Pharmacogenetic considerations in the treatment of co-infections with HIV/AIDS, tuberculosis and malaria in Congolese populations of Central Africa

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ABSTRACT

Background: HIV-infection, tuberculosis and malaria are the big three communicable diseases that plague sub-Saharan Africa. If these diseases occur as co-morbidities they require polypharmacy, which may lead to severe drug–drug-gene interactions and variation in adverse drug reactions, but also in treatment outcomes. Polymorphisms in genes encoding drug-metabolizing enzymes are the major cause of these variations, but such polymorphisms may support the prediction of drug efficacy and toxicity. There is little information on allele frequencies of pharmacogenetic variants of enzymes involved in the metabolism of drugs used to treat HIV-infection, TB and malaria in the Republic of Congo (ROC). The aim of this study was therefore to investigate the occurrence and allele frequencies of 32 pharmacogenetic variants localized in absorption distribution, metabolism and excretion (ADME) and non-ADME genes and to compare the frequencies with population data of Africans and non-Africans derived from the 1000 Genomes Project.
Results: We found significant differences in the allele frequencies of many of the variants when comparing the findings from ROC with those of non-African populations. On the other hand, only a few variants showed significant differences in their allele frequencies when comparing ROC with other African populations. In addition, considerable differences in the allele frequencies of the pharmacogenetic variants among the African populations were observed.
Conclusions: The findings contribute to the understanding of pharmacogenetic variants involved in the metabolism of drugs used to treat HIV-infection, TB and malaria in ROC and their diversity in different populations. Such knowledge helps to predict drug efficacy, toxicity and ADRs and to inform individual and population-based decisions.

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Background

HIV/AIDS, tuberculosis (TB) and malaria, the big three killlers, are diseases with high prevalences and considerable mortality in many developing countries. The world-wide prevalence of these diseases is over 250 million, with sub-Saharan Africa accounting for 71%, 25% and 92% of HIV/AIDS, TB and malaria, respectively (Chaudhry et al., 2016). As in many other sub-Saharan African countries, transmission of the malaria-causing parasite *Plasmodium falciparum* is high in the Republic of Congo (ROC) (WHO, 2015b), together with a high burden of TB and HIV-infection (Linguissi et al., 2017). These diseases overlap almost completely, leading to a high rate of concurrent occurrence of HIV/TB, TB/malaria and HIV/TB/malaria; however, incidences vary widely between individuals and communities. Comorbidities require a polypharmaceutical approach, which may lead to drug–drug-gene interations and drug-induced pathology, variations in adverse drug reactions (ADR) and therapeutic efficacy as well as the development of drug resistances (Alomar, 2014; Bassi et al., 2017; Rattan et al., 1998; Turner and Wainberg, 2006). It has recently been reported that the 25 most common drugs causing ADR in Africa are drugs used to treat TB, malaria and HIV-infections (Rajman et al., 2017).

Treatment of TB involves combinations of drugs to be taken over an extended period of time and in HIV-infection, the respective drug combinations are taken lifelong. The first-line treatment of HIV-infection includes highly active antiretroviral (HAART) drugs, with dolutegravir, efavirenz, raltegravir and nevirapine being recommended by WHO (2019). Standard first-line TB treatment consists of isoniazid, rifampicin, pyrazinamide and ethambutol for two months, followed by isoniazid and rifampicin for another four months. For the treatment of un-complicated malaria, artemisinin combination therapies (ACTs) such as artesunate-amodiaquine, artemether-lumefantrine and other combinations are the most commonly used drugs used in sub-Saharan Africa (WHO, 2015a). When multiple drugs are administered, the treatment outcomes, in particular the efficacy, ADRs and the degree of emergence of drug resistance vary at the individual and population level. These variations are influenced by the age, sex and weight of patients, dosage, comorbidities and genetic factors. It is known that up to 95% of variations in treatment outcomes can be explained by genetic factors alone (Ross et al., 2012). Genetic studies at the population level and the 1000 Genome Project Phase 3 indicate that African ethnic groups are the most genetically diverse populations (Tishkoff et al., 2009). Regional allele frequencies of African genomes also show a high degree of variation (Gurdasani et al., 2015; Ramos et al., 2014).

Genetic variations in drug absorbtion, distribution, metabolism and excretion (ADME) genes both within and between populations can influence the pharmacokinetics and pharmacodynamics of drugs (Ma et al., 2002; Wilson et al., 2001) and lead to differences in treatment outcomes, drug resistance and increased toxicity (Li et al., 2011). Pharmacogenomic studies of drugs used to treat the three diseases have been extensively conducted and variants in ADME and non-ADME genes have been found associated with treatment outcomes and toxicity. Some of the common genes include *CYP2B6, CYP3A5, CYP3A4, CYP2A6, ABCB1, NR1I3, UGT2B7, NAT2, CYP2E1, GSTMI, GSTTI, SLCOB1B1* and *UGT1A9* (Bains, 2013; Chaudhry et al., 2016). While translation of pharmacogenetic information into clinical practice has been slow, pharmacogenomic drug labels are increasingly being approved by the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), Swiss Agency of Therapeutic Products (Swissmedic) and the Pharmaceuticals and Medical Devices Agency, Japan (PMDA) (Whirl-Carrillo et al., 2012).

There are very few studies (Peko et al., 2019a, b) available so far on the allele frequencies of pharmacogenetic variants associated with the outcomes of the drugs used to treat the three big diseases in the Republic of Congo (ROC). The main objective of the present study is to identify allele frequencies of recognized pharmacogenetic variants that are associated with treatment outcomes of HIV/AIDS, TB and malaria in the ROC and to compare the frequencies with those in other African and non-African populations. Such knowledge helps to predict drug efficacy, toxicity and ADRs and inform individual and population-based decisions.

Materials and methods

Study population

Ethical approval was obtained from the Institutional Ethics Committee of the Fondation Congolaise pour la Recherche Médicale in Brazzaville, ROC. Following written informed consent from parents/guardians, a total of 67 children aged between one and ten years with fever were included randomly out of a cohort which was recruited for a previous study at the Marien Ngouabi Hospital in the north of Brazzaville (Etoka-Beka et al., 2016; Gampio Gueye et al., 2019). Two millilitres of venous blood were available in EDTA tubes stored at −20 °C.

SNP variants and selection criteria

SNPs with functional and clinical significance for drugs used to treat HIV-infection, TB and malaria were selected from the Pharmacogenomics Knowledge Base (PharmGKB; www.pharmgkb.org). SNPs with allele frequencies >10% known to occur in African populations based on the data from 1000 Genome Project were considered in our study.

The custom ordered TaqMan™ SNP panel which was designed and applied included 32 SNPs located in 18 ADME and non-ADME genes; (Table 1). ADME genes included those encoding ATP Binding Cassette Subfamily B Member 1 (*ABCB1*), cytochrome P450 family (*CYPs*) CYP2A6, CYP2B6, CYP2D6, CYP2C19, CYP2C8, CYP3A4, CYP3A5, N-acetyltransferase 2 (*NAT2*), solute carrier organic anion transporter family member 1B1 (*SLCO1B1*), UDP-glucuronosyltransferase family (*UGT1A1*). Non-ADME genes included those encoding glucose-6-phosphate dehydrogenase (*G6PD*), nuclear factor Kappa B subunit 1 (*NF-κB1*), tumor necrosis factor (*TNF-α*), nuclear receptor subfamily 1, group I, member 2 (*NR1I2*), cut like homeobox 2 (*CUX2*) and ATP/GTP binding protein like 4 (*AGBL4*).

DNA isolation, genotyping and quality control

Genomic DNA was extracted from the blood samples using the QIAamp DNA Minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quantity and quality of DNA was determined with the NanoDrop™ (Thermo Fisher Scientific Inc.) and Qubit™ 4 Fluorometer using the Qubit™ dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA concentrations ranged between 1–40 ng/μL. Genotyping of the 32 SNPs (Thermo Fisher assay IDs; Supplementary Table 1) were performed by allele discrimination with TaqMan® SNP Genotyping Assays on a Thermo QuantStudio5™ Real-Time 384 well PCR system (Thermo Fisher Scientific, Life Technologies, Carlsbad, USA) according to the manufacturer's protocol. 5 μL final reaction volume was used with a recommended DNA concentration of 0.2 ng/μL. Data obtained was loaded into the TaqMan® Genotyper Software (Thermo Fisher, Life Technologies, Carlsbad, USA) to automatically retrieve the genotyping data. Hardy–Weinberg Equilibrium (HWE) was calculated and only SNPs that passed underwent statistical analysis.

Table 1
ADME and non-ADME gene variants with clinical significance in drugs used to treat malaria, tuberculosis and HIV-infection.

Gene	SNP ID	Defining SNP	Phenotype/Association	HIV, TB and Malaria drug substrates	Refs
Absorption, distribution, metabolism and excretion (ADME)					
ABCB1	rs1045642	ABCB1*6_3435C>T (I1145I)	Null	Rifampicin, Efavirenz, Nevirapine, Nelfinavir, Atazanavir and Ritonavir	Ciccacci et al. (2010); Park et al. (2010); Yimer et al. (2011)
	rs3842	ABCB1_c193A>G (3'UTR)	Not defined	Efavirenz	Elens et al. (2010)
CYP2A6	rs28399433	CYP2A6*9_-48T>G (Promoter)	Reduced function	Efavirenz	Shahi et al. (1990); Soeria-Atmadja et al. (2017)
CYP2B6[a]	rs28399499	CYP2B6*18_c.983T>C (I328T)	Reduced function	Efavirenz and Nevirapine	Ciccacci et al. (2013); Gandhi et al. (2012)
	rs3745274	CYP2B6*6_c.516G>T (Q172H)	Reduced function	Efavirenz and Nevirapine	Cusato et al. (2016); Sinxadi et al. (2015)
CYP2D6[a]	rs1065852	CYP2D6*10_c.100T>C (S34P)	Intermediate function	Hydroxychloroquine, Tafenoquine and Primaquine	Bennett et al. (2013); Saito et al. (2018); St Jean et al. (2016)
CYP2C19[a]	rs12768009[b]	CYP2C19_c.168+3235G>A (Intron)	Reduced function	Nevirapine	Lehr et al. (2011)
	rs4244285	CYP2C19*2_19154G>A (P227P)	Reduced function	Nelfinavir	Haas et al. (2005); Kattel et al. (2015)
CYP2C8	rs11572103	CYP2C8*2_c.805A>T (I269F)	Reduced function	Amodiaquine	Parikh et al. (2007)
CYP3A5[a]	rs776746	CYP3A5*3_6986A>G (Splice defect)	Loss of function	Lumefantrine, Atazanavir, Ritonavir and Nevirapine	Brown et al. (2012); Castillo-Mancilla et al. (2016); Mutagonda et al. (2017)
	rs10264272	CYP3A5*6_14690G>A (K208K)	Reduced function		
	rs41303343	CYP3A5*7_27132insA (-346S)	Reduced function		
CYP3A4[a]	rs2740574	CYP3A4*1B_-392A>G (Intergenic)	Reduced function	Efavirenz, Atazanavir, Lumefantrine and Indinavir	Bertrand et al. (2009); Haas et al. (2005); Kile et al. (2012); Mutagonda et al. (2017)
NAT2[a]	rs1801280	NAT2*5_341T>C (I114T)	Slow acetylator function	Isoniazid/TB treatment	Ben Mahmoud et al. (2012); Chan et al. (2017); Shi et al. (2015); Singla et al. (2014)
	rs1799930	NAT2*6_590G>A (R197Q)	Slow acetylator function		
	rs1799929	NAT2*11_481C>T (L161L)	Association		
	rs1208	NAT2*12_803A>G (K268R)	Fast acetylator function		
	rs1041983	NAT2*13_282C>T (Y94Y)	Fast acetylation function		
	rs1495741	NAT2_18415371G>A (Tag SNP)	Association	Isoniazid/TB treatment	Chamorro et al. (2016); Ho et al. (2013)
SLCO1B1	rs2306283	SLCO1B1*1b_388A>G (N130D)	Enhanced function	Rifampicin and Efavirenz	Kwara et al. (2014)
	rs4149032	SLCO1B1*5_c.85-7793C>T (Intron)	Reduced function	Rifampicin and Isoniazid	Calcagno et al. (2019); Chigutsa et al. (2011)
UGT2B7	rs7439366[b]	UGT2B7*2_2100C>T (Y268H)	Reduced/Enhanced function (Substrate dependent)	Efavirenz	Cusato et al. (2016); Kwara et al. (2009)
UGT1A1[a]	rs3064744	UGT1A1*28_TA (Indel)	Reduced function	Atazanavir, Dolutegravir, Ritonavir and Raltegravir	Johnson et al. (2014); Vardhanabhuti et al. (2015)
	rs10929303	UGT1A1_1813C>T (3'UTR)	Reduced function	Atatanavir and Ritonavir	Nishijima et al. (2014)
Non-ADME					
G6PD[a]	rs1050828	G6PD_c.202G>A (V68M)	Loss of function	Chlorproguanil, Dapsone, Mefloquine, Amodiaquine, Pyrimethamine and Sulfadoxine	Fanello et al. (2008); Sicard et al. (1978)
	rs1050829	G6PD_c.376A>G (N126D)			
NF-κB1	rs4647992	NF-κB1_c.159+305C>T (Intron)	Association	TB treatment	Zhang et al. (2019)
TNF-α	rs1800629	TNF-α_-308G>A (Regulatory)	Association	Ethambutol, Isoniazid, Pyrazinamide and Rifampicin	Kim et al. (2012)
NR1I2	rs2472677	NR1I2_63396C>T (Intron)	Association	Atazanavir, TB and HIV drugs, and Isoniazid	Calcagno et al. (2019); Schipani et al. (2010); Siccardi et al. (2008)
CUX2	rs7958375	CUX2_c.64-11987G>A (Intron)	Association (GWAS)	Rifampicin	Petros et al. (2016)
AGBL4	rs319952	AGBL4_c.206+5387T>C (Intron)			
	rs320003	AGBL4_c.91+2046C>T (Intron)			

Note: All SNPs are reported in PharmGKB except rs4647992.
GWAS, genome wide association study.
[a] Pharmacogenetic labelling for TB/HIV/Malaria drugs approved by US Food and Drug Administration (FDA)/European Medicines Agency (EMA).
[b] LD SNPs used for genotyping (rs12768009 - rs4986894, rs7439366 - rs7438135, rs3064744 - rs887829).

Data analysis

Five SNPs failed in the assessment of Hardy–Weinberg Equilibrium (HWE). The allele frequencies of the remaining 27 SNPs were compared with those of non-African and African populations derived from data of the 1000 genome phase 3 project (www.ensembl.org). This database includes data of non-African populations, namely of mixed American (AMR), East Asian (EAS), European (EUR) and South Asian (SAS) groups and of African (AFR) populations. The latter include Yoruba in Ibadan, Nigeria (YRI), Luhya in Webuye, Kenya (LWK), Gambian in the West Coast Region, The Gambia (GWD), Mende in Sierra Leone (MSL), Esan in Nigeria (ESN), Americans of African descent in the South-West of the USA (ASW) and people of African-Caribbean descent from Barbados

(ACB). Pairwise comparisons of genotypes among the populations were done using Fisher's exact test, taking into account a p-value of <0.05.

Results

Using a custom-designed panel of SNPs we genotyped in 67 samples 32 SNPs localized in 12 ADME and 6 non-ADME genes which are known to have pharmacokinetic and pharmacodynamic relevance for drugs used to treat TB, HIV/AIDS and malaria. Details of all SNPs, genes, drug substrates and their pharmacogenetic relevance are given in Table 1. The allele frequencies of the 32 variants are shown in Table 2. Five out of the 32 SNPs failed in the assessment of HWE (CYP2B6*6, CYP2D6*10, rs1050828 and

rs1050829 [*G6PD*], rs4647992 [*NF-κB1*]) and were excluded from further statistical analysis. Failure of SNPs passing HWE could be due to the small sample size or errors in genotyping.

Allele frequencies in the ROC and non-African populations

Non-African populations included mixed American (AMR), East Asian (EAS), European (EUR) and South Asian (SAS) groups.

A pairwise comparison of the allele frequencies observed in ROC with these four non-African populations revealed significant differences in allele frequencies for the majority of SNPs tested (Table 2; p-values in Supplementary Table 2). 18 polymorphisms in the AMR group and 21 polymorphisms in the EAS, EUR and SAS populations showed significant differences. In the comparison of ROC with all non-African populations, the frequencies of 10 polymorphisms were significantly different, namely those of *ABCB1*6, *CYP2B6*18, *CYP2C8*2, *CYP3A5*3, *CYP3A5*6, *CYP3A5*7, *CYP3A4*1B, *SLCO1B1*5, *UGT1A1_1813C>T* and rs7958375-*CUX2*. Significant differences of allele frequencies applied also to 16 polymorphisms in some of the non-African populations only, namely rs3842-*ABCB1* (AMR, EUR), *CYP2A6*9 (EAS, EUR), rs12768009-*CYP2C19* (AMR, EAS, SAS), *CYP2C19*2 (AMR, EAS, SAS), *NAT2*5 (EAS), *NAT2*6 (EAS, EUR, SAS), *NAT2*11 (EAS, EUR), *NAT2*12 (EAS, SAS), *NAT2_18415371G>A* (EAS, EUR, SAS), *SLCO1B1*1b (AMR, EUR, SAS), *UGT2B7*2 (EUR, SAS), *UGT1A1*28 (AMR, EAS, EUR), rs1800629-*TNF-α* (AMR, EAS, SAS), *rs2472677-NR1I2* (EAS, EUR, SAS), rs319952-*AGBL4* (AMR, EUR, SAS) *and* rs320003-*AGBL4* (AMR, EUR, SAS). This is in concordance with published literature (Rajman et al., 2017; Xing et al., 2010), which describes significant genetic differences between non-African and African populations.

Allele frequencies in the ROC and other African populations

Other African populations with which comparisons were made included Yoruba in Ibadan, Nigeria (YRI), Luhya in Webuye, Kenya (LWK), Gambians in the West Coast Region, The Gambia (GWD), Mende in Sierra Leone (MSL), Esan in Nigeria (ESN), Americans of African descent in the South-West of the USA (ASW) and people of African-Caribbean descent from Barbados (ACB).

Pairwise comparisons of the allele frequencies of the 27 SNPs in the ROC population with the other African populations were made. Significant differences were found for only a few of the SNPs (Table 2; p-values in Supplementary Table 2). In detail, 5 polymorphisms in the ACB group, 3 polymorphisms in ASW, 5 polymorphisms in ESN, 2 polymorphisms in GWD, 5 polymorphisms in LWK, 5 polymorphisms in MSL and 4 polymorphisms in the YRI population showed significant differences of allele frequencies. Polymorphisms exhibiting significant differences in comparisons of the ROC with other African populations are rs3842-*ABCB1* (ACB, GWD), *CYP2A6*9 (ACB, GWD), *CYP2B6*18 (ACB, ESN, LWK), *CYP3A5*3 (ASW), *CYP3A5*6 (LWK), *NAT2*5 (MSL, YRI), *NAT2*6 (ACB, ASW, ESN, LWK), *NAT2*11 (ESN, MSL, YRI) *NAT2*13 (ACB, ESN, MSL, YRI), *SLCO1B1*5 (ESN), *UGT1A1_1813C>T* (LWK, MSL), rs1800629-*TNF-α* (ACB, GWD), and *rs2472677-NR1I2* (MSL, YRI). As expected, the number of SNPs with frequency differences in the ROC compared to other African populations was very small. Despite the low degree of differences, there are enormous

Table 2
Allele frequencies of 32 SNPs in Republic of Congo genotyped and allele frequencies in non-African and African populations from the 1000 Genome Project phase 3 data.

Gene	SNP ID	Risk Allele	Non-African populations				African populations								ROC (Genotyped)	HWE (P-values)
			AMR	EAS	EUR	SAS	AFR	ACB	ASW	ESN	GWD	LWK	MSL	YRI		
ABCB1	rs1045642	T	43	40	52	58	12–19	15	19	12	19	14	15	13	17	0.4
	rs3842	G	16	30	14	18	4–25	4	17	17	12	25	16	15	24	0.06
CYP2A6	rs28399433	G	10	24	7	15	6–11	6	11	10	6	9	7	10	14	0.51
CYP2B6	rs28399499	C	1	0	0	0	6–12	6	10	7	10	6	8	12	16	0.74
	rs3745274	T	37	22	24	38	35–41	38	35	41	35	36	35	40	36	0.02[a]
CYP2D6	rs1065852	T	15	57	20	17	4–16	15	16	9	12	4	17	11	8	0.01[a]
CYP2C19	rs12768009	A	10	37	15	37	14–23	16	14	22	14	23	19	17	19	0.79
	rs4244285	A	11	31	15	36	13–21	15	14	21	13	21	18	17	19	0.79
CYP2C8	rs11572103	A	1	0	0	1	14–24	21	15	20	24	14	17	20	21	0.13
CYP3A5	rs776746	G	20	29	6	33	69–89	75	69	89	77	88	88	83	84	0.86
	rs10264272	A	2	0	0	0	5–24	5	14	16	24	16	17	11	11	0.15
	rs41303343	AA	0	0	0	0	9–14	11	12	9	14	12	14	12	12	0.27
CYP3A4	rs2740574	G	10	0	3	4	66–83	66	67	77	79	83	83	76	75	0.96
NAT2	rs1801280	C	36	4	45	35	24–36	28	31	27	34	36	24	24	36	0.75
	rs1799930	A	17	26	28	36	19–29	26	29	26	19	27	22	20	16	0.47
	rs1799929	T	34	4	44	32	17–33	25	26	19	28	33	19	17	29	0.69
	rs1208	G	37	4	44	36	35–46	38	36	39	44	46	35	37	46	0.51
	rs1041983	T	29	44	31	43	39–53	50	45	53	39	43	50	50	36	0.75
	rs1495741	A	65	48	76	78	51–67	59	67	56	57	66	54	51	63	0.39
SLCO1B1	rs2306283	A	53	24	60	45	12–25	21	25	12	19	16	19	19	17	0.45
	rs4149032	C	61	40	66	49	19–36	30	36	19	23	28	27	28	24	0.82
UGT2B7	rs7439366	T	32	28	49	40	18–28	28	25	24	19	25	18	21	25	0.4
UGT1A1	rs3064744	(TA)8	36	13	29	40	38–46	43	42	40	38	46	41	46	47	0.44
	rs10929303	T	22	13	23	17	32–50	46	39	50	47	32	33	45	46	0.3
G6PD	rs1050828	A	1	0	0	0	4–21	13	17	16	4	18	7	21	19	<.0001[a]
	rs1050829	G	3	0	0	0	28–38	33	29	35	36	34	28	38	40	<.0001[a]
NF-κB1	rs4647992	T	3	4	5	4	8–24	10	9	10	8	24	8	10	9	0.03[a]
TNF-α	rs1800629	A	7	6	13	5	7–14	14	7	13	14	9	16	10	18	0.34
NR1I2	rs2472677	T	48	62	66	57	28–42	42	35	36	38	40	28	36	48	1
CUX2	rs7958375	G	98	100	100	99	76–86	83	86	85	84	79	76	81	80	0.19
AGBL4	rs319952	T	51	67	40	46	63–80	70	63	71	80	67	78	74	73	0.47
	rs320003	C	51	67	40	46	64–80	69	64	75	80	74	80	74	73	0.6

Bold and underlined: statistically significant (P < 0.05, Fisher exact test) pairwise comparison with congo population (P-values in Supplementary Table 2).
African (AFR), Yoruba in Ibadan, Nigeria (YRI), Luhya in Webuye, Kenya (LWK), Gambians in the West Coast Region, The Gambia (GWD), Mende in Sierra Leone (MSL), Esan in Nigeria (ESN), Americans of African Ancestry in SW USA (ASW), African Caribbeans in Barbados (ACB), Mixed American (AMR), East Asian (EAS), European (EUR), South Asian (SAS) and Republic of Congo (ROC).
[a] Failed Hardy–Weinberg equilibrium (HWE).

differences in allele frequencies between all African populations as can be seen in the AFR column of Table 2, which shows the lowest and highest allele frequencies for all genetic variants. For example, the allele frequency of rs3842 SNP ranges from 4% to 25%. For some SNPs the allele frequencies among African populations do not differ significantly however, due to the high burden of disease this may lead to considerable differences in treatment efficacy and drug toxicity.

Discussion

In this study, we performed Taqman genotyping assays of SNPs located in 18 genes in 67 samples of febrile children recruited in ROC (Etoka-Beka et al., 2016; Gampio Gueye et al., 2019).

CYP variations

Cytochromes P450 (CYPs) are a well characterized family of phase 1 drug metabolising enzymes involved in the metabolism of 70–80% of drugs. Polymorphisms in these genes are associated with altering the pharmacokinetics of several drugs and leading to ADRs (Phillips et al., 2001) or suboptimal clinical outcome. CYP2B6*6 and CYP2B6*18 alleles exhibit reduced enzymatic activity and increased plasma concentrations of efavirenz and nevirapine, which may lead to ADRs (Ciccacci et al., 2013; Cusato et al., 2016; Gandhi et al., 2012). Due to its clinical significance, CYP2B6*6 is approved for efavirenz drug labelling by FDA/EMA/Swissmedic/PMDA (Whirl-Carrillo et al., 2012). Studies have shown that in patients with the CYP2B6*6 homozygous allele, reduction in the daily dose of efavirenz from 600 to 200 mg/day was sufficient to maintain therapeutic levels and reduce ADRs (Dhoro et al., 2015). The frequency of the CYP2B6*6 allele in the ROC population is 36%. In this study, this particular allele CYP2B6*6 failed to pass HWE assessment. A previous study showed an allele frequency of 55% (Peko et al., 2019a), which is the highest compared to other African and to non-African populations.

CYP2C8*2 reduces the enzyme activity and alters the bioavailability of the antimalarial drug amodiaquine, leading to ADRs and possibly the emergence of drug resistance (Parikh et al., 2007). Amodiaquine is known to be the drug most commonly associated with ADRs in Africa (Cavaco et al., 2005; Kudzi et al., 2009). The allele frequency of this variant in ROC is 21%, and in other African populations the frequency ranges from 14 to 24%, while it is virtually non-existent in non-African populations. Since this variant occurs almost exclusively in African populations, respective genotyping in cases of ADRs is recommended for ROC as well as African populations treated with amodiaquine. CYP3A5*3, -*6 and -*7 reduce enzyme function and increase plasma concentrations of quinine, lumefantrine, nevirapine and atazanavir (Brown et al., 2012; Castillo-Mancilla et al., 2016; Mutagonda et al., 2017).

The CYP2A6*9 variant reduces enzyme activity and increases plasma levels of efavirenz (Soeria-Atmadja et al., 2017). This observation failed to be replicated in some studies. The allele frequency of this variant in ROC is 14%; the frequencies show significant differences in other African (6−11%) and non-African populations (7–24%). The allele frequency in the ROC is highest among all African populations, suggesting a higher risk of efavirenz-induced toxicity, and further studies are needed to strengthen the associations. The CYP2C19*2 and CYP2C19_G>A variants, which are in linkage disequilibrium, decrease enzyme function and increase plasma concentrations of nevirapine (Lehr et al., 2011). Only a few studies have been conducted so far and further studies are recommended. The allele frequency in ROC is 19% and significant differences among African and non-African

populations were observed. CYP3A4*1B reduces the enzyme function and increases the plasma concentrations of lumefantrine, nevirapine atazanavir, and efavirenz (Bertrand et al., 2009; Haas et al., 2005; Kile et al., 2012; Mutagonda et al., 2017). The frequency of the CYP3A4*1B allele in ROC is 75% and also high allele frequencies are seen in other African populations (66−83%) compared to non-African populations (0–10%). Since African populations have the highest risk of increasing plasma concentrations of drugs metabolized by CYP3A4 and subsequent toxicity; genotyping and dose adjustment could reduce toxicity and improve compliance.

NAT and UGT variations

N-acetyltransferase (NAT) and UDP-glucuronosyltransferase (UGT) are phase II metabolizing enzymes which play a key role in detoxification of drugs and xenobiotics. Isoniazid is metabolized by NAT2, and the slow acetylator variants are associated with anti-TB drug-induced hepatotoxicity (Ben Mahmoud et al., 2012; Chan et al., 2017; Shi et al., 2015; Singla et al., 2014). Due to its clinical importance it is approved for pharmacogenetic labelling by FDA. The NAT2 variants assessed in this study include the slow acetylation variants NAT2*5 and -*6, the fast acetylation variants NAT2*12 and -*13, and drug-induced liver injury associated variants NAT2_tag SNP and NAT2*11. The allele frequency of the slow acetylator NAT2*5 and -*6 variants in ROC is 36% and 17%, respectively, and their frequencies vary considerably across populations. UGT2B7*2, UGT1A1*28 and -*76 cause variable drug concentrations of efavirenz, atazanavir and ritanovir and toxicity (Johnson et al., 2014; Nishijima et al., 2014; Vardhanabhuti et al., 2015). Due to their clinical significance, FDA labelling has been approved for azatanavir (Du et al., 2019). The allele frequencies of UGT2B7*2, UGT1A1*28 and UGT1A1_1813C>T variants in ROC are 25%, 47% and 46% respectively. The frequencies vary significantly in other African populations, ranging from 18 to 28%, 38 to 46% and 32 to 50%, respectively and also differ significantly in non-African populations. The UGT2B7*2 allele frequency is generally lower in African than in non-African populations. The UGT1A1*28 and UGT1A1_1813C>T allele frequencies are higher in African than in non-African populations.

ABCB1 and SLCO1B1 variations

The P-glycoprotein (P-gp), encoded by ABCB1 and the solute carrier organic anion transporter family member 1B1 protein, encoded by the SLCO1B1 gene, are transporter proteins which facilitate transfer of drugs across cell membranes. Variants in these genes lead to toxicity or inefficacy and drug resistance.

The ABCB1*6, ABCB1_c193A>G, SLCO1B1*1b and SLCO1B1*5 variants are associated with altered efficacy, pharmacokinetics and toxicity of rifampicin, isoniazid, efavirenz, nevirapine, azatanavir and ritanovir (Calcagno et al., 2019; Ciccacci et al., 2010; Elens et al., 2010; Kwara et al., 2014; Park et al., 2010; Yimer et al., 2011). The allele frequency of ABCB1*6 and c193A>G in ROC is 17% and 24% respectively, and the frequencies differ significantly between all populations. The most extensively studied variant ABCB1*6 is associated with altered efficacy and toxicity in the treatment with anti-HIV and anti-TB drugs.

The frequencies of SLCO1B1*1b and -*5 in ROC are 17% and 24%, respectively. These variants occur significantly more frequently in non-African populations compared to African populations, indicating the relative risk for elevation of rifampicin and efavirenz plasma concentrations and isoniazid drug-induced toxicity. The SLCO1BI polymorphisms are common in South Africa and often associated with increased rifampicin clearance, leading to the recommendation of dose adaptation (Chigutsa et al., 2011).

Non-ADME gene variations

The non-absorption, distribution, metabolism and excretion (non-ADME) variants of *G6PD, NF-κB1, TNF-α, NR1I2, CUX2* and *AGBL4* are associated with toxicity and the pharmacokinetics of anti-HIV, TB and malaria drugs (Calcagno et al., 2019; Fanello et al., 2008; Kim et al., 2012; Petros et al., 2016; Schipani et al., 2010; Siccardi et al., 2008).

TNF-α is an inflammatory cytokine, which plays an important role in drug-induced immune reactions. NR1I2 (PXR) is a transcription factor that activates transcription of several ADME genes. *CUX2* and *AGBL4* gene variants have been found to be associated with anti-TB drug-induced hepatotoxicity; however, the causal mechanisms have not been identified so far. The variants rs1800629 (*TNF-α*), rs4647992 (*NF-κB1*), rs2472677 (*NR1I2*), rs7958375 (*CUX2*), rs319952 and rs320003 (*AGBL4*) are associated with anti-TB treatment-induced ADRs.

Study limitations include the small sample size, which needed to be kept small because of cost considerations. Also, variants with less than 10% allele frequency were not included despite their clinical significance. This high cutoff was kept, keeping in mind that most of the studies have low sample sizes and identifying statistical differences becomes difficult.

Conclusions

We have designed a custom SNP panel and successfully genotyped distinct pharamacogentic variants in ROC. When comparing the allele-frequencies of African with non-African populations, we found variations in allele frequencies among all populations. This study helps in predicting efficacy and ADRs at the population and individual level and underlines the importance of understanding the role and function of pharmacogenetic variants.

Ethics approval and consent to participate

Ethical approval was obtained from the Institutional Ethics Committee of the Fondation Congolaise pour la Recherche Médicale in Brazzaville, ROC. Following written informed consent from parents/guardians, a total of 67 children aged between one and ten years with fever were included.

Consent for publication

All authors agreed with the results and conclusions. All authors consented to this version of the manuscript to be published.

Availability of data and materials

All related data supporting the results reported in the article is available within the manuscript and in supplementary data file.

Conflict of interest

None declare.

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Author contributions

TPV designed and supervised the study, and wrote the manuscript. AAA, VBP, EAA, GM, MB, MPG, FN and PGK were involved in the study design. FN and PGK contributed to the study materials. SRP designed the SNP panel. SRP, DEA analyzed and wrote the first draft of the manuscript. CGM edited the manuscript. SRP, LTKL, CFN and KAF performed the experiments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2020.12.009.

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