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CYP2B6 516G>T Minor Allele Protective of Late Virologic Failure in Efavirenz-treated HIV-Infected Patients in Botswana

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Abstract

Background—*CYP2B6* polymorphisms that affect efavirenz (EFV) concentrations are common, but the effect of this polymorphism on HIV virologic failure in clinical practice settings has not fully been elucidated. Our objective was to investigate the relationship between the *CYP2B6* 516G>T genotype and late virologic failure in patients treated with EFV in Gaborone, Botswana.

Setting—We performed a case-control study that included 1,338 HIV-infected black Botswana on EFV-based antiretroviral therapy (ART). Patients were approached for enrollment during regular visits at one of the outpatient HIV clinics between 7/2013–4/2014.

Methods—Cases experienced late HIV failure, defined as plasma HIV RNA >1000 copies/mL after maintaining viral suppression (<400 copies/mL) for at least 6 months. For each case, a total of 4 control patients were randomly sampled from the same population. Controls had plasma HIV

RNA <400 copies/mL on ART for at least 6 months. Logistic regression was used to determine the adjusted odds of late HIV failure by 516G>T genotype.

Results—After adjustment for the confounding variables age and CD4 count, the *CYPB2B6* 516 T-allele was protective against late HIV virologic breakthrough, adjusted OR 0.70; 95%CI 0.50-0.97.

Conclusion—The *CYPB2B6* 516 T-allele was protective against late virologic breakthrough in patients with initial (6 month) HIV RNA suppression on EFV-based ART. Future studies are needed to assess long-term viral benefits of identifying and offering EFV containing ART to black African HIV-infected patients with *CYPB2B6* T-alleles, especially given the wider availability of a single pill EFV in this setting.

Keywords

Efavirenz; HIV; AIDS; Virologic Failure; Pharmacogenetics

Introduction

The rapid global expansion of antiretroviral therapy (ART) substantially impacted the HIV epidemic, resulting in an estimated 25% drop in HIV-related mortality between 2009 and 2013.¹ Botswana was the first sub-Saharan African country to provide universal free ART and currently covers 78% of HIV-infected adults.² Because of these successes, focus is shifting to long-term maintenance of viral suppression. Personalized medicine strategies on drug allocation based on biological determinants of adverse effects have already entered HIV care, as has occurred with abacavir and the genotyping of viruses to choose specific regimens.

The non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV) is frequently incorporated in mass roll-out programs, as was the case in Botswana. Pharmacokinetics and genetics studies have demonstrated a major metabolism role for cytochrome P450 (CYP) 2B6, the primary enzyme responsible for 8-hydroxylation of EFV.³ Specifically, a single nucleotide polymorphism (SNP) in *CYPB2B6* 516G>T (rs3745274) impairs EFV drug clearance and leads to increased plasma EFV concentrations.⁴⁻⁸ It remains unclear whether increased EFV exposure leads to improvements in virologic response, such as by maintaining adequate drug concentrations in the face of missed doses, or poses a risk for toxicity, such as CNS-related symptoms compromising virologic response. Understanding the clinical relevance of slow EFV metabolism on virologic response is of particular importance for populations in which the minor allele of *CYPB2B6* 516G>T is common, including patients of African descent.⁹⁻¹¹ Our goal was to determine whether the *CYPB2B6* 516G>T genotype is associated with differential rates of undetectable viral load after having achieved initial HIV suppression. We hypothesized that the 516G>T genotype would be associated with differences in late virologic failure, e.g., either increased rates due to greater CNS toxicity or lower rates due to higher drug concentrations being more permissive of missed doses.

Methods

Study Design

We performed a case-control study and included HIV-infected subjects who had virologic suppression with plasma HIV RNA <400 copies/mL with an EFV-based regimen at clinics in the larger Gaborone area, Botswana from July of 2013 to April of 2014. Participants were approached for enrollment during regular visits at one of the study sites. Criteria for inclusion were (i) confirmed HIV infection, (ii) black African origin, (iii) age ≥ 21 years, (iv) initial HIV treatment with EFV at 600 mg/day plus two NRTIs for at least 6 months, and (v) undetectable levels of plasma HIV RNA (<400 copies/mL) for at least 6 months upon ART initiation. Using medical health records, cases were patients who had any plasma HIV RNA >1000 copies/mL after 6 months on ART. For each case, a total of 4 control patients were randomly sampled from the same population with all plasma HIV RNA <400 copies/mL after the initial 6 months. To test the genotype-phenotype hypothesis we obtained whole blood DNA for genotyping of *CYPB2B6* 516G>T and genotyping was performed using the TaqMan® SNP Genotyping Assay from Applied Biosystems. To address the potential confounding of alcohol intake and CNS symptoms a modified Alcohol Use Disorders Identification Test (AUDIT) and the Mood Module of the Patient Health Questionnaire (PHQ-9) were administered.¹² The study was approved by the Ethics Board of the Health Research and Development Committee of the Botswana Ministry of Health and by the Committee on Human Subjects Research of the University of Pennsylvania. Each study participant provided written informed consent in English or Setswana.

Statistical Analyses

To assess baseline differences between cases and controls, the Student's T-test was used for normally distributed or transformable variables, the Wilcoxon rank sum test for skewed variables, and Chi-Square test for categorical variables. To test for potential confounders, the baseline characteristics were compared between patients with the *CYPB2B6* 516 GG, GT, or TT genotype. One-Way ANOVA was used to test for differences in normally distributed continuous variables between the genotypes, Kruskal-Wallis for skewed continuous variables, and Chi-Square for categorical variables. In a logistic regression model case-control status was the dependent variable. Genetic associations are shown for models assuming additive (e.g., allele dosage) and dominant effects (e.g., allele presence). Covariates that were differentially distributed between cases and control and the 516G>T genotypes at a p-value threshold of <0.1 were included as covariates in the model together with the 516G>T genotype and 95% confidence intervals were calculated. We targeted a sample size of 1,660 individuals to detect an OR of 2.0.

Results

We enrolled 1,338 patients, of whom 276 were cases and 1,062 controls (Table 1). A total of 1,167 patients provided a blood sample, of which 67 (5.7%) samples failed genotyping. Compared to controls, cases were more likely to be male, more likely to engage in hazardous drinking, have a lower BMI, were on ART for a shorter period of time, and more frequently reported depressive symptoms (Table 1). Of these patient characteristics, only age

and baseline CD4 count were differentially distributed for different 516G>T genotypes (Table S1, $P=0.049$ and $P=0.074$ respectively). There were no differences in baseline characteristics between patients that had genotyping data available versus those who didn't (Table S2). After controlling for age and baseline CD4 count, the *CYPB2B6* 516 T-allele showed a significant protective association with late virologic failure (Table 2).

Discussion

It is well known that *CYPB2B6* 516G>T genotype is a major source of variability in EFV pharmacokinetics and our study suggests that the *CYPB2B6* 516 T-allele is protective against late virologic failure in patients treated with regimens including EFV. Heterozygotes appear equally protected as homozygous variants despite of the additive effect the 516G>T variant has on EFV metabolism.⁸ This effect may be due to sufficiently higher concentrations in intermediate metabolizers to protect against decreases in exposure due to partial non-adherence. While one might expect the slowest metabolizers to be protected against the highest levels of non-adherence, the high correlation between non-adherence and retention in care suggests that these individuals may have dropped out of care and not been eligible for this study.¹³ Previously Ribaudo *et al.* showed that slow-metabolizer genotypes were associated decreased virologic failure among African American patients in a study with over 3.5 years of follow-up data.¹⁴ This protective effect was not observed amongst whites and white Hispanics. Haas *et al.* did not find an association between *CYPB2B6* 516T-allele in 504 participants in the Adult AIDS Clinical Trials Group Study.¹⁵ Similarly, Lehmann *et al.* did not identify a genome-wide association between over 8 million genotyped and imputed SNPs and virologic response in over 500 African American patients treated on similar EFV-based protocols.¹⁰ Dickinson *et al.* recently showed a slight difference in rates of virologic failure after 96 weeks of ART between *CYPB2B6* 516G>T genotypes, however the failure rates were only between 2.1% and 3.7% and were not statistically significant.⁹ Our current case-control study allowed us to effectively enroll a sufficient number of patients with late virologic failure and demonstrate a long-suspected statistically significant association with *CYPB2B6* 516G>T genotype.

Limitations of our study included the absence of pharmacy records on drug refills to potentially address a mediating effect of differential adherence by genotype. Therefore, we are unable to definitively determine if the reason for the protective effect of the slow metabolism was due to making EFV more forgiving of missed doses in that subset.¹⁶⁻¹⁸ We did not assess CNS-related symptoms although we did assess for depression.^{12,19} We emphasize that the cases in the current study are representative of late failure, and so the findings cannot be generalized to early events. In this regard, we previously observed a risk-increasing effect of the *CYPB2B6* 516 T-allele on composite treatment endpoint after 6 months, indicating that late and early virologic failure might have substantially different underlying mechanisms.¹¹ Furthermore, plasma HIV RNA measurements not captured in medical health records may have led to study participants being misclassified as controls. This phenomenon is likely to bias our effect estimate toward the null. Finally, we observed an unexpected genotypic difference in age and baseline CD4 count. We included these confounders as covariates in our multivariate analyses.

A major strength of the current study is the large sample size in an African country experiencing a high HIV burden and in which slow EFV metabolism alleles are common. To limit confounding of non-virologic factors study eligibility was restricted to individuals exposed to EFV-based ART for at least 6 months while maintaining HIV RNA below 400 copies/mL. We doubt that selection bias explains these results. The variant T allele conferring slow EFV clearance has in some studies been associated with CNS adverse effects and discontinuation in other settings. Therefore, if slow metabolism caused early treatment discontinuation and was selected against in this study of longer-term therapy, then the variant T allele conferring slower clearance would have been less frequent in our population. However, the T-allele frequency was comparable to that observed in individuals of African ancestry in the 1000 Genomes Project²⁰ and actually slightly higher than that of our prior cohort study of individuals initiating therapy.¹¹ Finally, the chosen case-control approach enriched for virologic failure has provides us with sufficient statistical power to detect a genotype-phenotype association.

Conclusion

We found that slow efavirenz metabolizers of African descent were less likely to experience virologic failure. Additional prospective investigations are required to evaluate whether consideration should be given to maintaining slow metabolizers on EFV given the observed protective effect in this setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. UNAIDS. Global Report: UNAIDS Report on the Global AIDS Epidemic 2014. Geneva: UNAIDS; 2014.
2. UNAIDS. Prevention Gap Report. Geneva: UNAIDS; 2016.
3. Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J Pharmacol Exp Ther*. 2003; 306(1):287–300. [PubMed: 12676886]
4. Kwara A, Lartey M, Sagoe KW, Rzek NL, Court MH. CYP2B6 (c.516G-->T) and CYP2A6 (*9B and/or *17) polymorphisms are independent predictors of efavirenz plasma concentrations in HIV-infected patients. *Br J Clin Pharmacol*. 2009; 67(4):427–436. [PubMed: 19371316]
5. Ngaimisi E, Mugusi S, Minzi O, et al. Effect of rifampicin and CYP2B6 genotype on long-term efavirenz autoinduction and plasma exposure in HIV patients with or without tuberculosis. *Clin Pharmacol Ther*. 2011; 90(3):406–413. [PubMed: 21814190]

6. Nyakutira C, Roshammar D, Chigutsa E, et al. High prevalence of the CYP2B6 516G-->T(*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. *Eur J Clin Pharmacol*. 2008; 64(4):357–365. [PubMed: 18057928]
7. Dhoru M, Zvada S, Ngara B, et al. CYP2B6*6, CYP2B6*18, Body weight and sex are predictors of efavirenz pharmacokinetics and treatment response: population pharmacokinetic modeling in an HIV/AIDS and TB cohort in Zimbabwe. *BMC Pharmacol Toxicol*. 2015; 16:4. [PubMed: 25889207]
8. Csajka C, Marzolini C, Fattinger K, et al. Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. *Clin Pharmacol Ther*. 2003; 73(1):20–30. [PubMed: 12545140]
9. Dickinson L, Amin J, Else L, et al. Comprehensive Pharmacokinetic, Pharmacodynamic and Pharmacogenetic Evaluation of Once-Daily Efavirenz 400 and 600 mg in Treatment-Naive HIV-Infected Patients at 96 Weeks: Results of the ENCORE1 Study. *Clin Pharmacokinet*. 2016; 55(7): 861–873. [PubMed: 26715213]
10. Lehmann DS, Ribaud HJ, Daar ES, et al. Genome-wide association study of virologic response with efavirenz-containing or abacavir-containing regimens in AIDS clinical trials group protocols. *Pharmacogenet Genomics*. 2015; 25(2):51–59. [PubMed: 25461247]
11. Gross, R., Bellamy, SL., Ratshaa, B., et al. Slow Efavirenz Metabolism Alleles Are Associated with Antiretroviral Failure in Botswana. 32nd International Conference on Pharmacoepidemiology; Dublin, Ireland. 2016.
12. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001; 16(9):606–613. [PubMed: 11556941]
13. Bonacci RA, Frasca K, Swift L, et al. Antiretroviral Refill Adherence Correlates with, But Poorly Predicts Retention in HIV Care. *AIDS Behav*. 2016; 20(5):1060–1067. [PubMed: 26400078]
14. Ribaud HJ, Liu H, Schwab M, et al. Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: an AIDS Clinical Trials Group study. *J Infect Dis*. 2010; 202(5):717–722. [PubMed: 20662624]
15. Haas DW, Smeaton LM, Shafer RW, et al. Pharmacogenetics of long-term responses to antiretroviral regimens containing Efavirenz and/or Nelfinavir: an Adult Aids Clinical Trials Group Study. *J Infect Dis*. 2005; 192(11):1931–1942. [PubMed: 16267764]
16. Liu H, Golin CE, Miller LG, et al. A comparison study of multiple measures of adherence to HIV protease inhibitors. *Ann Intern Med*. 2001; 134(10):968–977. [PubMed: 11352698]
17. Achieng L, Musangi H, Billingsley K, et al. The use of pill counts as a facilitator of adherence with antiretroviral therapy in resource limited settings. *PLoS One*. 2013; 8(12):e67259. [PubMed: 24339861]
18. Hardon, AP., Davey, S., Gerrits, T., et al. From access to adherence: The challenges of antiretroviral treatment: Studies from Botswana, Tanzania and Uganda. Geneva: World Health Organization; 2006.
19. Clifford DB, Evans S, Yang Y, et al. Impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals. *Ann Intern Med*. 2005; 143(10):714–721. [PubMed: 16287792]
20. Genomes Project C, Auton A, Brooks LD, et al. A global reference for human genetic variation. *Nature*. 2015; 526(7571):68–74. [PubMed: 26432245]

Table 1
Baseline Characteristics by Outcome

Characteristic	Late Virologic Failure		
	Control	Case	P-value
<i>N</i>	1062	276	
CYP2B6 516 T-alleles, [*] n (MAF)	557 (38.3%)	127 (34.5%)	0.129
Age, y, mean (IQR)	43.9 (37-50)	44.9 (39-51)	0.090
Male, n (%)	619 (58.5%)	203 (74.6%)	<0.001
Years on EFV, median (IQR)	4.0 (1.6-6.8)	1.5 (0.8-3.0)	<0.001
CD4 count at study entry, n (%)			0.078
0-200 cells/mm ³	298 (31.6%)	90 (39.0%)	
200-499 cells/mm ³	580 (61.5%)	125 (54.1%)	
500+ cells/mm ³	65 (6.9%)	16 (6.9%)	
BMI, mean (IQR)	22.6 (20.1-25.7)	21.6 (19.4-24.4)	0.003
Hazardous drinking, [†] n (%)	110 (10.4%)	39 (14.2%)	0.085
Baseline plasma HIV RNA log copies/mL, [‡] median (IQR)	5.38 (4.77-5.80)	5.28 (4.72-5.68)	0.464
Mood Module (PHQ-9), n (%)			<0.001
None	929 (87.6%)	214 (78.4%)	
1 symptom	92 (8.7%)	38 (13.9%)	
2 symptoms	28 (2.6%)	16 (5.9%)	
3 symptoms	7 (0.7%)	3 (1.1%)	
4+ symptoms	5 (0.5%)	2 (0.7%)	

^{*} Blood samples were not available for 171 people, and an additional 67 patients failed genotyping.

[†] Hazardous drinking is defined as >7 drinks weekly or >4 drinks per session for women, and >14 drinks weekly or >5 drinks per session for men.

[‡] Plasma HIV RNA concentrations pre EFV-therapy initiation.

N, number; MAF, minor allele frequency; IQR, inter-quartile range; PHQ, Patient Health Questionnaire.

Table 2

Association between *CYP2B6* 516G>T Genotype and Late Virologic Failure using an Additive and Dominant Genotypic Effect Model.

Genotypic Effect		Additive Model			Dominant Model		
Characteristic		OR (95%CI)	OR (95%CI)*	P*	OR (95%CI)	OR (95%CI)*	P*
<i>CYP2B6</i> 516G>T				0.06	<i>CYP2B6</i> 516G>T		0.03
GG		1.00 (Ref)	1.00 (Ref)		GG	1.00 (Ref)	
GT		0.74 (0.54-1.01)	0.69 (0.49-0.97)		GT/TT	0.75 (0.56-1.01)	0.70 (0.50-0.97)
TT		0.79 (0.48-1.27)	0.71 (0.41-1.18)				
Age			1.02 (1.00-1.03)	0.08	Age	1.02 (1.00-1.03)	0.08
CD4 count				0.41	CD4 count		0.41
0-200 cells/mm ³			1.00 (Ref)		0-200 cells/mm ³	1.00 (Ref)	
200-499 cells/mm ³			0.76 (0.54-1.08)		200-499 cells/mm ³	0.76 (0.54-1.08)	
500+ cells/mm ³			1.05 (0.54-1.94)		500+ cells/mm ³	1.05 (0.54-1.94)	

* Logistic regression model includes the independent variables of *CYP2B6* 516G>T, age, and CD4 count at study entry