CYP2B6 983T>C polymorphism is prevalent in West Africa but absent in Papua New Guinea: implications for HIV/AIDS treatment

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What is already known about this subject

- The novel CYP2B6 functional polymorphism 983T>C (either alone as CYP2B6*18 or linked with 785A>G as the CYP2B6*16 allele) was found in Africans and African-Americans but not in Caucasians and Asians.
- The polymorphism by itself and together with 516G>T (the key polymorphism in the most frequent variant allele CYP2B6*6) was associated with significantly higher mean plasma efavirenz concentrations in the African HIV patients.
- In Papua New Guinea, the HIV/AIDS epidemic is escalating, CYP2B6*6 is highly prevalent, and the prevalence of 983T>C is not known.

What this study adds

- CYP2B6 983T>C is absent in the Papua New Guinea population.
- The outcome of treatment with efavirenz may prove different in Papua New Guineans ([CYP2B6*6 +] [983T>C -]) compared with Africans or African-Americans ([CYP2B6*6 +] [983T>C +]).

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Keywords

antiretroviral drugs, CYP2B6, efavirenz, North America, Papua New Guinea, West Africa

Aims

To determine the prevalence of the novel CYP2B6 functional polymorphism 983T>C in Papua New Guinea where HIV/AIDS poses a significant health problem.

Method

We genotyped Papua New Guineans (PNG, $n = 174$), West Africans (WA, $n = 170$), and North Americans (NA, $n = 361$).

Results

The polymorphism was absent in PNG, while its overall frequency was 4.7% in WA. Among NA, the polymorphism was present in African-Americans (7.5%) and Hispanic-Americans (1.1%) but not in Caucasian-Americans and Asian-Americans. Haplotype analysis indicated that 983T>C was present alone as the CYP2B6*18 allele in WA and African-Americans.

Conclusions

Significant interethnic differences occur at the CYP2B6 locus, which may influence treatment outcomes with efavirenz.

Received

9 November 2006

Accepted

18 December 2006

Published OnlineEarly

28 March 2007

Introduction

HIV/AIDS poses significant health threats to the individuals living in West Africa and North America. Papua New Guinea (PNG) has the highest incidence of HIV/AIDS in the Pacific region, and is the fourth country in the Asian region to fit the criteria for a generalized HIV/AIDS epidemic (<http://www.who.int/hiv>). As of December 2005, the estimated HIV prevalence in PNG was 57 000 (23 000–91 000), or 2% (0.8–3.2%) of the 15–49 year old population. According to WHO and UNAIDS estimates (March 2006), <500 HIV/AIDS patients in PNG are receiving antiretroviral therapy, and 7300 (4100–12 000) are in need of treatment.

Antiretrovirals efavirenz (EFV) and nevirapine (NVP) are part of the major global HIV/AIDS treatment programs [1, 2]. These drugs are principally metabolized by the human hepatic cytochrome P450 2B6 (CYP2B6) [3, 4]. The *CYP2B6* gene (chromosome 19) is highly polymorphic. So far, 24 nonsynonymous single nucleotide polymorphisms (SNPs), a SNP resulting in a premature termination codon (R378X), and several SNPs in the *CYP2B6* promoter region have been reported. This represents a total of 28 alleles (*CYP2B6**1A [wild type] to *CYP2B6**28, <http://www.cypalleles.ki.se/cyp2b6.htm>). Many of these alleles significantly influence the CYP2B6 protein expression level and/or enzyme activity [5, 6]. The most frequent variant allele *CYP2B6**6 (516G>T [Q172H] and 785A>G [K262R]) (23–62%) [7] has shown a 65% reduction in the mean protein expression and 50% reduction in the mean enzyme activity in the homozygous state [5]. In many HIV/AIDS clinical studies, which have included patients of various ethnicities, the *CYP2B6**6 allele (or its key SNP 516G>T) was associated with two- to three-fold higher plasma EFV concentrations, 50–60% lower EFV clearance, and increased neuropsychiatric side-effects [8–11]. In one South-east Asian patient, who was homozygous for 516G>T, the plasma EFV concentration was ~30-fold higher [12]. In addition, the 516TT genotype may predict increased risk of developing drug resistance after discontinuation of EFV-containing regimens [13].

Recently, the novel *CYP2B6* SNP 983T>C (I328T, exon-7) was reported in Africans (Ghanaians, 6.6%; Tanzanians, 6.9%) [14, 15], African-Americans (4.4% and 19%) [14, 16], and Turks (4.1%) [15] but was not found in Caucasians, Swedes, Chinese, Japanese, Taiwanese, Koreans, or South-east Asians [14–16]. The SNP when present alone defines the *CYP2B6**18 allele [14] but when linked with 785A>G, defines the *CYP2B6**16 allele [15]. The SNP caused pronounced reduction in the CYP2B6 protein expression [14, 15],

and was associated with three-fold higher mean plasma EFV concentrations in African HIV patients [15]. Moreover, 516G>T and 983T>C together were associated with up to five-fold higher mean plasma EFV concentrations, suggesting an additive effect of these polymorphisms [15].

Recently, we reported high frequencies of the *CYP2B6**6 allele in PNG (62%) and West African (42%) populations [7]. We found significant differences in the *CYP2B6**6 allele and *6/*6 genotype frequencies between the two populations [7], suggesting that these populations are significantly different at the *CYP2B6* locus. In this study, we were interested to determine whether 983T>C is present in PNG, where the HIV/AIDS epidemic is escalating, since this SNP is present in African [14, 15] but absent in many Asian populations [14, 16]. We also examined West African and North American populations for comparison.

Methods

Study populations

The study populations and sample collection procedures have been previously described [7, 17]. Blood samples from Papua New Guineans ($n = 174$, from the Wosera area, East Sepik Province), DNA samples from West Africans ($n = 170$) (Ghana [$n = 33$, three villages], Guinea [$n = 32$, one village], Ivory Coast [$n = 45$, six villages], Sierra Leone [$n = 52$, six villages], and Senegal [$n = 8$, one village]), and blood samples from four major North American ethnic groups (Caucasian, $n = 93$; African, $n = 87$; Asian, $n = 87$; and Hispanic, $n = 94$) were collected under protocols including the procedures for informed consent approved by the corresponding institutional review boards. DNA extraction from the blood samples was performed as previously described [7, 17]. PNG samples were obtained from the Abelam linguistic group, while all West African samples were obtained from the West Atlantic Niger-Congo linguistic subgroup. The North American samples were collected from random blood donors, whose race/ethnicity was self-identified.

Genotyping

We used PCR primers described by Wang *et al.* [15] to amplify the genomic DNA region containing 983T>C. Following PCR, genotyping was carried out by using an oligonucleotide ligation detection reaction-fluorescent microsphere assay (LDR-FMA) on a Bio-PlexTM suspension array system (Bio-Rad Laboratories) [7, 17]. The LDR primers were: T allele 5'-CTTTTCAAATCAATACTCAACTTTAGAGAGTCTACAGGGAGAT-3', C allele 5'-CAATTAACATACATAACATACAGA

GAGTCTACAGGGAGAC-3', and common 5'-/5Phos/TGAACAGGTGATTGGCCAC/3Bio/-3'. The LDR conditions were 95°C 1 min, 95°C 15 s and 60°C 2 min (32×). Mean and 95% confidence interval values of the 983T and 983C allelic ratios were calculated to determine genotypes (983TT = 1.63 and 0.01 ($n = 674$), 983TC = -0.29 and 0.02 ($n = 31$)) [7, 17].

Statistical analysis

Differences in allele frequencies between two populations were measured using the Fisher's exact test (<http://www.matforsk.no/ola/fisher.htm>). Linkage disequilibrium (LD, D' and r^2 values) and haplotype analyses were performed by using SHEsis (<http://202.120.7.14/analysis/myAnalysis.php>).

Results

Table 1 shows the prevalence of the 983C allele (%) and 983TC genotype (number) in various populations. We did not detect 983C in any of the samples from PNG. The overall frequency of 983C in West Africa was 4.7%, comparable with previous reports [14, 15]. When further analyzed by country, the frequency of 983C ranged from 1.6% (Guinea) to 7.6% (Ghana). Small sample sizes (from 8 to 52) and/or subethnic differences among the West African populations undoubtedly contributed to the allele frequency differences observed. Among North Americans, 983C was present in African-Americans (7.5%) and Hispanic-Americans (1.1%) but not in Caucasian-Americans and Asian-Americans confirming previous observations [14–16]. Data for the Hispanic-American ethnic group have not been reported before.

Table 1

Prevalence of 983T>C allele and genotype in various populations

Population/country	<i>n</i>	983C (%)	983TC (n)
Papua New Guinean	174	0	–
West African	170	4.7	16
Ghana	33	7.6	5
Guinea	32	1.6	1
Ivory Coast	45	5.5	5
Sierra Leone	52	3.8	4
Senegal	8	6.2	1
North American	361		
Caucasian	93	0	–
Asian	87	0	–
African	87	7.5	13
Hispanic	94	1.1	2

The difference in the 983C frequencies between West Africans and African-Americans was not significant ($P = 0.23$).

Since 983T>C is in complete linkage with 785A>G defining the *CYP2B6**16 allele [15], and 785A>G is in complete linkage with 516G>T defining the *CYP2B6**6 allele [5], we performed LD and haplotype analyses for positions 516, 785, and 983 in West Africans and African-Americans using our previous 516G>T and 785A>G [7] and current 983T>C genotype data. In both groups, high D' and high r^2 values for the 516–785 pair indicated strong linkage but high D' and low r^2 values for the 516–983 and 785–983 pairs indicated no significant linkage (Table 2); 983T>C does not seem to be in linkage with either 516G>T or 785A>G, which is also reflected in our haplotype analysis. We observed 516G-785A-983T (*1), 516T-785G-983T (*6), 516T-785A-983T (*9), and 516G-785A-983C (*18) as major haplotypes in both groups (Table 2). However, there was no indication of haplotype 516G-785G-983C (*16). Based on the haplotype analysis, genotypes *1/*18, *6/*18, *9/*18, and *6/516T-785A-983C in West Africans and *1/*18 and *6/*18 in African-Americans were inferred (Table 2).

Discussion

We did not observe 983T>C in Papua New Guineans. Our results confirm that *CYP2B6* 983T>C is prevalent in Africans and African-Americans [14–16]. In five West African countries and in African-Americans, the SNP is present alone as the *CYP2B6**18 allele. Our haplotype results are concordant with those of Klein *et al.* [14], who observed the *18 allele in Ghanaians (3.1%) and African-Americans (2.9%). As reported by Wang *et al.* [15], in Central, Western, and Southern Africa, the SNP is linked with 785A>G and is present as the *CYP2B6**16 allele. This suggests ethnic as well as intraregional differences among African populations. Although comparative pharmacokinetic studies are yet to be performed, our genetic observations indicate that EFV treatment outcomes may be different in Papua New Guineans ([*CYP2B6**6 +] [983T>C –]) compared with Africans or African-Americans ([*CYP2B6**6 +] [983T>C +]).

*CYP2B6**6, *16, and *18 are considered the key functional alleles, since they cause significant reduction in *CYP2B6* protein expression [5, 14, 15] and enzyme activity [5, 14]. They have functional significance in HIV/AIDS treatment. In various HIV/AIDS clinical studies, *CYP2B6**6 (or its key SNP 516>T) and *CYP2B6**16 alleles, separately and together, were associated with significantly higher plasma EFV concentra-

Table 2Linkage disequilibrium values (D' and r^2), haplotype and genotype frequencies

		West African ($n = 170$)	African-American ($n = 87$)
<i>SNP pair</i>			
516-785	D'	1.000	0.923
	r^2	0.719	0.853
516-983	D'	0.688	0.997
	r^2	0.025	0.047
785-983	D'	0.995	0.997
	r^2	0.037	0.047
<i>Haplotype (allele)</i>			
516G-785A-983T (*1)		46.1%	54%
516T-785G-983T (*6)		41.5%	34.8%
516T-785A-983T (*9)		7.3%	1.8%
516G-785A-983C (*18)		4.2%	7.5%
516G-785G-983T (*4)		n.o.	1.8%
516T-785A-983C (?)		0.8%	n.o.
<i>Genotype</i>			
*1/*18		2.9%	10%
*6/*18		4.1%	4.6%
*9/*18		1.8%	n.o.
*6/516T-785A-983C		0.6%	n.o.

? new haplotype, warrants validation by cloning and sequencing; n.o. not observed.

tions [8–11, 15]. The mean plasma EFV concentrations were 20–30 μM in *6/*6 HIV patients [9] and were ~40 μM in *6/*16 HIV patients [15]. Plasma EFV concentrations $\geq 9 \mu\text{M}$ may predict increased risk of experiencing neuropsychiatric side-effects [18].

Previously, we observed 43% of Papua New Guineans, 17% of West Africans, and 11% of African-Americans with a *6/*6 genotype [7]. In this study, we observed 4.1% of West Africans and 4.6% of African-Americans with a *6/*18 genotype. The effect of *6/*18 genotype on plasma EFV concentrations has yet to be determined. Since CYP2B6*18 protein expression and enzyme activity were undetectable in COS-1 cells [14], we anticipate that *6/*18 would be associated with a poor EFV metabolism phenotype, and might cause elevated plasma EFV concentrations in West Africans and African-Americans.

Finally, results from this and previous studies [7, 14–16] suggest that CYP2B6 alleles may have a global impact on HIV/AIDS treatment with the widely used EFV. Considering the high worldwide prevalence of the CYP2B6*6 allele, EFV effectiveness may be compromised to a greater extent in Africans who carry both *6 and *16 or *18 alleles than in Asians, including Papua New Guineans, and those Africans who carry only the *6 allele. If that is the case, then steps need to be taken to

monitor closely for adverse outcomes of EFV, so that this drug continues to be a useful weapon in the arsenal for fighting HIV/AIDS.

We are thankful to Dr Carolyn Myers, Dr Charles King, Dr Brian Grimberg, and Mr David McNamara for their comments on the manuscript. This work was supported by grants from the National Institutes of Health (AI-52312) and the James B. Pendleton Charitable Trust to PAZ. RKM was supported by the Fogarty International Center and in part by a grant (AI-36478) from the National Institutes of Health.

Competing interests: None declared.

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