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Research Article

Characterization of inter-ethnic genetic variability of *CYP2D6, CYP2C19, CYP2B6, NAT2* and *GSTs* in the Bantu and Nilotic populations of Kenya and implications for the chemotherapy of infectious diseases

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Background: Drug metabolism genes are variable in populations. African populations are highly genetically differentiated. Analysis of drug metabolism genes offers opportunities to enhance drug efficacy and reduce toxicity.

Objectives: We characterized SNPs of *CYP2D6*, *CYP2C19*, *CYP2B6*, *NAT2* and *GST* genes in Kenyans.

Methodology: Genotyping of *CYP2C19 (*2, *3); CYP2B6 (*6)*; *CYP2D6 (*2,*4, *17, *29)*; *NAT2 (*5, *6, *7, *14); GSTM1* and *GSTT1* by PCR-RFLP.

Results: *CYP2D6*4* was higher in Eastern Nilotes (9%) compared to Western Nilotes (2.5%) and Bantus (1.7%) (P = 0.002). *CYP2D6*17* was higher in Bantus (34%) compared to Nilotes (18 – 23%) (P = 0.003). *GSTM1del* was higher in Western Nilotes and Bantus (29% -31%) compared to Eastern Nilotes (16%) (P = 0.009). *GSTT1del* was higher in Eastern Nilotes (41%) compared to Bantus and Western Nilotes (22 - 26%) (P = 0.005). CYP2C19*3 was undetected in Bantus but was >1.0% in Nilotes ((P <0.01). *CYP2C19*2* (10 – 18%), *CYP2B6*6* (35 – 37%), *NAT2*5* (30 – 42%), *NAT2*6* (20 – 27%), *NAT2*7* (2 – 6%), *NAT2*14* (8-14%) were similar in Kenyans. Kenyan frequencies were comparable to other Africans but different from Caucasians and Asians.

Discussion: Variability was evident for *CYP2D6*4*, *CYP2D6*17*, *GSTM1del* and *GSTT1del.* Findings provide a framework for Pharmacogenomic optimization of therapeutic outcomes.

Key Words: Pharmacogenomics, Drug metabolism, inter-ethnic variability, Kenyans

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1. Introduction

Genes encoding drug metabolizing enzymes exhibit significantly variable distribution among ethnic populations (Lamba et al, 2002). African populations reportedly show the greatest amount of genetic diversity and may not be taken as a homogenous group (Yu et al, 2002). Genetic studies show that some African

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populations are highly genetically differentiated especially those found in Eastern Africa where Kenya is situated (Tishkoff et al, 2009). Analysis of genes responsible for variable drug response in different populations could offer an opportunity to enhance drug efficacy and reduce toxicity. However, the scarcity of genetic data from African countries puts these populations at a distinct disadvantage of receiving the potential benefits of genetically tailored therapy. Of greater concern to tropical countries, however, is the optimization of therapeutic outcomes in the treatment of endemic infectious diseases such as malaria, tuberculosis (TB) and HIV. Equally important are genes that influence the disposition of drugs for increasingly resurgent chronic diseases such as cancer and cardiovascular diseases as well as those involved in the control of exposure to environmental toxicants such as aflatoxin.

The selection of drug metabolism genes for this study was based on their clinical relevance in the disposition of anti-infective agents for the therapy of HIV, malaria and tuberculosis (TB) (WHO, 2011). Cytochrome P450 (CYP) *2B6* and N-acetyltransferase 2 (*NAT2)* are involved in the metabolism of first line anti-retroviral and antituberculous drugs respectively. *CYP2C19* influences the metabolism of the antiretroviral agent nelfinavir, antifungal agents voriconazole and fluconazole as well as the prophylactic anti - malarial drug, proguanil (Birkett et al, 1994). *CYP2D6* is of great importance to pharmacogenetic research as it affects the metabolism of over 25% of all drugs in clinical practice (Zhou et al, 2009). Glutathione - S-transferase (*GST*) is involved in the detoxification pathway of the common analgesic, paracetamol, and the environmental toxicant, aflatoxin B_1 **(**Johnson et al, 1997; Zuppa et al, 2011).

So far, there is limited pharmacogenetic data with regard to the distribution of these genes in Kenyan populations. We aimed at characterizing the genetic variability of clinically relevant single nucleotide polymorphisms (SNPs) of *CYP2D6, CYP2C19, CYP2B6, NAT2* and *GST* in the Bantu and Nilotic populations of Kenya. Kenyan populations may be divided into three major ethno-linguistic groups, namely, the Bantu (67%), the Nilotes (30%) and the Cushites (3%) (Lewis, 2009; KNBS, 2010). This study covered the two major ethno-linguistic populations of Kenyan and may reveal new insights into the genetic diversity of Kenyan populations and the interplay between genes and disease as well as the variable response to medications.

2. Methods

2.1 Study population and sample collection

A cross-sectional study was conducted to recruit potential study subjects for this population based study. A purposive sampling strategy was adopted to recruit study subjects belonging to the Kikuyu (Bantu, who mainly live in the highland area of central Kenya), the Luo (Western Nilotes, who mainly live in western Kenya along Lake Victoria) and the Maasai (Eastern Nilotes, who mainly live in southern Kenya along the Great Rift Valley). The Bantu and the Western Nilotes were recruited from a population of medical students of the College of Health Sciences (CHS) University of Nairobi or health workers at the Kenyatta National Hospital (KNH). The Eastern Nilotes were from members of the general population residing around the Kitengela Township or Kajiado District Hospital. For each ethnic group, a sample size of 100 individuals was calculated to be sufficient to detect SNPs with frequencies higher than 0.01 at a power of 80% (Liu, 1997). Potential study subjects were interviewed using a questionnaire to collect demographic characteristics, medical and medication history, smoking and use of alcohol. Information pertaining to ethnicity up to that of grandparents was also collected. Subjects meeting study criteria were recruited sequentially till the required sample size was attained. On recruitment, a 1.0 mL blood sample was collected into an EDTA vacutainer and stored at minus twenty degrees centigrade until analyzed.

2.2 Inclusion and Exclusion criteria

Study subjects were above 18 years of age and not related to a subject already recruited into this study. They stated their ethnicity up to the first generation grandparents. They were healthy without an acute or known chronic illness, signedd a voluntary consent form and provided a blood sample for genetic studies. Subjects of mixed parentage, those on medication for treatment of any acute or chronic disease and those not willing to provide a blood sample for genetic analysis were excluded.

2.3 DNA extraction and genotyping procedure

Genomic DNA was extracted from 200 µL of whole blood using the Eppendorf Perfect gDNA Blood Mini Kit (Eppendorf AG, Hamburg, Germany) according to manufacturer's instructions. The presence of the *CYP2C19**2 and *CYP2C19**3 alleles was detected by a PCR-RFLP method described by de Morais et al, (1994 and 1994b). Genotyping for *CYP2B6* *6 was done according to the method described by Klein et al (2005). Detection of *NAT2**5, *NAT2**6, *NAT2**7 and *NAT2**14 alleles was done according to the method described by Dandara et al (2003). *GSTM1* and *GSTT1* were genotyped according to the method of Dandara et al (2002). Genotyping for *CYP2D6**4, *CYP2D6**17 and *CYP2D6**29 was performed by a PCR-RFLP method as described by Gaedigk et al (1999). The amplified products were analyzed by agarose gel electrophoresis and the molecular weight of the DNA fragments was assessed using GeneRuler™ 100 bp molecular weight marker (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.4 Data Analysis

Study subjects were categorized as either belonging to the Bantu or Nilotic ethnic groups according to the major ethno-linguistic families of Africa described by Tishkoff et al (2009) and Lewis (2009). Deviation of observed genotypes from Hardy-Weinberg equilibrium (HWE) was tested by the chi-square goodness of fit test. *GSTM1 or GSTT1* genotypes were coded as positive or as negative (deletion), thereby making direct calculation of Hardy - Weinberg equilibrium impossible. Allele and genotype frequencies were calculated for individual Kenyan populations then averaged across the three populations to derive the population mean and 95%

confidence interval (CI). Allele frequencies for Kenyan populations were compared to those reported in literature for other African, Caucasian and Asian populations. All comparisons between ethnic groups and populations were performed by the Chi-square or Fisher's exact test using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). A P value of less than 0.05 was considered as statistically significant.

2.5 Ethical considerations

Ethical approval was obtained from the Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (KNH/UoN-ERC, **Approval Reference No P72/7/2003**). Study subjects were furnished with comprehensive verbal and written information about the study. This included details about study aims, procedures and potential benefits and risks. Upon verbal consent, each subject signed a voluntary consent form before enrollment into the study.

To ensure confidentiality of study subjects, filled questionnaires were identified by a code and held in safe custody.

3. Results

3.1 Demographic characteristics of study population

Demographic characteristics of study population are shown in **Table 1**. The study population consisted of 354 healthy, unrelated adult Kenyans aged between 18 to 55 years of whom 229 (65%) were males. Majority of the study subjects were non-smokers 296 (84%) and did not take alcohol 333 (94%). In terms of ethnicity, the majority were Nilotes 252 (70%) made up of Western Nilotes (Luo) 100 (28%) and Eastern Nilotes (Maasai) 152 (43%). The Bantus were 102 (30%) and were made up of the Kikuyu.

3.2 Genotype and allele frequencies

All genotypes were found to be in Hardy-Weinberg equilibrium (data not shown). Allele frequencies for the SNPs detected in the three Kenyan populations are shown in **Table 2**.

Table 1: Demographic characteristics of the Bantu and Nilotic populations in this study

Characteristic	N	%	Tribe	Region	Linguistic family	Historic origin
Ethnicity						
Bantu	102	28.8	Kikuyu	Central	Niger-Kordofanian	West Africa
Eastern Nilotes	152	42.9	Maasai	Rift-valley	Nilo-Saharan	Sudan/Chad
Western Nilotes	100	28.2	Luo	Nyanza	Nilo-Saharan	Sudan/Chad
Gender						
Male	229	64.5				
Female	125	35.3				
Age						
Range (18 - 55 yrs)						
Age group (yrs)						
<20	32	9.0				
21 – 24	211	59.6				
25 – 29	62	17.5				
>30	49	13.8				
Alcohol						
Yes	21	5.9				
No	333	94.1				
Smoking						
Yes	58	16.4				
No	296	83.6				
Total	**354**					

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3.3 *CYP2D6* and *GSTs* inter-ethnic variability

The prevalence of *CYP2D6* SNPs detected in the three Kenyan populations is shown in **Table 2**. The null allele *CYP2D6*4*, was significantly higher in the Eastern Nilotes at 9% compared to the Western Nilotes (2.5%) and the Bantus (1.7%) (P = 0.002). The reduced activity allele *CYP2D6**17, was significantly more prevalent in the Bantus (34%) compared to the Nilotes (Western and Eastern Nilotes, 18 – 23%, P = 0.003). Another reduced activity variant, *CYP2D6**29, was detected in all the three Kenyan populations at a frequency of 7 – 11%. The most common CYP2D6 genotypes were *CYP2D6**1/*17, *CYP2D6**2/*17 and *CYP2D6**17/*17. *CYP2D6**17*17 was significantly more prevalent in the Bantu (17%) compared to the Nilotes (6.3 – 7.5%) (P = 0.042).

The inter-ethnic distribution of *GSTM1del* and *GSTT1del* in the three Kenyan populations is shown in **Table 2**. The frequency of *GSTM1del* in the Eastern Nilotes was 16% which was nearly half that found in the Western Nilotes and the Bantu (29%-31%) (P = 0.009). The frequency of *GSTT1del* was also significantly higher in the Eastern Nilotes (41%) compared to that found in the Bantu and the Western Nilotes (22 - 26%) (P = 0.005).

Table 2: The frequency of allelic variants of *CYP2D6*, *CYP2C19*, *CYP2B6*, *NAT2* and *GST* observed in the Bantu and Nilotic populations of Kenya

Allele	Prevalence (%)					
	Bantus	**Eastern Nilotes**	**Western Nilotes**	***P***	**Mean**	**95% CI**
CYP2D6						
*4	1.7	8.8	2.5	**0.002**	4.2	(2.8-6.4)
*5	3.4	5.0	3.8	0.742	4.0	(2.6-6.2)
*17	33.5	18.1	22.5	**0.003**	25	(21.4-29.0)
*29	10.8	8.1	6.9	0.424	8.7	(6.5-11.5)
N	176	160	160			
CYP2C19						
*2	15.8	10.7	17.9	0.0781	14.3	(11.8-17.2)
*3	0	0.4	0.5	**<0.01**	0.32	(0.09-1.2)
N	184	262	184			
CYP2B6						
*6	34.5	35.2	37.1	0.871	35.5	(31.8-39.5)
N	168	256	178			
NAT2						
*5	38.3	41.6	32.6	0.15079	38.1	(34.4-41.9)
*6	22.9	26.6	22.8	0.54584	24.5	(21.3-27.9)
*7	6.1	3.7	3.3	0.29434	4.3	(3.0-6.1)
*14	10.7	8.8	14.1	0.19326	10.9	(8.7-13.5)
N	196	274	184			
GST						
*M1**0	29.2	15.6	30.9	**0.0089**	23.6	(19.4-28.5)
N	89	147	94			
*T1**0	26.4	40.6	21.9	**0.0049**	31.3	(26.5-36.5)
N	87	143	96			

N = Total number of alleles

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Table 3: Comparison of allele frequencies of *CYP2D6*, *CYP2C19*, *CYP2B6*, *NAT2* and *GST* variants in Kenyans with other African, Caucasian and Asian populations

Allele	Prevalence %					
	Bantus	Eastern .Nilotes	Western Nilotes	Zimbabweans$^{\Phi}$	Germans$^{\Phi}$	Han Chinese$^{\Phi}$
$^{¥}$*CYP2D6*						
*4	1.7	8.8	2.5	2	20	1
*17	33.5	18.1	22.5	34	2.1	0
*29	10.8	8.1	6.9	17	0	0
§*CYP2C19*						
*2	15.8	10.7	17.9	13	15	26
*3	0	0.4	0.5	0	0	5.2
$^{\phi}$*CYP2B6*						
*6	34.5	35.2	37.1	49	25	21
$^{\Psi}$*NAT2*						
*5	38.3	41.6	32.6	31	47	5
*6	22.9	26.6	22.8	21	28	30
*7	6.1	3.7	3.3	6	1.3	12
*14	10.7	8.8	14.1	14	0	0
†*GST*						
M1del	29.2	15.6	30.9	24	51	54
T1del	26.4	40.6	21.9	26	21	58

3.4 The distribution of *CYP2C19*, *CYP2B6* and *NAT2* variant alleles

The frequencies of the variant alleles *CYP2C19* (*2 and *3), *CYP2B6* (*6) and *NAT2* (*4,*5, 6*,*7,*14) are shown in **Table 2**.

*CYP2C19**3 was undetected in the Bantus while occurring at low frequencies of less than 1.0% in the Nilotic populations (P<0.01). There were no significant differences in the prevalence of the other variant alleles between the three Kenyan populations. The frequency of the null allele *CYP2C19**2 was in the range of 10 – 18% in the three Kenyan populations. *CYP2B6*6* occurred at a frequency of 35 – 37% whereas *CYP2B6*6/*6* was found at 12 – 16% across the three Kenyan populations. The prevalence of *NAT2*5* (30 – 42%), *NAT2*6* (20 – 27%), *NAT*7* (3-6%) and *NAT*14* (8-14%) was similar in the three Kenyan populations. In terms of genotypes, *NAT2**4/*5 was the most abundant rapid acetylator genotype with a frequency of 18 - 20% whereas the slow acetylator genotypes of *NAT2*5/*5* (13 – 18%) and *NAT2*5/*6* (11 – 19%) were the most prevalent.

3.5 Inter-population comparison of allelic variants

The frequencies of allelic variants of *CYP2D6*, *CYP2C19*, *CYP2B6*, *NAT2* and *GST* detected in Kenyan populations were compared to those reported in literature for other African, Caucasian and Asian populations (**Table 3**). *CYP2D6*4* was low in Kenyans (2 – 9%) and Zimbabweans (2%) compared to the high frequencies in Caucasians (Germans, 20%). *CYP2D6*17* was high in Kenyans (18 – 33.5%) and Zimbabweans (34%) but has not been detected in Caucasians and Asians.

The *CYP2C19*2* was lower in Kenyans (11% - 18%), Zimbabweans (13%) and Caucasians (15%) compared to Asians (the Han Chinese, 26%). *CYP2C19*3* was higher in Asians (5%) compared to >1% in Kenyan Nilotes. *CYP2B6*6* was higher in Kenyans and other Africans (35 - 49%) compared to Caucasians (26%) and Asians (21%). The prevalence of *NAT2* variant alleles was similar in Kenyans and other African populations but was variable between Kenyans, Caucasians and Asians. *NAT2*5* was comparable in Kenyans (33-42%) and Caucasians (51%) but much lower in Asians (5%). *NAT2*7* was higher in Asians (12%) compared to Kenyans (3 - 6%) and Caucasians (1.3%). *NAT2*14* has not been reported in Caucasians and Asians.

4. Discussion

4.1. Population pharmacogenetic diversity

We analyzed SNPs of genes involved in the disposition of drugs commonly used in the chemotherapy of endemic infectious diseases in Kenya. We report significant variability in the distribution of *CYP2D6*4, CYP2D6*17, GSTM1del and GSTT1del* between the Bantu and Nilotic populations of Kenya. To our knowledge, this is the first study to assess the variability of drug metabolizing genes between the Nilotic and Bantu

populations of East Africa. Previous pharmacogenetic studies in Eastern Africa have targeted the Bantu and Cushitic populations (Masimirembwa et al, 1993, Aklillu et al, 1996, Dandara et al, 2001). The Bantu and the Nilotes are the two major ethnic populations of Kenya which are deemed different due to linguistic and geographic divide.

We observed a high prevalence of *CYP2D6*4* in the Eastern Nilotes compared to other East African populations (Dandara et al, 2001) but in similarity to African Americans (7%) (Gaedigk et al, 2005) and Ghanaians (7%) (Yen-Revollo et al, 2009). *CYP2D6*4* is a splice-site mutant allele that is reported to be the most frequent null allele in Caucasians, occurring at a high frequency of 17.2% (Sistonen et al, 2007). Its presence in the Eastern Nilotes (Maasai) seems to point to gene flow from Caucasoid populations, possibly due to the intense focus on the Maasai in international tourism.

CYP2D6 variant alleles exhibited great inter-ethnic variability in our study population which confirms previously reported observations that the *CYP2D6* locus is subject to remarkable inter - ethnic and inter-population genetic diversity (Zhou et al, 2009). The *CYP2D6*17* (T107I, R296C) is a variant allele that exhibits substrate dependent diminished affinity and activity of up to 50% (Oscarson et al, 1997). We report a high prevalence of *CYP2D6*17* in Kenyan Bantus in conformity with the landmark study in Zimbabweans (Masimirembwa et al, 1996). The clinical implication of this African specific allele on treatment outcomes of substrate drugs in African populations remains to be explored.

Our study also revealed a disparity in the distribution of *GSTM1del* and *GSTT1del* in the Eastern Nilotes on the one hand and the apparent convergence of the Bantu and the Western Nilotes on the other. This distribution is believed to be a reflection of the high levels of admixture among some Nilo-Saharan speakers such as the Western Nilotes (Luo) and geographically diverse Bantu populations (Niger-Kordofanian speakers) as reported by (Tishkoff et al, 2009). In this study, the observed frequency of *GSTM1del* in Kenyan Bantus and the Western Nilotes (Luo) was in agreement with that reported for other African populations such as Tanzanians (36%) (Dandara et al, 2002) but different from Egyptians (55%) (Hamdy et al, 2003) reflecting the pharmacogenetic diversity of African populations.

Traces of *CYP2C19*3* was observed in Kenyan Nilotes in this study. *CYP2C19*3* is a predominantly Asiatic allele and its presence in Kenyan Nilotes could be attributed to gene flow between the Nilotes and the Cushitic populations (Afro-Asiatic speakers) possibly during the period of Nilotic migration from Chad and Sudan to East Africa as has been observed in other studies (Tishkoff et al, 2007).

4.2 Clinical relevance of pharmacogenetic variability to the therapy of infectious diseases

The high prevalence of the *CYP2B6*6* (T) and the homozygous genotype *CYP2B6*6/*6* (516TT) in the three Kenyan populations is consistent with reports from most sub-Saharan African populations (Mukonzo et al, 2009). The clinical relevance of *CYP2B6* 516 G>T in patients exposed to substrate drugs continue to attract intense interest. Studies covering HIV patients within East Africa point to the influence of *CYP2B6* 516 G>T not only on the pharmacokinetics of efavirenz and nevirapine but also on clinical outcomes. (Ngaimisi et al, 2010; Ngaimisi et al, 2013). Incorporation of *CYP2B6* 516 G>T genotypes in pharmacokinetic modeling of efavirenz dosage has led to suggestions of a dosage reduction from 600 mg to 450 mg for Africans in general and 300 mg for those who are homozygous to the mutation (*CYP2B6* 516 TT) (Nyakutira et al, 2008, Mukonzo et al, 2014). These observations should be of great interest since the majority of HIV patients in Kenya are on either nevirapine or efavirenz based first - line antiretroviral therapy.

NAT2 acetylation as well as *GSTM1del* and *GSTT1del* genotypes reported in this study bears potential clinical implications for Kenyan patients with TB and or HIV. Studies have associated *NAT2* slow acetylation and *GSTM1* deletion genotypes with increased incidence of isoniazid induced hepatitis (Yimer et al, 2008; Wang et al, 2011). NAT2 slow acetylation has also been associated with increased incidence of serious adverse drug reactions during treatment with co-trimoxazole, a drug currently used by a large number of Kenyan HIV patients as an anti-infective prophylactic agent (Kim et al, 2010). A recent study in a Zimbabwean HIV-TB co-infected cohort has explored the role of *NAT2* in isoniazid induced peripheral neuropathy (Dhoro et al, 2013) which has sparked further interest in follow up studies.

Results from this study showed that 29% of Kenyan populations bear *CYP2D6* intermediate metabolizer (IM) genotypes. The commonly used drugs that are metabolized by CYP2D6 enzyme include analgesics such as codeine; antidepressants such as amitriptyline; cardiovascular agents such as captopril and anticancer drugs such as tamoxifen (Ingelman-Sundberg, 2005). The high prevalence of *CYP2D6* intermediate metabolizer genotype in Kenyan populations therefore has implications for the optimization of therapy with these categories of drugs. CYP2D6 inhibitors are also commonly used in Kenyan populations for the therapy of tropical diseases such as helminthiasis and malaria. Amodiaquine, an antimalarial drug has been reported to cause significant inhibition of CYP2D6 together with its active metabolite N-desethylamodiaquine, both in vivo (Wennerholm et al, 2006) and in vitro (Bapiro et al, 2001). The IM metabolizer status becomes a matter of clinical concern during concurrent use of CYP2D6 enzyme inhibitory drugs which could convert CYP2D6 extensive metabolizers (EM) to poor metabolizers (PM) in a phenomenon known as phenocopying (Ebner and Eichelbaum, 1993).

4.3 Study strengths and limitations

A major strength for this study was the adequate sample size of the populations studied which were estimated to detect SNPs occurring at frequencies higher than 0.01. Sample sizes for the studied populations were not similar, but increasing the sample size does not affect variability of the SNPs detected within each population. It may nonetheless have an impact on the precision of the frequencies of the SNPs

detected in the underlying ethnic population (Holsinger and Weir, 2009). Thus, it is not expected that the higher sample size of the Maasai (152) would provide more representative SNPs compared to the Luo (100) or the Kikuyu (102). A limitation for this study was the lack of sequencing facilities to explore novel SNPs in Kenyan populations.

5. Conclusion

Our findings have revealed interethnic variability in clinically relevant SNPs of *CYP2D6, GSTM1* and *GSTT1* in Kenyan populations. We have provided new pharmacogenetic data on African populations which is deemed timely to position African populations to benefit from recent advancements in technology and genomic knowledge. For example, the approval of various pharmacogenomic tests by the FDA could herald the search for population specific diagnostic tests covering SNPs such as *CYP2D6*17*, *CYP2B6*6* and *NAT2*14* which are specific to African populations. Optimization of efavirenz dosage based on the high prevalence of *CYP2B6* 516 T calls for multi-centre clinical studies in affected African populations.

Conflict of Interest declaration

The authors declare no conflict of interest

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