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Effect of ABCB1 C3435T Polymorphism on Clinical Outcomes in Kenyan HIV Patients on Lopinavir-Based Regimens

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Abstract: ATP Binding Cassette sub-family B member 1 (ABCB1) affects disposition of many drugs and thus affects the pharmacokinetics of drugs and ultimately treatment response. Polymorphisms of ABCB1 especially ABCB1 C3435T polymorphism may thus affect pharmacokinetics of antiretroviral drugs and hence CD4 treatment response and other clinical outcomes of HIV patients. Methods: The study design was a historical cohort study and entailed collection of patient data. PureLink® genomic DNA extraction mini kit was used for the extraction and purification of genomic DNA. TaqMan® drug genotyping assay and protocol was used in the DNA amplification and genotyping. Data analysis was done using STATA software version 10. Results: Study participants with the CT genotype had lower creatinine levels after 6 months on lopinavir-based regimens compared with those with the CC genotype (p = 0.001). In addition, the study participants with the CT genotype had consistently higher CD4 cell counts compared with those with the CC genotype from the time of ART switch but this was not statistically significant. However, there was no significant association between the ABCB1 C3435T genotypes and haemoglobin and ALT levels. Conclusion: There was a significant association between ABCB1 C3435T polymorphism and creatinine levels 6 months after therapy on lopinavir-based regimens.

Key words: ABCB1 C3435T polymorphism, lopinavir-based regimens, creatinine levels, CD4 cell counts, ALT.

1. Introduction

ABCB1 belongs to the ABC transporter superfamily. ABC transporters use energy generated by ATP hydrolysis to carry out transmembrane movement of substrates [1]. ABCB1 binds many structurally unrelated compounds, however, most of its substrates are generally hydrophobic amphipathic compounds [2]. ABCB1 transport protein confers drug resistance and affects pharmacokinetics and pharmacodynamics of protease inhibitors by decreasing bioavailability, reducing sequestration in tissues and target organs and increasing accumulation at sanctuary locations. ABCB1 interferes with intracellular concentrations of lopinavir and its oral bioavailability in the blood-testis barrier, brain, and intestine [3]. ABCB1 is important in reducing transport of atazanavir in the testes and brain [4]. ABCB1 may also affect the disposition of other drugs like lamivudine [5] and efavirenz [6]. It also affects the entry of antiepileptic drugs through BBB [7]. Some drugs normally inhibit ABCB1 and thus interfere with the pharmacokinetics of ABCB1 substrates like verapamil, valspodar, and quinidine.

The structure and model of transport of ABCB1 is shown in Fig. 1.

The highly polymorphic ABCB1 or MDR1 gene which has three insertion/deletion and more than 50 SNPs reported encodes for ABCB1. The ABCB1 gene is localized to chromosome 7p21.1, contains 28 (or 29) exons, spans a region of ~200 kb and is highly polymorphic [8]. The SNPs that occur commonly

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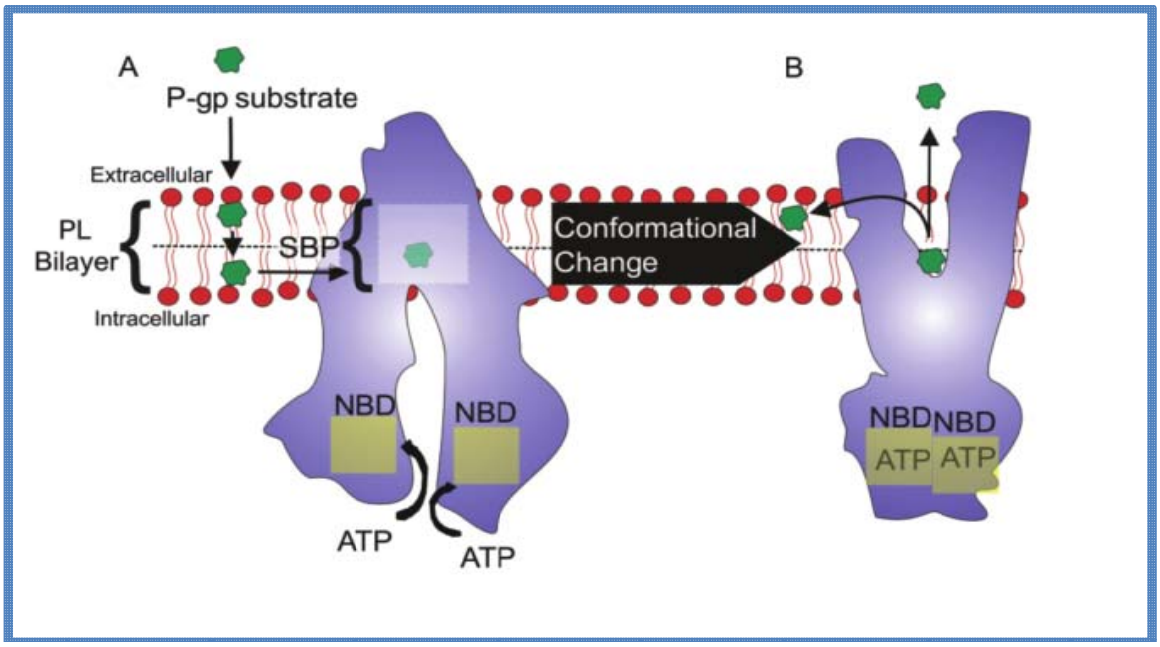

Fig. 1 Model of ABCB1 substrate transport [2].

include c. 1236C > T (rs1128503), the c. 3435 C > T (rs1045642) and the c. 2677 G > A/T (rs2032583). The c.2677G > A/T (rs2032583) polymorphism is a non-synonymous mutation giving rise to a change in amino acid sequence p. A893T (2677 G > A) SNP or p. A893S (2677 G >T) SNP. Haplotypes consisting of 1236C > T, 2677G > A/T, and 3435C > T have been reported and they are in strong linkage disequilibrium [9].

Studies conducted on effects of ABCB1 1236T > C polymorphism on drug response found inconsistent results; some reported increased drug exposure or response associated with the 1236 CC genotype and others with 1236 TT genotype [10]. Other studies found inconsistent results with regard to effect of 2677T > G/A variant [10]. ABCB1 3435 C > T SNP results in reduced expression and function and has also been associated with decreased mRNA and protein levels, via unknown mechanisms [11]. Patients with the ABCB1 3435TT homozygous mutant genotype show low expression of ABCB1 indicating that 3435 C>T SNP leads to reduced activity of ABCB1 [12].

The frequency of 2677 GG genotype is 10-32% in Mexicans, Caucasians, Asians, Italians and American Indians but more than 81% in African populations [10]. There are different frequencies of variant alleles of ABCB1 in different African populations [13], for example, the prevalence of ABCB1 c. 4036A > G genotypes is significantly higher in Tanzanians compared to Ethiopians [6].

A study in the Henan cohort in China reported that genotyping for ABCB1 genotypes may assist in predicting HIV treatment response [5]. 3435 C > T genotypes was significantly associated with CD4 + T cell count. Patients with CT or CC genotype tended to have significantly lower CD4 cell count than those with the TT genotype. However, viral load was not significantly associated with the genotype [5]. In another study in South India, differences in the ABCB1 3435 C > T genotypes distribution was reported to impact on ARV response and progression of HIV-1 disease; higher plasma efavirenz concentrations was associated with the CC genotype [11]. Lower viral loads and greater CD4 cell counts have been reported in several studies among HIV-positive individuals with 3435TT genotype of ABCB1 than those with CT or CC genotype probably due to lack of efflux of the antiretroviral drugs from sanctuary sites by ABCB1

[12]. However, another study in Germany reported that immunological and virological response were not affected by ABCB1 2677TT, 3435TT genotypes and 2677/3435 haplotype [14].

Association of ABCC2 and/or ABCB1 polymorphisms with concentrations of drug in blood is difficult to interpret and correlate effects of ABCB1 3435 genotypes with regard to drug activity in the body. However, immunosupressants and ARVs intralymphocyte concentrations demonstrated a direct influence by ABCB1 polymorphisms on intracellular or target tissue drug concentrations [15].

ABCB1 3435 C > T variant was likely to cause reduced risk of hepatotoxicity in patients on nevirapine [12]; however, another study reported an increase in liver enzymes among patients on nevirapine-based regimens [16]. A study done in Africans concluded that 3435T alleles resulted in a higher effective renal plasma flow, glomerular filtration rate and lower renal resistance [17].

Effects of ABCB1 C3435T variants have not been evaluated in Kenyan populations. This study helps to identify if ABCB1 C3435T genotypes affect treatment response in Kenyan populations. The findings could be used to justify genetic screening before treatment initiation and this would be used to guide dosing decisions

2. Methods

2.1 Study Design, Site and Population

The design was a retrospective cohort study and entailed collection of patient data from the time of initiation of therapy to the time when blood samples were obtained for genotyping. It was conducted at the Comprehensive Care Centre (CCC) of the Kenyatta National Hospital. Kenyatta National Hospital is the largest teaching and referral hospital in Kenya and East and Central Africa, with a diverse inter-ethnic population of patients. The study population was HIV patients on any lopinavir-based second-line ART regimen who were seen at the KNH CCC between January 2016 and April 2016.

2.2 Inclusion and Exclusion Criteria

The patients included in the study were HIV infected patients on any lopinavir-based second-line ART for at least 6 months, aged above 18 years, of either sex and gave informed consent to participate in the study. The patients excluded from the study were those who declined to give consent, were on second-line ART for less than 6 months and aged below 18 years.

2.3 Sample Size

The expected main outcome of interest is change of CD4 levels. Consequently the Twisk (2003) formula for estimation of sample size of a continuous outcome variable in a cohort study was used. Therefore, the sample size needed to make a 0.3 difference in a continuous outcome variable with a power of 80% and statistically significant on a 5% level with different within-subject association coefficients (ρ) of 0.5 and four repeated measurements was 59. To accommodate for expected missing files or incomplete data entries of about 20%, the calculated sample size was inflated by 20%. Therefore, a minimum sample size of 71 participants was targeted.

2.4 Data Collection

Equipment

The equipment used included a heat block, sterile micro centrifuge tubes, pipettes, spin columns and collection tubes (supplied with the kit), a vortexing machine (Thermal Electron Corporation, Denley VibroMix), a centrifuge (Biofuge Pico, Heraeus Instruments) and a water bath.

Materials and Reagents

The PureLink® Genomic DNA Kit (K182002) was used for DNA extraction according to manufacturer's instructions and it was composed of Proteinase K (20 mg/mL in storage buffer), RNAse A (20 mg/mL in 50 mM Tris-HCL, pH 8.0, 10 mM EDTA) and buffers. The buffers included PureLink® Genomic Wash buffer

1 and PureLink® genomic Wash buffer 2 which were dissolved in ethanol; PureLink® Genomic Lysis/Binding buffer and PureLink® Genomic Elution buffer (10 mM Tris-HCL, pH 9.0, 0.1 mM EDTA).

Participant medical records were retrieved. Data was abstracted from the files. Demographic data collected included sex, age, body mass index, marital status, occupation, education, ethnicity, alcohol use and smoking. Clinical data that was collected included first line ART regimen and duration, second line ART regimen and duration, WHO staging, CD4 cell counted at different time points, viral load, adherence, haemoglobin, ALT (alanine transaminase) and creatinine levels.

Blood collection was done in the bleeding room of the CCC laboratory. From each patient 5 mL of blood was obtained from the antecubital vein and was immediately transferred to an EDTA containing tube. The blood was centrifuged; 3 mL plasma was separated and stored at -20 °C and approximately 2 mL of whole blood was used for genotyping.

DNA extraction was done at the African Institute of Biomedical Science and Technology (AiBST) laboratory, University of Nairobi situated at the Department of Pharmacology and Pharmacognosy, School of Pharmacy. It was done from thawed whole blood using PureLink® Genomic DNA Kit described above as per the manufacturer's protocol (Life Technologies Carlsbad CA). About 200 µL of the thawed whole blood was pipetted into sterile micro centrifuge tubes. Twenty microlitre of proteinase K was added to the 200 µL of whole blood. This lysed the proteins. This was followed by hydrolysis of RNA by the addition of 20 µL of RNAse A. The mixture was vortexed and incubated at room temperature for 2 minutes to ensure that the contents were well mixed. Two hundred microlitres of PureLink® Genomic Lysis/Binding buffer was added and vortexed further to obtain a homogenous solution, and the lysate incubated at 55 ^{o}C for ten minutes to promote protein digestion. To the lysate, 200 µL of ethanol (96-100%) was added and mixed well by vortexing for 5 seconds to obtain a homogenous solution.

Six hundred and fifty microlitre of the lysate was added to sterile PureLink® Spin Columns in collection tubes provided with the kit. The contents were centrifuged at 10,000 x g for 1 minute at room temperature. The collection tubes were discarded and the spin columns placed into a clean PureLink® Collection Tube supplied with the kit.

Five hundred microlitre of wash buffer 1 was added to the spin columns and the columns centrifuged at room temperature at 10,000 x g for 1 minute. The collection tubes were then discarded and the spin columns placed into clean PureLink® Collection tubes also provided with the kit. Five hundred microliter of wash buffer 2 was added to the spin columns and the columns centrifuged at maximum speed for 3 minutes at room temperature and the collection tubes discarded.

Fifty microlitre of PureLink® Genomic Elution Buffer was added to the spin columns which were already transferred to sterile 1.5 mL micro centrifuge tubes. The contents were then incubated at room temperature for approximately 1 minute and the columns centrifuged at maximum speed (13,000 x g) for 1 minute at room temperature to complete the elution of the DNA. The eluted purified genomic DNA was contained in the 1.5 mL micro centrifuge tube. The spin columns were discarded. The DNA was stored in a freezer at -20 ^{o}C for further processing.

2.5 DNA Amplification

Genotyping was done at the US Army Medical Research Unit in Kenya Medical Research Institute (KEMRI) and was achieved on 7500 Fast Real Time PCR machine (Applied Biosystems, Foster City, California).

The reaction mix was composed of 20X TaqMan Drug Metabolism Genotyping Assay, TaqMan Genotyping Master Mix and nuclease-free water. The 2X TaqMan Genotyping Master Mix was swirled gently to mix the contents. The 20X TaqMan Drug

Metabolism Genotyping Assay was vortexed and centrifuged so as to mix properly. A total volume of 105 µL of 20X TaqMan Drug Metabolism Genotyping Assay, 1.05 mL of 2X TaqMan Genotyping Mix and 777 µL of nuclease-free water were pipetted into sterile tubes and capped then vortexed briefly to mix the components. The air bubbles were eliminated from the solution by centrifuging and spinning down the contents. The reaction mix for each well contained 12.50 µL of 2X TaqMan® Genotyping Master Mix, 1.25 µL of 20X TaqMan® Drug Metabolism Genotyping Assay, 9.25 µL of nuclease free water and 2 µL of genomic DNA. This was sealed with transparent adhesive tape and gently tilted and vortexed to ensure uniform mixing of the reaction solution.

The reaction plate was then introduced into the reaction chamber of the 7,500 fast real time PCR machine and the sequence detection software activated. Allelic discrimination pre-read test in a reaction volume of 25 µL was carried out at 60 ^{o}C for 1 minute. DNA amplification was achieved under the following conditions: an initial hold cycle for 10 minutes at 95 ^{o}C, followed by 50 cycles of denaturation at 92 ^{o}C for 15 seconds and then annealing and extension at 60 ^{o}C for 1 minute and 30 seconds. After real time PCR amplification was over, allelic discrimination post-read using sequence detection software was performed at 60 ^{o}C for 1 minute to characterize the distribution of ABCB1 C3435T alleles in the study population.

2.6 Variables

The main outcome variable in this study was clinical outcomes which included CD4 cell count, ALT levels, creatinine levels and haemoglobin. The main predictor variable of interest was the influence of ABCB1 C3435T genotypes.

2.7 Data Analysis

All variables were subjected to descriptive data analysis. Shapiro-wilk test was used to determine if the continuous variables were normally distributed. Mean and standard deviation were used to summarize variables that were normally distributed and those that were not normally distributed were expressed as the median and inter- quartile range.

Linear regression was done to assess the effect of ABCB1 C3435T genotypes on various clinical outcomes like CD4 cell count, ALT levels, creatinine levels, haemoglobin and BMI (body mass index). Data analysis was conducted using STATA version 10 software. The level of significance was set at 0.05. Hardy-Weinberg Equilibrium was done to test if ABCB1 3435 CC, CT and TT genotypes conformed to the Hardy-Weinberg proportions.

2.8 Ethical Considerations

Permission to conduct the study was granted by the KNH/UoN Research and Ethics committee (Ref: KNH-ERC/A/499). The nature of the study was fully disclosed to the participants. Patients signed informed consent to participate in the study and data collected was handled with confidentiality.

3. Results

There were 84 study participants of whom 50 (59.5) were female and 34 (40.5) male. There were 53 (63.1%) participants from the Bantu ethno-linguistic group, 30 (35.7%) from the Nilotes and finally Cushites with 1(1.2%) participant. Median baseline body weight was 61 [IQR (interquartile range) 54.2-71.2]. Median age was 36 years [IQR 32-44].

3.1 Prevalence of ABCB1 C3435T Alleles and Genotypes

The frequency of the ABCB1 C3435T alleles was 147 (87.5%) for the C allele and 21 (12.5%) for the T allele as shown in Table 1. The prevalence of the ABCB1 3435CC homozygous wild-type genotype was 64 (76.2%) while that of the heterozygous CT genotype was 19 (22.6%), and the TT homozygous variant genotype was 1 (1.2%). Comparison between

observed and expected genotype frequencies was in conformity with Hardy-Weinberg equilibrium indicating that they conformed to the Hardy-Weinberg proportions (p = 0.755) as presented in Table 1.

The frequency of ABCB1 3435C allele was 90 (86.5%) among Bantus while that of the T allele was 14 (13.5%). The frequency of the C allele was 55 (88.7%) among Nilotes while that of the T allele was 7 (11.3%). The frequency of the C allele was 2 (100%) among Cushites. There were no statistically significant differences with respect to frequency of the C and T alleles among Bantus, Nilotes, and Cushites.

There were more females than males with the wild-type CC genotype. The males who expressed the CC genotype were 25 (73.5%) while those with the CT genotype were 9 (26.5%). The females with the CC genotype were 39 (78%) and those with the CT genotype were 10 (20%). For the homozygous mutant allele, only one female expressed it. The distribution of the ABCB1 C3435T genotypes in the two genders conforms to the Hardy-Weinberg equilibrium. There was no statistically significant difference in the distribution of ABCB1 C3435T genotypes by sex (p = 0.575).

3.2 Effect of ABCB1 C3435T Genotypes on CD4 Cell Count

The participants with ABCB1 CC genotype had a median baseline CD4 cell count of 86 [19-276] and those with ABCB1 CT genotype had a median baseline CD4 cell count of 150.5 [43-361] as presented in Table 2. Only one participant had ABCB1 TT genotype and the participant had the lowest baseline median CD4 cell count of 18. Therefore, the study participants with the CT genotype had the highest baseline CD4 cell counts. On univariate regression analysis, participants with the CT genotype had higher log baseline CD4 cell counts by 0.218 units [crude β = 0.218(-0.658, 1.096)] compared with those with the CC genotype. However, this was not statistically significant (p = 0.620).

At ART switch, the study participant with the TT genotype had a high CD4 cell count of 624. The study participants with the CC genotype had higher CD4 cell counts with a median CD4 cell count of 275[85.5-435] compared with those with the CT genotype who had a median CD4 cell count of 173[89-264] as illustrated in Table 2. On univariate regression analysis, participants with the CT genotype had lower square root of CD4

Table 1 C3435T allele and genotype frequencies in the study population.

ABCB1 C3435T	n	%	p-value*
Allele			
C	147	87.5	
T	21	12.5	
Total	168	100	
Genotype			
CC	64	76.2	
CT	19	22.6	0.755
TT	1	1.2	
Total	84	100	

* Test for Hardy-Weinberg equilibrium of the genotype.

Table 2 Effect of ABCB1 C3435T genotypes on CD4 cell count.

ABCB1 C3435T Genotypes	Baseline CD4 cell counts, median [IQR]	CD4 cell counts at ART switch, median [IQR]	CD4 cell counts 6 months after ART switch, median [IQR]	Current CD4 cell counts, median [IQR]
CC genotype	86[19-276]	275[85.5-435]	303[125-464]	414[244-617]
CT genotype	150.5[43-361]	173[89-264]	244[204-806]	454.5[304.5-749.5]
TT genotype	18[18-18]	624[624-624]	346[346-346]	346[346-346]
p-value	0.620	0.789	0.230	0.636

cell counts by 0.629 units [crude β = -0.629 (-5.341, 4.083)]. However, this was not statistically significant ($p = 0.789$).

After 6 months of antiretroviral therapy with lopinavir-based regimens, the study participants with the CC genotype had a higher median CD4 cell count of 303 [125-464] compared to those with the CT genotype who had a median CD4 cell count of 244 [204-806] as illustrated in Table 2. On univariate regression analysis, participants with the CT genotype had higher square root of CD4 cell counts by 2.572 units [crude β = 2.572(-1.681, 6.826)]. However, this was not statistically significant ($p = 0.230$).

The study participants with the CT genotype have higher current CD4 cell counts with a median of 454.5[304.5-749.5] compared to those with the CC genotype who have a median of 414 [244-617]. The participant with the TT genotype had a decrease in CD4 cell count compared to the CD4 cell count at ART switch as shown in Table 2. On univariate regression analysis, participants with the CT genotype had higher square root of CD4 cell counts by 0.802 units [crude β = 0.802 (-2.566, 4.169)]. However, this was not statistically significant ($p = 0.636$).

In order to control the effect of education, two different plots of the rate of change of CD4 cell counts vs. C3435T genotype were generated; the first for participants with tertiary education and the second for those without tertiary education. The study participants with the heterozygous CT genotype and no tertiary education had consistently higher log CD4 cell counts compared to those with the CC genotype and no tertiary education. However, the rate of change of CD4 cell count appeared to be similar as illustrated in Fig. 2. The study participants with the heterozygous CT genotype and tertiary education and above had consistently higher log CD4 cell counts compared to those with the CC genotype and tertiary education and above. However, the rate of change of CD4 cell count appeared to be similar between the two groups as illustrated in Fig. 2. The slopes were parallel and thus indicate that the rate of change was constant regardless of the genotype.

3.3 Effect of ABCB1 C3435T Genotypes on ALT Levels

The study participants with the CC genotype had a higher median baseline ALT level of 23 [17-35.5] compared to those with the CT genotype who had a median of 22.7 [16-37]. The study participant with the TT genotype had a baseline ALT level of 14. On univariate regression analysis, the effect of ABCB1 C3435T genotypes was not statistically significant ($p = 0.515$)

At ART switch, the study participants with the CC

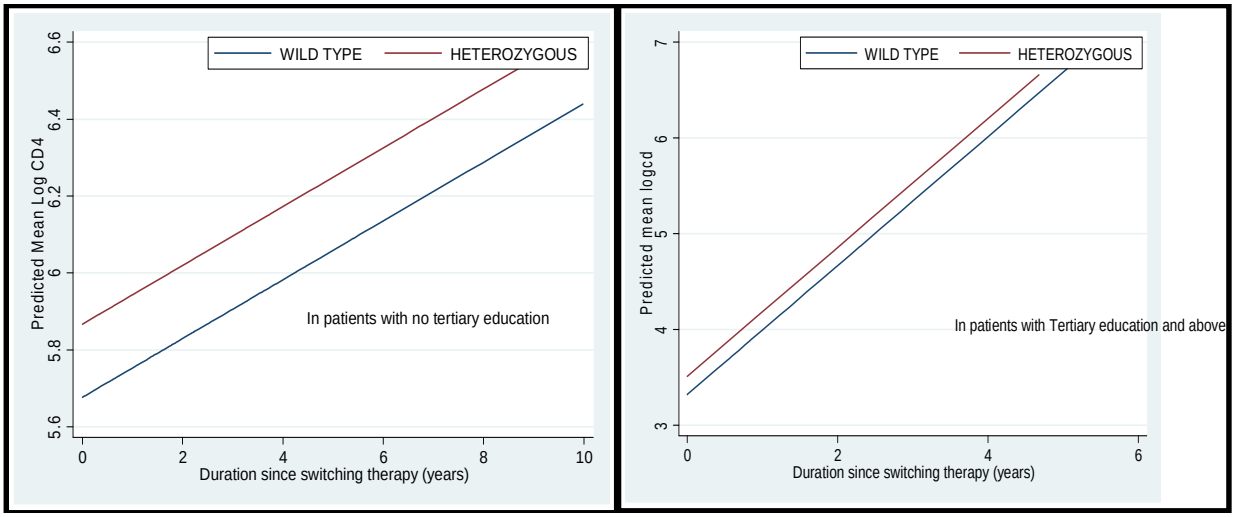

Fig. 2 Effect of ABCB1 C3435T genotypes on rate of change of CD4 cell count.

genotype still had a higher median ALT level of 24 [22-43] compared to those with the CT genotype who had a median ALT level of 20.5 [13.5-27]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on log ALT levels was not statistically significant (p = 0.081). When adjusted for baseline ALT levels, the effect of ABCB1 C3435T genotypes on log ALT levels was not statistically significant (p = 0.122).

After 6 months of therapy with lopinavir-based regimens (second-line regimen), the study participants with the CC genotype had lower median ALT levels of 16.7 [13-21] compared to those who had the CT genotype with a median ALT level of 25.5 [17-39]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on log ALT levels was not statistically significant (p = 0.257). Compared to levels at ART switch, the study participants with the CC genotype had a decrease in median ALT levels while those with CT genotype had an increase in median ALT levels as presented in Table 3.

3.4 Effect of ABCB1 C3435T Genotypes on Creatinine Levels

The study participants with the CC genotype had a lower median baseline creatinine level of 81 [72-101] compared to those with the CT genotype who had a median of 88 [63-97.3]. The study participant with the TT genotype had a baseline creatinine level of 82. On univariate regression analysis, the effect of ABCB1 C3435T genotypes was not statistically significant (p = 0.200)

At ART switch, the study participants with the CC genotype still had a lower median creatinine level of 83.3 [72.5-117] compared to those with the CT genotype who had a median creatinine level of 86 [81.75-103.5] as presented in Table 4. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on creatinine levels was not statistically significant (p = 0.479).

After 6 months of therapy with lopinavir-based regimens (second-line regimen), the study participants with the CC genotype had higher median creatinine levels of 87.5 [72-101] compared to those who had the CT genotype with a median creatinine level of 67.5 [56.5-73.15]. Compared to levels at ART switch, the study participants with the CC genotype had an increase in median creatinine levels while those with CT genotype had a decrease in median creatinine levels. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on creatinine levels was statistically significant (p = 0.001). When adjusted for baseline creatinine levels, the effect of ABCB1 C3435T genotypes was still statistically significant (p = 0.009);

Table 3 Effect of ABCB1 C3435T genotypes on ALT levels.

ABCB1 C3435T Genotypes	Baseline ALT levels, median [IQR]	ALT levels at ART switch, median [IQR]	ALT levels 6 months after failure, median [IQR]
CC genotype	23 [17-35.5]	24 [22-43]	16.7 [13-21]
CT genotype	22.7 [16-37]	20.5 [13.5-27]	25.5 [17-39]
TT genotype	14 [14-14]	-	-
p-value	0.515	0.122*	0.257

* when adjusted for confounding by baseline ALT levels.

Table 4 Effect of ABCB1 C3435T genotypes on Creatinine levels

ABCB1 C3435T Genotypes	Baseline creatinine levels, median [IQR]	Creatinine levels at ART switch, median [IQR]	Creatinine levels 6 months after failure, median [IQR]
CC genotype	81 [72-101]	83.3 [72.5-117]	87.5 [72-101]
CT genotype	88 [63-97.3]	86 [81.75-103.5]	67.5 [56.5-73.15]
TT genotype	82 [82-82]	-	-
p-value	0.200	0.479	0.009*

* when adjusted for confounding by baseline creatinine levels.

Table 5 Effect of ABCB1 C3435T genotypes on haemoglobin levels.

ABCB1 C3435T Genotypes	Baseline haemoglobin, median [IQR]	Haemoglobin at ART switch, median [IQR]	Haemoglobin 6 months after failure, median [IQR]
CC genotype	12.4 [11-14.3]	12.2 [10-14.2]	12.85 [11-14.5]
CT genotype	12.7 [11.7-14.3	12.55 [11.35-14.2]	12.5 [10.8-15.3]
TT genotype	13.5 [13.5-13.5]	-	
p-value	0.154	0.827	0.878

the participants with the CT genotype had a decrease of creatinine levels by 25.093 units [crude β = -25.093 (-42.985, -7.202)] compared to those with CC genotype.

3.5 Effect of ABCB1 C3435T Genotypes on Hemoglobin Levels

The study participants with the CC genotype had a slightly lower median baseline haemoglobin of 12.4 11-14.3] compared to those with the CT genotype who had a median of 12.7[11.7-14.3] as presented in Table 5. The study participant with the TT genotype had baseline haemoglobin of 13.5. On univariate regression analysis, the effect of ABCB1 C3435T genotypes was not statistically significant (p = 0.154).

At ART switch, the study participants with the CC genotype still had a slightly lower median haemoglobin of 12.2 10-14.2] compared to those with the CT genotype who had a median haemoglobin of 12.55 11.35-14.2]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes on was not statistically significant (p = 0.827).

After 6 months of therapy with lopinavir-based regimens (second-line regimen), the study participants with the CC genotype had higher median haemoglobin of 12.85 [11-14.5] compared to those who had the CT genotype with a median haemoglobin level of 12.5 [10.8-15.3]. On univariate regression analysis, the effect of ABCB1 C3435T genotypes was not statistically significant (p = 0.878).

4. Discussion

4.1 ABCB1 C3435T Single Nucleotide Polymorphism Variability

The allele frequency of the variant T allele was 12.5% while the frequency of the C allele was 87.5%. Regarding the ABCB1 C3435T genotypes, the prevalence of ABCB1 3435CC wild-type genotype was 76.2% while that of the heterozygous CT genotype was 22.6% and the TT variant genotype was 1.2%. This was in agreement with other studies done in some African populations. The prevalence in Burundi was 78.4% CC (160 patients), 20.1% CT (41 patients) and 1.5% TT (three patients) while the prevalence in Ethiopia was 4.9% (13 patients) TT genotype, 34.1% (90) CT, 61% (161 participants) CC genotype and in Tanzania it was 1.1% (2 patients) TT genotype, 29.0% (53) participants CT genotype and 69.9% (128 participants) CC genotype.

Therefore, this study indicated that our study participants were similar to those in Burundi and Tanzania but a little different compared to Ethiopians. The allele frequencies conformed to the Hardy-Weinberg equilibrium. A study done in South India reported seventy eight (44%) of the study population with the TT genotype [11]. This was very different from our findings and thus indicated racial differences in the distribution of ABCB1 C3435T polymorphism.

4.2 Effect of ABCB1 C3435T Genotypes on Clinical Outcomes

ABCB1 C3435T genotypes had no significant association with rate of change of CD4 cell count, baseline CD4 cell count, CD4 cell count at ART switch and current CD4 cell count. However, participants with ABCB1 3435CT genotype had consistently higher CD4 cell counts compared to those with the CC genotype. This is probably because a mutation on ABCB1 at position 3435 would reduce the activity of the MDR protein and thus prevent efflux of lopinavir

from sanctuary sites. This increase in lopinavir in sanctuary sites would lead to higher CD4 cell counts. Though our study did not find a significant association, a study done in China found a significant association between ABCB1 C3435T polymorphism and CD4 cell count [5]. Another study found a significant relationship between ABCB1 C3435T polymorphism and virological failure especially for those on regimens containing protease inhibitors [18].

In this study, ABCB1 C3435T genotypes had a significant association with creatinine levels six months after switching to second-line regimen. Patients with the ABCB1 3435 CT genotype had significantly lower creatinine levels compared to those with the CC genotype. This is probably because those with the CT genotype had reduced activity of the MDR1 protein. Since MDR1 is found in the apical membrane of the proximal and distal tubule epithelium, it would limit the flow of the drug to the tubule for those with the CC genotype and hence reduce excretion of lopinavir leading to reduced kidney function. The participants with the CT genotype would have reduced function of the MDR1 protein and thus lopinavir would easily flow to the tubule and hence excreted. This is in agreement with a study done among Africans which concluded that 3435T allele was associated with a higher glomerular filtration rate and effective renal plasma flow and lower renal resistance compared to the reference genotype (CC) [17].

In this study, ABCB1 C3435T genotypes had no significant association with ALT levels. This is unlike other studies done on participants on nevirapine-based regimens; some studies reported that ABCB1 3435 C > T variant was likely to cause reduced risk of hepatotoxicity in patients on nevirapine [12] while others reported an increase in liver enzymes among patients on nevirapine-based regimens [16]. This is probably because lopinavir does not have an effect on the liver while nevirapine does.

5. Conclusions

There is a significant association between ABCB1 C3435T genotypes and creatinine levels of patients on lopinavir-based regimens. Study participants with the CT genotype had lower creatinine levels compared to those with the CC genotype. In addition, study participants with the ABCB1 3435CT genotype had consistently higher CD4 cell counts compared to those with the CC genotype.

This study indicates that genotyping for the ABCB1 C3435T polymorphism would assist in identifying patients who would respond effectively to lopinavir-based regimens. Therefore, genotyping for ABCB1 C3435T polymorphism is important in actualizing personalized therapy.

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