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Impact of Single Nucleotide Polymorphisms on Plasma Concentrations of Efavirenz and Lopinavir/ritonavir in Chinese Children Infected with the Human Immunodeficiency Virus

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Abstract

Background—Single-nucleotide polymorphisms (SNPs) in the genes that encode the cytochrome P450 (CYP) drug metabolizing enzymes and drug transporters have been reported to influence antiretroviral drug pharmacokinetics. While primarily metabolized by CYP2B6 and -3A, efavirenz (EFV) and lopinavir/ritonavir (LPV/r) are substrates of P-glycoprotein and solute carrier organic (SLCO) anion transporter, respectively.

Objective—To investigate the association between SNPs and efavirenz (EFV) or lopinavir/ritonavir (LPV/r) concentrations in Chinese children infected with the human immunodeficiency virus (HIV).

Methods—Genotyping was performed on *CYP2B6* 516G→T, -1459C→T, and -983T→C, *ABCB1* 3435C→T, and *SLCO1B1* 521T→C in 229 HIV-infected Chinese pediatric patients with an age range from 4.0 to 17.5 years old. Plasma concentrations of EFV and LPV/r were measured using a validated high performance liquid chromatography coupled with mass spectrum (HPLC-MS) method among 39 and 69 children who received EFV- and LPV/r-containing regimens, respectively.

Results—The frequencies of *CYP2B6* 516G→T in the study participants were 71%, 25% and 4% for G/G, G/T, and T/T genotypes, respectively. Among the children under therapeutic drug

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monitoring, 21% and 39% experienced EFV and LPV concentrations above the upper threshold of the therapeutic window. *CYP2B6* 516G→T was significantly associated with EFV concentrations (p<0.001). Older children (>10 years) were more likely to have significantly higher EFV concentrations than the younger ones (p=0.0314).

Conclusion—*CYP2B6* genotyping and EFV concentration monitoring may help optimize antiretroviral therapy in pediatric patients who initiate an EFV-based regimen.

Keywords

HIV; pediatrics; efavirenz; lopinavir/ritonavir; single-nucleotide polymorphisms; drug concentrations

The National Highly Active Antiretroviral Therapy Program significantly reduced mortality and disease progression among HIV-1 infected children and adolescents in China since 2005.[1–3] By the end of 2014, a total of 5275 HIV-infected Chinese children had received antiretroviral therapy (ART) through this program. Unlike the standard ART regimens in the United States or Europe for HIV-infected pediatric patients, the currently preferred treatment options in China include efavirenz- (EFV) and lopinavir/ritonavir (LPV/r)-based regimens in line with the World Health Organization (WHO) guidelines, commonly used in the low and middle-income countries. [4–6] Clinical, immunological and virologic outcomes of ART in this program have been reported previously.[7] Despite a high response rate to ART (~75%), a considerable number of children with HIV still experience treatment failure and drug toxicity.[8, 9]

While the exposure-response relationship, particularly toxicity, has been suggested for certain antiretroviral agents in adult patients, it remains largely unclear among HIV-infected children.[10] The process of puberty and growth has a substantial impact on drug metabolism, therefore, it has been considered a practical approach to perform therapeutic drug monitoring and subsequent dosage adjustment in order to optimize the antiretroviral treatment in children.[11]

Several single-nucleotide polymorphisms (SNPs) in the genes encoding drug transporters and cytochrome P450 (CYP) metabolizing enzymes have been shown to affect gene expression and/or protein activity, as well as related pharmacokinetics.[12] EFV and LPV/r are the most important agents as a part of the preferred regimens recommended by WHO and Chinese pediatric HIV treatment guideline.[13] While primarily metabolized by CYP2B6 and -3A, efavirenz (EFV) and lopinavir/ritonavir (LPV/r) are substrates of P-glycoprotein and solute carrier organic (SLCO) anion transporter, respectively. *CYP2B6* 516 G→T and 983T→C have been significantly associated with plasma concentrations of EFV, which is primarily metabolized by CYP2B6.[14–17] P-glycoprotein, encoded by ATP Binding Cassette Subfamily B Member 1 (*ABCB1*), has been suggested in drug transport, but previous studies investigating the association of *ABCB1* 3435C→T and drug concentrations have been inconclusive.[18–21] *SLCO1B1* (also known as *OAT1B1*) encodes organic anion transporting polypeptide 1B1, and several previous studies have indicated an association of *SLCO1B1* 521T→C with plasma LPV/r exposure.[22–24] The primary objective of the present study was

to investigate effects of the genotypes on antiretroviral drug concentrations in HIV-1 infected Chinese children.

Materials and methods

Study Design and Participants

This was a single center cohort analysis of 229 HIV-infected Chinese children receiving long-term care at the Chinese Center for Disease Control Clinic in Shangcai County, Henan Province, between 2010 and 2013. This site was selected because of its long history of providing ART to the largest cohort of HIV-infected children, accounting for nearly 20% in China. Among the 229 patients, 21 were treatment naïve and 208 received a standard ART regimen including nevirapine, EFV or LPV/r in combination with lamivudine and zidovudine, stavudine or abacavir. The standardized dosage was based on subject weight bands as defined in the China Free ART Manual.[13] After treatment initiation, patients were followed up every 3 months for 6 months, with whole blood samples (5 mL) collected at each visit for CD4+ T cell count and clinical laboratory tests to monitor efficacy and toxicity. Plasma samples for drug concentration monitoring were collected from patients who received EFV- or LPV/r-based regimens at least 1 month after ART initiation at the steady state and during the follow-up visits. The samples were collected before the patients received their morning dose. The resulting concentrations represented trough (Ctrough) for LPV/r, and mid-dosing interval for EFV, given at the bedtime without food.

All patients on EFV or LPV/r were selected for the drug concentration monitoring study. However, if they took their morning dose of LPV/r before the sample collection (n=19) or received EFV with food or missed the dose at bedtime (n=2), their samples were excluded from the analysis. Time since last dose was calculated based on the dosing history retrieved from the medical records. This study was approved by the Institutional Review Board at the National Center for AIDS/STD Control and Prevention in China. Both patients and parents provided informed consent.

Genotyping

DNA was extracted from the whole blood by phenol-chloroform extraction using the commercially available Qiagen QIAamp Blood Mini kit according to manufacturer's protocol (Qiagen, Hilden, Germany). Genotypic analyses were performed on the following SNPs: *CYP2B6* 516G→T (Exon4, rs3745274), -1459C→T (Exon9, rs3211371), and -983T→C (exon7, rs28399499), *ABCB1* 3435 C→T (Exon 26, rs1045642) and *SLCO1B1* 521T→C (rs4149056) by polymerase chain reaction (PCR), followed by ABI PRISM SNaPshot Multiplex Kit and GeneMapper4.0 (Applied Biosystems, Foster City, CA).

Antiretroviral Drug Concentration Measurement

Plasma concentrations of EFV, LPV, and ritonavir (RTV) were measured by a validated HPLC-MS method. [25] Inter- and intra-assay variation ranged between 1% and 10%. Linear ranges were 50–10,000 ng/mL for EFV, and 10–10,000 ng/mL for LPV and RTV. For patients with multiple measurements, mean concentrations of multiple measurements were used in the final analysis. The therapeutic window used in the present study for EFV was at

a mid-interval concentration range of 1000–4000 ng/mL [26, 27] and 1000–10,000 ng/mL for LPV [28, 29] to ensure efficacy and minimize the risk of toxicity. Absolute CD4+ T cell counts were analyzed by FACS Calibur (BD Biosciences, San Jose, CA).

Statistical Analysis

Clinical characteristics including age, gender, route of transmission, CD4+ T cell count, plasma HIV-1 RNA, and treatment duration were described by medians and inter quartile range (IQR) or proportions. Allelic frequencies were calculated as number of alleles divided by all alleles in the population. Chi-square test was used to compare allelic frequencies and genotypic frequencies. The effect of potential influencing factors, such as gender, age (≥10 y vs <10 y), *ABCB1* 3435 C→T, *CYP2B6* 516G→T, and *SLCO1B1* 521T→C, on plasma concentrations of EFV, LPV or RTV were examined using the nonparametric Wilcoxon signed-rank test. The selection of 10-year old breakpoint was primarily based on findings from the ARROW trial, which demonstrated age-dependent viral suppression in pediatric patients such that long-term suppression was better with EFV in children younger than 10 and with nevirapine in those older than 10.[30] A multivariable linear regression was performed for multiple factors with p values less than 0.10. Post-hoc pairwise comparisons of drug concentrations and genotype groups, as well as adjustment for significant factors such as age and gender, were performed for confirmation. A p value less than 0.05 was considered statistically significant. Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Results

Demographic Characteristics

A total of 229 participants were enrolled in the study with an age range from 4.0 to17.5 years of age, of whom 39 and 69 received EFV- or LPV/r-based regimens, respectively, and had plasma concentrations monitored. Clinical characteristics of these participants are presented in Table 1.

Genotype Frequencies

Genotypes for *CYP2B6* 516G→T, -1459C→T, -983T→C, *ABCB1* 3435C→T, and *SLCO1B1* 521T→C were obtained in 229 patients. The genotype data from the study population did not significantly deviate from the Hardy-Weinberg equilibrium. As shown in Table 2, homozygous variants of *CYP2B6* 983 T→C and -1459C→T were not identified in this study population. The allelic frequencies are also summarized in Table 2, with a T allele frequency of 16.8% in *CYP2B6* 516G→T.

Inter-individual (CV_{inter}) and intra-individual (CV_{intra}) variability

The weight-based median (IQR) dosage was 12.2 (10.8–13.4) mg/kg and 10.5 (9.2–11.8) mg/kg for EFV and LPV, respectively. Time since last dose was 13.3±0.7 hours for EFV and 13.4±0.7 hours for LPV/r. We collected 93 blood samples from 39 patients receiving EFV-based regimens. Only one patient experienced low EFV concentration (978.5 ng/mL), whereas 8 patients (21%) experienced EFV concentration greater than 4000 ng/mL. We collected 143 blood samples from 69 patients receiving LPV/r-containing regimen. No

sample was below 1000 ng/mL, whereas LPV concentrations from 27 patients (39%) were above 10,000 ng/mL. Intra- and inter-individual variabilities are summarized in Supplement Table 1. EFV demonstrated the highest inter-individual variability (109%) but the lowest intra-individual variability (12%), whereas LPV had the lowest inter-individual variability (45%) and RTV had the highest intra-individual variability (38%).

Associations Between Genotypes and Drug Concentrations

The effect of genotypes (*CYP2B6* 516G→T, *ABCB1* 3435C→T, and *SLCO1B1* 521 T→C), gender, and age on EFV, LPV, and RTV concentrations were included in the univariable analysis with the nonparametric Wilcoxon signed-rank test. Because of the low frequencies of *CYP2B6* 983T→C and -1459C→T variants, these SNPs were excluded from the association analysis. A significant association was identified between *CYP2B6* 516 G→T and EFV concentrations (p=0.0009) (Table 3). The median EFV concentration (IQR) was 2322 ng/mL (1932–2761 ng/mL) for GG (n=26), 3394 ng/mL (2760–4442 ng/mL) for GT (n=12) and 23,668 ng/mL for TT (n=1) (Figure 1). The univariable analysis identified significant effects of gender, age and *CYP2B6* 516 G→T on EFV concentrations (p<0.05, Table 3). An association between *ABCB1* 3435C→T and EFV concentrations was suggested (p=0.0827) without statistical significance. *CYP2B6* 516 G→T remained significant in the multivariable analysis (p<0.0001), but not age (p=0.1299) and gender (p=0.2798) did not. Post-hoc pairwise comparisons of EFV concentrations and genotype groups confirmed significantly higher EFV concentrations in GT and TT than those in GG after adjusting for age and gender. All of these factors had no significant effects on the concentrations of LPV or RTV in the univariable analysis (p>0.05, Table 4), even though a higher median LPV concentration was observed with CT (8627 ng/mL) in comparison to that with TT (6952 ng/mL) suggesting an association of *SLCO1B1* 521 T→C and LPV concentrations without reaching statistical significance (p=0.0918).

Discussion

To our knowledge, this is the first study to evaluate effects of genotypes on antiretroviral drug concentrations in HIV-infected Chinese children. Despite a number of previous studies in sub-Saharan African countries, [31, 32] limited data are currently available on antiretroviral pharmacogenomics in pediatric Asian populations.

The current dosing recommendations of antiretroviral agents in China for pediatric patients are based on body weight bands in line with the WHO guidelines. [13, 33] Wide inter-patient differences in antiretroviral drug concentrations have been associated with fixed dose regimens. [34] For example, while 95% of an American adolescent cohort had adequate EFV concentrations[35], sub-therapeutic EFV concentrations have been reported in 40% and 64% pediatric patients in South Africa and Germany, respectively. [36, 37] A therapeutic drug monitoring study in adult patients receiving EFV also reported 37% below the therapeutic window, but 6% above the upper threshold.[29] In our study, almost all of the measured EFV and LPV concentrations (except one EFV concentration) were above the lower threshold. However, 20.5% and 39.1% of the patients experienced high EFV and LPV concentrations above the upper threshold, suggesting that the current EFV and LPV/r dosage

recommendation might provide sufficient therapeutic control of HIV infection for the majority of Chinese children, but overdose might occur in a considerable portion of these pediatric patients. The recent randomized, double-blind, placebo-controlled, non-inferiority ENCORE1 study showed that EFV at a reduced 400 mg daily dose remained non-inferior to 600 mg daily dose in treatment naïve adult patients.[38, 39] Additionally, numerous reports have demonstrated safety and effects of EFV dose reduction to prevent discontinuations as a result of EFV-related adverse drug reactions.[40, 41] The new WHO treatment guideline has therefore recommended EFV 400 mg daily as the alternative first line regimen in adults and adolescents older than 12 years of age.[4] Our findings suggested that EFV dose reduction might be needed for pediatric patients in China.

The present study demonstrated low intra-patient variability for EFV (12%), which might be due to its convenient once-daily dosing and long half-life, and high inter-patient variability (109%), likely associated with the variation in EFV metabolism. A number of SNPs in *CYP2B6*, including 516G→T, 983T→C and 1459C→T, have been associated with plasma EFV concentrations. [42] *CYP2B6* 983T→C and 1459C→T were excluded from the analysis because of their low frequencies in the Chinese population, particularly people with Han nationalities. A significant association was revealed among Chinese pediatric patients between *CYP2B6* 516G→T and EFV plasma concentrations in the present study, which was consistent to the previous results in both pediatric and adult populations.[15, 43] The frequency of variant T allele varies among different ethnicities with 14%, 15%, 26%, and 49% in Japanese, Korean, Caucasian, and African populations, respectively. [44, 45] The T allele frequency was 16.8% in the present study, similar to that observed in Japanese, Koreans and Han Chinese (21%), but lower than 34.5% reported in 507 Southern Chinese from Hong Kong. [46] In addition, the frequency of 516TT genotype (4.4%) in this study population, mainly with Han nationality and from Central China, was significantly lower than that reported in Southern Chinese (23.1%), suggesting regional differences in *CYP2B6* 516G→T frequencies in China. Because *CYP2B6* 516G→T genotype was significantly associated with EFV metabolism and nearly 20% of patients having EFV concentrations above the upper threshold, a genotype-based EFV dosage adjustment might be needed to optimize treatment outcomes and avoid concentration-related adverse drug effects.

Age might be an influencing factor for drug metabolism because hepatic enzyme activity is significantly higher in children compared with adults. [43, 47] Consistent with findings in our study, older children (>10 years old) had significantly higher EFV concentrations than the younger ones (p=0.0314) in the univariate analysis, but not in the multivariable analysis, suggesting a more important role of *CYP2B6* in determining EFV pharmacokinetics. Gender appeared to be the other confounding factor for EFV concentrations where significantly higher concentrations were observed among males than females in the univariable analysis (p=0.0325) but not in the multivariable analysis. This might reflect the gender differences in hepatic expression of CYP2B6 where significantly higher expression of *CYP2B6* (>1.7 fold) and activity (>1.6 fold) have been reported in females in comparison to males. [48]

The Chinese HIV/AIDS Treatment Guidelines recommend LPV/r as the second-line treatment for pediatric patients with HIV-1 infection. [13] The present study found nearly one third (25.9%) of the LPV concentrations higher than the upper threshold of the therapeutic

window (10,000 ng/mlL), and high intra-patient variability of LPV/r among Chinese pediatric patients, possibly due to a twice daily dosing regimen and relatively wide range of sampling time, ranging from 11.5 to 15 hours. Recent studies have demonstrated that *SLCO1B1* 521T→C was significantly associated with lower LPV clearance and higher concentrations in adults. [23, 49] A prospective cohort study in 50 children (4–18 years old) on stable antiretroviral therapy with LPV/r indicated a significant association between *SLCO1B1* 521T→C and increased LPV exposure, but not with viral load. [50] The present study revealed a low frequency of *SLCO1B1* 521CC genotype (<1%) in Chinese children, and 21.4% heterogeneous 521TC genotype. A trend for high LPV concentrations among 521C allele carriers was observed without statistical significance (p=0.0918). Further studies to confirm the clinical importance of *SLCO1B1* polymorphisms in LPV pharmacokinetics might be warranted.

There were certain limitations of the present study. This was a single center, cross-sectional, retrospective study design and all of the study participates were from the Han nationality. Therefore, the genetic profiles, particularly the frequencies of the SNPs, might not represent the characteristics of the Chinese population. Due to its small sample size, analyses of associations between antiretroviral drug concentrations and adverse effects or virologic response were not performed. Although a significant difference in EFV concentrations was noted among genotypes (Table 3), much of the concentration data appeared to be driven by only one subject with the homogeneous mutant genotype of *CYP2B6* 516G→T (Figure 1). Thus, the relationship between genotypes and drug concentrations would be further reinforced if more study subjects with the TT genotype and high EFV concentrations were identified. Despite these limitations, the findings from this study have provided additional evidence to support genotype-based EFV dose adjustment as a part of precision medicine for not only Chinese, but also Asian, children with HIV infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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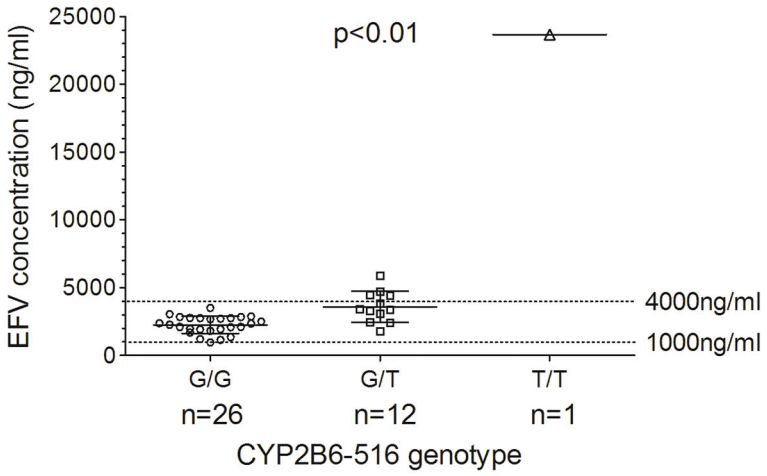

Figure 1.

Efavirenz (EFV) plasma concentration of 39 children, divided into *CYP2B6*-516 genotype G/G, G/T, T/T. The horizontal line in the scatters indicates the median and interquartile ranges. The dotted lines of 1000 ng/mL and 4000 ng/mL indicate the lower and upper limit of the therapeutic range of EFV. CC genotype is shown by circle shape. CT genotype is shown by square shape. TT genotype is shown by triangle shape.

Table 1

Demographic Characteristics

	Genotype Analysis (n=229)	EFV Group (n=39)	LPV/r Group (n=69)
Male, n (%)	155 (67.7)	22 (56.4)	48 (69.6)
Age, year	11.7 (8.9~14.4)	13.3 (9.0~14.5)	12.0 (9.6~14.5)
Transmission route, n (%)			
Mother to child	167 (72.9)	25 (64.1)	52 (75.4)
Blood transfusion	44 (19.2)	10 (25.6)	15 (21.7)
Unknown	18 (7.9)	4 (10.3)	2 (2.9)
Regimen, n (%)			
Nevirapine	79 (34.5)		
EFV	41 (17.9)		
LPV/r	88 (38.4)		
Naïve	21 (9.2)		
Dosage, mg/kg		12.2 (10.8~13.4)	10.5 (9.2~11.8)
Treatment duration, months		26.8 (10.5~40.3)	34.0 (15.9~50.9)
Treatment duration of current regimen, months		20.9 (10.5~34.1)	17.9 (15.7~18.3)
$CD4^+$, cells/mm^3		435 (282~630)	511 (257~683)

Data were summarized in median (interquartile range, IQR).

Table 2

Frequencies of the SNP Genotype and Allelic Variant

Genotype		**rs1045642** ***ABCB1*** **3435 C→T**		**rs4149056** ***SLCO1B1*** **521 T→C**		**rs28399499** ***CYP2B6*** **983 T→C**		**rs3211371** ***CYP2B6*** **1459 C→T**		**rs3745274** ***CYP2B6*** **516 G→T**
Wild type	CC	65 (28.4%)	TT	178 (77.7%)	TT	226 (98.7%)	CC	227(99.1%)	GG	162 (70.7%)
Heterozygous variant	CT	124 (54.1%)	TC	49 (21.4%)	TC	3 (1.3%)	CT	2(0.9%)	GT	57 (24.9%)
Homozygous variant	TT	40 (17.5%)	CC	2 (0.9%)	CC	0	TT	0	TT	10 (4.4%)
Allelic frequency		T		C		C		T		T
		204 (44.5%)		53 (11.6%)		3 (0.66%)		2 (0.44%)		77 (16.8%)
Hardy-Weinberg equilibrium (p)		0.1462		0.491		0.9205		0.9471		0.0956

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Table 3

Effects of Genotypes, Age and Gender on Plasma Antiretroviral Concentrations (ng/mL) in the Univariate Analysis

	Efavirenz				Lopinavir			Ritonavir		
		n	Concentration	*p*	n	Concentration	*p*	n	Concentration	*p*
CYP2B6 516G→T	GG	26	2322 (1932–2761)	0.0009 *	55	7721 (5477– 10573)	0.1415	55	302 (206 – 571)	0.1807
	GT	12	3394 (2760–4442)		12	7324 (4754 – 9060)		12	252 (190 – 435)	
	TT	1	23668		2	12746 (12389 – 13103)		2	713 (615 – 811)	
ABCB1 3435C→T	CC	11	3403 (2124 – 4446)	0.0827	21	5855 (4944 – 10554)	0.1966	21	276 (159 – 498)	0.512
	CT	21	2384 (1932 – 2761)		37	8251 (6073 – 11292)		37	307 (224 – 571)	
	TT	7	2687 (2275 – 3047)		11	8214 (4553 – 10195)		11	276 (206 – 584)	
SLCO1B1 521T→C	TT	30	2734 (2124 – 3306)	0.639	55	6952 (5034 – 10195)	0.0918	55	287 (198 – 557)	0.3951
	CT	8	2178 (1879 – 3008)		14	8627 (7055 – 11251)		14	319 (224 – 617)	
	CC	1	2839							
Gender	Male	22	2773 (2370 – 3842)	0.0325	48	8052 (5314 – 10923)	0.6528	48	340 (213 – 564)	0.1709
	Female	17	2124 (1818 – 2839)		21	6391 (5414 – 10195)		21	227 (193 – 581)	
Age	<10y	14	2032 (1366 – 3047)	0.0314	18	7004 (5554 – 10554)	0.5522	18	286 (175 – 445)	0.6422
	>=10	25	2754 (2370 – 3507)		51	8230 (5365 – 11081)		51	302 (206 – 615)	

*Concentrations summarized as median (interquartile range). The univariate analysis with the Wilcoxon test revealed that gender, age and *CYP2B6* 516 G→T had a significant impact ($p < 0.05$) on EFV concentrations. *CYP2B6* 516 G→T remained significant in the multivariable linear regression ($p<0.0001$), but not age ($p=0.1299$) or gender ($p=0.2798$).

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