PHARMACOGENETIC ASPECTS OF HIV/AIDS, TUBERCULOSIS AND MALARIA: EMPHASIS ON UGANDAN POPULATION

Jackson Kijumba B. Mukonzo

MAKERERE UNIVERSITY

Kampala and Stockholm 2011

ABSTRACT

Infectious diseases such as HIV, tuberculosis and malaria are endemic in Africa and often require concomitant treatments that may result into subsequent drug–drug interactions. Inter-individual variability in the pharmacokinetics and pharmacodynamics of drugs used in infectious diseases, as a result of genetic polymorphism, has been reported. Pharmacogenetics of HIV, TB and malaria treatments is inadequately described in the African population. This thesis describes the pharmacogenetic aspects of HIV, TB and malaria treatment focusing on the Ugandan population.

Studies were conducted among Ugandan adult healthy volunteers (n=161) and HIV patients (n = 263), some of whom were co-infected with TB. Healthy volunteers were examined for the effect of sex and different single nucleotide polymorphisms (SNPs) in *ABCB1*, *CYP2B6* and *CYP3A5* genes on single dose pharmacokinetics of efavirenz (n=30) and quinine (n=20). Patients were examined for effects of rifampicin and CYP2B6 (*6 &*11), CYP3A5 (*3,*5 & *7) and ABCB1 (c.3435C>T & c.4036A>G) on enzyme induction, efavirenz clearance and efavirenz related CNS toxicities.

Apparent efavirenz oral clearance in subjects homozygous to CYP2B6*6 and *11 was 21 and 20% lower than extensive metabolizers respectively, while efavirenz relative bioavailability was 26% higher in subjects homozygous for *ABCB1* (rs3842). A two-fold increase in apparent peripheral volume of distribution was associated with female sex. Comparisons of efavirenz pharmacokinetics between HIV and healthy volunteers revealed 30% decrease in its bioavailability with HIV/AIDS disease. Long term enzyme induction during efavirenz treatment was greater without rifampicin than during rifampicin co-treatment and it was majorly driven by CYP2B6 polymorphism rather than rifampicin treatment. Rifampicin co-treatment influenced neither efavirenz plasma concentrations nor incidence of efavirenz CNS toxicities (p = 0.8). Additionally, efavirenz plasma concentration dependent CNS side effects are common in HIV/AIDs patients.

Thirty and thirteen fold variations in plasma quinine concentrations and quinine-to-3-hydroxyquinine metabolic ratio respectively, were observed. Plasma quinine concentration was significantly influenced by ABCB1 haplotype, CYP3A5 genotype / haplotype as well as sex.

CYP2B6 is the major predictor of efavirenz pharmacokinetics and pharmacodynamics with or without rifampicin co-treatment. CYP3A5 influences quinine but not efavirenz disposition while ABCB1 plays a role in disposition of both drugs. Pharmacogenetics rather than rifampicin co-treatment may determine efavirenz treatment outcomes in TB co-infected HIV patients treated with efavirenz and rifampicin based regimens.

LIST OF PUBLICATIONS

I.   **Mukonzo JK**, Röshammar D, Waako P, Andersson M, Fukasawa T, Milani L, Svensson JO, Ogwal-Okeng J, Gustafsson LL, Aklillu E. A novel polymorphism in ABCB1 gene, CYP2B6*6 and sex predict single-dose efavirenz population pharmacokinetics in Ugandans. Br J Clin Pharmacol. 2009;68:690-99

.

II. **Mukonzo JK,** Waako P**,** Ogwal-Okeng J, Gustafsson LL, Aklillu E**.** Genetic variations in ABCB1 and CYP3A5 as well as sex influence quinine disposition among Ugandans. Ther Drug Monit. 2010;32:346-52

III. **Mukonzo JK**, Nanzigu S, Rekić D, Waako P, Röshammar D, Ashton M, Ogwal-Okeng J, Gustafsson LL, Aklillu E.. HIV/AIDS patients display lower efavirenz relative bioavailability than healthy subjects. Clin Pharmacokinet. 2011; 50: 531-40

IV. **Mukonzo JK**, Nanzigu S, Ma Q, Waako P, Ogwal-Okeng J, Morse G, Gustafsson LL, Aklillu E. CYP2B6 genetype but not rifampicin co-administration explains variability in long term efavirenz clearance and plasma exposure among HIV patients in Uganda. Submited to Clin Pharmacol Ther.

V.   **Mukonzo JK**, Okwera P , Nakasujja N, Luzze H, Sebuwufu D, Waako P, Ogwal-Okeng J, Gustafsson LL, Aklillu E. Rifampicin co-treatment exhibits no effect on efavirenz central nervous system toxicity among HIV-1 infected Ugandans. In manuscript

The papers will be referred to in the text by their roman numerals (1-V)

CONTENTS

LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapies
AIDS	Acquired immunodeficiency syndrome
AL	Artmether - lumefantrine
ART	Antiretroviral therapy
CAR	Constitutive androstane receptor
CD	Custer of differentiation
CDC	Center for disease control
CL	Clearance
CYP	Cytochrome P-450 enzyme
DNA	Deoxyribonucleic acid
F_{rel}	Relative bioavailability
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
NCDs	Non-communicable diseases
ICH	International Conference on Harmonization
NRTIs	Nucleoside reverse transcriptase inhibitors
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
PCR	Polymerase chain reaction
P-gp	P-glycoprotein
PI	Protease Inhibitors
PXR	Pregnane X receptor
RFLP	Restricted fragment length PCR
SP	Sulphadoxine-pyrimethamine
SNP	Single nucleotide polymorphism
TB	Tuberculosis
WHO	World Health Organization

1.0   INTRODUCTION

1.1 BACKGROUND

Infectious diseases remain the biggest killer and accounts for more than 25% global disease burden. The African, Southeast Asian, and eastern Mediterranean regions bear the most burden(1). HIV, Malaria, Tuberculosis, Lower respiratory tract infections constitute most of the world's infectious disease burden. Nearly all of the 15 million people who die each year from infectious diseases live in developing countries.

1.2 THE HIV BURDEN AND TREATMENTS

UNAIDS estimates that there were 33.3 million [31.4 million–35.3 million] people living with HIV at the end of 2009 compared with 26.2 million [24.6 million– 27.8 million] in 1999 indicating 27% increase. Although the annual number of new HIV infections has been steadily declining since the late 1990s, this decrease is offset by the reduction in AIDS-related deaths due to the significant scale up of antiretroviral therapy over the past few years. Sub-Saharan Africa still bears an inordinate share of the global HIV burden. In 2009, 68% of the global total number of HIV infected people was living in Africa 22.5 million [20.9 million–24.2 million]. The estimated 1.3 million [1.1 million–1.5 million] people who died of HIV related illnesses in sub-Saharan Africa in 2009 comprised 72% of the global total of 1.8 million [1.6 million– 2.0 million] deaths attributable to the epidemic. While HIV infection was, globally estimated in about 15% of TB cases (2), in some countries particularly in sub-Saharan Africa, which accounts for 78% of HIV related TB, the HIV prevalence among people with TB was often as high as 80%.

Highly active antiretroviral therapy (HAART) utilizes three main classes of drugs to neutralize the functions of the virus' reverse transcriptase; nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and non-NRTIs (NNRTIs) and protease inhibitors (PIs). Most current national HIV treatments guidelines recommend; zidovudine (AZT) + lamivudine (3TC) + efavirenz or nevirapine (NVP), tenofovir (TDF) + 3TC or emitricitabine (FTC) + efavirenz or NVP although the relatively inexpensive stavudine (d4T) based regimens are still being used particularly in some developing countries. Due to its potentially life-threatening toxicity, use of d4T has been discouraged according to both WHO 2010 and CDC 2011 HIV treatment

1

guidelines. Recommended second line ART regimens include TDF + 3TC (or FTC) + atanazavir / ritonavir (ATV/r) or lopinavir / ritonavir (LPV/r) and AZT + 3TC (or FTC) + ATV/r or LPV/r for patients whose first line ART included d4T or tenofovir respectively. During TB co-treatment, AZT or TDF + 3TC (or FTC) + EFV is recommended as first line ART while TDF + 3TC (or FTC) + ATV/r or LPV/r and AZT + 3TC (or FTC) + ATV/r or LPV/r are used accordingly with or without dose modification of the protease base depending upon whether the rifamycins co-administered is rifabutin or rifabutin.

1.3   THE MALARIA BURDEN AND TREATMENTS

Approximately 40% of the world is at risk for malaria infection, rendering it an internationally devastating disease. Nearly 600 million new malaria infections occur each year, with at least 1 to 3 million of them resulting into death(3, 4). The burden of malaria disease falls heaviest on sub-Saharan Africa. Nearly 30% of the annual mortality in this population is attributable to malaria.

Malaria is caused by four species of a unicellular protozoan *plasmodium* including *P. falciparum, P. Malarie, P.Vivax* and *P. ovale*. Of these species, *P. falciparum* accounts for the preponderance of morbidity and mortality globally. The protozoan is transmitted through the bite of the female anopheles mosquito. Although the proportion of the population at risk of malaria infection has reduced from 77% to 50% over the years 1900 to 2000, the absolute numbers of people estimated at risk for malaria increased steadily from 0.8 billion to 3.4 billion because of population increases in malaria-endemic regions (5).

By the end of 2009, artemisinin-based combination therapies (ACTs) had been adopted as national policy for first-line treatment of malaria in 90% and 97.6% of countries endemic to *P. falciparum* worldwide and in Africa respectively. ACTs are recommended for treatment of all uncomplicated malaria with an additional primaquine single dose as a gametocidal agent. For severe malaria, a parenteral artemisinin derivative or quinine, followed by a complete course of an effective ACT may be used. Due to fairly high costs and possible spread of the already reported artemisinin resistance (6, 7), however, quinine continues to play a critical role in treatment of malaria.

1.4   UGANDA

Uganda, a country located in the East African region is inhabited by at least 17 different tribes with Bantu or Nilotic origin. People of the Bantu and Nilotic origin constitute majority of the current African tribes in addition to the Khoi-san and the Niger –cong tribes of south western and West Africa respectively. Uganda's population is estimated at 32 million with a population growth of 3.4% and a total fertility rate of about 6.7 in 2006. Uganda's healthcare system works on a referral basis beginning with a village heath team to health centers II, III, IV, district, regional referral and national hospitals. Ugandan Government invests about US$19 per capita on health (7 -9 % of the national budget)(8). The biggest burden of disease in Uganda remains predominantly infectious diseases although there are increasing trends in burden of non-communicable diseases (NCDs) over the years. HIV, malaria and TB are among the five commonest causes of death in Uganda.

Uganda is often held up as a model for Africa in the fight against HIV and AIDS as a result a of successful reduction of the HIV prevalence from > 30% in 1990s to the current 6% to 7%. Estimates from the MoH HIV/AIDS epidemiological surveillance system indicate that there were approximately 1.2 million HIV-infected individuals in Uganda by December 2009. Of these, 43% are male and 57% females. Overall children (0 -14 years) contribute 12.5%. By March 2010, there were 398 active accredited facilities providing ART services in the public and private sector in Uganda. A total of 218,359 were by 2010 active on ART, 97.2 % of them on standard first-line ART regimens. Another 432,986 individuals were estimated to be in need of ART in the country, based on a cluster of differentiation (CD)-4 T-cell count cut off of < 250 CD-4 T-cells /µl. This figure increases to 528,172 individuals if the <350 CD-4 T-cells /µl WHO-recommended ART eligibility criteria is used.

Being a country endemic of infectious diseases, with a TB incidence rate is 402 in 100,000, HAART is often given concomitantly with anti-tuberculous and other antimicrobial agents in Uganda. The recommendations for HIV treatment ensure that the first-line regimen for adults and adolescents contain two NRTIs plus one NNRTI(9). Efavirenz remains a major constituent of first line ART with or without rifampicin co-treatment in Uganda (Table 1)

Figure 1: Map of Uganda indicating the country's district as of 2010. Districts where study participants were recruited are marked with arrows on the map.

Table 1: The preferred first and second line ART regimens as recommended by Uganda's National antiretroviral treatment guidelines of 2009

	Preferred ART Regimens	
	First line ART	**Second line ART**
Patients not being co-treated for TB	ZDV/3TC + NVP or EFV	TDF+3TC+LPV/r
	TDF+3TC or FTC + NVP or EFV	ZDV+3TC + LPV/r
Patient co treated with rifampicin for TB	ZDV/3TC/EFV	TDF+3TC+*LPV/r
	TDF/3TC/EFV	ZDV+3TC +* LPV/r

*Lopinavir 400mg/ritonavir400mg twice daily or SQV 400 mg/RTV 400mg with close laboratory monitoring for hepatotoxicity

Malaria remains Uganda's number one killer disease particularly among children less than 5 years killing 70,000 to 100,000 annually. Overall, malaria prevalence rate in Uganda is 250 in 100,000. All four human plasmodia species occur in Uganda although *Plasmodium falciparum* is by far the most common contributing 90-98% of the parasite population. The second most common species is *P. malariae* with 1-3% as mono-infection but is more commonly found as a mixed infection with *P. falciparum* (up to 16% of childhood infections in highly endemic areas). Both *P. vivax* and *P.ovale* are rare and do not exceed 1-1.5% of malaria cases.

Following reports of malaria resistance to chloroquine(10) and sulphadoxine-pyrimethamine (SP)(11-13) in Uganda and the entire region, a national artemisinin combination therapy (ACT) policy was launched in 2006, using artemether-lumefantrine (AL) as first-line treatment for uncomplicated malaria. In spite of all efforts to reduce malaria infections and prevent progression of uncomplicated malaria to complicated forms of the disease, severe malaria requiring treatment with quinine still occur in Uganda.

1.5   PHARMACOKINETICS

Pharmacokinetics entails the quantitative analysis of the processes of drug absorption and mechanisms of absorption, distribution, metabolism and excretion [ADME]. Pharmacokinetics forms basis for optimization of drug outcomes both in terms outcomes efficacy and dose related adverse drug reactions. Pharmacokinetics however, can differ substantially between individuals. Drug-metabolizing enzymes (e.g., cytochrome P450 enzymes) and transport proteins (e.g P-glycoprotein) have an important role in breakdown, intestinal absorption, distribution, and renal or hepatic excretion of drugs. Inherited determinants of drug disposition due to genetic polymorphism in drug metabolizing enzymes and transporters, or drug targets, remain the major cause of inter-individual differences and thus form basis for individual characterization such as poor metabolizers, extensive metabolizers, Ultra rapid metabolizers and the associated clinical consequences. Variation in pharmacokinetics and pharmacodynamics with genetic make-up, currently constitute most of pharmacogenetics. Differences by ethnicity may have a profound impact on important drug pharmacokinetic parameters such as clearance (CL) and bioavailability (F) that may in turn affect drug efficacy, drug interactions and effectiveness sometimes necessitating dose or regimen modification. In the context of Africa, the situation may be even more serious due to the genetic heterogeneity and co-infections with endemic infectious diseases that require multiple drug therapies.

2 PHARMACOGENETICS

2.1   COMMON TERMINOLOGY

Deoxyribonucleic acid (DNA) consists of four bases: adenine (A), guanine (G), thymine (T), and cytosine (C). Any combination of three nucleic acids can form a codon, which is transcribed into messenger RNA and translated into a particular amino acid (e.g., ACG encodes for threonine). The stop codon, TAG, terminates protein synthesis. An incorrectly placed stop codon in a gene, caused by mutation, prematurely truncates an amino acid chain and may form a nonfunctional protein. Many of the variations in the human genome are single base changes, termed single nucleotide polymorphisms (SNPs). A mutation of one nucleotide of a codon may result in either a change in the coded amino acid (nonsynonymous SNP) or no change (silent polymorphism or synonymous SNP). Since each person has a pair of each chromosome, he or she has two alleles for each gene, one on each chromosome. Two identical alleles result in a homozygous dominant or homozygous recessive trait of that gene. A combination of two different alleles leads to a heterozygous trait thus the three possible genotypes for a particular character. On the contrary phenotype refers to the outward appearance of an individual in all of their anatomical, biochemical, physiological and behavioral characteristics as dictated by their genetic and environmental influences. Pharmacogenetics was first defined by Friedrich Vogel as the study of variability in drug response due to heredity[14], while pharmacogenomics is a relatively newer term that refers to whole genome effect on drug response.

2.2   THE CYTOCHROME P450 SYSTEM

The cytochrome P450s (CYPs), are heme-containing membrane bound metabolic enzymes. CYPs are localized on the cytosolic side of the endoplasmic reticulum with a few located on the matrix of mitochondrial inner membrane. In humans xenobiotic and drug metabolizing CYPs are mainly located in the liver and intestines but can also be found in the kidney, skin, brain and lungs. CYPs utilize NADPH as an electron source whose transfer is mediated by the co-enzyme NADPH- cytochrome P450- reductase (equation 1).

$$DH + NADPH + H^+ + O_2 \longrightarrow DOH + NADP^+ + H_20 \quad (equation\ ...1)$$

Overall twelve CYP families in humans have been described so far. However, most drug metabolizing enzymes belong to the highly polymorphic CYP1, CYP2, and CYP3 which are responsible for up to 80% of all phase 1 metabolism of drugs in clinical use. Human CYPs constitutes several subfamilies with varying expression, activity and substrate specificity (Table2).

Table 2: Substrates and functions of human *CYP* gene families

Family	Number of sub-families	Number of genes	substrates and functions
CYP1	2	3	***Drugs,*** foreign chemicals, arachidonic acid, eicosanoids
CYP2	13	16	***Drugs,*** foreign chemicals, arachidonic acid, eicosanoids
CYP3	1	4	***Drugs,*** foreign chemicals, arachidonic acid, eicosanoids
CYP4	5	12	Fatty acids, arachidonic acid, eicosanoids
CYP5	1	1	Thromboxane A_2 synthase
CYP7	2	2	Cholesterol, bile acid synthesis
CYP11	2	3	Steroidogenesis
CYP17	1	1	Steroid 17α-hydroxylase, 17/20-lyase
CYP19	1	1	Aromatase to form oestrogen
CYP21	1	1	Steroid 21-hydroxylase
CYP24	1	1	Vitamin D_3 24-hydroxylase
CYP26	3	3	Retinoic acid hydroxylation

2.2.1 CYP2B6

CYP2B6 which catalyses up to about 70 structurally different xenobiotics and drugs including pro-carcinogens (15) and antiretroviral drugs (16) has been mapped to the CYP2 gene cluster on chromosome 19. Due to its low hepatic composition (2 - 10%), CYP2B6 was until recent mistakenly regarded to be of minor importance in drug metabolism (17, 18). To date CYP2B6 is the primary metabolizing enzyme for efavirenz and nevirapine. In addition, CYP2B6 is involved in the metabolism of other drugs and environmental factors such as artemisinin and derivatives (19), vitamin D

and its analogues (20), bupropion (21), methadone (22, 23), nicotine (24), and propofol (25). Overall, it is involved in the metabolism of approximately 4% of the most used drugs(26)

Other CYP2 sub-families that play a key role in human drug metabolism include CYP2C9, CYP2C19 and CYP2D6.

2.2.2 CYP3A

CYP3A sub-family participates in the metabolism of about 50% of all marketed prescription drugs (27, 28). Four identified members in the CYP3A sub-family that contribute to its overall activity include CYP3A4, CYP3A5, CYP3A7 and CYP3A43. Of these CYP3A4 and CYP3A5 are the major enzymes responsible for drug metabolism in human adults (29-31). Although CYP3A4 is considered the major enzyme expressed in human liver (31), up to 50% metabolic activity contribution is associated with the highly polymorphic CYP3A5 (32-34) that significantly contributes to both inter-individual and population differences in metabolism of CYP3A substrates.

2.3   DRUG TRANSPORTERS

Adenosine triphosphate (ATP) – binding cassette (ABC) proteins and solute-linked carrier (SLC) proteins, are responsible for transportation of majority of drugs as well as endogenous substrates. ABC transporters are generally efflux pumps, that naturally offer protection to the body by limiting entry of xenobiotics (35) and drugs while solute carrier (SLC) proteins are typically influx transporters that mediate facilitated diffusion of their substrates(36). The mammalian ABC super family of transporters comprises a large number of functionally diverse transmembrane proteins which have been subdivided into seven families designated A to G constituting 48 sub-families. Activity or substrate efflux capacity of ABC-transporters is highly variable with different genotypes of the protein

2.3.1 ABCB1

ABCB1 sometimes referred to as MDR1, which is so far the best characterized, was the first of the ABC transporters to be cloned. *ABCB1* gene encodes the transporter protein P-gp. It transports xenobiotics from the cytoplasm to the extracellular space thus, providing a barrier against accumulation of drugs and xenobiotics inside the cells and entire body. Although it was first recognized in tumor cells, P-gp was

recently identified in many normal tissues as part of a mechanism to prevent the intracellular concentration of potential harmful substances (38). It is particularly located at the apical membrane of many secretory cell types such as kidney, liver, intestine, adrenal gland, and the blood–brain barrier where the normal function involves the excretion of drugs and their metabolites (39).

ABCB1 transports several classes of drugs, including antiretroviral drugs such as efavirenz. Genetic differences of ABCB1are known to exist, and more than 100 variations have been identified, most of them, single nucleotide polymorphisms (SNPs). These single nucleotide polymorphisms are associated with altered oral bioavailability of ABCB1 substrates, drug resistance, and susceptibility to some human diseases(40-42).

2.4   ABCB1, CYP3A AND CYP2B6 GENE POLYMORPHISM

ABCB1 is polymorphic and a number of SNPs have been documented with varying allelic frequencies in different ethnic groups. Although population specific ABCB1 SNP have been reported, overall, ABCB1 SNPs are found in all studied populations (43-45).Chances of carrying particular mutant alleles however vary between different populations (46). Effects of some of the widely studied ABCB1 SNPs such as 3435C>T, G2677T/A and C1236T on individual drug pharmacokinetics including digoxin, fexofenadine, cyclosporine, efavirenz and protease inhibitors are well documented (47-49). Additionally, ABCB1 is expressed in CD56, CD8, CD4, CD19, and other subpopulations in peripheral blood monocytes (50). Because CD4cells are a major target for HIV, their ABCB1 activities can affect intracellular concentration of many HIV protease inhibitors (47). In a Swiss study the clinical role of ABCB1 polymorphism was further highlighted by a significantly higher increase in CD4 cell counts and marked improvement in viral infection among patients carrying ABCB1 3435TT genotype as compared to those with the CT or CC genotypes (51).

Both the wide substrate spectrum and the propensity to induction or inhibition of CYP3A raise interest in its pharmacogenetics. . Although more than 40 *CYP3A4* alleles have been defined by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee, the actual number of true haplotypes is much smaller and the most frequent of them are common to all major ethnic groups (52, 53). Most variants of CYP3A4 have no functional consequences or are too rare to contribute significantly to the

10

CYP3A4 variability(54, 55) except the relatively frequent *CYP3A4* variant, *CYP3A4*1B*, which is associated with higher grade prostate cancer (56).

The polymorphic expression of CYP3A5 becomes detectable for some CYP3A substrates with decreasing concomitant expression of CYP3A4 (32, 57). Overall, CYP3A5 is a highly polymorphic enzyme as a consequence of mutations that severely reduce the synthesis of functional CYP3A5 protein (CYP3A5* 3, *6 and *7) (34, 58). *CYP3A5*3* is the most common defective allele with an allele frequency of about 90%, 75% and 20% in Caucasians, Asians and Africans, respectively, whereas *CYP3A5*6 a*nd *CYP3A5*7* are not present in Caucasians and Asians and have a 17% and 8% frequency in Africans, respectively(59). Unlike, CYP3A4, the hepatic expression of CYP3A5 is bimodal, with 'high expressors' exhibiting 10 − 30 higher CYP3A5 protein expression levels than CYP3A5 'low expressors' (34, 58, 60).

The *CYP3A5*3* variant which disrupts the correct splicing of CYP3A5 transcripts is common to all ethnic groups. *CYP3A5*6(34)*, and the frameshift mutation, *CYP3A5*7(58)*, have been identified in Africans and African-Americans. Due to the considerable allelic frequencies of 10 − 20%, *CYP3A5*6* and -*7* need to be considered together with *CYP3A5*3* via haplotypes analysis to achieve an accurate prediction of CYP3A5 expression in these ethnic groups (61). The frequency of CYP3A5 'high expressors' generally decreases with the distance from the equator; thus, the highest is observed in Africans (70%) and the lowest in European Caucasians (10%) (52, 62).

CYP2B6 is a member of the cytochrome P450 family that constitutes approximately 2−10% of the total hepatic CYP content(18). It is also expressed in the brain, and may be an important factor in the metabolism of drugs acting on the central nervous system (CNS) and neurological side effects of drug treatments(63). CYP2B6 is responsible for the metabolism of 4% of the most commonly used 200 drugs (26) and is highly inducible by several drugs and other xenobiotics(64). There is high inter-individual variation in CYP2B6 mRNA expression, ranging from 20 to 250-fold, which may be attributed to differential transcriptional regulation and inherited genetic variation (19, 26, 65, 66). A large number of variants have been reported, including many that are present at high frequencies and several that show linkage resulting in multiple haplotypes. Both CYP2B6 haplotypes and genotypes exhibit high racial and ethnic population frequency differences(67). The enzyme metabolic activity varies with the different allelic variants. Subjects homozygous for combinations of the alleles

CYP2B6*6, *CYP2B6*16* and *CYP2B6*18* may exhibit lower capacity for metabolism of CYP2B6 substrates, like efavirenz, than expected from a linear gene–dose relationship (68, 69).

2.4.1 CYP2B6 and CYP3A enzyme induction

CYP enzymes are generally inducible through a process involving de novo RNA and protein synthesis that has been demonstrated in studies using transcription and translation inhibitors (70). The commonest mechanism of induction is by ligand activation of key receptor transcription factors including pregnane X receptor (PXR) and constitutive androstane receptor (CAR) leading to increased transcription. PXR and CAR were originally established as the predominant regulators of xenobiotic-induced CYP3A and CYP2B hepatic expression, respectively. More recent reports suggest cross-talk between PXR and CAR resulting in reciprocal activation of rodent and human *CYP2B* and *CYP3A* genes (71, 72) but with preferential induction of CYP2B6 over CYP3A4 by hCAR activation (73)

2.5 PHARMACOGENETICS OF INFECTIOUS DISEASES TREATMENTS

Pharmacogenetics of drug therapy of infectious diseases constitute the basis of the subject with modern pharmacogenetics having its origin in the 1950s (74) when genetic factors were found to play a role in the pharmacodynamics and pharmacokinetics of the anti-malarial primaquine and the anti-TB isoniazid respectively (75, 76). Recently pharmacogenetic studies have been conducted on antiretrovirals and other drugs with particular focus on metabolizing enzymes and transporter proteins in the cell membrane.

2.5.1 Pharmacogenetics of antiretroviral drugs

Since the approval of the first antiretroviral drug, zidovudine in 1987, more than 20 drugs have been approved for the treatment of HIV infection. Although use of antiretroviral drugs as combination regimens leads to complete suppression of viral replication in most cases, inter-individual differences have been cited in their pharmacokinetics and pharmacodynamics possibly accounting for variations in ART outcomes: incidence of drug–related toxicities, drug-drug interactions and treatment failure. Given that HIV infection remains incurable requiring life-long treatment once

initiated, the genetic factors determining the inter-individual variability in drug exposure, drug interactions and efficacy need to be explicitly described.

The CYP enzyme system is the main metabolizer involved in liver clearance of two major categories of antiretroviral drugs; non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI). Several polymorphisms at different CYP isoenzymes are known to influence the metabolism of drugs, including ARVs, causing increased or decreased clearance. In addition, the activity of the P-glycoprotein (P-gp) cell membrane efflux pump influences the intracellular disposition of some antiretroviral drugs. Polymorphisms at the genes encoding protein transporters are known to influence both their expression and activity (46, 77, 78).

2.5.1.1 Efavirenz

Efavirenz is a widely used NNRTI in the treatment of HIV infected patients as a result of its potency and convenience. Efavirenz however, exhibits a narrow therapeutic window, as plasma drug levels greater than 4 µg/ml have been associated with more central nervous system (CNS) toxicity while the rate of virologic failure increases with concentration below 1 µg/ml (79) . Inter-patient variations in efavirenz hepatic metabolism and P-gp mediated movement across plasma membranes are some of the main causes of important inter-individual variations in the plasma concentration of the drug. Efavirenz undergoes oxidative hydroxylation primarily by CYP2B6 to 8-hydroxyefavirenz, which is its major metabolite, and to 7-hydroxyefavirenz, which is the minor metabolite (80).

CYP2B6 516 G>T polymorphisms has been associated with differences in efavirenz plasma exposure and CNS toxicities in most populations suggesting that prescription of lower efavirenz doses could be warranted in subjects harboring the T/T genotype in order to minimize side effects without compromising the efficacy of the drug. *CYP2A6*, *CYP3A4/3A5*, and uridine-glucuronyl-transferases constitute minor efavirenz metabolic pathways (81) with both CYP2A6 and CYP3A4/A5 accessory pathways influencing efavirenz elimination independent of CYP2B6 (82).

Although there are conflicting reports on whether efavirenz is a substrate for P-gp (51, 83), lower efavirenz plasma levels, better immune recovery in Caucasians who carried the 3435T/T genotype and lack of EFV resistance have been associated with polymorphisms in the ABCB1 gene (51, 84).

2.5.1.2   Efavirenz – rifampicin interactions

Rifampicin induces expression and activity of CYP2B6, the main metabolic enzyme for efavirenz. In primary human hepatocytes, the increase in CYP2B6 activity due to rifampicin varies widely from 2.5 fold to 13-fold (85) (86). I*n vivo*, co-administration of efavirenz with rifampicin may lead to 22 – 26%   reduction in efavirenz plasma exposure (87-89) , although more recent studies report no significant differences in efavirenz concentrations with or without rifampicin co-treatment (90, 91).   Inter-individual differences in the inducibility of efavirenz metabolizing enzymes by co-administered rifampicin have also been reported (92). The variability in efavirenz concentrations was found to be greater in the presence of rifampicin than without rifampicin (93, 94), a probable manifestation of inherent differences in the inducibility of *CYP2B6* variants. The possible inter-individual differences in enzyme induction on the clearance of efavirenz when co-administered with rifampicin further complicate decisions about dose adjustments of efavirenz in the setting of concurrent rifampicin-containing TB therapy (92). Efavirenz dose adjustment has been suggested, particularly during co-administration with rifampicin among patients weighing >50 kgs, in order to maintain optimal therapeutic concentrations. However, the need for balance to avoid supra-therapeutic efavirenz plasma concentrations in individuals who are genetically predisposed still challenges the practice of the recommendation, particularly among the African populations who are known to exhibit significant intra-population genetic differences.

2.5.2 Pharmacogenetics of other infectious disease treatments

Although HIV pharmacogenetics has undergone tremendous development and attracted most research in the field, it was treatment with anti-malarial drugs that led to wider recognition of the importance of the subject. During World War 2, African–American soldiers developed more acute haemolytic crises following treatment with primaquine compared to their white counterparts. Pharmacogenetic research into anti-malarial drugs however has remained at its infancy with their metabolic pathways being established only recently, while their efflux and uptake mechanisms remain largely unexplored.

2.5.2.1 Quinine

Quinine, one of the oldest and commonly used anti-malarial, is metabolized by CYP3A4/-A5 to its primary metabolite, 3-hydroxyquinine (95, 96), which has been shown, ex vivo, to contribute 5–10% of the anti-malarial activity (97). Quinine has a narrow therapeutic window (98) indicating the need for dose optimization through evidence based population tailored dose adjustments or therapeutic drug monitoring. Cinchonism a syndrome characterized by tinnitus, high-tone hearing loss, nausea, dizziness and dysphoria, resulting from quinine use, is a major cause for poor adherence and related treatment failures (98).

In-vitro studies suggest that quinine at therapeutic levels is an inhibitor and substrate of ABCB1 (99, 100). Increased brain concentrations of quinine has previously been reported in ABCB1 -knockout mice and in wildtype mice after inhibition of P-glycoprotein with cyclosporine, verapamil, and mefloquine (101). Inter-individual variation in quinine disposition exist among malaria patients as well as among healthy volunteers with the CYP3A5*3/*3 exhibiting substantially lower 3-hydroxylation (96) .

3   THIS THESIS

3.1   RATIONALE OF THESIS

Several studies have demonstrated the critical role played by gene based ethnic differences in HIV treatments outcomes. Over the past few years however, as a result of reduced costs of antiretrovirals and the global initiative to improve access, marked ART program up scaling has occurred resulting into at least 40% of people in need receiving ART by end of 2010. Majority of the new entrants into the ART program are found in the developing countries particularly the sub-Saharan African region. ART scale up is based on the experiences from the developed countries in Europe and North America yet genetic variations relevant to ART have been reported between populations. As a result, differences in terms of drug pharmacokinetics, pharmacodynamics, drug interaction and toxicity profiles are expected to occur. This may indicate a possible need for population based modifications of the existing HIV treatments.

Some gene polymorphisms of great relevance to antiretroviral therapy, and particularly efavirenz such as CYP2B6 have been highlighted. CYP2B6 polymorphism may substantially explain inter-individual differences in the pharmacokinetic and dynamics of efavirenz. However, clinical relevance of the efavirenz pharmacogenetics has not been adequately demonstrated in Africans, a population that is uniquely different in terms of: SNP frequency, intra-population variability, spectra of co-morbidities and associated co-treatments, socio-economic and environmental factors.

On the other hand the pharmacogenetics of quinine an important anti malarial drug has not been well described, particularly in the sub-Saharan Africa population where malaria disease is most endemic.

This thesis offers relatively comprehensive explanation for inter-individual efavirenz pharmacokinetics using a population pharmacokinetic / pharmacogenetic modeling and simulation approaches that integrate of 30 SNPs in CYP2B6, 3A5 and ABCB1 genes, including six novel ones, HIV disease, demographic and biochemical factors as covariates. While the study contextualizes role of pharmacogenetics in efavirenz pharmacokinetics among Ugandans, findings of this study may be extrapolated to communities with similar characteristics.

Majority of the drugs used during HIV/AIDS disease are evaluated in clinical trials conducted among healthy volunteers. Little is known on whether they exhibit normal pharmacokinetics in HIV patients. This thesis demonstrates the need for caution when extrapolating pharmacokinetic findings from healthy volunteer trials to HIV/AIDS patients. The thesis, in addition explores efavirenz clearance as well as the clinical relevance of genetic and demographic factors on efavirenz treatments by linking the effects of such factors and the drug's pharmacokinetics to CNS toxicities.

Additionally, the thesis describes the role of pharmacogenetics and key demographic factors such as sex on quinine disposition among in the Ugandan population.

Conduct of the current study among Ugandans, a diverse population in terms of ancestry may form basis for the wide application of findings of these studies especially in people from sub-Saharan African region that share similar characteristics with Ugandans.

3.2 OBJECTIVES

3.2.1 General objective

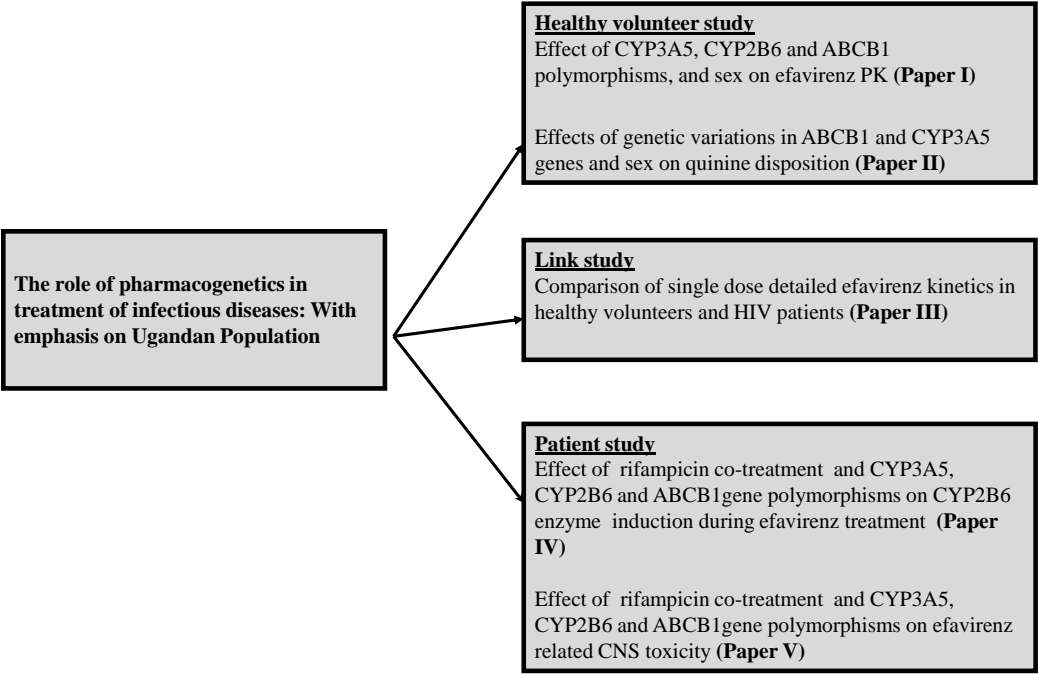To describe the pharmacogenetic aspects of TB-HIV/AIDS and malaria treatments in Ugandans

3.2.2 Specific objectives

1. To assess the effect of CYP3A5, CYP2B6 and ABCB1 polymorphisms on efavirenz population pharmacokinetics
2. To examine the effect of ABCB1 and CYP3A5 gene polymorphism on quinine deposition
3. To assess the effect of HIV disease on efavirenz pharmacokinetics
4. To assess the effect of rifampicin co-treatment and CYP3A5, CYP2B6 and ABCB1 gene polymorphisms on CYP2B6 enzyme induction during efavirenz
5. To assess the effect of rifampicin co-treatment and CYP3A5, CYP2B6 and ABCB1gene polymorphisms on efavirenz related CNS toxicity

4 MATERIALS AND METHODS

4.1 STUDY DESIGN

The study was conducted among healthy volunteers and HIV/AIDS patients. A link sub-study was integrated into the design to enable comparative analysis of efavirenz pharmacokinetics between HIV/AIDS patients and healthy volunteers. All healthy volunteer sub-studies **(Paper I) (Paper II)** and **(Paper III)** were cross-sectional while sub-studies involving TB-HIV/AIDS patients were prospective cohort studies with a follow up period of twelve weeks **(Paper V)** thirty two weeks **(Paper IV) (figure 2)**

Figure2: A schematic presentation of the study design indicating healthy volunteer, the link and patient sub-studies

4.2 STUDY AREA AND POPULATION

The study was conducted in three districts including two urban districts (Kampala and Mukono) and one rural district (Kasese) as shown in figure 1. Kampala and Mukono districts are located in central Uganda. Kampala District hosts the administrative headquarters of the central Government of Uganda. It also hosts two national referral hospitals. The population in Kampala and Mukono districts is estimated at 3.0 and 1.07 million people respectively. The population in both districts is largely drawn from the various rural parts of Uganda, bringing all tribes together.   Kasese district, is on the other hand located in western part of the country bordering with the Democratic Republic of Congo. The district population that is estimated at 660,000 people is largely of the Konjo tribe.

4.3 DATA COLLECTION

4.3.1 Healthy volunteer studies

Adult healthy volunteers (n = 161) including Bwera and Mulago Hospital staff, medical students and local residents were recruited from Kasese, Mukono and Kampala districts. Clinical examination combined with HIV and hepatitis B serology, liver and renal function tests were performed to establish the participant's health status. Participants were advised to abstain from medications including herbal preparations a week before and throughout the study period. Participant ancestral tribes, and age were recorded as self declared.   Eligible participants were each requested to give a blood sample for genotyping before receiving quinine 600mg and efavirenz 600mg as single oral doses at least two days apart. Sixteen hour blood samples were collected following quinine administration for determination of plasma concentrations of quinine and its major metabolite; 3-hydroxy quinine (Paper II).

Following efavirenz administration, all participants contributed sparse efavirenz plasma samples at 4 and 24 hours. Thirty two of the participants were in addition intensively sampled for efavirenz up to 72 hours.

 Plasma samples for quinine, 3-hydroxy quinine and efavirenz determination and whole blood samples were stored at -70^{0}C until laboratory analysis was performed at Karolinska Institute, Stockholm. Participants were genotyped for selected SNPs in CYP3A5, CYP2B6 and ABCB1 genes (table 3)

Table 3: 30 single nucleotide polymorphisms in ABCB1, CYP2B6 and CYP35A genes determined in 161 healthy volunteers.

Gene	Position	rs number	Allele	Relevance
CYP2B6	c. 785 A→G	rs2279343	*CYP2B6*4, *6, *7, *13, *16 *19, *20*	Reduced expression and activity
	c.516 G→T	rs3745274	*CYP2B6*6, *7, *9, *13, *19, *20*	Reduced expression and activity
	c.136A→G	rs35303484	*CYP2B6*11*	Phenotypic null allele
	c.983 T→C	rs28399499	*CYP2B6*16, *18*	Phenotypic null allele
	c.64 C→T	rs8192709	*CYP2B6*2*	Phenotypic null allele
	c.1282 C→T	rs35010098	*CYP2B6*21*	Phenotypic null allele
	exon 8/−6 C→T	rs35449271	New SNP	Undetermined
	296 G→A	rs36060847	*CYP2B6*12*	Reduced expression
	1375 A→G	rs3211369	*CYP2B6*23*	Unknown
	c.1172 T→A	rs35979566	*CYP2B6*15*	Reduced expression
CYP3A5	g.27289C→A	rs28365083	*CYP3A5*2*	Unknown
	g.6986A→G	rs776746	*CYP3A5*3*	Phenotypic null allele
	g.14665A→G		*CYP3A5*4*	Unknown
	g.14690G→A		*CYP3A5*6*	Phenotypic null allele
	g.27131-27132insT	rs241303343	*CYP3A5*7*	Phenotypic null allele
	g.3699C→T	rs28371764	*CYP3A5*8*	Phenotypic null allele
	g.19386G→A	rs28383479	*CYP3A5*9*	Decreased activity
ABCB1	c.1236 C→T	rs1128503		Phenotypic null allele
	c.2677 G/A→T	rs2032582		Phenotypic null allele
	c.3435 T/C	rs1045642		Phenotypic null allele
	c.4036 A/G	rs3842	New SNP	Undetermined
	c.1659 G→C	rs2235012		
	exon 6/+139 C→T	rs1202168	New SNP	Undetermined
	exon 19/−88 T→C	rs4728699	New SNP	Undetermined
	c.781A→G	rs36008564		
	c.239C→A	rs9282565		
	exon 12/+44 C→T	rs20328588	New SNP	Undetermined
	c.1199G→A	rs2229109		
	c.1795C→T	rs2235036		
	exon 20/+24 G→A	rs2235040	New SNP	Undetermined

4.3.2 Patient studies

ART naïve HIV/AIDS patients were recruited at the HIV clinics at Butabika National referral hospitals in Kampala (n= 60) and Bwera Hospital, Kasese (n= 47). 152 TB co-infected HIV patient's naïve to both ART and anti-TB therapy were recruited from the TB-HIV clinic at Mulago National Referral Hospital. All patients were treated for HIV with efavirenz 600mg daily doses in combination with zidovudine and Lamivudine. Follow up period was 32weeks. Patients co-infected with TB received rifampicin based anti-TB regimen for 6 month (2EHRZ/4HR) in addition to ART. CD4 analysis was performed to ensure eligibility for HIV treatment. All patient participants were genotyped for CYP2B6 (*6 and *11), CYP3A5 (*3,*6 and *7) and ABCB1 (rs 3842 and 3435C>T). Mid-dose plasma efavirenz concentrations samples were drawn on days 1 and 3 then weeks 1, 2, 4, 6, 8, 12, 16, 20, 24, 28 and 32. Twenty nine of the patients were intensively sampled within 24 hours of treatment initiation and compared with healthy volunteers (Paper III). Patients were also evaluated for adverse events including neuropsychotic ones (sleep disorders, hallucinations and cognition impairment)

4.4 LABORATORY ANALYSIS

4.4.1 HPLC quantification of drug levels

4.4.1.1 *HPLC determination of efavirenz (**Paper I, III, IV &V**)*

Plasma was prepared from blood samples by centrifugation at 3000 g for 10 min and stored at -70°C until high performance liquid chromatography (HPLC) analysis was performed. Plasma efavirenz was determined by reverse-phase with ultraviolet (UV) detection. The HPLC machine, Agilent series 1100, consisting of column compartment G1316A, Degasser G132A, Quat pump G1311A, an auto-sampler ALS, G1329A, and G1315B diode array detector was used. The mobile phase consisted of 30% acetonitrile, 30% methanol, 4 mmol l^{-1} potassium hydroxide and 10 mmol l^{-1} acetic acid (pH 4.3). Plasma proteins were precipitated with acetonitrile before centrifuging. Elution was performed at 0.80 ml min-1 for 3.5 min. The retention time for efavirenz was 2.42 min as detected at UV-VIS 1, 210 nm, UV-VIS 2,220 nm. The method was linear, with a within-day coefficient of variation of 3.2, 3.3 and 5.1% at concentrations of 2.0 mM (n = 17), 8.0 mM (n = 17), and 20 mM (n = 16), respectively, and a between-day coefficient of variation of 4.1% (n = 50). The limit of quantification for the method was set at 0.35 mM.

*4.4.1.2   HPLC determination of Quinine and 3-Hydroxy quinine (**II**)*

HPLC determination of quinine and its metabolite 3-hydroxy quinine was performed as previously reported (102) by mixing 100µl of plasma with 200 µl of cold methanol and vortexing the mixture for 10 seconds before centrifugation at 3000g for 10 minutes. The supernatant was transferred to HPLC injection vials. 10 µl to 30 µl was injected into an HPLC system consisting of a rheos 4000 gradient pump (Flux Instruments AB Karlskoga, Sweden) a Gilson 231 XL injector (Pretech Instruments, Stockholm, Sweden) a waters 474 detector (Millipore, Milford, MA, USA) and a computer with Chemstation software for data registration and calibration (Agilent Technologies, USA). Excitation and emission wavelengths were set at 350 and 450 nm respectively. Zorbax Eclipse XDB phenyl 150 X 4.6 mm I.D. 5µm (Chrom Tech, Hagersten, Sweden) was used.   Gradient elution was performed with two mobile phases A (consisting of 8% acetonitrile in 0.1M acetate buffer, pH 3.9) and   B (consisting of 24% acetonitrile in 0.1 M acetate buffer, pH 4.2).

4.4.2   Genotyping

4.4.2.1   Restricted Fragment Length PCR

RFLP was used to genotype for C3435T, C1236T and G(A)2677T in the ABCB1 gene according to Tang et al and Cascorbi et al (103) (104). PCRs were performed in a reaction mixture (25 ml) containing buffer X10, 0.125 ml Smart Taq hot DNA polymerase, 1.6–2.0 ml MgCl2 (25 mM l-1), 6.25 mM dNTPs, and primers. Endonucleases Bsp1431, Eco01091 (Drall) and BshNi(HgiCl) were used to digest PCR products for C3435T, C1236T and G(A)2677T, respectively, followed by gel electrophoresis.

4.4.2.2   Micro-array Assay

Genomic DNA was isolated using QIAgen kit was genotyped for SNPs in the three genes: CYP2B6, CYP3A5 and ABCB1 by minisequencing using micro-tag arrays method (105). Cyclic minisequencing reactions with fluorescently labeled dideoxynucleotides were performed using multiplex polymerase chain reaction (PCR) product as template and detection primers, designed to anneal immediately adjacent to and upstream of the SNP site. Primer sequences are available upon request. The microarrays were prepared using detection primers carrying unique 5′ tag sequences and oligonucleotides complementary to the tag sequence of the minisequencing primers, immobilized on a microarray. Hybridization was performed as according

Lindroos et al and Lovmar et al (105, 106). The QuantArray file was exported and analysed using the SNPSnapper analysis software, version 4.0 beta.

4.4.2.3 TaqMan Methods

SNPs identified to be of clinical importance by the healthy volunteer sub-studies (I) and (II), including CYP2B6 (*6 and *11), CYP3A5 (*3,*6 and*7) and ABCB1 (rs3842 and 3435C>T) were determined on patient genomic DNA using this method. Allelic discrimination reactions were performed using TaqMan (Applied Biosystems, CA, USA) genotyping assays: (C___7586657_20 for *ABCB1* 3435C>T, C___7817765_60, for *ABCB1* rs3842T>C, C__29560333_20, for *CYPB6* 516G>T [*CYP2B6*6*], for *CYP2B6* 136A>G [*CYP2B6*11*], C__26201809_30 for *CYP3A5* 6986A>G [*CYP3A5*3*], C__30203950_10 for *CYP3A5* 14690G>A [*CYP3A5*6*]) and C__32287188_10 for *CYP3A5* g.27131_27132insT [*CYP3A5*7*] on ABI 7500 FAST (Applied Biosystems, Foster City, CA). The final volume for each reaction was 10μl, consisting of 2x TaqMan Universal PCR Master Mix (Applied Biosystems), 20 X drug metabolising genotype assay mix and 10 ng genomic DNA. The PCR profile consisted of an initial step at 50°C for 2 min and 50 cycles with 95°C for 10 minutes and 92°C for 15 sec

4.5 DATA MANAGEMENT AND ANALYSIS

4.5.1 Data management

Data was entered into an excel sheet. Primary sources of data were also kept in electronic form for reference. Prior to data modeling using Nonlinear Mixed Effect Modeling **(Paper I & III)** data was exported to SAS for cleaning. Before analysis with Statistica, Graph Pad Prism version 5.0 for Windows or SPSS version 9 **(Paper II, IV & V)**, appropriate data was extracted cleaned and formatted

4.5.2 Nonlinear mixed effect modeling (NONMEM)

NONMEM, a one-stage analysis that simultaneously estimates mean parameters, fixed effect parameters, individual variability and residual random effects was used for the population pharmacokinetic analysis. First-order conditional estimation in NONMEM VI (Icon Development Solutions, Ellicott City, MD, USA) was applied **(Paper I & III)** and the resulting models and model output were managed using the Census software. The structural model was parameterized in terms of apparent oral clearance (CL/*F*), apparent central and peripheral oral volumes of distribution (*Vc*/*F*

and Vp/F), apparent inter-compartmental clearance (Q/F), first-order absorption rate constant (ka), duration of zero-order input (D) and lag times for first- and zero-order absorption ($ALAG$). To be included in the model at the exploratory stage, a covariate had to account for a significant difference, P < 0.05. All retained covariates were then removed from the full model in stepwise backward manner using a more stringent criterion of $P < 0.01$

4.5.3 Statistical analysis

All data was initially entered into excel spreadsheets. For sub-studies **I** and **III** data was exported to SAS software and processed into a NONMEM format. For sub-study II, CYP3A5 activity was determined using the 16-hour plasma molar concentration of quinine to that of 3-hydroxyquinine ratio as an index. Analysis of covariance was used to determine covariate effects on drug pharmacokinetics parameters **(V)** and its metabolic ratio **(II)** while path analysis using ANOVA was employed to determine associations of genotypes, rifampicin treatment, efavirenz concentrations and various demographic factors on CNS toxicity **(V).**

4.6   ETHICAL CONSIDERATIONS

Studies were conducted in conformity with the Helsinki declaration and the International Conference on Harmonization (ICH) recommendations. Written informed consent were sought and obtained from all participants **(Paper 1 - V)**. No incentives were given for participation while withdrawal from the studies was voluntary without retribution. Participant identities were kept anonymous accept to the principal investigator and primary study team. Ethical clearance was sought from the Higher Degrees Research Committee of Makerere University, the Uganda National Council for Science and Technology **(Paper I - V)** and the Ethical Review Board at Karolinska Institute, Stockholm Sweden **(Paper I, III, IV & V)**

5 RESULTS

5.1 EFFECT OF SEX AND *CYP2B6, CYP3A5* AND *ABCB1* GENE POLYMORPHISM ON SINGLE DOSE EFAVIRENZ PHARMACOKINETICS (PAPER I)

SNP frequencies among Ugandans were comparable to what has been reported for other African populations. CYP2B6*6 and *11 influenced apparent oral clearance (CL/F) of efavirenz with slower metabolizers exhibiting 21% and 20% less than the faster metabolizers respectively. Efavirenz relative bioavailability was 26% higher in persons homozygous to ABCB1 (c4036A>G). The three SNPs found to influence efavirenz pharmacokinetics: CYP2B6 *6 and *11 and ABCB1 c.4036A>G SNPs were observed at frequencies of 55%, 13.6% and 16.8% respectively

5.1.1 Sex effect on efavirenz pharmacokinetics

The study reports two fold higher apparent volume of distribution (Vp/F) among women compared to men signifying an increased likelihood of efavirenz toxicity or treatment failure among females than men respectively. Efavirenz lipophilicity offers a plausible explanation for the higher Vp/F among women who are known to have bigger fat deposits compared to their male counterparts (107).

5.1.2 Genotype - sex effects on efavirenz pharmacokinetics

While participants homozygous for all clinically relevant mutations (CYP2B6 *6, *11 and ABCB1 c.4036A>G) exhibited 1.5 fold longer efavirenz half-life and about two fold greater area under the curve (AUC) ($943 \mu Mh^{-1}$) than wild type participants ($475 \mu Mh^{-1}$), complimentary sex effects resulted into a 3 and 2 fold increase in terminal half-life among mutant women (108.9 hours) compared to typical wild type (37.3 hours) and mutant men (54.7 hours) respectively. A 24-h AUC (AUC24) for efavirenz of 190–380 μMh; (60–120 mg h/l 1μM = 0.315 mg/l) represents the 50th to twice the 50th percentile of AUC24 values in adults receiving the recommended dose of 600 mg/day (108). High AUC ($943 \mu Mh^{-1}$) was reported for individuals homozygous for the three functional alleles.

5.2 EFFECT OF GENE POLYMORPHISM ON QUININE DISPOSITION (PAPER II)

Assessment of ABCB1 and CYP3A5 gene and sex effect on weight normalized quinine plasma concentrations revealed that while women metabolize quinine 1.2 times faster than men (p =0.013), they also exhibit higher drug bioavailability resulting into significantly higher 16-hour plasma quinine concentrations (5.98± 0.28µMolL^{-1}) compared to men (4.43± 0.30µMolL^{-1}) (p= 0.0003). The findings also revealed that ABCB1 polymorphism at Exon 6/ +138C>T (rs 1202168), c2677G/A>T (rs 2032582) and Exon 20/+24G>A, that were observed frequencies 16.8%, 3.7% and 4.6% respectively, significantly influenced and may partially explain the 30 fold variation in plasma quinine concentrations reported by this sub-study. Individuals exhibiting wild type haplotype (68.5%) for SNPs in ABCB1 that were studied, had significantly low weight-normalized quinine plasma concentration (mean ± SE, 4.33 ± 0.29 mmol/L) compared with those heterozygous (mean ± SE, 5.9 ± 0.49 mmol/L) or homozygous (mean ± standard deviation, 7.5 ± 0.64 mmol/L) (P = 0.0003). Similarly, individuals with 2 CYP3A5 functional alleles exhibited significantly low quinine MR followed by those with one (P = 0.030).

5.3 EFFECT OF HIV INFECTION ON EFAVIRENZ BIOAVAILABILITY (PAPER III)

This study, in which single dose efavirenz pharmacokinetics was compared between HIV/AIDS patients and healthy volunteers, confirmed a higher peripheral volume of distribution (108%) and two fold longer half-life (78hours) among compared women to men, while HIV/AIDS patients exhibited 30% lower relative bioavailability compared to healthy volunteers.

5.4 EFFECT OF RIFAMPAICIN AND CYB2B6, CYP3A5, ABCB1 GENE POLYMORPHISM ON ENZYME INDUCTION DURING EFAVIRENZ TREATMENT (PAPER IV)

In this study the CYP2B6, CYP3A5, ABCB1 gene polymorphism and rifampicin co-treatment effect on enzyme induction in patients receiving efavirenz treatment was assessed. Both short and long–term induction was predicted by CYP2B6*6 on all drug analysis days (p <0.01) while CYP2B6*11 predicted efavirenz clearance on days 14 and 56; p = 0.002 and 0.019 respectively. Rifampicin co-treatment is associated with short-term CYP2B6 enzyme induction in HIV/AID patients receiving efavirenz and

rifampicin co-treatment. Patients co-treated with rifampicin exhibited higher efavirenz clearance on day 14 (p= 0.01) while effect of efavirenz auto induction on efavirenz clearance was lower in patients co-treated with rifampicin.

5.5 EFAVIRENZ CENTRAL NERVOUS SYSTEM TOXICITY DURING RIFAMPICIN CO-TREATMENT (PAPER V)

Evaluation of the effect of rifampicin and CYP2B6, CYP3A5 and ABCB1 gene polymorphism on efavirenz related CNS toxicity: sleep disorders, hallucinations and cognition, in HIV patients revealed that efavirenz CNS toxicity is a major problem in Ugandan HIV-1 infected patients. At least 7 in every 10 of the patients (75.6%) suffer some form of CNS toxicity during the initial twelve weeks of efavirenz treatment. The commonest form of CNS disorder exhibited is sleep disorders 60.5% (n= 124), followed by hallucination 30.7% (n = 63). Neither rifampicin co-treatment (p=0.7) nor sex (p= 0.4) influence efavirenz CNS toxicity. CYP2B6 (*6 & *11) and ABCB1 rs 3842 predict efavirenz concentrations, which in turn influenced CNS toxicity. CNS toxicity is predicted by efavirenz concentrations on days 3, 28, 42 and 84 (p = 0.02, 0.023, 0.025 and 0.032).

5.5.1 Sleep disorders

Sleep disorders are a major symptom of efavirenz CNS toxicity whose incidence increased 4 fold from 18.2% (n =36) at baseline, [15.2% (n =9) in non-rifampicin treatment group and 19.7% (n=27) in rifampicin treatment group] to 60.5% (n= 124) over the initial 12 weeks of efavirenz treatment. Incidence of sleep disorders was not affected by rifampicin co-treatment [36.4% (n = 20) non-rifampicin versus 35.5% (n = 49) rifampicin treatment groups], p= 0.46. Efavirenz related sleep disorders were neither determined by sex nor CYP2B6 (*6 & *11), ABCB1 (c.4036A>G & c.3435C>T), CYP3A5*1 genotypes but rather by efavirenz plasma concentrations at day1 (p = 0.01), day 14 (p = 0.035), day 28 (p= 0.003) day 42 (p =0.006) day 56 (p=0.17) and day 84 (p=0.02). Sleep disorders were evaluated in terms of insomnia, sleep walking and vivid dreams. Majority of patients who suffered sleep disorders, 93 % (115) experienced vivid dreams. Insomnia and sleep walking were reported at rates of 36.3% (n= 45) and 8.1% (n= 10) respectively.

5.5.2 Hallucinations

Efavirenz treatment increased incidence of hallucinations among HIV patients from 1.6% (n=3) before treatment to 30.7% (n=63) during 12 weeks of initial treatment. All hallucinations except one occurred by week two of efavirenz treatment. Although isolated cases of paranoid hallucinatory symptoms have previously been report as symptoms for HIV/AIDS disease (109, 110), findings in this thesis associate hallucinations with efavirenz HIV treatment.

5.5.3 Cognition

HIV/AIDS disease is associated with cognitive dysfunction. According to our study 73 % of HIV infected patients initiating ART have a cognition disorder, 23.3% (n =46) exhibited severely impaired cognition, MMSE < 19. Although efavirenz is generally associated CNS toxicity, cognition improved with efavirenz based antiretroviral therapy, with the number of patients scored <19 on the MMSE reducing from 46 (23.3%) at ART initiation to 22 (10.7%) at twelve weeks of treatment. This finding is in agreement with results from several studies including those conducted in Uganda, Thailand Brazil and USA, which according Joska et al. (111) reported a significant improvement in neurocognitive status profile with ART.

6 DISCUSSION

In this thesis, the role of multiple SNPs (30) on efavirenz pharmacokinetics was initially determined **(Paper I)**. The thesis further examined the effect of SNPs found to play a role in efavirenz population pharmacokinetics and rifampicin co-treatment on CYP2B6 enzyme induction and efavirenz clearance **(Paper IV)** and efavirenz related CNS toxicity **(Paper V)**.   Based on possible physiological and anatomical changes associated with HIV infection, the effect of the disease on efavirenz pharmacokinetics was also assessed.   Furthermore, the thesis examined the effect of various SNPs in CYP3A5 and ABCB1 on quinine disposition. Findings from this first experiment apart from further enriching scientific knowledge, guided the subsequent clinical studies with regard to SNPs that were likely to be clinically important. CYP2B6 *6, CYP2B6 *11, ABCB1   (c.4036A>G),   sex   and   HIV/AIDS   disease   influenced   efavirenz pharmacokinetics **(Paper I & III)**. Although, from the healthy volunteer study, ABCB1 c.3435C>T did not significantly influence efavirenz pharmacokinetics **(Paper 1)** due to its previously documented role in HIV treatments (112), this thesis controlled for its possible effect on efavirenz disposition during assessment of the effect of HIV infection on efavirenz pharmacokinetics **(Paper III)** and examined its possible role in enzyme induction and efavirenz related CNS toxicity among HIV patients.

Overall sex plays a role in pharmacokinetics of both quinine and efavirenz. Given that quinine has a narrow therapeutic window with its therapeutic and toxic concentration profiles overlapping(98), women are more likely to experience acute cinchonism and other quinine concentration related side effects. Higher quinine bioavailability in women   may   explain   the   previously   reported   higher   ototoxicity   exhibited   by women(113).   This result indicates need for steady state quinine pharmacokinetic–pharmacodynamic studies that consider sex differences. On the other hand, the sex effect   on   efavirenz   pharmacokinetics   seem   to   weaken   at   steady   state   conditions implying that except in cases of existing complimentary factors, it may not have significant pharmacodynamic implications **(Paper IV & V).** This is further supported by the finding of no significant sex difference in efavirenz related CNS toxicity **(Paper V).**

In agreement with previous findings the results herein indicate that CYP2B6*6 predicts both efavirenz pharmacokinetics as well as treatment outcomes. The thesis further demonstrated the role of CYP2B6*11 and ABCB1 c.4036A>G in both efavirenz pharmacokinetics **(Paper I & IV)** and pharmacodynamics **(Paper V)**. Although not as significant as for *CYP2B6*6,* the demonstrated role of *CYP2B6*11* and *ABCB1 c.4036A>G* in both efavirenz pharmacokinetics and pharmacodynamics signify their clinical significance regarding HIV treatment with efavirenz. Furthermore *CYP2B6*11* has been confirmed to influence intracellular efavirenz concentrations (114) a fact that supports its clinical relevance considering that its antiviral activity is exhibited intracellularly.

The thesis reports no effect of rifampicin co-treatment on long term efavirenz induction as well as efavirenz related CNS toxicity. While the observed lack of rifampicin effect on long term enzyme induction is in agreement with other recent reports, lack of rifampicin effect on efavirenz related CNS toxicity reported by this thesis (Paper **V**) may be attributed to similar efavirenz concentrations without and during rifampicin co-treatment. According to findings of this thesis, the rifampicin / efavirenz interactions that have long been speculated during co-treatment with the two drugs may not have significant clinical relevance but rather the CYP2B6 genotypes. TB-HIV co-infections remain the biggest challenge in HIV care, yet optimal treatment regimens for HIV/TB co-infection are not yet clearly defined (115) largely due to fear for sub-therapeutic drug concentrations resulting from possible alterations in the activities of the hepatic metabolizing enzymes and protein transporters. While CYP2B6 genotypes might form basis for population specific efavirenz dosing recommendations for HIV patients on or off rifampicin, CYP3A5, ABCB1 genotypes and sex need to be considered for quinine dose recommendations although additional studies are still required. Although clinical data available at conception of this thesis suggest that, for the majority of individuals, rifampicin-based regimens could be successfully combined with the non-nucleoside reverse transcriptase inhibitor efavirenz, dosage modification was recommended. According to this thesis however, rifampicin co-treatment neither affects steady state efavirenz plasma concentrations nor modifies efavirenz related CNS toxicities. Conversely CYP2B6 and ABCB1 genotypes influence efavirenz pharmacokinetics as well as pharmacodynamics. These findings support TB-HIV co-treatment with rifampicin and efavirenz based regimens respectively. Additionally, the primary role of CYP26 genotype in efavirenz

disposition as well as dynamics demonstrated in this thesis and other reports, clearly indicate a possibility to optimize efavirenz use in management of HIV with or without rifampicin co-treatment by genotyping for the CYP2B6.

This thesis, further reports 30 % lower efavirenz bioavailability among HIV/AIDS patients compared to healthy volunteers indicating the need for pharmacokinetic studies of relevant drugs in HIV patients. Control for the effect of several biological factors in determination of effect of HIV/AIDS disease on efavirenz bioavailability render findings reported herein reliable although they may not directly apply for all drugs used by HIV/AIDS patients. Notable is that similar findings have been reported for other drugs including ketoconazole. Both physiological (116) and anatomical changes related to HIV/AIDS disease(117) are probable causes for reduction in bioavailability of efavirenz and other drugs among HIV patients. Although this finding may not be generalized to all drugs, pharmacokinetic studies of existing and new drugs in HIV/AIDS patients may be informative especially for drugs relevant for HIV care. Finally, the genotype effect on both efavirenz, with or without rifampicin, and quinine pharmacokinetics clearly indicate the critical role of pharmacogenetics in malaria, HIV and TB treatments.

Although assessment of virological and immunologic recovery during rifampicin co-treatment and with CYP2B6, CYP3A5 and ABCB1 gene polymorphism was outside the scope of this thesis, findings herein clearly demonstrate the clinical relevance of the studied genotypes. Importantly, equal sex participation in the studies (50 – 60 % female representation) enabled assessment for possible sex differences in efavirenz and quinine disposition and efavirenz pharmacodynamics. Furthermore, Ugandans among whom the constituent studies of this thesis were performed are known to have a diverse ancestry giving these findings wide application particularly in sub-Saharan Africa.

7   CONCLUSIONS AND RECOMMENDATIONS

7.1   CONCLUSIONS

- Quinine disposition is influenced by sex and ABCB1 (rs2235040, rs1202168 and rs36008564) and CYP3A5 genotypes.

- Both quinine plasma concentrations and metabolic ratios vary significantly in Ugandans.

- Women are faster quinine metabolizers and display higher quinine bioavailability than men and exhibit than men

- *CYP2B6*6*, *CYP2B6*11,* ABCB1 (rs3842) and sex are major predictors of efavirenz plasma exposure both after single-dose administration and at steady state

- Efavirenz auto induction effect on its own clearance is greater in patients off than during rifampicin co-treatment.

- CYP2B6 genotypes rather than rifampicin predict long–term enzyme induction in patients treated with or without rifampicin

- Rifampicin co-treatment predicts short term enzyme induction during co-treatment with efavirenz

- Efavirenz related CNS side effects are common among HIV/AIDS patients receiving efavirenz based treatments

- Efavirenz related CNS toxicity is not affected by rifampicin co-treatment but by plasma efavirenz concentrations.

7.2 RECOMMENDATIONS

7.2.1 Quinine

1. In the short term, pharmacogenetic research can help to better understand the pharmacokinetics and pharmacodynamics of anti-malarial drugs and particularly quinine. A prospective, randomized trial where comprehensive clinical data, from large numbers of patients to systematically assess pharmacokinetics in relation to dosage and clinical outcomes considering host genetic factors and sex need to be conducted among Ugandan malaria patients. Replication of such a trial in independent cohorts based on endemicity of malaria and likely differences in host genetic factor may go a long way in optimization of quinine in malaria treatment.

7.2.2 Efavirenz

1. Dose prediction and pharmacokinetics of efavirenz and other such drugs that are commonly used among HIV infected persons need to be conducted in HIV/AIDS patients.

2. Integration of pharmacogenetics into patient care is important for HIV/TB treatments. Genotyping CYP2B may particularly optimize efavirenz dosing and treatment outcomes in both HIV and TB co-infected HIV patients. Resource limited settings might benefit from dose stratification based on well designed pharmacogenetics studies.

3. Efavirenz – rifampicin co-treatments may be administered to TB- HIV patients without dose modification

8 ACKNOWLEDGEMENTS

9 REFERENCES

1.   Hotez PJ, Remme JH, Buss P, Alleyne G, Morel C, Breman JG. Combating tropical infectious diseases: report of the Disease Control Priorities in Developing Countries Project. Clin Infect Dis. 2004 15;38(6):871-8.

2.   Naidoo K, Padayatchi N, Abdool Karim Q. HIV-Associated Tuberculosis. Clin Dev Immunol. 2011;2011.

3.   Breman JG, Alilio MS, Mills A. Conquering the intolerable burden of malaria: what's new, what's needed: a summary. Am J Trop Med Hyg. 2004;71(2 Suppl):1-15.

4.   WHO. World Health Malaria Report. 2009.

5.   Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW. The global distribution and population at risk of malaria: past, present, and future. Lancet Infect Dis. 2004;4(6):327-36.

6.   Omari AA, Gamble C, Garner P. Artemether-lumefantrine (six-dose regimen) for treating uncomplicated falciparum malaria. Cochrane Database Syst Rev. 2005(4):CD005564.

7.   Simba DO, Warsame M, Kakoko D, Mrango Z, Tomson G, Premji Z, et al. Who gets prompt access to artemisinin-based combination therapy? A prospective community-based study in children from rural Kilosa, Tanzania. PLoS One. 2010;5(8).

8.   Mason C. Delivering health care on US$19 per capita. Canadian Medical Association Journal. 2008 178:135–7.

9.   MOH. National Antiretroviral Treatment Guidelines for Adults, Adolescents and Children. In: Uganda, editor. 3rd ed. Kampala2009.

10.   Wellems TE, Plowe CV. Chloroquine-resistant malaria. J Infect Dis. 2001 Sep 15;184(6):770-6.

11.   Mberu EK, Mosobo MK, Nzila AM, Kokwaro GO, Sibley CH, Watkins WM. The changing in vitro susceptibility pattern to pyrimethamine/sulfadoxine in Plasmodium falciparum field isolates from Kilifi, Kenya. Am J Trop Med Hyg. 2000;62(3):396-401.

12.   Nzila AM, Mberu EK, Sulo J, Dayo H, Winstanley PA, Sibley CH, et al. Towards an understanding of the mechanism of pyrimethamine-sulfadoxine resistance in Plasmodium falciparum: genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites. Antimicrob Agents Chemother. 2000;44(4):991-6.

13.   Lynch C, Pearce R, Pota H, Cox J, Abeku TA, Rwakimari J, et al. Emergence of a dhfr mutation conferring high-level drug resistance in Plasmodium falciparum populations from southwest Uganda. J Infect Dis. 2008 1;197(11):1598-604.

14.   Vogel F. [Future problems of medical genetics]. Bull Schweiz Akad Med Wiss. 1978;34(4-6):411-23.

15.   Ekins S, Wrighton S. The role of CYP2B6 in human xenobiotic metabolism Drug metabolism Reviews. 1999;31:719 - 54.

16.   Ward BA, Gorsk JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The Cytochrome P450 2B6 (CYP2B6) Is the Main Catalyst of Efavirenz Primary and Secondary Metabolism: Implication for HIV/AIDS Therapy and Utility of Efavirenz as a Substrate Marker of CYP2B6 Catalytic Activity JPET 2003;306(387 - 300).

17.   Hesse L, Ping H, Krishnaswamy S, Qin H, Hogan K, Moltke L, et al. Pharmacogenetic determinants of interindividual variability in bupropion hydroxylation by cytochrome P450 2B6 in human liver microsomes Pharmacogenetics. 2004;14:225 - 38.

18.   Wang H, Tompkins LM. CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme. Curr Drug Metab. 2008;9(7):598-610.

19.   Thorn CF, Lamba JK, Lamba V, Klein TE, Altman RB. PharmGKB summary: very important pharmacogene information for CYP2B6. Pharmacogenet Genomics. 2010;20(8):520-3.

20.   Drocourt L, Ourlin JC, Pascussi JM, Maurel P, Vilarem MJ. Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. J Biol Chem. 2002 12;277(28):25125-32.

21.   Kirchheiner J, Klein C, Meineke I, Sasse J, Zanger UM, Murdter TE, et al. Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6. Pharmacogenetics. 2003;13(10):619-26.

22.   Crettol S, Deglon JJ, Besson J, Croquette-Krokkar M, Gothuey I, Hammig R, et al. Methadone enantiomer plasma levels, CYP2B6, CYP2C19, and CYP2C9 genotypes, and response to treatment. Clin Pharmacol Ther. 2005;78(6):593-604.

23.   Bunten H, Liang WJ, Pounder DJ, Seneviratne C, Osselton D. OPRM1 and CYP2B6 gene variants as risk factors in methadone-related deaths. Clin Pharmacol Ther. 2010;88(3):383-9.

24.	Ring HZ, Valdes AM, Nishita DM, Prasad S, Jacob P, 3rd, Tyndale RF, et al. Gene-gene interactions between CYP2B6 and CYP2A6 in nicotine metabolism. Pharmacogenet Genomics. 2007;17(12):1007-15.

25.	Restrepo JG, Garcia-Martin E, Martinez C, Agundez JA. Polymorphic drug metabolism in anaesthesia. Curr Drug Metab. 2009;10(3):236-46.

26.	Zanger UM, Turpeinen M, Klein K, Schwab M. Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. Anal Bioanal Chem. 2008 392(6):1093-108.

27.	Wrighton S, Schuetz E, Thummel K, Shen D, Korzekwa K, Watkins P. The human CYP3A subfamily : Practical considerations Drug metabRev. 2000;32 (3-4):339 – 61.

28.	Guengerich FP. Cytochrome P450 3A4: regulation and role in drug metabolism. Ann Rev PhramocolToxicol. 1999; 39:1-17.

29.	Lin YS, Dowlingn ALS, Quigley SD, Farin FM, Zhang J, J Lamba, et al. Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism Mol Pharmacol. 2002;62(1):162 - 72.

30.	Williams JA, Ring JB, Cantrell EV, Jones RD, Eckstein J, Ruterbories K, et al. Comparative metabolic capacities of CYP3A4, CYP3A5 and CYP3A7. drug Metab Dispos. 2002;30(8):883 - 91.

31.	Lamba J, Lin Y, Schuetz E, Thummel K. Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev 2002;54(10):1271 - 94.

32.	Huang W, Lin YS, McConn DJ, 2nd, Calamia JC, Totah RA, Isoherranen N, et al. Evidence of significant contribution from CYP3A5 to hepatic drug metabolism. Drug Metab Dispos. 2004 32(12):1434-45.

33.	Yamaori S, Yamazaki H, Iwano S, Kiyotani K, Matsumura K, Honda G, et al. CYP3A5 Contributes significantly to CYP3A-mediated drug oxidations in liver microsomes from Japanese subjects. Drug Metab Pharmacokinet. 2004;19(2):120-9.

34.	Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet. 2001;27(4):383-91.

35.	Fromm M. P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. Int J Clin Pharmacol Ther. 2000;38(2):69 - 74.

36.   Kindla J, Fromm F, Konig J. In vitro evidence for the role of OATP and OCT uptake transporters in drug–drug interactions. Expert Opin Drug Metab Toxicol. 2009;5:489 - 500.

37.   Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci U S A. 2000 28;97(7):3473-8.

38.   Fromm MF. P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. Int J Clin Pharmacol Ther. 2000;38(2):69-74.

39.   Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci U S A. 1987;84(21):7735-8.

40.   Magliulo L, Dahl ML, Lombardi G, Fallarini S, Villa LM, Biolcati A, et al. Do CYP3A and ABCB1 genotypes influence the plasma concentration and clinical outcome of donepezil treatment? Eur J Clin Pharmacol. 2011;67(1):47-54.

41.   Takahashi N, Miura M, Scott SA, Kagaya H, Kameoka Y, Tagawa H, et al. Influence of CYP3A5 and drug transporter polymorphisms on imatinib trough concentration and clinical response among patients with chronic phase chronic myeloid leukemia. J Hum Genet. 2010;55(11):731-7.

42.   Balcerczak E, Panczyk M, Piaskowski S, Pasz-Walczak G, Salagacka A, Mirowski M. ABCB1/MDR1 gene polymorphisms as a prognostic factor in colorectal cancer. Int J Colorectal Dis. 2010;25(10):1167-76.

43.   Cavaco I, Gil JP, Gil-Berglund E, Ribeiro V. CYP3A4 and MDR1 alleles in a Portuguese population. Clin Chem Lab Med. 2003;41(10):1345-50.

44.   Kaya P, Gunduz U, Arpaci F, Ural AU, Guran S. Identification of polymorphisms on the MDR1 gene among Turkish population and their effects on multidrug resistance in acute leukemia patients. Am J Hematol. 2005;80(1):26-34.

45.   Pechandova K, Buzkova H, Slanar O, Perlik F. Polymorphisms of the MDR1 gene in the Czech population. Folia Biol (Praha). 2006;52(6):184-9.

46.   Zhou SF. Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. Xenobiotica. 2008;38(7-8):802-32.

47.   Woodahl EL, Yang Z, Bui T, Shen DD, Ho RJ. MDR1 G1199A polymorphism alters permeability of HIV protease inhibitors across P-glycoprotein-expressing epithelial cells. AIDS. 2005 14;19(15):1617-25.

48.   Drescher S, Schaeffeler E, Hitzl M, Hofmann U, Schwab M, Brinkmann U, et al. MDR1 gene polymorphisms and disposition of the P-glycoprotein substrate fexofenadine. Br J Clin Pharmacol. 2002;53(5):526-34.

49.   Foote CJ, Greer W, Kiberd B, Fraser A, Lawen J, Nashan B, et al. Polymorphisms of multidrug resistance gene (MDR1) and cyclosporine absorption in de novo renal transplant patients. Transplantation. 2007 27;83(10):1380-4.

50.   Klimecki WT, Futscher BW, Grogan TM, Dalton WS. P-glycoprotein expression and function in circulating blood cells from normal volunteers. Blood. 1994 1;83(9):2451-8.

51.   Fellay J, Marzolini C, Meaden ER, Back DJ, Buclin T, Chave JP, et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. Lancet. 2002 5;359(9300):30-6.

52.   Thompson EE, Kuttab-Boulos H, Witonsky D, Yang L, Roe BA, Di Rienzo A. CYP3A variation and the evolution of salt-sensitivity variants. Am J Hum Genet. 2004;75(6):1059-69.

53.   Schirmer M, Toliat MR, Haberl M, Suk A, Kamdem LK, Klein K, et al. Genetic signature consistent with selection against the CYP3A4*1B allele in non-African populations. Pharmacogenet Genomics. 2006;16(1):59-71.

54.   Sata F, Sapone A, Elizondo G, Stocker P, Miller VP, Zheng W, et al. CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. Clin Pharmacol Ther. 2000;67(1):48-56.

55.   Lamba JK, Lin YS, Thummel K, Daly A, Watkins PB, Strom S, et al. Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. Pharmacogenetics. 2002;12(2):121-32.

56.   Rodrigues IS, Kuasne H, Losi-Guembarovski R, Fuganti PE, Gregorio EP, Kishima MO, et al. Evaluation of the influence of polymorphic variants CYP1A1*2B, CYP1B1*2, CYP3A4*1B, GSTM1*0, and GSTT1*0 in prostate cancer. Urol Oncol. 2010 28.

57.   Kamdem LK, Streit F, Zanger UM, Brockmoller J, Oellerich M, Armstrong VW, et al. Contribution of CYP3A5 to the in vitro hepatic clearance of tacrolimus. Clin Chem. 2005;51(8):1374-81.

58.   Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, et al. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics. 2001;11(9):773-9.

59.   Lee SJ, Usmani KA, Chanas B, Ghanayem B, Xi T, Hodgson E, et al. Genetic findings and functional studies of human CYP3A5 single nucleotide polymorphisms in different ethnic groups. Pharmacogenetics. 2003;13(8):461-72.

60.   Lin YS, Dowling AL, Quigley SD, Farin FM, Zhang J, Lamba J, et al. Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism. Mol Pharmacol. 2002;62(1):162-72.

61.   Wojnowski L, Turner PC, Pedersen B, Hustert E, Brockmoller J, Mendy M, et al. Increased levels of aflatoxin-albumin adducts are associated with CYP3A5 polymorphisms in The Gambia, West Africa. Pharmacogenetics. 2004;14(10):691-700.

62.   Wojnowski L, Kamdem LK. Clinical implications of CYP3A polymorphisms. Expert Opin Drug Metab Toxicol. 2006;2(2):171-82.

63.   Miksys S, Tyndale RF. The unique regulation of brain cytochrome P450 2 (CYP2) family enzymes by drugs and genetics. Drug Metab Rev. 2004;36(2):313-33.

64.   Code EL, Crespi CL, Penman BW, Gonzalez FJ, Chang TK, Waxman DJ. Human cytochrome P4502B6: interindividual hepatic expression, substrate specificity, and role in procarcinogen activation. Drug Metab Dispos. 1997;25(8):985-93.

65.   Lang T, Klein K, Fischer J, Nussler AK, Neuhaus P, Hofmann U, et al. Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. Pharmacogenetics. 2001;11(5):399-415.

66.   Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, et al. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther. 2003;307(3):906-22.

67.   Zanger UM, Klein K, Saussele T, Blievernicht J, Hofmann MH, Schwab M. Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. Pharmacogenomics. 2007;8(7):743-59.

68.   Wang J, Sonnerborg A, Rane A, Josephson F, Lundgren S, Stahle L, et al. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. Pharmacogenet Genomics. 2006;16(3):191-8.

69.   Rotger M, Tegude H, Colombo S, Cavassini M, Furrer H, Decosterd L, et al. Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. Clin Pharmacol Ther. 2007;81(4):557-66.

70.   Daujat M, Clair P, Astier C, Fabre I, Pineau T, Yerle M, et al. Induction, regulation and messenger half-life of cytochromes P450 IA1, IA2 and IIIA6 in primary

cultures of rabbit hepatocytes. CYP 1A1, 1A2 and 3A6 chromosome location in the rabbit and evidence that post-transcriptional control of gene IA2 does not involve mRNA stabilization. Eur J Biochem. 1991 1;200(2):501-10.

71.   Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, et al. Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. J Biol Chem. 2000 19;275(20):15122-7.

72.   Smirlis D, Muangmoonchai R, Edwards M, Phillips IR, Shephard EA. Orphan receptor promiscuity in the induction of cytochromes p450 by xenobiotics. J Biol Chem. 2001 20;276(16):12822-6.

73.   Faucette SR, Sueyoshi T, Smith CM, Negishi M, Lecluyse EL, Wang H. Differential regulation of hepatic CYP2B6 and CYP3A4 genes by constitutive androstane receptor but not pregnane X receptor. J Pharmacol Exp Ther. 2006;317(3):1200-9.

74.   Gardiner SJ, Begg EJ. Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. Pharmacol Rev. 2006;58(3):521-90.

75.   Alving AS, Carson PE, Flanagan CL, Ickes CE. Enzymatic deficiency in primaquine-sensitive erythrocytes. Science. 1956 14;124(3220):484-5.

76.   Hughes HB, Biehl JP, Jones AP, Schmidt LH. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. Am Rev Tuberc. 1954;70(2):266-73.

77.   Liu L, Fan L, Peng X, Hu D, Zhou H. MDR1 C2005T polymorphism changes substrate specificity. Cancer Chemother Pharmacol. 2010;66(3):617-23.

78.   Choudhuri S, Klaassen CD. Structure, function, expression, genomic organization, and single nucleotide polymorphisms of human ABCB1 (MDR1), ABCC (MRP), and ABCG2 (BCRP) efflux transporters. Int J Toxicol. 2006 J;25(4):231-59.

79.   Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS. 2001 5;15(1):71-5.

80.   Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. J Pharmacol Exp Ther. 2003;306(1):287-300.

81.		Desta Z, Saussele T, Ward B, Blievernicht J, Li L, Klein K, et al. Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro. Pharmacogenomics. 2007;8(6):547-58.

82.		Arab-Alameddine M, Di Iulio J, Buclin T, Rotger M, Lubomirov R, Cavassini M, et al. Pharmacogenetics-based population pharmacokinetic analysis of efavirenz in HIV-1-infected individuals. Clin Pharmacol Ther. 2009;85(5):485-94.

83.		Dirson G, Fernandez C, Hindlet P, Roux F, German-Fattal M, Gimenez F, et al. Efavirenz does not interact with the ABCB1 transporter at the blood-brain barrier. Pharm Res. 2006;23(7):1525-32.

84.		Haas DW, Smeaton LM, Shafer RW, Robbins GK, Morse GD, Labbe L, et al. Pharmacogenetics of long-term responses to antiretroviral regimens containing Efavirenz and/or Nelfinavir: an Adult Aids Clinical Trials Group Study. J Infect Dis. 2005 1;192(11):1931-42.

85.		Faucette SR, Wang H, Hamilton GA, Jolley SL, Gilbert D, Lindley C, et al. Regulation of CYP2B6 in primary human hepatocytes by prototypical inducers. Drug Metab Dispos. 2004;32(3):348-58.

86.		Hesse LM, Sakai Y, Vishnuvardhan D, Li AP, von Moltke LL, Greenblatt DJ. Effect of bupropion on CYP2B6 and CYP3A4 catalytic activity, immunoreactive protein and mRNA levels in primary human hepatocytes: comparison with rifampicin. J Pharm Pharmacol. 2003;55(9):1229-39.

87.		Csajka C, Marzolini C, Fattinger K, Decosterd LA, Fellay J, Telenti A, et al. Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. Clin Pharmacol Ther. 2003;73(1):20-30.

88.		Lopez-Cortes LF, Ruiz-Valderas R, Viciana P, Alarcon-Gonzalez A, Gomez-Mateos J, Leon-Jimenez E, et al. Pharmacokinetic interactions between efavirenz and rifampicin in HIV-infected patients with tuberculosis. Clin Pharmacokinet. 2002;41(9):681-90.

89.		Manosuthi W, Kiertiburanakul S, Sungkanuparph S, Ruxrungtham K, Vibhagool A, Rattanasiri S, et al. Efavirenz 600 mg/day versus efavirenz 800 mg/day in HIV-infected patients with tuberculosis receiving rifampicin: 48 weeks results. AIDS. 2006 2;20(1):131-2.

90.		Ramachandran G, Hemanth Kumar AK, Rajasekaran S, Kumar P, Ramesh K, Anitha S, et al. CYP2B6 G516T polymorphism but not rifampin coadministration influences steady-state pharmacokinetics of efavirenz in human

immunodeficiency virus-infected patients in South India. Antimicrob Agents Chemother. 2009;53(3):863-8.

91.   Cohen K, Grant A, Dandara C, McIlleron H, Pemba L, Fielding K, et al. Effect of rifampicin-based antitubercular therapy and the cytochrome P450 2B6 516G>T polymorphism on efavirenz concentrations in adults in South Africa. Antivir Ther. 2009;14(5):687-95.

92.   Kwara A, Ramachandran G, Swaminathan S. Dose adjustment of the non-nucleoside reverse transcriptase inhibitors during concurrent rifampicin-containing tuberculosis therapy: one size does not fit all. Expert Opin Drug Metab Toxicol. 2010;6(1):55-68.

93.   Friedland G, Khoo S, Jack C, Lalloo U. Administration of efavirenz (600 mg/day) with rifampicin results in highly variable levels but excellent clinical outcomes in patients treated for tuberculosis and HIV. J Antimicrob Chemother. 2006 58(6):1299-302.

94.   Matteelli A, Regazzi M, Villani P, De Iaco G, Cusato M, Carvalho AC, et al. Multiple-dose pharmacokinetics of efavirenz with and without the use of rifampicin in HIV-positive patients. Curr HIV Res. 2007;5(3):349-53.

95.   Zhang H, Coville PF, Walker RJ, Miners JO, Birkett DJ, Wanwimolruk S. Evidence for involvement of human CYP3A in the 3-hydroxylation of quinine. Br J Clin Pharmacol. 1997;43(3):245-52.

96.   Mirghani RA, Sayi J, Aklillu E, Allqvist A, Jande M, Wennerholm A, et al. CYP3A5 genotype has significant effect on quinine 3-hydroxylation in Tanzanians, who have lower total CYP3A activity than a Swedish population. Pharmacogenet Genomics. 2006;16(9):637-45.

97.   Pukrittayakamee S, Looareesuwan S, Keeratithakul D, Davis TM, Teja-Isavadharm P, Nagachinta B, et al. A study of the factors affecting the metabolic clearance of quinine in malaria. Eur J Clin Pharmacol. 1997;52(6):487-93.

98.   Krishna S, White NJ. Pharmacokinetics of quinine, chloroquine and amodiaquine. Clinical implications. Clin Pharmacokinet. 1996;30(4):263-99.

99.   Hayeshi R, Masimirembwa C, Mukanganyama S, Ungell AL. The potential inhibitory effect of antiparasitic drugs and natural products on P-glycoprotein mediated efflux. Eur J Pharm Sci. 2006;29(1):70-81.

100.   Crowe A, Ilett KF, Karunajeewa HA, Batty KT, Davis TM. Role of P glycoprotein in absorption of novel antimalarial drugs. Antimicrob Agents Chemother. 2006;50(10):3504-6.

101.   Pussard E, Merzouk M, Barennes H. Increased uptake of quinine into the brain by inhibition of P-glycoprotein. Eur J Pharm Sci. 2007;32(2):123-7.

102.   Mirghani RA, Ericsson O, Cook J, Yu P, Gustafsson LL. Simultaneous determination of quinine and four metabolites in plasma and urine by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 2001 15;754(1):57-64.

103.   Tang K, Ngoi SM, Gwee PC, Chua JM, Lee EJ, Chong SS, et al. Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. Pharmacogenetics. 2002;12(6):437-50.

104.   Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clin Pharmacol Ther. 2001;69(3):169-74.

105.   Lindroos K, Liljedahl U, Syvanen AC. Genotyping SNPs by minisequencing primer extension using oligonucleotide microarrays. Methods Mol Biol. 2003;212:149-65.

106.   Lovmar L, Syvanen AC. Genotyping single-nucleotide polymorphisms by minisequencing using tag arrays. Methods Mol Med. 2005;114:79-92.

107.   Takano R, Sugano K, Higashida A, Hayashi Y, Machida M, Aso Y, et al. Oral absorption of poorly water-soluble drugs: computer simulation of fraction absorbed in humans from a miniscale dissolution test. Pharm Res. 2006;23(6):1144-56.

108.   Fletcher CV, Brundage RC, Fenton T, Alvero CG, Powell C, Mofenson LM, et al. Pharmacokinetics and pharmacodynamics of efavirenz and nelfinavir in HIV-infected children participating in an area-under-the-curve controlled trial. Clin Pharmacol Ther. 2008;83(2):300-6.

109.   Grehl H, Kaschka WP. [Paranoid hallucinatory psychoses as the first manifestation of HIV infection]. Fortschr Neurol Psychiatr. 1994;62(11):413-6.

110.   Bernhard H, Frommberger U, Weber KC, Philipp M, Ramadori G, Meyer zum Buschenfelde KH. [Chronic paranoid-hallucinatory psychosis as the initial manifestation of an HIV infection?]. Dtsch Med Wochenschr. 1989 Mar 31;114(13):503-6.

111.   Joska JA, Gouse H, Paul RH, Stein DJ, Flisher AJ. Does highly active antiretroviral therapy improve neurocognitive function? A systematic review. J Neurovirol. 2010;16(2):101-14.

112.   de la Tribonniere X, Broly F, Deuffic-Burban S, Bocket L, Ajana F, Viget N, et al. ABCB1 allele polymorphism is associated with virological efficacy in

naive HIV-infected patients on HAART containing nonboosted PIs but not boosted PIs. HIV Clin Trials. 2008;9(3):192-201.

113.	Tumwikirize WA. Drug information and monitoring services in Uganda; Studies on drug information sources and adverse drug reactions [PhD]. Kampala: Makerere, Uganda 2009.

114.	Elens L, Vandercam B, Yombi JC, Lison D, Wallemacq P, Haufroid V. Influence of host genetic factors on efavirenz plasma and intracellular pharmacokinetics in HIV-1-infected patients. Pharmacogenomics. 2010;11(9):1223-34.

115.	Breen RA, Swaden L, Ballinger J, Lipman MC. Tuberculosis and HIV co-infection: a practical therapeutic approach. Drugs. 2006;66(18):2299-308.

116.	Lake-Bakaar G, Elsakr M, Hagag N, Lyubsky S, Ahuja J, Craddock B, et al. Changes in parietal cell structure and function in HIV disease. Dig Dis Sci. 1996;41(7):1398-408.

117.	Brenchley JM, Douek DC. HIV infection and the gastrointestinal immune system. Mucosal Immunol. 2008;1(1):23-30.