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Review Article

CLINICAL RELEVANCE OF EFAVIRENZ PHARMACOKINETICS AND PHARMACOGENETICS IN HIV/AIDS THERAPY

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ABSTRACT

Individuals respond to the same medications in diverse manners. Polymorphism in drug-metabolizing enzymes plays a very important role in interindividual variations in drug medical care. Hence, the aim was to review reported cases of genetic polymorphisms among the antiretrovirals, especially efavirenz, a non-nucleoside reverse transcriptase inhibitor, used in the management of human immunodeficiency virus infection/acquired immune deficiency syndrome, which is metabolized primarily by the CYP2B6 enzyme. Several previous publications on genetic polymorphism associated with the antiretrovirals of patients on highly active antiretroviral therapy were selected and carefully reviewed to evaluate their correlation and or conflict of interest among different authors. The existence of polymorphisms on the CYP2B6 gene that encodes for expression of the enzyme, among other factors responsible for efavirenz metabolism, is a significant determinant of inter-individual variability in pharmacokinetics and pharmacodynamic response to the drug used in clinical practice. Furthermore, plasma levels of efavirenz and phenotypic difference were observed, are contributing factors as to the rate of antiretroviral adverse drug reactions. Following the review, studies showed similar outcomes relating to efavirenz pharmacokinetics and polymorphism; hence, patients that display genetic polymorphism on efavirenz may likely develop the same on other therapeutic agents metabolized by the CYP450 enzymes or other polymorphic enzymes.

Keywords: Polymorphism, HIV/AIDS, CYP450, Pharmacogenetics, Pharmacokinetics.

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INTRODUCTION

Acquired immunodeficiency syndrome is a spectrum of conditions caused by the human immunodeficiency virus [1,2]. Following the invasion of the virus into the host immune system, an infected individual may not notice any symptoms or may just notice a brief period of influenza-related symptoms [3]. This initial stage is followed by a long period, also without symptoms [4]. As the virus replicates more in the system, the infection progresses and interferes with the cells of the host immune system. This increases the risk of developing certain infections, known as "opportunistic infections," including tuberculosis and certain kind of cancerous tumors that affect people who had stable immune systems [3]. The late phase of infection has accompanying symptoms and is referred to as AIDS, which is usually linked with unexplainable loss of weight [4]. It has been reported that AIDS can be prevented through the use of a condom (protected sex), advocacy on the safe use of needles and sharp objects especially in the hospital and medical laboratories, male circumcision to prevent abhorring of bacteria or other infectious organisms in the male genitals and immediate treatment of infected persons [4].

In the Joint United Nations Programme on HIV/AIDS global fact sheet of 2016, it was reported that about 36.7 million people were living with HIV and this resulted in about 1 million mortalities globally [5,6].

AIDS is well-defined in terms of either CD_4^+ T cell count, which is <200/μL cells or the occurrence of specific diseases related to the infection. In the absence of antiretroviral therapy (highly active antiretroviral therapy), it is known that about half of people infected with the virus may develop AIDS within 10 years period following viral infection. Some of the frequent symptoms or conditions that show the development of AID include cachexia (20%), pneumonia (40%), esophageal candidiasis, and respiratory tract infection that are persistent [7]. People infected with the AIDS stage have an increased tendency to develop certain cancers induced by the virus such as Kaposi's sarcoma, primary central nervous system lymphoma, conjunctival, and cervical cancer [7-9]. Various reports have it that HIV is rapidly transmitted by raw sexual contact, homosexuals, significant exposure to body fluids from HIV-infected persons and from mother-to-child during pregnancy, at birth, or during breastfeeding, which is known as vertical HIV transmission [10]. It was reported in 2008 that vertical HIV transmission accounted for almost 90% of HIV/AIDS cases in children. With proper antiretroviral therapy, the risk of mother-to-child transmission of HIV infection can be reduced to about 1% [10-12].

Current HIV/AIDS treatment and gene mutation

The World Health Organization recommends the use of highly active antiretroviral therapy (HAART) in sub-Saharan African countries, including Nigeria [13]. HAART has been reported to slow the progression of HIV/AIDS infection. More than 6.6 million people in 2010 were reported to be taking HAART regimens in developing countries. The HAART regimen tackles all the stages of the HIV life cycle and these antiretrovirals are classified on this basis. Some of the commonly used classes of antiretroviral drugs include fusion inhibitors, nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors, protease inhibitors, and maturation inhibitors [14-16]. HIV life cycle is as short as 1½ day from point of viral entry into the cells of the host, throughout replication, during cell assembly, and release of new viral particles to infect other surrounding immune cells [17]. The short life cycle of the virus and its high error rate cause the virus to mutate very rapidly, resulting in the high genetic variability of HIV. Hence, HIV has different subtypes such as HIV-1 and HIV-2. Although most of the mutations are inferior to the parent virus and do not convey any advantage, some of them have natural selection superiority to their parent and this can enable HIV to break the human immune system defense and resist some antiretroviral drugs [18].

Combinations of antiretrovirals are the mainstay of HIV/AIDS therapy because it creates multiple hindrances to HIV replication at a different

stage of the viral life cycle, thereby keeping the number of new viral particles low and also reduce the possibility of viral cell mutation. If a mutation that conveys resistance to one of these drugs arises, the other drugs in the regimen will continue to suppress the replication of that mutation. With rare exceptions, no individual antiretroviral drug has been demonstrated to suppress an HIV infection for a long duration; therefore, these agents must be taken in combinations to have a lasting effect. As a result, the standard of HIV/AIDS care is to use combinations of antiretroviral therapy known as a triple cocktail [19,20]. It has been reported that the primary causes of death from HIV/AIDS are due to the HIV-related opportunistic infections and tumors as a result of uncontrolled genetic polymorphism, both of which are frequently the result of the progressive failure of the immune system [8,21,22].

Pharmacogenetics and polymorphism

The concept of pharmacogenetics is concerned with individual variations to drug or medications response, which could be due to insertions, deletions, duplications, or multiplication, and point mutations within the genes coding for the enzymes responsible in metabolizing the drug in question. These genetic changes can give rise to enzymes with altered or deficient or increased activity resulting in compromised drug metabolism as well as environmental carcinogens such as poly saturated or chlorinated hydrocarbons [23]. Pharmacogenetic polymorphism has also been reported to occur, but within a population, a single gene responsible for producing a metabolizing enzyme has a variant allele at the same locus and more than one phenotype with regards to drug interaction with the host receptors; the frequency of the least common allele being >1% [24].

Gene polymorphism is a distinction in deoxyribonucleic acid (DNA) sequence among individual patients, groups, races or ethnicity. Sources such as single nucleotide polymorphisms (SNPs), sequence duplications, insertions, deletions, and recombination are the likely cause of gene polymorphism [25,26]. Genetic polymorphism is also the result of probability processes or could be induced by external agents such as radiation or other microorganisms such as viruses [27-29]. These genetic mutations are some forms of genetic polymorphism that involve a permanent modification within the sequence of that polymer that produces the gene, specified that the sequence differs from what is found in the majority of individuals in a given population [30]. Mutations change polymer (DNA) building block (base pair) at any point to an oversized phase of a chromosome that features multiple genes [31,32].

Genetic polymorphism of enzymes involved in drug metabolism has been reported to play key roles for inter-individual variations in clinical practice. This variability is of current significance throughout the drug development and importance for routine drug prescription. Many different drugs enclose pharmacogenomic labels where genotyping before prescription has been necessary or recommended based on individual differences to drug response. Predictive genotyping of drug-metabolizing genes can thus help to design individualized, less toxic, efficacious, and more cost-effective pharmacotherapy [33].

Efavirenz pharmacokinetics and the cytochrome P450 enzyme system

Both the NRTIs and NNRTIs inhibit the reverse transcriptase enzyme (Fig. 1). The reverse transcriptase is an essential viral enzyme that transcribes proviral ribonucleic acid into DNA. NRTIs such as zidovudine and lamivudine act by binding to the enzyme active site, while the NNRTIs like efavirenz act allosterically by binding to a distinct site away from the enzyme's active site known as the NNRTI pocket. Efavirenz is metabolized chiefly by the CYP2B6 enzyme [34] and it has been reported to also play a major role in the biotransformation of some other therapeutically important drugs, including cyclophosphamide, bupropion, ketamine, propofol, and artemisinin [35-38]. Efavirenz is stable, as there is no reported significant change in drug content and drug release after prolonged storage [39].

Efavirenz is known to induce its biotransformation (metabolism) and this auto-induction process was observed to give a three-fold increase in the oral clearance after multiple efavirenz administration [40]. It has also been shown that mild-dosing interval plasma concentration of efavirenz <1 mg/L has been associated with therapeutic failure and this could lead to the development of HIV viral resistance, on the other hand, efavirenz plasma concentration >4 mg/L can increase the risk of adverse effects, including neuropsychiatric effects such as drowsiness, dizziness, and confusion [41].

A Swiss HIV cohort study, conducted by Margalida *et al.* (2005), evaluated the CYP2B6 polymorphic allele as a pharmacogenetic marker of efavirenz and nevirapine pharmacokinetics and efavirenz toxicity in about 167 subjects receiving efavirenz and 59 receiving nevirapine. The drug concentrations were measured in plasma and peripheral blood mononuclear cells from the same sample. Neuropsychological toxicities of efavirenz (sleep disorders, mood disorders, and fatigue) were assessed using a standardized questionnaire. They concluded that CYP2B6 516TT was associated with greater efavirenz intracellular plasma concentration and greater plasma exposure to nevirapine [42].

In another study, the CYP2D6*3*4*9*10*17 and *29 alleles in a Nigerian population subjected to dextromethorphan and its O-demethylated metabolite, dextrorphan was reported by Ebeshi *et al*., 2011. The frequency of the CYP2D6*4 allele was found to be 2%, 8%, and 3% in Hausa, Igbo, and Yoruba speaking subjects, respectively. The average frequency of 4% for CYP2D6*4 implies that there is a very low likelihood of poor metabolizers due to this allele in Nigerian populations. The most common alleles were CYP2D6*17 and CYP2D6*29. The frequency of the CYP2D6*17 allele was not significantly different in the three populations (p>0.05) occurring at 18%, 14%, and 22% in the Hausa, Igbo, and Yoruba, respectively. Furthermore, the frequency of the CYP2D6*29 allele was found to be 10%, 20%, and 10% in the Hausa, Igbo, and Yoruba population, respectively [43].

The Cytochrome P4502B6 enzyme

The CYP2B6 is a highly polymorphic isoenzyme encoded by the *CYP2B6* gene. Numerous allelic forms have been reported to be responsible for the proteins with varying degrees of enzyme activity. In particular, the *CYP2B6*6* allele variant of the *CYP2B6* gene is associated with its decreased enzyme activity. An example of SNPs with *CYP2B6* with decreased enzymatic activity is the variant *516G/T* relating to the *CY2B6*6* allele. Phenotypically, the *TT* homozygotes are reported to be poor metabolizers, (highly toxic drugs – reduction of the dose is recommended), the *GT* heterozygotes have been characterized by immediate activity (intermediate metabolizers) of the CYP2B6 enzyme (normal therapeutic dose of the drug is recommended), and the *GG* homozygotes are extensive or rapid metabolizers (increased dose of

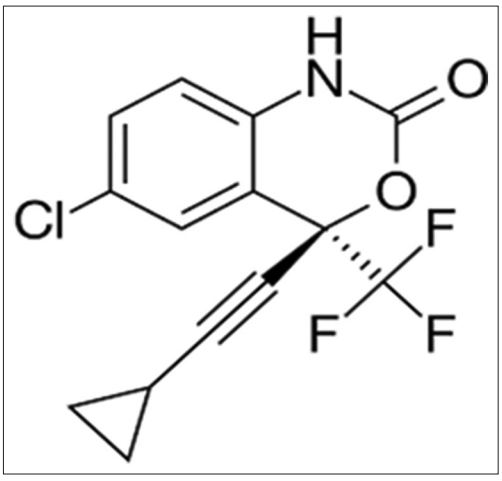

Fig. 1: Structure of efavirenz

Table 1: Genotypes and alleles frequencies of CYP2B6*6 in Nigerian populations (Ebeshi *et al*., 2011)

CYP2B6*6 genotype/allele frequencies	Hausa N/total (%)	Ibo N/total (%)	Yoruba N/total (%)	Mean pooled N/pooled total (%)
EM (G/G)	37/98 (38)	43/101 (43)	34/101 (34)	114/300 (38)
IM (G/T)	39/98 (40)	40/101 (40)	50/101 (50)	129/300 (43)
PM (T/T)	20/98 (20)	18/101 (18)	17/101 (17)	55/300 (18)
Allele (T)	82/196 (42)	72/202 (36)	84/202 (42)	238/600 (40)

EM: Extensive metabolizers, IM: Intermediate metabolizers, PM: Poor metabolizers, G: Guanine, T: Thymine

Table 2: The observed and expected distribution of CYP2B6 516 G/T pharmacogenetically important polymorphism Ukrainian populations, distribution of the 516G/T polymorphism of the *CYP2B6* gene

	Males, n	Female, n	Total, n (%)
GG	27	30	57 (56)
GT	16	22	38 (37)
TT	5	2	7 (7)

Statistics: x^2=0.656, df=2, p>0.05 (χ^2=Pearson's criterion, df-degree of freedom, p-significant level) [50]

Table 3: Frequency of the G and T allele of the *CYP2B6* gene (516G/T polymorphism)

	Alleles	
	G	T
Males	0.73	0.27
Females	0.76	0.24
Total	0.75	0.25

Table 4: Genotype frequencies of the 516G/T polymorphism of the *CYP2B6* gene

	Genotypes		
	GG	GT	TT
Males	0.54	0.39	0.07
Females	0.58	0.36	0.06
Total	0.56	0.38	0.06

Table 5: The observed and expected genotype frequencies of the 516G/T polymorphism of the *CYP2B6* gene

	Expected genotype frequencies	Observed genotype frequencies
GG	56	57
GT	37	38
TT	6	7

Statistics: χ^2=0.054, df=2, p>0.05

Table 6: The frequency of allelic variants of CYB2B6 observed in the Bantu and Nilotic populations of Kenya [51]

Allele	Prevalence (%)					
CYP2B6	Bantus	Eastern Nilotes	Western Nilotes	p	Mean	95% CI
*6	34.5	35.2	37.1	0.871	35.5	31.8–39.5
n	168	256	178			

n=Total number of alleles

the drug is recommended). The CYP2B6 enzyme metabolizes several currently prescribed medications, including efavirenz, bupropion, and cyclophosphamide [44].

Ethnicity variability of efavirenz metabolism

In a population pharmacokinetic study, Csajka *et al*. (2003) reported pronounced interindividual differences in efavirenz bioavailability and an inverse correlation between average drug exposure and HIV viral load, as well as the trend with central nervous system toxicity [45]. These same investigators found that black populations had higher efavirenz plasma concentrations due to a significant reduction in clearance rate compared to other ethnic groups. Efavirenz is a key drug in the first-line management of HIV/AIDS treatment (HAART), the availability of CYP2B6 allele frequency data in different populations, especially in populations with high HIV infection risk (such as Africans, Asians, and African-Americans) is of great interest (http://www.unaids.org). CYP2B6 polymorphism has been shown to have clinical relevance in HIV-infected patients treated with efavirenz, but it is increasingly being recognized for other drug substrates metabolized by this enzyme [46].

Several methods have been developed, including high-performance liquid chromatography, polymerase chain reaction, and restriction fragment length polymorphism, to quantify the serum levels and polymorphic nature of antiretrovirals among patients on HAART [47,48].

RESULTS SUMMARY AND DISCUSSION

Several journal articles were reviewed on pharmacogenetic polymorphism, observed causes of major polymorphism and phenotypic differences are presented Tables 1-6.

They concluded that the allelic distribution of *CYP2B6*6* appears comparable in Nigerian populations and other African populations but significantly higher than the Caucasian and Asian populations, indicating that Nigerian populations may be at risk of adverse reactions if given the similar or same dose of efavirenz or other substrates of CYP2B6. They further stated that CYP2B6 genotyping may be useful to complement an individualization strategy based on plasma drug determinations to increase the safety and tolerability of efavirenz. The *CYP2B6*6* genotype and allele frequencies showed no significant deviations from Hardy–Weinberg equilibrium (p>0.05) in Nigeria Population. However, the 40% obtained in this study was seen to be consistent with those found in other African populations with frequencies up to 49% and 47% in Ghanaian and African-American population, respectively, but higher when compared to frequencies of 25% and 18% in Caucasians and Orientals population [43,49].

It was concluded that the observed and expected genotype and allele frequencies did not show statistically significant differences compared to those expected under the Hardy–Weinberg equilibrium and that genetic polymorphism revealed in the Ukrainian population is the basis for recommending genetic testing for the 516G/T polymorphism for drug therapy optimization with drugs which are *CYP2B6* gene substrates [50].

They observed that the high prevalence of the *CYP2B6*6* (T) and the homozygous genotype *CYP2B6*6/*6 (516TT) in the Kenyan populations were consistent with reports from most sub-Saharan African populations [52]. Studies covering HIV/AIDS patients in East Africa showed the influence of *CYP2B6* 516 G>T not only on the pharmacokinetics of efavirenz and nevirapine but also on clinical outcomes [53,54]. Previous studies has seen the incorporation

of *CYP2B6* 516 G>T genotypes in pharmacokinetic modelling of efavirenz dosing interval which have led to dosage reduction in African population. These observations should is of great interest since the majority of HIV/AIDS patients in Kenya are on efavirenz-based first-line antiretroviral therapy [52,55].

CONCLUSION

Efavirenz's clinical relevance cannot be overemphasized. Following the above reviews, several studies have been conducted around the world on efavirenz pharmacokinetics and polymorphism with similar outcomes, including Nigeria; hence, patients with a polymorphism on efavirenz pharmacokinetics may likely develop the same on other pharmacotherapeutic agents metabolized by the CYP450 enzymes or other metabolic enzymes.

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AUTHORS' CONTRIBUTIONS

Data were collected and written by Dr. Samuel J. Bunu and it was edited by Dr. Ere D. and Dr. O. Miediegha.

CONFLICTS OF INTEREST

The authors hereby declare that there are no conflicts of interest relating to all the articles reviewed.

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