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Combined Effect of *CYP2B6* and *NAT2* Genotype on Plasma Efavirenz Exposure During Rifampin-based Antituberculosis Therapy in the STRIDE Study

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In STRIDE, slow metabolizer *CYP2B6* and *NAT2* genotypes were each associated with increased plasma efavirenz concentrations during antituberculosis therapy. Concentrations were greater on therapy than off therapy in 58% with *CYP2B6* and 93% with *NAT2* slow metabolizer genotypes. Individuals with slow metabolizer genotypes in both genes had markedly elevated concentrations.

Keywords. HIV/AIDS; tuberculosis; efavirenz; rifampin; pharmacogenetic.

Efavirenz is recommended in first-line regimens for human immunodeficiency virus (HIV)-infected patients with tuberculosis [1, 2], in whom concurrent treatment of both infections reduces risk of HIV disease progression [3, 4] and death in patients with advanced HIV disease [5]. Efavirenz is primarily metabolized by cytochrome P450 (CYP) 2B6, with minor contributions by CYP2A6 and CYP3A isoforms. Rifampin, a potent CYP inducer and a key antituberculosis drug, reduced plasma efavirenz exposure in HIV-negative volunteers [6]. However, STRIDE and several other studies showed that multidrug antituberculosis regimens that included rifampin were associated with paradoxically increased efavirenz concentrations [7–9]. This paradoxical increase appears to be influenced by *CYP2B6* loss-of-function polymorphisms that predict increased plasma efavirenz exposure [10–13]. In addition, isoniazid appears to contribute to increased efavirenz concentrations by inhibiting CYP2A6, which may be a particularly important elimination pathway in *CYP2B6* slow metabolizers [14–17]. Isoniazid is metabolized by N-acetyl transferase 2 (*NAT2*), and *NAT2* loss-of-function polymorphisms are associated with increased plasma isoniazid exposure. Thus, *NAT2* genotype may also contribute to increased plasma efavirenz exposure with antituberculosis therapy, as seen in HIV-infected South African pregnant women with slow *NAT2* genotypes, who demonstrated elevated efavirenz concentrations during treatment with isoniazid [9].

In the CAMELIA study, among Cambodians with *CYP2B6* slow metabolizer genotypes (ie, 516 TT) treated for HIV-1 and tuberculosis, concomitant *NAT2* slow metabolizer genotype was associated with decreased plasma efavirenz clearance [8]. Data are limited regarding the combined influence of *CYP2B6* and *NAT2* polymorphisms in populations representing other race/ethnicities. Frequencies of *CYP2B6* loss-of-function polymorphisms vary by ancestry, with 516G→T (rs3745274) more frequent with African or Asian ancestry, 983T→C (rs28399499) found only with African ancestry, and 15582C→T (rs4803419) more frequent with Asian or European ancestry [11–13, 18].

We previously reported paradoxically elevated efavirenz concentrations during combination tuberculosis treatment in the STRIDE study, which prospectively evaluated earlier vs later ART in HIV-infected individuals with <250 $CD4^+$ cells/mm^3 and initiating tuberculosis treatment [3, 19]. The present study examined the extent to which *CYP2B6* and *NAT2* polymorphisms were associated these increased efavirenz concentrations in black and Hispanic patients enrolled from sub-Saharan Africa and South America.

METHODS

Patient Population and Study Design

We conducted a nested pharmacogenetics analysis using data from the larger STRIDE study. In STRIDE, 809 HIV-infected, antiretroviral-naive patients with <250 $CD4^+$ cells/mm^3 and

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confirmed or probable tuberculosis were randomized to either early initiation of antiretroviral therapy (within 2 weeks after starting antituberculosis therapy) or later initiation of antiretroviral therapy (between 8 and 12 weeks after starting antituberculosis therapy). Additional eligibility criteria for the STRIDE study are described elsewhere [3]. Participants received once-daily efavirenz 600 mg without dose adjustment for weight, and a coformulated tablet containing emtricitabine 200 mg and tenofovir disoproxil fumarate 300 mg. The study protocol was approved by institutional review board or ethics committee at each participating site and was registered under clinicaltrials.gov NCT00108862. The pharmacogenetic study population comprised a subgroup of STRIDE participants who had at least one efavirenz minimum concentration (C_{min}) assayed during rifampin-based antituberculosis therapy and at least one efavirenz C_{min} measured a minimum of 4 weeks after stopping antituberculosis therapy, and who provided written informed consent for genetic research under ACTG protocol A5243.

Efavirenz Assays

Efavirenz C_{min} was measured by high performance liquid chromatography (HPLC, lower limit of quantitation 0.1 μg/mL) in plasma samples obtained between 20 and 28 hours post-dose, and with no missed dose by self-report in prior 3 days. For each participant, the on antituberculosis therapy C_{min} concentration was the mean of available C_{min} concentrations at weeks 4, 8, 16, and 24, and the off antituberculosis therapy efavirenz C_{min} concentration was the mean of available C_{min} concentrations at weeks 4 and 8 after antituberculosis therapy.

Genetic Testing

Three *CYP2B6* polymorphisms (15582C→T, 516G→T, and 983T→C) were genotyped by MassARRAY iPLEX Gold (Sequenom, Inc). Based on these polymorphisms, metabolizer status was categorized as extensive, intermediate, or slow as follows (haplotypes correspond to positions 15582-516-983): *CYP2B6 extensive*, CC-GG-TT or CT-GG-TT; *CYP2B6 intermediate*, TT-GG-TT, CC-GT-TT, CC-GG-TC, CT-GT-TT, or CT-GG-TC; *CYP2B6 slow*, CC-TT-TT, CC-GT-TC, or CC-GG-CC [10]. Four *NAT2* polymorphisms, rs1801279 (*NAT2*14*), rs1801280 (*NAT2*5*), rs1799930 (*NAT2*6*), and rs1799931 (*NAT2*7*), were genotyped by TaqMan (Applied Biosystems, Inc., Foster City, California), and categorized as *slow*, homozygous for the variant allele at any of the four loci (ie, AA, CC, AA, AA, respectively), or heterozygous at 2 or more loci; *intermediate,* heterozygous at a single locus; or *extensive,* not variant allele at any locus (ie, GG, TT, GG, GG, respectively) [20].

Statistical Analysis

Efavirenz C_{min} concentrations within genotype groups are summarized by median, 25th and 75% percentiles (Q1 and Q3) and range. Within-participant on- and off-antituberculosis therapy differences in efavirenz C_{min} concentrations were evaluated by the Wilcoxon signed-rank test. Within-participant differences by metabolizer group were compared using the Wilcoxon rank sum test (without continuity correction). Tests comparing metabolizer groups within the on- or off-antituberculosis therapy condition were not performed.

RESULTS

Forty-two participants from the STRIDE study were included in this pharmacogenetics analysis, of whom 52% were male; 71% black non-Hispanic, 29% Hispanic; 52% from South Africa, 29% from Peru, and 19% from Uganda. The median efavirenz C_{min} while on antituberculosis therapy was 1.96 mg/L (range 0.05 mg/L to 19.71 mg/L), and off antituberculosis therapy was 1.85 mg/L (range 0.73 mg/L to 11.69 mg/L; the median within-participant difference was 0.11 mg/L, WSR *P*-value = .17).

Among the 42 participants, 11 (26%), 19 (45%), and 12 (29%) had *CYP2B6* extensive, intermediate, and slow metabolizer genotypes, respectively. Participants with slow metabolizer genotypes had higher efavirenz C_{min} concentrations in comparison to those with intermediate and extensive metabolizer genotypes, regardless of whether on antituberculosis therapy (median efavirenz C_{min} 7.82 μg/mL (range 2.73 to 19.71) or off antituberculosis therapy (median efavirenz C_{min} 4.84 μg/mL (range 0.89 to 11.69); see Figure 1, panel *A*). Among participants with *CYP2B6* extensive, intermediate and slow metabolizer genotypes, 55%, 63% and 58% respectively had higher efavirenz C_{min} concentrations while on antituberculosis therapy than off antituberculosis therapy.

Among the 42 participants, 8 (19%), 19 (45%), and 15 (36%) had *NAT2* extensive, intermediate, and slow metabolizer genotypes, respectively. Among participants with *NAT2* extensive, intermediate, and slow metabolizer genotypes, 25%, 47%, and 93% of participants had higher efavirenz C_{min} concentrations while on antituberculosis therapy than off antituberculosis therapy, respectively. Although 93% of slow metabolizers exhibited higher efavirenz C_{min} concentrations while on antituberculosis therapy, and while differences in C_{min} on vs off therapy were statistically significant, the differences were small in magnitude for most participants (Figure 1, panel *B*).

We next examined whether *CYP2B6* and *NAT2* genotypes in combination better explained efavirenz C_{min} concentrations. Changes in efavirenz C_{min} concentrations according to *CYP2B6* genotype, and further stratified by *NAT2* genotype, are shown in Figure 1, panel *C*. In participants with *CYP2B6* extensive and intermediate metabolizer genotypes, only small differences between efavirenz C_{min} concentrations on antituberculosis therapy and off antituberculosis therapy were seen for all *NAT2* metabolizer

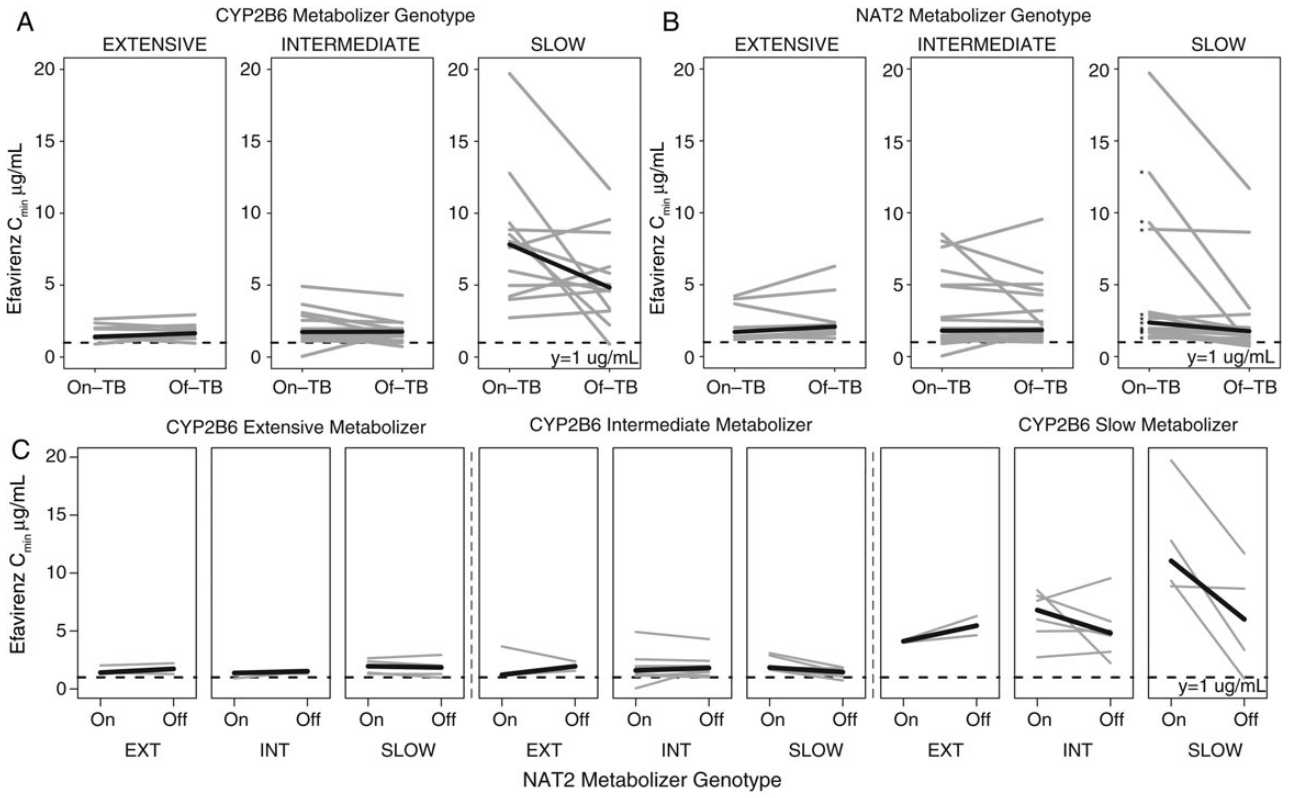

Figure 1. Efavirenz C_{min} µg/mL by *A, CYP2B6* genotype, *B, NAT2* genotype, and *C*, Both *CYP2B* and *NAT2* genotypes: Grey lines connect within-participant efavirenz C_{min} on-antituberculosis therapy and off-antituberculosis therapy. Solid black lines indicate median on-antituberculosis therapy and off-antituberculosis therapy C_{min}. *Indicate the 10 participants with 2 intermediate (INT) *NAT2* alleles (categorized in this analysis as slow metabolizers). Abbreviations: EXT, extensive; TB, tuberculosis.

genotypes. In contrast, among the 4 participants with both *CYP2B6* and *NAT2* slow metabolizer genotypes, efavirenz C_{min} concentrations were substantially elevated on antituberculosis therapy compared to off antituberculosis therapy, with differences exceeding 8 µg/mL in 3 of these 4 participants; this was not statistically significant in this subset. One individual with slow *CYP2B6* and intermediate *NAT2* metabolizer genotypes had a considerably larger efavirenz C_{min} concentration on vs off antituberculosis treatment.

DISCUSSION

Among STRIDE participants who were included in pharmacogenetic analyses, the majority of participants with *CYP2B6* slow metabolizer genotypes had higher efavirenz C_{min} concentrations on antituberculosis therapy than off antituberculosis therapy. Our data suggest that increased efavirenz C_{min} concentrations during concomitant antituberculosis therapy are driven largely by *CYP2B6* slow metabolizer genotypes. *NAT2* slow metabolizer genotypes appeared to associated with considerable further increases in efavirenz C_{min} concentrations on antituberculosis therapy. This elevation in plasma efavirenz exposure likely reflects the combined effect of several factors. Carriage of 2 major *CYP2B6* loss-of-function alleles markedly reduces efavirenz clearance by CYP2B6, which makes clearance more dependent on CYP2A6. Concomitant isoniazid interferes with the alternative metabolic pathway, with the effect most apparent in individuals with *NAT2* slow metabolizer genotypes, who are predicted to have higher plasma isoniazid concentrations.

The CAMELIA study, which enrolled HIV-infected patients in Cambodia, found a similar association between *NAT2* slow metabolizer genotype and decreased plasma clearance of efavirenz among *CYP2B6* slow metabolizers [8]. The present study supports this association and extends these findings to STRIDE participants that included black and Hispanic participants from sub-Saharan Africa and South America. These data provide further evidence for 2 pharmacogenetic pathways that may be contributing to the elevated efavirenz levels reported during tuberculosis therapy in several African studies [7, 21, 22]. The cumulative data suggest that HIV-infected individuals on efavirenz-based therapy who carry both *CYP2B6* and *NAT2* slow metabolizer genotypes may experience marked elevations

in efavirenz plasma exposure if prescribed antituberculosis therapy that includes isoniazid, potentially including isoniazid preventative therapy alone. This is clinically relevant because higher plasma efavirenz concentrations have been associated with increased central nervous system symptoms [21, 23, 24]. The role of screening for *CYP2B6* and *NAT2* genotypes in clinical practice is not known.

Notes

Authorship. Contribution to authorship: All authors are members of the New Works Concept Sheet Team 364 and all contributed to study design and manuscript preparation. A. F. L., B. G., J. S., I. S., and D. V. H. are members of the A5221 protocol study teams. D. W. H. is a member of the A5243 study team and was responsible for pharmacogenetic analyses. S. L. R. and D. L. were responsible for statistical analysis.

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