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PHARMACOGENETICS

CYP2B6 genotype is a strong predictor of systemic exposure to efavirenz in HIV-infected Zimbabweans

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Abstract
Objective Efavirenz, an antiretroviral medicine, is extensively metabolized by cytochrome P450 2B6 (CYP2B6), UDP-glucuronosyltransferase 2B7 (UGT2B7), and CYP2A6. In this study, we investigated the association of single nucleotide polymorphisms (SNPs) in these genes with plasma efavirenz levels in Zimbabwean human immunodeficiency virus (HIV)-positive patients treated with efavirenz.
Methods The exon regions of the *CYP2B6*, *CYP2A6*, and *UGT2B7* genes were re-sequenced in 49 HIV-infected Zimbabwean patients treated with a combination therapy including efavirenz. Associations of SNPs in these three genes with efavirenz plasma concentrations 11–16 h after the administration of treatment were evaluated.
Results Eight patients carrying *CYP2B6*6/*18* showed the highest plasma efavirenz levels, with a fourfold higher concentration than patients who carried *CYP2B6*1/*1*. Patients with *CYP2B6*6/*6* also showed higher efavirenz plasma concentrations than those with *CYP2B6*1/*1*. Among the 17 and 12 SNPs identified in *CYP2A6* and *UGT2B7*, respectively, no SNP showed a significant association with the plasma efavirenz concentration.
Conclusion Although based on only a limited number of subjects, our results suggest that the *CYP2B6*6* and *CYP2B6*18* alleles should affect hepatic metabolic activity and elevate the systemic circulation level of efavirenz, which may lead to toxicity in Zimbabwean HIV patients.

Keywords CYP2A6 · CYP2B6 · Efavirenz · HIV/AIDS · UGT2B7 · Zimbabwe

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Introduction

In Southern Africa, the first-line antiretroviral therapy consists of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI), namely, nevirapine or efavirenz [1, 2]. Based on affordability, nevirapine is the drug of choice compared to the relatively more expensive efavirenz. Efavirenz is consequently used in patients who develop hypersensitivity reactions to nevirapine or in patients who are on human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and tuberculosis (TB) treatment. The switch to efavirenz in patients undergoing HIV/AIDS and TB treatment is carried out to avoid drug–drug interactions between nevirapine and one of the anti-TB drugs, rifampicin [3]. Due to the very high incidence of HIV and TB co-infections in southern Africa, with 60–80% patients with TB also testing HIV

positive, the number of people on HIV/AIDS and TB treatment is very high, resulting in a large number of patients also receiving efavirenz therapy. The use of efavirenz has been associated with two major side effects, hepatoxicity [4] and adverse effects (AEs) on the central nervous system (CNS) [5]. High plasma efavirenz concentrations have been associated with both an increasing frequency and increasing severity of CNS AEs [5].

The plasma concentration of efavirenz has been reported to show large inter-individual variability, which is mainly caused by the inter-individual variation of its metabolism. Ogburn et al. [6] investigated the primary and secondary metabolic pathways of efavirenz in vitro and in vivo. In vitro metabolism by human the liver microsomes 7- and 8-hydroxyefavirenz accounted for 22.5 and 77.5% of the overall metabolites of efavirenz, respectively. The cytochrome P450 2B6 (*CYP2B6*) 516 G>T gene polymorphism is associated with high efavirenz plasma levels and low efavirenz clearance, as well as the increased probability of CNS AEs [5]. In individuals with impaired CYP2B6 function, efavirenz metabolism might be directed to other pathways. Hence, in addition to CYP2B6 gene polymorphism, those in other drug-metabolizing enzymes, including CYP2A6 [6] and CYP3A4 [7], both of which are involved in the 7-hydroxylation pathway, and UDP-glucuronosyltransferase 2B7 (UGT2B7) [8], which is involved in *N*-glucuronidation, may influence efavirenz pharmacokinetics. The inter-individual variability in efavirenz pharmacokinetics is therefore not entirely explained by the commonly known *CYP2B6* 516 G>T gene polymorphism. The aim of this study was therefore to investigate the effects of polymorphisms in *CYP2A6*, *UGT2B7*, and *CYP2B6* on plasma efavirenz concentration in Zimbabwean HIV-positive patients treated with efavirenz.

Materials and methods

HIV/AIDS outpatients

Of 74 black Zimbabwean HIV-positive outpatients described in a previous study [9], 49 (20 males, 29 females) receiving efavirenz (600 mg, once a day) in combination with two NRTIs (one patient was on a combination of zidovudine and lamivudine) were enrolled in this study. Clinical data on side effects were not available and were also not collected during this cross-sectional study. Blood samples at 11–16 h post-treatment administration were obtained from the patients treated for at least 3 months at clinics in Harare, Zimbabwe. The median time of treatment duration for patients in this study was 6 months (minimum 3 months, maximum 27 months). Over this period of treatment, any autoinduction of efavirenz metabolism [16] should have reached its maximal effect in all patients studied, thus having minimal effects on inter-individual variation to drug exposure. Of the 74 blood original samples, blood samples for DNA preparation were only available for the 49 patients included in this study. Plasma efavirenz concentrations were determined using a high-performance liquid chromatography–UV system as described in our previous report [9]. This study was approved by the Ethical Committee of the RIKEN Yokohama Institute, Japan, and the Medical Research Council of Zimbabwe (MRCZ), Zimbabwe. Written Informed consent was obtained from all participants prior to the study.

Genotyping and linkage disequilibrium analysis

Patients were genotyped for *CYP2B6*, *CYP2A6*, and *UGT2B7* according to the methods proposed by Lang et al. [10], Kiyotani et al. [11], and Mehlotra et al. [12], respectively. The genotyping of the *CYP2A6* deletion was performed as described by Gyamfi et al. [13]. Briefly, PCR was performed using the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) in a total reaction volume of 20 μL with 10 ng of genomic DNA and Ex Taq DNA polymerase (Takara Bio, Shiga, Japan). PCR conditions consisted of an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. Sequencing was carried out using the 3730*xl* DNA Analyzer (Applied Biosystems).

Linkage disequilibrium (LD) and haplotype analyses were performed by SNPAlyze software (ver. 3.2; Dynacom, Yokohama, Japan). The designation of each haplotype followed that previously reported in Human CYP Allele Nomenclature Committee database (http://www.cypallele.ki.se).

Statistical analysis

Kruskal–Wallis and Mann–Whitney's *U* tests were performed to evaluate any potential association between genotype and plasma efavirenz concentration. A significance level after Bonferroni's correction for multiple testing was 0.00156 (0.05/32). All polymorphisms evaluated in this study were tested for deviation from Hardy–Weinberg equilibrium (HWE) with the use of a chi-square test. All statistic analyses were performed using SPSS (ver. 12.0; SPSS, Chicago, IL).

Results

To investigate the effects of *CYP2A6*, *CYP2B6*, and *UGT2B7* polymorphisms on the efavirenz plasma concentration in 49 subjects of Zimbabwean origin, we re-sequenced all exons in these three genes. Among the 49 subjects, the minor allele

Table 1 Associations of single nucleotide polymorphisms in *CYP2A6*, *CYP2B6*, and *UGT2B7* with steady state efavirenz concentrations in plasma of HIV-infected Zimbabweans

Gene	Position (cDNA)	Variation (1/2)	Location	Chromosome position[a]	rs ID	Amino acid changes	Genotype			MAF	HWE *P* value	Kruskal–Wallis test *P* value	Mann–Whitney *U* test *P* value	
							11	12	22				11 vs. 12+22	22 vs. 11+12
CYP2B6	516	G/T	Exon 4	46204681	rs3745274	Gln172His	15	27	7	0.42	3.55E-01	6.08E-04	7.87E-03	6.74E-04
	785	A/G	Exon 5	46207103	rs2279343	Lys262Arg	15	27	7	0.42	3.55E-01	6.08E-04	7.87E-03	6.74E-04
	983	T/C	Exon 7	46210061	rs28399499	Ile328Thr	40	9	0	0.09	4.79E-01	3.60E-02	3.60E-02	NA
CYP2A6	–1680	A/G	5′FR	46049851	rs2644914	–	10	20	17	0.43	3.74E-01	9.91E-01	8.97E-01	9.82E-01
	–1569	T/C	5′FR	46049740	rs2644911	–	40	8	0	0.08	5.29E-01	3.91E-01	3.91E-01	NA
	–1301	A/C	5′FR	46049472	rs7260629	–	24	21	4	0.30	8.42E-01	2.09E-01	3.91E-01	8.36E-02
	–1289	G/A	5′FR	46049460	rs7259706	–	23	20	6	0.33	6.14E-01	3.10E-01	4.02E-01	1.36E-01
	–1013	A/G	5′FR	46049184	rs4803381	–	14	21	13	0.49	3.88E-01	3.58E-01	4.21E-01	4.37E-01
	–420	T/C	5′FR	46048591	rs10418304	–	38	11	0	0.11	3.76E-01	9.12E-02	9.12E-02	NA
	–48	T/G	5′FR	46048219	rs28399433	–	43	5	1	0.07	1.06E-01	6.94E-01	6.69E-01	3.96E-01
	22	C/T	Exon 1	46048150	rs8192720	Leu8Leu	47	2	0	0.02	8.84E-01	5.78E-01	5.78E-01	NA
	51	A/G	Exon 1	46048121	rs1137115	Val17Val	3	20	25	0.27	7.03E-01	1.99E-01	2.77E-01	9.46E-02
	291	G/A	Exon 2	46047615	rs28609092	Glu97Glu	47	2	0	0.02	8.84E-01	1.00E+00	1.00E+00	NA
	312	A/G	Exon 2	46047594	rs72549434	Gln104Gln	48	1	0	0.01	9.42E-01	2.58E-01	2.58E-01	NA
	328	G/C	Exon 2	46047578	rs72549435	Val110Leu	48	1	0	0.01	9.42E-01	2.58E-01	2.58E-01	NA
	459	G/A	Exon 3	46046393	rs28399441	Ala153Ala	38	9	2	0.13	1.58E-01	2.40E-01	9.12E-02	5.44E-01
	771	C/T	Exon 5	46044680	rs1809811	Arg257Arg	45	4	0	0.04	7.66E-01	7.98E-01	7.98E-01	NA
	1093	G/A	Exon 7	46043107	rs28399454	Val365Met	38	10	0	0.10	4.20E-01	1.13E-01	1.13E-01	NA
	1191	T/C	Exon 8	46042488	rs28399461	Ser397Ser	31	12	3	0.20	2.46E-01	4.20E-01	1.89E-01	5.48E-01
	1224	C/T	Exon 8	46042455	rs28399462	Pro408Pro	34	10	3	0.17	9.07E-02	5.60E-02	2.95E-02	1.00E+00
UGT2B7	–327	G/A	5′FR	69996501	rs7662029	–	25	22	2	0.27	2.88E-01	6.56E-01	4.72E-01	4.79E-01
	–161	T/C	5′FR	69996667	rs7668258	–	26	21	2	0.26	3.71E-01	4.96E-01	4.79E-01	2.79E-01
	–138	G/A	5′FR	69996690	rs73823859	–	40	9	0	0.09	4.79E-01	8.36E-01	8.36E-01	NA
	–125	T/C	5′FR	69996703	rs7668282	–	47	2	0	0.02	8.84E-01	7.24E-01	7.24E-01	NA
	372	A/G	Exon 1	69997199	rs28365063	Arg124Arg	41	8	0	0.08	5.34E-01	3.94E-01	3.94E-01	NA
	735	A/G	Exon 2	69998860	rs28365062	Thr245Thr	36	11	1	0.14	8.83E-01	8.33E-02	5.23E-02	1.21E-01
	801	A/T	Exon 2	69998926	rs7438284	Pro267Pro	26	20	2	0.25	4.41E-01	4.37E-01	5.02E-01	2.22E-01
	802	T/C	Exon 2	69998927	rs7439366	His268Tyr	26	20	2	0.25	4.41E-01	4.37E-01	5.02E-01	2.22E-01
	1059	C/G	Exon 4	70007538	rs4292394	Leu353Leu	33	12	3	0.19	2.14E-01	9.34E-01	9.49E-01	7.55E-01
	1062	C/T	Exon 4	70007541	rs4348159	Tyr354Tyr	26	19	3	0.26	8.48E-01	3.60E-01	2.18E-01	2.97E-01
	1191	C/T	Exon 5	70008510	rs57913007	Ala397Ala	39	9	1	0.11	5.83E-01	6.47E-01	7.85E-01	4.37E-01
	1506	A/G	Exon 6	70012959	rs72551393	Ala502Ala	46	3	0	0.03	8.25E-01	9.00E-01	9.00E-01	NA

HIV, Human immunodeficiency virus; CYP, cytochrome P450; MAF, Minor allele frequency; HWE, Hardy-Weinberg equilibrium; UGT2B7, UDP-glucuronosyltransferase 2B7; 5′FR, 5′ flanking region; NA, not available

[a] Based on NCBI36 genome assembly.

frequencies of 516 G>T, 785 T>C, and 983 T>C of *CYP2B6* single nucleotide polymorphisms (SNPs) were 42, 42, and 9%, respectively. Both 516 G>T and 785 T>C, which were absolutely linked each other (r^2 of LD=1.00), were significantly associated with plasma efavirenz concentration (P=0.000608; Table 1); however, no significant association was found for 983 T>C, although those patients heterozygous for the C allele had a fourfold higher efavirenz concentration than those homozygous for the T allele (P=0.036; Table 1).

Three *CYP2B6* haplotypes, corresponding to the *CYP2B6*1* (516 G, 785A, 983 T), **6* (516 T, 785 G, 983 T), and **18* (516 G, 785A, 983 C) alleles, were inferred in the 49 Zimbabwean subjects. The frequencies of *CYP2B6*1*, *6, and *18 were 49, 41, and 10%, respectively. Among five inferred *CYP2B6* genotype groups, plasma efavirenz concentrations were significantly different (P=0.00017; Fig. 1). Patients carrying *CYP2B6*6/*18* showed a fourfold higher plasma efavirenz concentration than those carrying *CYP2B6*1/*1*. Patients with *CYP2B6*6/*6*, who were defined as homozygous carriers of both 516 T>C and 785A>G, also showed higher plasma efavirenz concentrations. Taken together, these results suggest the possibility of comparable treatment outcomes for patients carrying *CYP2B6*6/*6* and *CYP2B6*6/*18*.

To examine the effects of polymorphisms of the *CYP2A6* gene, we re-sequenced this gene and found 17 SNPs. The numbers of subjects with each individual genotype are given in Table 1. Minor allele frequencies varied between a low of 4% for *CYP2A6* 771 C>T and a high of 49% in *CYP2A6*-1013A>C. None of these SNPs showed a significant association with the plasma efavirenz concentration (Table 1). None of our subjects carried the *CYP2A6*4A* allele, which is a whole gene deletion variant. In addition no significant association was observed based upon our analysis of haplotype (data not shown). For *UGT2B7*, minor allele frequencies were between 2 and 27% (Table 1), and no association between *UGT2B7* allele frequencies and efavirenz plasma concentration was found in the SNP- and haplotype-based analysis.

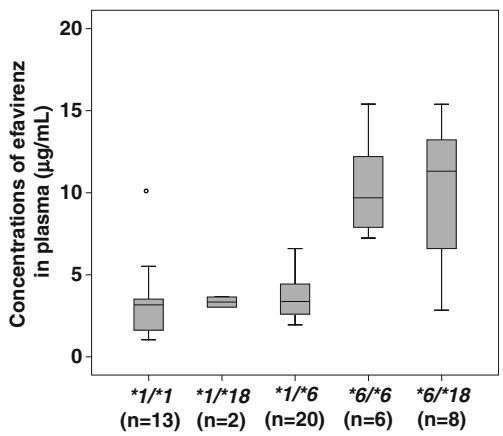

Fig. 1 Steady state plasma efavirenz concentrations of 49 Zimbabwean human immunodeficiency virus (HIV) patients with different cytochrome P450 2B6 (*CYP2B6*) genotypes. *Horizontal line* Median concentration, *box* 25–75 percentiles. *Maximum length of each whisker* equals 1.5-fold the interquartile range; *dot outside the whiskers* is an outlier

Discussion

In this study, we analyzed the associations between plasma efavirenz concentration and polymorphisms in *CYP2B6*, *CYP2A6*, and *UGT2B7*, which code for the three main enzymes involved in the metabolism of this drug. Our results reveal a significant association between SNPs in *CYP2B6* and plasma concentration of efavirenz. In addition to the 516 G>T and 785A>G genetic polymorphisms, the effects of which on plasma efavirenz concentration are well-investigated [5], our patients heterozygous for the minor allele of 983 T>C showed higher plasma efavirenz levels than those homozygous for the major allele. The 983 T>C allele in exon 7, which results in the Ile328Thr amino acid substitution and is predicted to cause reduced activity of CYP2B6 [14], was found at a high frequency in our Zimbabwean patients. Haplotype analysis demonstrated that patients carrying *CYP2B6*6/*18* showed extremely high plasma efavirenz concentrations compared to those carrying either *CYP2B6*1/*1* or *CYP2B6*6/*6* (Fig. 1). These results support the high plasma efavirenz concentration in Zimbabwean HIV patients reported earlier [9].

Despite previous reports [15] demonstrating that *CYP2B6*16* (516 G, 785 G, 983 C) is commonly found in African populations (6.9% in 92 Tanzanians), this allele was not found in Zimbabwean patients in this study; in contrast, *CYP2B6*18* (516 G, 785A, 983 C) was found at a relatively high frequency, namely, 10%, in the Zimbabwean population. Other studies [12, 15] have shown that *CYP2B6*16* is more common in Central, Western, and Southern Africa, while *CYP2B6*18* is more commonly found in Ghanaians and African Americans. These results imply inter-population differences in LD structure even within African populations. Since small sample sizes might have caused these differences, further analyses using a large number of samples are required. The allele frequencies of 516 C>T and 785A>G in our Zimbabwean population are comparable to those reported previously [9]. The overall frequency of the *CYP2B6*1* allele has been calculated to be 24.6% in Zimbabweans, which is lower compared to the 50.7% in Caucasians or 68.4% in Asians [14].

In conclusion, our genotyping results reveal that *CYP2B6*6* and *CYP2B6*18* are key functional alleles for improving the treatment of Zimbabwean HIV-positive

patients. Especially in this new era, genotype-guided efavirenz therapy will become an important strategy in the development of HIV treatment guidelines in Zimbabwe and elsewhere in Africa.

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