6*6 was biased either toward or away from the null. That is, if CYP2B6*6 homozygosity confers either a survival advantage (or disadvantage) compared with other genotypes, the prevalence of the slow metabolism genotype would be higher (or lower) than the general population of Botswana. However, if this is the case, then the clinical importance of the polymorphism in this population would be even greater than anticipated.

In conclusion, slow efavirenz metabolism genotype is common in Botswana. Studies are needed in such a population to determine if the CYP2B6*6 homozygous genotype affects treatment outcome. As suggested by recent work adjusting doses,¹⁵ useful HIV management changes may be implemented in response to genetic testing for CYP2B6 genotype.

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