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A pilot study on cytochrome p450 2B6 single nucleotide polymorphism among Fulani ethnic group of Northwest Nigeria.

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Abstract

Cytochrome 2B6 is one of the most polymorphic human cytochromes that metabolizes a wide range of clinically important drugs used as antimalarial, anti-retroviral, anesthetics, statins and anti-cancer. About 40-70% of patients demonstrate a lack of efficacy of drug response or adverse drugs reactions due to high intra and inter-individual variability and the greater majority of this variability is accounted for by genetic polymorphism of genes encoding the drugs metabolizing cytochrome p450 enzymes. Fulani constitutes a major ethnic group not only in Nigeria but the whole of West Africa where malaria is endemic and poses major health challenge. Participants were drawn from Wamakko and Dange- Shuni Local Government Areas of Sokoto State. Five milliliters of whole blood from each participant were collected for DNA extractions. PCR and Gel electrophoresis were carried out at Centre for Advanced Medical Research and Training, Usmanu Danfodiyo University, Sokoto using the QIAamp DNA blood mini kit using manufacturer's instructions. All the participants were Muslims, with mean age of 24.6±1.1 and mean body weight of 59.1±1.9 Kg. Fifty percent were intermediate metabolizers, 33.3 and 16.7% were extensive and poor metabolizers respectively. A good percentage of the participants were poor metabolizers. More elaborate studies involving large sample sizes are recommended.

Keywords: Cytochrome p450 2B6, Fulani, Polymorphism, Artemisinin

Introduction

Cytochrome 2B6 is one of the most polymorphic human cytochromes that metabolizes clinically important drugs such as artemisinins, efavirenz which are vital in the treatment of malaria and management of Acquired Immune Deficiency Syndrome (HIV/AIDs). Over 30 non-synonymous variants have been reported that influence intra and inter population variability in drug response (Langmia, 2021). About 40-70% of patients demonstrate a lack of efficacy of drug response or adverse drugs reactions due to high Intra and inter-individual variability and the greater majority of this variability is accounted for by genetic polymorphism of genes encoding the drugs metabolizing cytochrome p450 enzymes .

Artemisinins-based combination therapy (ACT) is the gold standard for the treatment of uncomplicated *Plasmodium falciparum* malaria as recommended by the World Health Organization since 2001 in areas with proven chloroquine, amodiaquine, sulfadoxine-pyrimethamine resistance. The ACT consists of rapid-acting artemisinin derivatives that clear parasitemia combined with a partner drug that is much longer acting that eliminates the remaining parasites and guard against the establishment of a new infection for a period of post-prime infection . Artemether-lumefantrine and artesunate-amodiaquine are the most commonly prescribed anti-malaria in Africa .

The existence of these SNPs in drug-metabolizing enzymes most specifically cytochromes P450 results in a variation of these enzymes activities classified phenotypically as

poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultra-rapid metabolizers .

At present no such study has been done to the best of our knowledge on the ethnic group. Fulani no doubt constitutes a major ethnic group not only in Nigeria but the whole of West Africa where malaria is endemic and poses major health challenge. Knowledge gained from this study would be valuable to the patients, health practitioners and policy makers.

Materials and Methods

Study Population, Settings and Eligibility Criteria

Participants were drawn from Gidan-Fulani in Wamakko LGA and Bade in Dange- Shuni LGA all of Sokoto State. Blood samples and labeling were done in the Laboratory of Department of Pharmacology & Therapeutics of College of Health Sciences while storage, DNA extractions, PCR and Gel electrophoresis were carried out at Centre for Advanced Medical Research and Training, Usmanu Danfodiyo University, Sokoto. Only participants of Fulani extraction as declared and confirmed by one of the researchers (MT) were included in the study. The participants must be healthy as confirmed by physical examination, vital signs measurements and routine laboratory assessments.

Sample Size, Ethical Clearance and Blood Collection

The sample size of 40 was based on the convenient sampling for clinical preliminary studies (Machin *et al.*, 2018). The study was carried out according to the Declaration of Helsinki. All the participants read and signed a study-specific informed consent form before participating in the study procedures (Appendix I). The study protocol, investigators, study site, informed consent form, and recruiting materials were presented for approval to the Ethics Committee of Sokoto State Ministry of Health. Trained personnel collected 5 milliliters of whole blood from each participant through veno-puncture at the fore arm using a syringe into a labeled EDTA vacuum container. After the collection, participants were discharged uneventfully, and blood samples stored at -20^{O}C till analysis.

DNA Extraction

The DNA extraction was done using the QIAamp DNA blood mini kit. The frozen samples were equilibrated to room temperature. Proteinase K 20µl was aliquot into the bottom of each 1.5ml microcentrifuge tubes. This was followed by the addition of 200µl of blood sample and vortex for 15 seconds. Then 200µl of lysis buffer (AL buffer) was added and vortexed for another 15 seconds and subsequently incubated at 56^{O}C for 10 minutes. After brief centrifuge, 200µl ethanol (96-100%) was added and pulse vortex for 15 seconds. The mixture was loaded to QIAamp spin column in a 2ml collection tube and centrifuge at 8000rpm for 1 minute. The spun column was opened and 500µl of buffer (AW1) was added without wetting the rim and cap was closed and centrifuged at 8000rpm for 1 minute. Afterward, 500µl buffer (AW2) was added and spin at 14,000rpm for 3 minutes and after the spinning, 200µl of buffer AE added and incubated at room temperature for 5 minutes and subsequently, centrifuge at 8000rpm for 1 minute.

Polymerase chain reaction (PCR)

Method of Rotger *et al* (2005) was adopted in genotyping. PCR reaction condition was set up according to manufacturer's instruction followed by optimization to select the optimum conditions using forward 5'-GGTCTGCCCA TCTATAAAC-3' and reverse 5'-CTGATTCT TCACATGTCTGCG-3' primers. BioEdit software was used for sequence editing and alignment. The specificity of the primers was confirmed by basic local alignment search tool (BLAST) analysis and compare the genomic sequences in the National Centre for Biotechnology Information (NCBI) data base.

Table 1: Amplification Cycling Conditions

Cycle	Time	Operation	Temperature
	3 minutes	Denaturation	94^{O}C
35	30 seconds	Denaturation	94^{O}C
	30 seconds	Primer Annealing	60^{O}C
	1 minute	Primer Extension	72^{O}C
	6 minute	Final Extension	72^{O}C

Results

Forty (40) volunteers participated in the study, of both genders. Results of demographics of the respondents demonstrated that 20(50%) were females. All the volunteers were Muslims with mean age of 24.6±1.09, and body mean body weight 59.08±1.95.

Representative chromatograms

CYP2B6*6 (15631G>T; rs3745274)
Size: 526bp

GGTCTGCCCATCTATAAACTGGAGCTAAT
AATCAAATTGCATCTGCCTCACATTGTTG
TAGTGAGAGTTCAATGGAA
TTACGCGTGACGTGCTGGTACATAATTAG
CTGTTACGGTTATTCTCATGTTTACCATTA
CTGAGTGATGGCAGACAATCACACAGAG
ATAGGTGACAGCCTGATGTTCCCCAGGC
ACTTCAGTCTGTGTCCTTGACCTGCTGCT
TCTTCCTAGGGGCCCTCATGGACCCCACC
TTCCTCTTCCAGTCCATTACCGCCAACAT
CATCTGCTCCATCGTCTTTGGAAAACGAT
TCCACTACCAAGATCAAGAGTTCCTGAA
GATGCTGAACTTGTTCTACCAGACTTTTT
CACTCATCAGCTCTGTATTCGGCCAGGTC
AGGGAGACGGAGAGGGACAGGGGGTGT
GGGGGTGAGGTGAACACCCAGAACACA
CGAGAAAAGGATGACCTGTCTTGGGGGC
TCAGAAATGCAGCTTATCCTTGGAAGAA
ACGCAGACATGTGAAGAATCAG

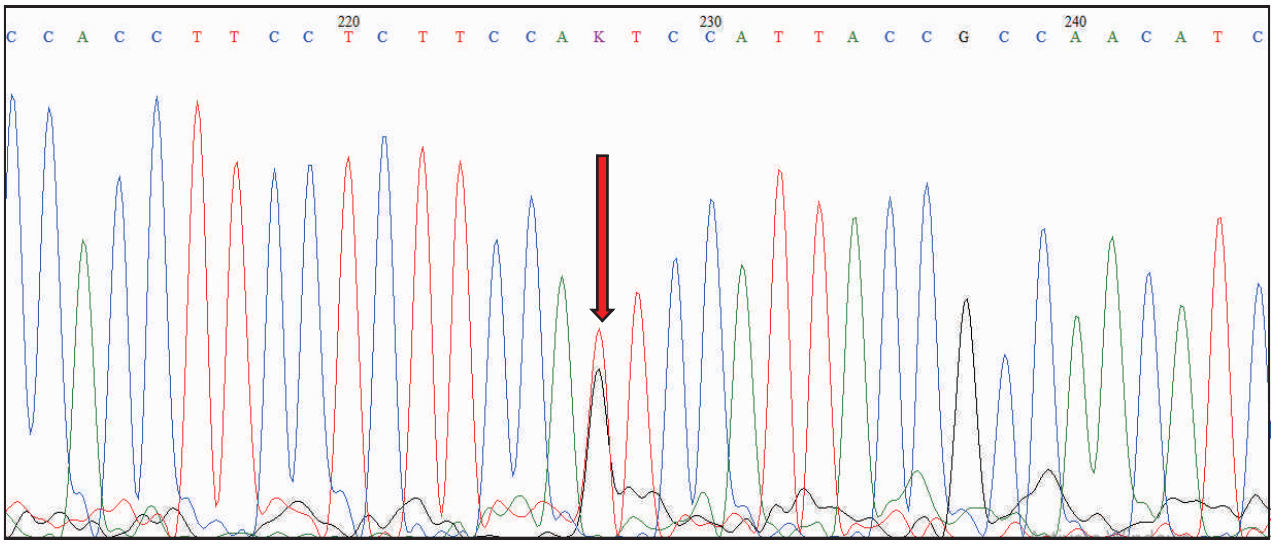

Figure 1: Results indicating the presence of heterozygous *CYP2B6*6 G/T variant (*presence of the "T" allele variant).

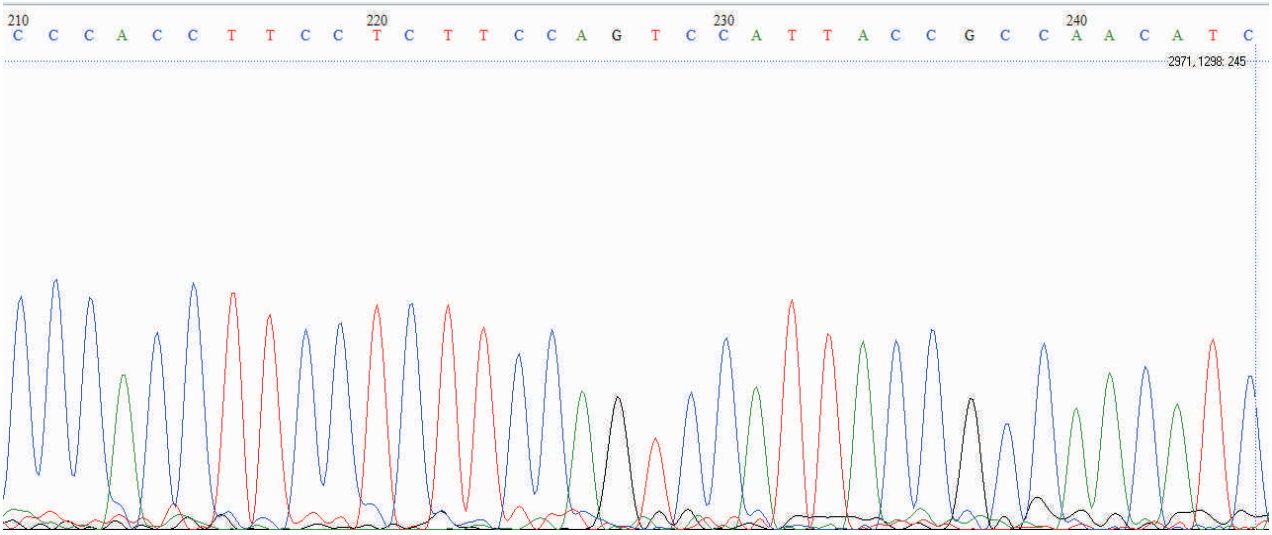

Figure 2: Results indicating the presence of wild *CYP2B6*6 G/G (*presence of the "G" nucleotide only).

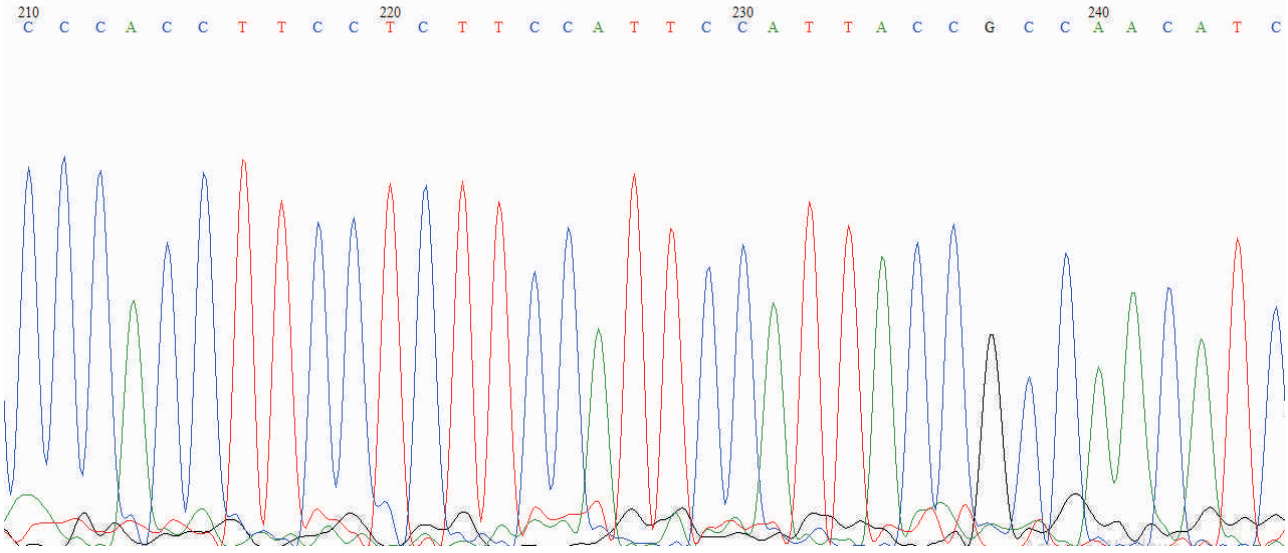

Figure 3: Results indicating the presence of homozygous *CYP2B6*6T/T (*presence of the variant "T" nucleotide only).

Table 2: Genotypes and allele frequencies of CYP2B6*6(15631G>T; rs3745274)

CYB2B6*6 genotypes/allele frequencies	n	Percentage
EM (G/G)	4	33.3
IM (G/T)	6	50
PM (T/T)	2	16.7
Allele (T)	8	66.7

Discussion

Out of the forty (40) blood samples collected, DNA extractions, PCR and Gel electrophoresis. were done, however only 12 were successfully sequenced.

This pilot study is the first attempt at unraveling the activity of this cytochrome enzyme among Nigerian Fulani ethnic group. The finding in this study on the genotypic poor metabolizer's frequency is the same with that of Yoruba ethnic group but lower than what was reported in Ibos and Hausa (Benjamin, 2011). This finding is surprising considering the closeness of Hausa ethnic group to Fulani through intermarriages and other long standing social demographics. This study' report on the intermediate metabolizers (G/T) frequency is higher than what was observed in an Indonesian, Egyptian and Mozambican population who documented 40.6, 28.8 and 42.6% respectively (Hananta, 2018; Ellison, 2012; Arnold, 2013). Inter-individual and inter- ethnic variations are well documented phenomena globally (Natalla, 2017) and what informed the drive for personalized medicine to tailor therapy to individuals bearing in mind genetic make-up as against the currently practice of "one size fits all".

Similar studies in Germany, Turkish and Swiss populations documented 32.1, 25.3 and 24.8 % respectively (Giardina, 2018; Schurig, 2018). These values are lower than the frequencies reported by the index study. Cytochrome 2B6 is highly polymorphic and this has tremendous impact on their biotransformation activities and subsequent effects either as toxicity or therapeutic failure.

The poor metabolizers tend to have high concentration of the drug in the system and for longer duration which may predispose to toxicity even at therapeutic doses. On the other hand, rapid and ultra-rapid metabolizers have a tendency to metabolize the drug faster than necessary and may result to therapeutic failure also at therapeutic doses. These called for dosage adjustments in respective cases to achieve optimum therapeutic goal (Baldwin, 2008).

*CYP2B6*6* is highly polymorphic and has been reported to have a higher frequency in African populations (38%) than in Caucasians and Asians with 14-27% and 10-21% frequencies respectively . This variability has a tremendous impact on the management and treatment of malaria which is endemic as well as HIV/AIDS patients of African descent as clearance of efavirenz is decreased by about 20% .

Conclusion

This study demonstrated frequencies of cytochrome p450 2B6 alleles in Fulani ethnic group with high proportion of poor metabolizers. Because it is a pilot study, a full-scale study is recommended to reveal a true picture.

Conflict of interest: None

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