Research Article

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Allelic variants of CYP2B6 gene expression and its implication on the pathogenesis of malaria among a cohort of outpatients in North-Central Nigeria

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Abstract

Background – Human cytochrome P450 2B6 (CYP2B6) is fortified with the biotransformation of the antimalarial, artemisinin combination therapy (ACT). Owing to emerging reports of *Plasmodium* species resistance to ACT in other climes, CYP2B6*6 genotype significantly alters ACT metabolism. In North-Central Nigeria, the distribution of the CYP2B6*6 variant is poorly documented. This study investigated the distribution of CYP2B6 c.516G>T variants and its relationship with certain malaria pathogenesis among a cohort of clinical-malaria outpatients in Ilorin, Nigeria.

Methods – A total of 50 symptomatic *P. falciparum* malaria-positive samples were genotyped for CYP2B6 c.516G>T using restriction fragment length polymorphism and a specific haplotype population was established. The allele frequencies and genotype distributions were analyzed. Haplotypes were clustered using Ward's method. Correlations determined include defective CYP2B6 versus parasitemia densities and thrombocytopenia, respectively.

Results – Forty-five samples show genotypic ratios and nine CYP2B6 genetic single nucleotide polymorphisms were identified. The following haplotypes (64C>T = *1/*2, 785A>G = *1/*4, and 1459C>T = *1/*5) occurred and *2, *3, and *18 alleles harbor 64C>T, 777C>A, −82T>C, and 499C>G. The codon 64CT, 516GT, and 785AG; −82TC and 777CA; and 499CG, 516GT, and 785AG were identified as *2/*6 heterozygotes. Samples with 516GT and 785GG genotypes occurred with *4/*6 heterozygotes. Mutant trait alleles recorded high parasitemia 72 h post-ACT regimen. Only individuals with CYP2B6*6 alleles had severe malaria and thrombocytopenia.

Conclusion – This report contributes to the growing knowledge of CYP2B6*6 genotype frequency and its relationship with malaria pathogenesis among a Nigerian population.

Keywords: cytochrome P450 2B6*6, *Plasmodium falciparum*, haplotypes, artemisinin combination therapy, thrombocytopenia

1 Background

Malaria accounts for 2.6% of the total disease burden of the world and the disease is responsible for the loss of more than 35 million disability-adjusted life-years [1]. The emergence of resistance against different antimalarial drugs is a major challenge to malaria control and elimination [2]. During the 1990s, *Plasmodium falciparum* acquired resistance to chloroquine and sulfadoxine-pyrimethamine throughout all malaria endemic countries [3]. Owing to the aforementioned, the World Health Organization (WHO) recommended artemisinin combination therapy (ACT) as first-line therapeutic agent for the treatment of all uncomplicated *falciparum* malaria [4]. Today, there are reports of ACT failure in some parts of the world and the apicomplexan, *P. falciparum* phenotype is already becoming insensitive to artemisinin (ART) [5]. The cytochrome P450 2B6 (CYP2B6) enzymes are one of the super-family of xenobiotic-metabolizers involved with the therapeutic biotransformation of ACT and some other drugs [6]. These enzymes have been identified as the most important catalysts among all drug-metabolizing agents and predominantly belong to the phase 1 motifs [7]. Some ART derivatives are metabolized by polymorphic CYP2B6 and owing to emerging ACT resistance by *Plasmodium* species, there is an increasing interest in CYP2B6 genetic polymorphism. The presence of these

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defective genes manifests in man as delayed parasite clearance time [8,9]. Polymorphisms occurring as a result of CYP2B6 are a major contributor to the inter-individual variability associated with the expression, pharmacokinetics, and activities of these drug substrates. The average relative contribution of CYP2B6 to total hepatic CYP content ranges from 2 to 10% with a considerable increasing substrate list for the enzymes' polymorphic and ethnic variations in expression levels. The aforementioned is evident in the cross-regulation with another isoenzyme referred to as CYP3A4 and UGT1A1, respectively [10,11].

CYP2B6*6 (CYP2B6 c.516G>T; rs3745274) is a genetic factor that alters drug metabolism in antimalarial, antiretroviral, antidepressant, and anti-tuberculosis first-line drugs [12]. An estimated 25–30% variability in drug disposition and effect is absolutely due to genetic factors resident in the DNA sequence, basically called genetic polymorphisms, and are due to single base-pair mutations [13,14]. The established variants of CYP2B6 alleles occur with relatively high frequencies across different populations. Particularly, the CYP2B6*6 genotype is associated with decreased protein expression terminating in an increase or decrease activity depending on the drug substrate being metabolized. For instance, efavirenz-mediated drug–drug interactions (DDI) on lumefantrine pharmacokinetics in African pediatric population groups demonstrated a significant difference in lumefantrine target day 7 concentrations (C_{d7}) being more apparent in the *6/*6 compared to *1/*1 population group [15]. The CYP2B6 gene comprises nine exons and it is located on chromosome 19 at position 19q13.2 encoding 38 variants [16]. The variants associated with CYP2B6 are collectively referred to as star alleles on the Pharmacogene Variation website with designated clinical functions as normal, decrease, increase, no, or uncertain function [17]. DNA sequence variation occurs when a single nucleotide (adenine, thymine, cytosine, or guanine) in the genome sequence is altered. Sequence nucleotide variants (SNVs) may be rare or common in a population, but usually present in at least 1% of the population with marked positions of insertion and deletions (indels). Indels are highly abundant in human genomes, second only to single nucleotide polymorphisms (SNPs), and make up 15–21% of human polymorphisms [18]. Polymorphic variants of CYP2B6 star alleles possess a growing number of sub-alleles all sharing specific key-defining SNVs, but none has been reported so far in the study area. Therefore, this study investigated the distribution of CYP2B6 c.516G>T variants and related the established variants with certain pathogenesis among a cohort of clinical malaria outpatients in Ilorin, Nigeria.

2 Methods

2.1 Sample collection and study design

The subjects for this study were randomly picked from a cohort of clinical malaria outpatients specifically presenting with *P. falciparum* infections. The subjects were enrolled voluntarily without bias to gender, age, or any socio-demographic influence. We collected occult blood samples randomly from 50 unrelated subjects manifesting signs and symptoms of febrile malaria. Peripheral blood samples of 3 mL were simultaneously collected with ethylene diamine tetraacetic acid (EDTA) screw-cap bottles from the volunteers. Four drops of occult blood were spotted on the sample pads of the 903 protein saver cards to make dried blood spot (DBS) and subsequently stored carefully in a plastic container with desiccants at room temperature.

2.2 Malaria parasite test by microscopy

Thin and thick blood films were prepared following the methods of Gilles [20]. The slides were fixed in methanol in order to allow lysis of red blood cells. The slides were stained with 10% stock solution of Giemsa for about 30 min, and washed in gentle tap water drops. Dried thin and thick films were screened under an oil immersion (X1000Mg) Olympus microscope for *Plasmodium* spp. Parasite density were determined following the guidelines in WHO, 2001 as the number of parasites per 200 leukocytes (WBC).

2.3 DNA extraction from DBS

Individual genomic DNA was extracted from the blood impregnated 903 protein saver cards (Pittsburgh, PA), using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the labels in the manufacturer's instructions. Briefly, each frozen blood sample was thawed at room temperature and transferred to a clean polypropylene tube after pre-purification. Four sets of tubes were labeled for each sample using a convenient labeling system and each set was arranged on a separate tube rack. For the third set of tube, a column was added while for the fourth set a secure label was made by pasting cello tape on the label (tubes and columns were provided in the kit). After cell lysis, cellular proteins were precipitated by salt precipitation leaving the high molecular weight genomic DNA in the solution, and were allowed to

concentrate and were desalted by isopropanol precipitation. The DNA was bound to a column/resin, then washed using concentrated ethanol and eluted or dissolved in a buffer solution AE (10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0), provided in the isolation kit. After elution stage, the purity of DNA sample was determined by measuring its concentration using a UV spectrophotometer. DNA samples at 4°C prior to genotyping analysis and aliquot of the samples were stored at –20°C for long-term use. Genotyping of CYP2B6*6 was carried out using PCR-restriction fragment length polymorphism technique in accordance with the method of Daniels et al. [21].

2.4 CYP2B6 genotyping

SNP analysis of the CYP2B6 genotype was carried out using the allele-specific fluorogenic 5-nuclease chain reaction assay with predesigned primers (Fwd.: GGTCTGCCCATCTA TAAAC; Rev.: CTGATTCTTCACATGTCTGCG) and TaqMan Minor groove binder probes (TaqMan SNP Genotyping Assay; Applied Biosystems, Foster City, CA). The following CYP2B6 polymorphism coding regions were determined using validated TaqMan™ Drug Metabolism Genotyping Assays (Thermo Fisher Scientific), *viz*., −82T>C, 64C>T, 499C>T, 516G>T, 777C>A, 785A>G, 983T>C, 1375A>G, and 1459C>T. Briefly, PCR reactions were carried out in a 25 µL volume containing the following assays: 20 ng genomic DNA, drug metabolism genotyping mix (20×), and 2X TaqMan universal master mix (Applied Biosystems) or 40× assay mix (Applied Biosystems). All the PCR reactions were run with drug metabolism genotyping assay mix (Applied Biosystems) stepwise: 95°C for 10 min, followed by either 50 cycles of 92°C (15 s) and 60°C (90 s) or with assay mix at 40 cycles of 92°C (15 s) and 60°C (60 s) [22].

2.5 Determination of platelet and hemoglobin count

The following hematological parameters were obtained from the blood of all the subjects: hemoglobin and platelet counts, respectively, following the methods of Bain [23] with slight modifications. Briefly, 3 mL of blood sample was collected aseptically from each subject into a tripotassium ethylene-diamine tetra-acetic acid anticoagulant bottle. The anticoagulant bottle was inverted and mixed gently to ensure complete blood count analysis. Individual blood samples were analyzed using the hematology auto analyzer (Sysmex KX-21N).

2.6 Data analysis

Allele and genotype frequencies were obtained by direct counting with respect to identified numbers of minor alleles at each polymorphic locus. All genotype distributions were subjected to Hardy–Weinberg equilibrium on the basis of chi square (χ^2) test of observed versus predicted using the Stata intercooled statistical software version 9.0. The null hypothesis was rejected if $p < 0.05$. Allele and haplotype descriptions were executed according to the published recommendation of the Committee on Human Cytochrome P450 Allele Nomenclature (https://www.pharmacogenomics.pha.ulaval.ca/ugt-alleles-nomenclature/criteria-for-inclusion-of-alleles-and-snps/). Observed frequencies of CYP2B6 genotype in the subjects were reported using the 95% confidence interval. The agglomerative algorithm in SPSS 21 hierarchical cluster analysis using Ward's method was employed to designate the CYP2B6 star alleles population among the subjects. The linkage function specifies the distance between the two clusters as the increase in "error of squares" after fusing two clusters into a single cluster. The categorical data are presented as frequency (percentages), and the Chi-square test statistics was used to test the association between the variables. The Mantel–Haenszel test of trend was used to examine the rates across the levels of parasitemia. The trend across age ranges was evaluated by the Jonckheere trend test followed by Kendall's tau-b to measure the strength and direction of association between the variables on an ordinal scale. Continuous variables were presented as mean ± standard error of mean for parametric variables and median (interquartile range) for nonparametric variables.

3 Results

A total of 50 febrile samples were randomly picked for CYP2B6 gene analysis out of which 20 (40.0%) had the recessive mutant traits of CYP2B6*6 allele. Mutant population with respect to gender and ethnic group was not statistically significant ($p > 0.05$). However, the defective CYP2B6*6 allele in comparison with the wild dominant allele was significant with respect to distribution among the respective age groups sampled ($p = 0.001$) (Table 1).

The genotype and allele frequencies were estimated prior to the SNP analysis and subjected to Hardy–Weinberg equilibrium calculator (http://www.perinatology.com/calculators/Hardy-Weinberg.htm). The genotype frequencies obtained obeyed the assumptions for the principle. For instance, the allele frequency for this generation was done by pooling together the alleles from each genotype of the same generation according to the expected contribution from the homozygote

Table 1: Baseline characteristics of samples taken for CYP2B*6 screening

Factor	No.	Allele frequency (%)			
	Examined (%)	Mutants	Wild type	Not amplified	*p*-value
Total	50	20 (40.0)	25 (50.0)	5 (10.0)	
Gender					NS
Male	30	12 (60.0)	16 (64.0)	2 (40.0)	
Female	20	8 (40.0)	9 (36.0)	3 (60.0)	
Age group					<0.001
6–15	10	2 (10.0)	3 (12.0)	5 (100.0)	
16–25	10	2 (10.0)	8 (32.0)	0	
26–35	10	5 (25.0)	5 (20.0)	0	
36–45	10	6 (30.0)	4 (16.0)	0	
>45	10	5 (25.0)	5 (20.0)	0	

Calculated at http://www.perinatology.com/calculators/Hardy-Weinberg.htm.

and heterozygote genotypes. Forty-five individuals showed genotypic ratios out of the 50 samples subjected to screening for the CYP2B6 gene but no genetic determinants (i.e., haplotype) were explored at this stage. Children 6–15 years of age had the highest mutant autosomal recessive allele CYP2B6*6 (0.577). The carrier frequency was however higher among the age group of 36–45 years (50.0%) (Table 2).

Genomic DNA was extracted from 45 individual blood samples and the genotypes of nine SNPs were identified. The coding region for the SNP analysis of the CYP2B6 gene is as shown in Table 3. There was however no CYP2B6 genetic polymorphism detected in six individuals and their haplotype was determined to be *1/*1. The haplotypes of single-SNP carriers with 64C>T, 785A>G, and 1459C>T were reported to be *1/*2, *1/*4, and *1/*5, respectively, whereas those of homozygous carriers with both 516TT and 785GG were determined to be *6/*6. Once it was established that *2, *3, and *18 were the only alleles harboring 64C>T, 777C>A, −82T>C, and 499C>G, individuals with 64CT, 516GT, and 785AG; −82TC and 777CA; and 499CG, 516GT, and 785AG were identified as *2/*6 heterozygotes. Individuals carrying both 516GT and 785GG genotypes but not in other polymorphisms were shown to have *4/*6 heterozygotes. Allele and haplotype descriptions were performed according to the published recommendation of the Human CYP-P450 Allele Nomenclature Committee (https://www.pharmvar.org/gene/CYP2B6).

Dendrogram gives a visual summary of the cluster solution; therefore, the phylogenetic relatedness of the randomly selected samples used for genomic analysis of CYP2B6*1-6 and 18 were subjected to hierarchical cluster analysis among the malaria febrile group (>37.5°C). Four related clusters were identified using Ward's method (Figure 1).

The asexual parasitemia density reduction over the course of ACT treatment for 72 h regimen and also the reduction in parasite density was subsequently monitored for 7 days and compared between malaria patients with autosomal recessive mutant traits of CYP2B6*6 allele (MTA,

Table 2: Genotypes for CYP2B6*6 and T allele frequency with respect to age groups

Age groups	CYP2B6*6 (rs71337213, A>T)						$p + q = 1$	
	Genotype frequencies				Allele frequency		*p	^q
	N	AA	AT	TT	T	Carrier %		
Total no. genotyped	45	0.603	0.347	0.050	1 in 3	34.72	0.776	0.224
6–15	5	0.179	0.488	0.333	1 in 2	48.80	0.423	0.577
16–25	10	0.418	0.457	0.125	1 in 2	45.71	0.646	0.354
26–35	10	0.306	0.494	0.200	1 in 2	49.44	0.553	0.447
36–45	10	0.25	0.500	0.250	1 in 2	50.0	0.500	0.500
>45	10	0.306	0.494	0.200	1 in 2	49.44	0.553	0.447

*Frequency of the wild type allele; ^Freq. of mutant allele.
Calculated at http://www.perinatology.com/calculators/Hardy-Weinberg.htm.

Table 3: CYP2B6 allele, nucleotide position, and genotype frequency derived from the SNP analysis

CYP2B6	Nucleotide position coding region									Frequency (%)	95% CI
	−82	64	499	516	777	785	983	1375	1459		
Allele (*n* = 45)											
*1	T	C	C	G	C	A	T	A	C	6(3.0)	2.991–3.009
*2	T	T	C	G	C	A	T	A	C	4(2.0)	1.991–2.009
*3	T	C	C	G	A	A	T	A	C	3(1.5)	1.491–1.509
*4	T	C	C	G	C	G	T	A	C	1(0.05)	0.041–1/059
*5	T	C	C	G	C	A	T	A	T	2(1.0)	0.991–1.009
*6	T	C	C	T	C	G	T	A	C	8(4.0)	3.991–4.009
*18	T	C	C	T	C	G	T	A	C	2(1.0)	0.991–2.009
No allele	—	—	—	—	—	—	—	—	—	19	—
Genotype (*n* = 26)											
*1/*1	TT	CC	CC	GG	CC	AA	TT	AA	CC	2(2.0)	0.009–2.009
*1/*2	TT	TC	CC	GG	CC	AA	TT	AA	CC	2(2.0)	0.009–2.009
*1/*3	TT	CC	CC	GG	CA	AA	TT	AA	CC	2(2.0)	0.009–2.009
*1/*4	TT	CC	CC	GG	CC	AG	TT	AA	CC	2(2.0)	0.009–2.009
*1/*5	TT	CC	CC	GG	CC	AA	TT	AA	CT	6(6.0)	2.991–6.009
*1/*6	TT	CC	CC	GT	CC	AG	TT	AA	CC	1(1.0)	0.991–1.009
*2/*6	TT	TC	CC	GT	CC	AG	TT	AA	CC	1(1.0)	0.991–1.009
*4/*6	TT	CC	CC	GT	CC	GG	TT	AA	CC	1(1.0)	0.991–1.009
*6/*6	TT	CC	CC	TT	CC	GG	TT	AA	CC	8(8.0)	6.091–8.213
*1/*18	TT	CC	CC	GT	CC	AG	TT	AA	CC	1(1.0)	0.991–1.009

−82T>C, 64C>T, 499C>T, 516G>T, 777C>A, 785A>G, 983T>C, 1375A>G, and 1459C>T SNPs genotyped with allele-specific fluorogenic 5′-nuclease chain reaction assay. 95% CI, 95% confidence intervals.

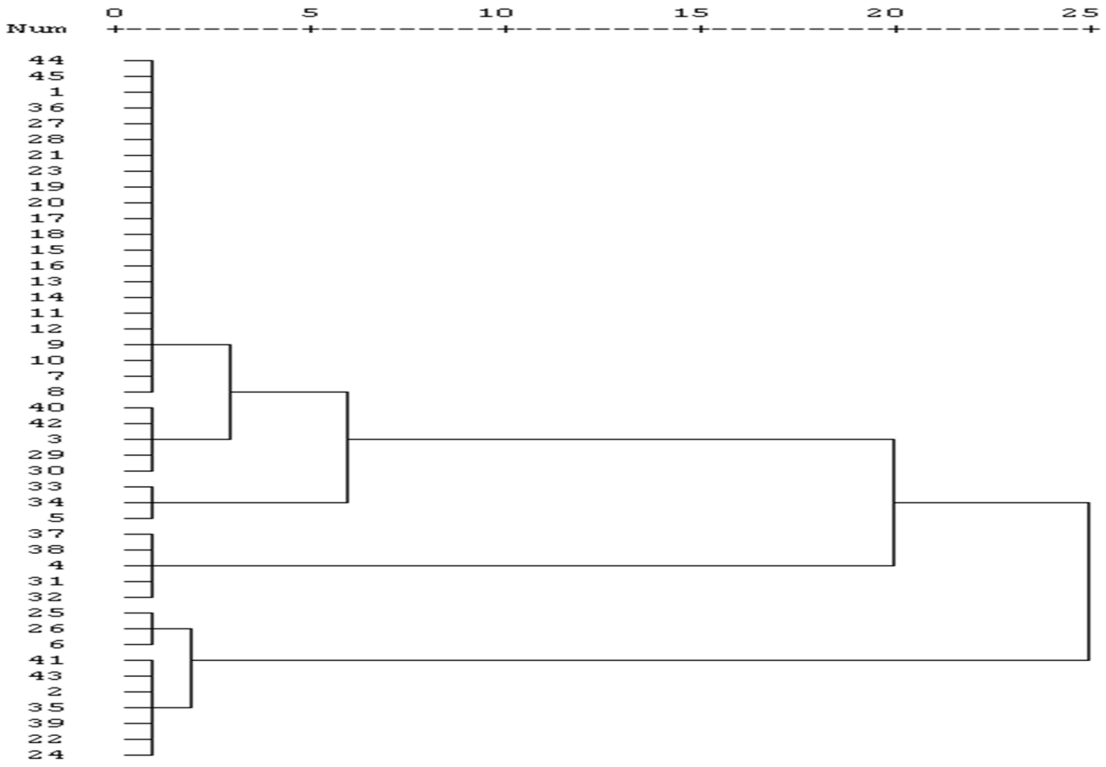

Figure 1: Chart showing hierarchical clusters of plot dendrogram VICICLE for CYP2B6*1-*6 and *18 alleles among the malaria febrile samples.

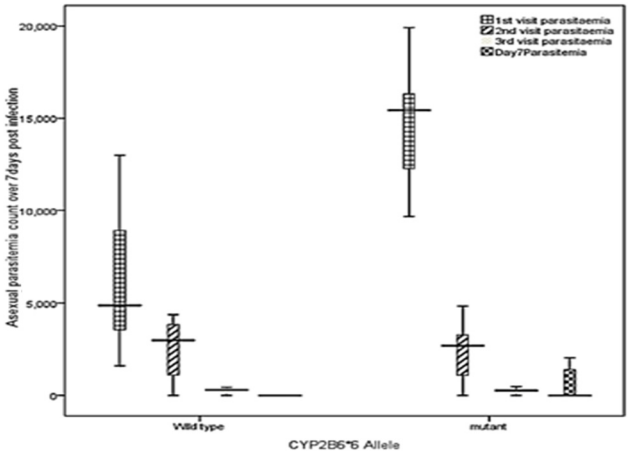

Figure 2: The geometric mean asexual parasitemia density between the CYP2B6*6 MTA and WTA over the course of day 1, 24, 48, 72 h, and 7 days post-ACT treatment regimen. Boxes represent 25–75th percentiles, solid bar within boxes represents median values, within the bars represent non-outlier maximum and minimum. There were no outliers. (Alpha levels for all mean parasitemia of *Pf* malaria-positives categories were significantly different at $p < 0.05$.).

20, 44.4%) and the other population with the dominant wild type allele (25, 55.6%). The mean parasitemia density on the first day of presentation was MTA – 5922.00 ± 746.78; 95% CI: 7485.03–4358.97 and WTA – 146000.00 ± 579.63; 95% CI: 158000.00–134,000. Both the MTA and the WTA recorded a steady reduction in mean parasitemia density after 24, 48, and 72 h, but the MTA group after 72 h still recorded a high mean parasitemia density post-treatment (446.40 ± 147.87; 95% CI: 751.58–141.22), while the WTA group recorded zero mean parasitemia (Figure 2).

The nucleotide positions for the genotypes obtained from the SNP analysis showed that all seven CYP2B6*:1-6 to *18 recorded varying degrees of WHO classified malaria infection (Figure 3). Samples with the absence of allele showed light infection (9, 47.4%), moderate infection (8, 42.1%), and severe infection (2, 10.5%). CYP2B6*1, *2, *3, *4, and *5 did not fall under light and moderate infections. However, all samples with CYP2B*1-*18 allele recorded severe infections with samples exclusive to CYP2B6*6 having the highest population with severe infections (7, 87.5%). Samples with CYP2B6*18 recorded moderate (1, 50%) and severe (1, 50%). The association was highly statistically significant ($p < 0.05$).

The samples with no alleles had (18, 56.2%) normal platelet counts (>150,000 (10^9/L)) and only one sample (1, 7.7%) had thrombocytopenia (<150,000 (10^9/L)). T, samples with CYP6*2, *5, and *18 had ties in their platelet status. The association was however marginally statistically significant ($p = 0.04$) (Figure 4).

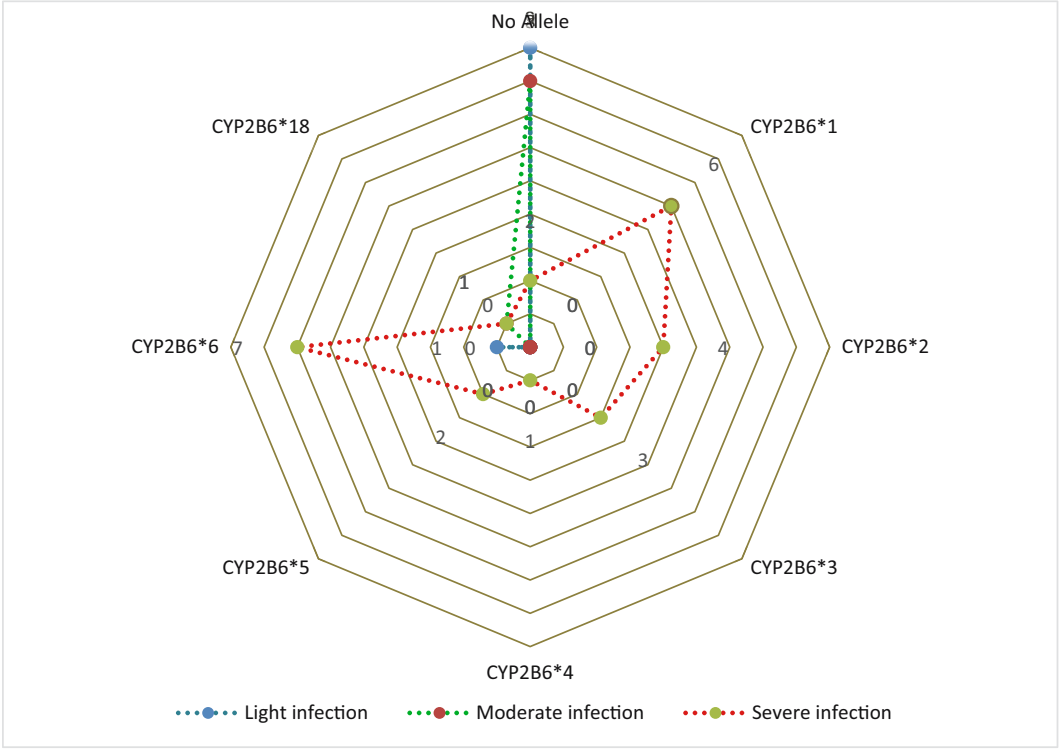

Figure 3: Distribution of SNP identified CYP2B6*1-*6 and *18 alleles with respect to degree of malaria infections among the samples taken for CYP2B6 genes ($p < 0.005$).

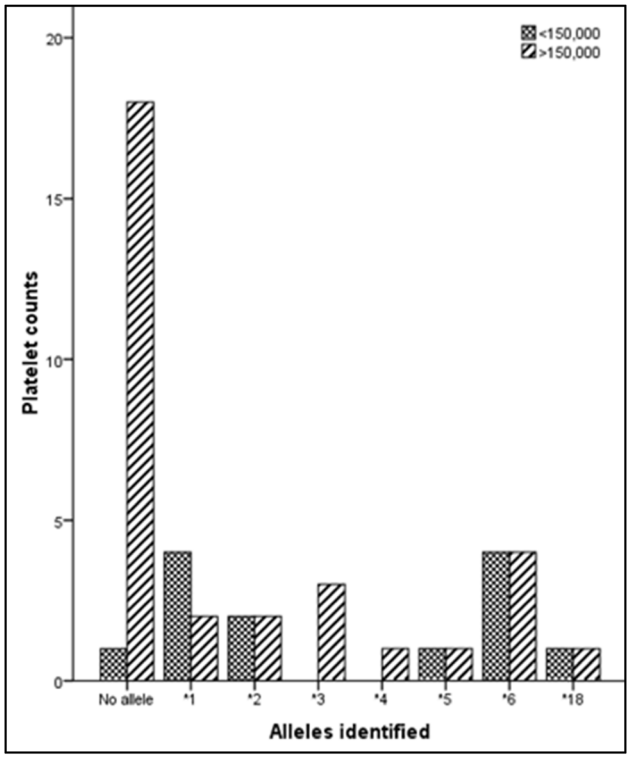

Figure 4: Distribution of platelet level among the CYP2B6*1-6, *18 alleles. The platelet count was designated by <150,000 – thrombocytopenia and >15,000–450,000 – normal platelet count.

The pyramidal chart in Figure 5 shows the distribution of the CYP2B6* allele types with respect to hemoglobin levels (<10.5 – low hemoglobin, 10.6–15.5 – normal hemoglobin). Only three out of the eight individuals with CYP2B6*6 allele had low hemoglobin (4, 37.5%) among the CYP2B6*6 allele population. Between the two individuals with CYP2B6*18 allele, only one (1, 50%) had low hemoglobin, but in all, the association was not statistically significant ($p > 0.05$).

4 Discussion

The relevance of CYP2B6 to the ACT drug moiety as one of the most polymorphic CYP genes in humans and their variants have been shown to affect transcriptional regulation, splicing, mRNA and protein expression, and catalytic activity [24]. In this study, the polymorphic nature of the CYP2B6 genes in humans and its variants have been successfully isolated by genomic DNA probes. Nine genotype SNP positions were established from 50 subjects with a plethora of haplotypes. The inter-individual variability associated with the pharmacokinetics, protein expression, and catalytic activity of the ACT drug-induced CYP2B6 was further substantiated in the current research and this is

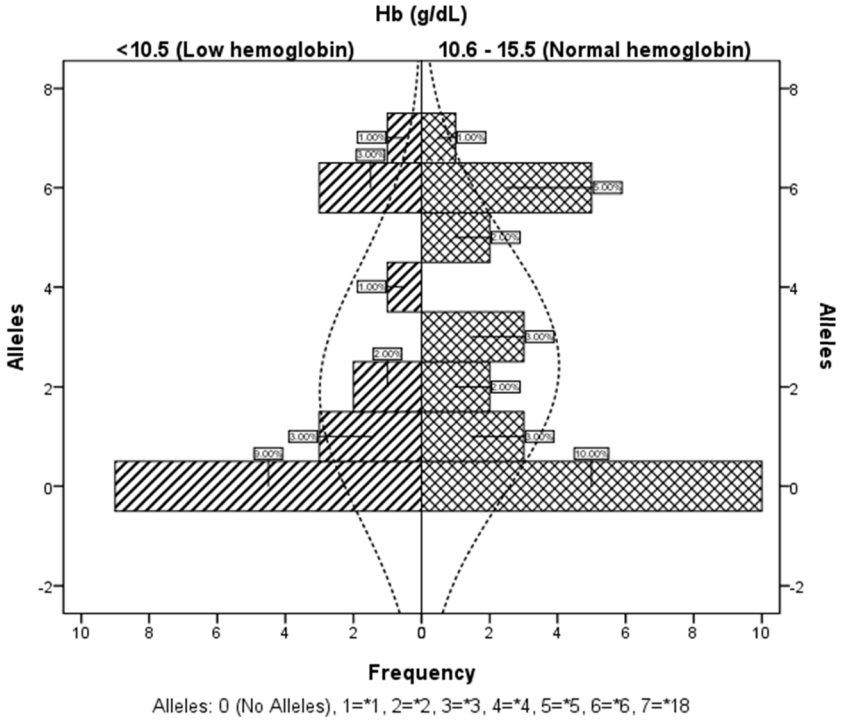

Figure 5: Pyramidal chart showing distribution of the CYP2B6* allele types with respect to hemoglobin levels (<10.5 – low hemoglobin, 10.6–15.5 – normal hemoglobin).

similar to earlier reports [11,12,22]. The different haplotype populations identified in this findings attest to the diversity of the human genetic factors resident in the DNA sequence, and this phenomenon is associated with single base-pair mutations [14]. The CYP2B6*6 gene is common to African descents and this study posited to its presence among the subjects evaluated [16,25,26]. The TaqMan PCR assay employed for this study is accurate, sensitive, and rapid for SNV determination for CYP2B6 gene and haplotype estimation which were till now believed to be difficult to achieve. We determined CYP2B6-selective assays and differentiated their occurrence by standardized codons [26]. CYP2B6*6/*6 genes occurred higher than other SNVs which further ascertains decrease protein expression indicating a drop in ACT-mediated DDI among individual subjects screened for in our research. In a related investigation, efavirenz-mediated DDI on lumefantrine target day 7 concentrations was apparent in the CYP2B6*6/*6 as compared to the CYP2B6*1/*1 group [15]. In our findings, all the star alleles with *1/*1 carriers were wild type alleles, a similar finding by Abdullahi et al. [14]. Other haplotypes manifesting the star alleles *2, *3, *18 had 64C>T, 777C>A, and 499C>G and were heterozygous as compared with the homologous *6 alleles, similar to the reports of Hananta et al. [27]. CYP2B6*6 allele appears to cause both high and low activity of CYP2B6 in several discourse, while it is plausible that the 516G>T and 785A>G mutations, causing the amino acid substitutions, Q172H and K262R are linked to other mutations, giving rise to specific haplotypes associated with high or low activity of CYP2B6 [13]. Our findings revealed that the individuals with the CYP2B6*6 gene metabolize drugs slowly and exhibit more pathogenesis associated with malaria infections than the other individuals with different CYP variants. This affirmation is also evident in earlier studies [13,27].

5 Conclusion

This study identified genetic variability in CYP2B6 alleles among unrelated subjects living in the same geographic location hitherto earlier described as malaria endemic region. These subjects access same ACTs and primary health centers for the treatment of symptomatic malaria but manifest variance in their haplotypes and SNVs. The outcome of malaria in the subjects examined vary with respect to parasitemia counts and obviously the star allele of CYP2B6*6 suffered severe forms of malaria together with remarkable pathogenesis. Furthermore, the established variations in the expression of the SNVs may promote retardation in the efficacy of the ACTs administered at the primary health center and may also narrow the drugs therapeutic index. Of course, we may not shy away from individual's response to ACTs because drug efficacies can also be altered by the following factors, *viz*, natural immune competence of the host, parasite resistance, concomitant diseases, and the ACT's pharmacokinetics. Finally, this report made an attempt to contribute to the growing knowledge of CYP2B6*6 genotype frequency and its relationship with malaria pathogenesis among Nigerian population.

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Author contributions: O.S.H. designed the protocols for the study, carried out field evaluation, and wrote the first draft of the manuscript. M.O.O. and T.A.O. collected relevant literature and supervised the molecular aspect of the study. M.O.O. edited the first draft of the manuscript and T.A.O. supervised the entire exercise. All authors contributed immensely to the final draft and approved the submitted copy.

Conflict of interest: The authors state no conflict of interest.

Informed consent: Subjects were enrolled in the study after obtaining written informed consent, and for patients aged ≤18 years informed consent was obtained from their parents/guardians. Demographic information of the volunteers was recorded anonymously.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance with the tenets of the Helsinki Declaration [19], and has been approved by the Kwara State Ministry of Health, and the University of Ilorin, Ilorin, Nigeria with ref. No.: MOH/KS/EU/777/377.

Data availability statement: All data generated or analyzed during this study are included in this published article and its supplementary information file.

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