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A PRELIMINARY STUDY ON CYTOCHROME 2E1 GENE SINGLE NUCLEOTIDE POLYMORPHISMS AMONG ETHNIC FULANI IN NORTH WESTERN NIGERIA

Pharmacology

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ABSTRACT

Single nucleotide polymorphisms of xenobiotics metabolizing enzymes are critical in inter-individual variability in drug response. Cytochrome P450 2E1, which is highly polymorphic belongs to these enzymes and ethnicity significantly determine their expressions. Volunteers from Fulani extractions were recruited through their expressed consents. Five mls of blood sample was collected in EDTA container used for DNA extraction, PCR and sequencing. The consensus sequence generated for individual participants were located to the Cyp 2E1 gene and spanned both target *rs 2031920* to *rs 3813867* polymorphism region. Chromatograms were validated with Bioedit software. Three (3) volunteers (15%) revealed new *rs 147346389* A>C polymorphism at nucleotide position 4029. Also, three (3) other volunteers (15%) revealed *rs 35806299* A>G polymorphism at position 3661. One (5%) new (unlabelled) A>C polymorphism was revealed at position 3906.

KEYWORDS

Polymorphism, Cytochrome 2E1, Fulani, Nigeria

INTRODUCTION

Single nucleotide polymorphisms along the sequence of genes encoding proteins responsible for absorption, distribution, biotransformation, and excretion of administered drugs are considered as the single most important factor in inter-individual variability in drug response.[1] These polymorphisms profoundly alter gene activity and give rise to important clinical implications from adverse drug reactions to lack or decrease efficacy.[2] Cytochrome P450 enzymes are key players on variability observed in the drug pharmacokinetics responses among individuals or populations.[3] The expression of each enzyme is determined primarily by genetic polymorphism, regulation by cytokines, induction by xenobiotics, disease states, gender, age and hormones.[4]

Communicable diseases still constitute a major public health challenge in Africa.[5] Most of these diseases are treated with drugs that are subject to metabolism by CYP450 enzymes, which are in turn susceptible to extensive gene polymorphism.[6] Majority of the drugs used in clinical practice currently are effective in only 25-60% of patients while adverse drug reactions arising from these therapies were estimated to cost billions of US dollars and tens of thousands of deaths globally.[7]

Cytochrome P450 2E1, belongs to phase I xenobiotics metabolizing enzymes, though it metabolizes only about 3% of clinically used drugs, it is highly polymorphic and this polymorphism corresponds to the replacement of cytosine and thymine at position -1019.[8] High expression and the activity of the enzyme have been linked to non-alcoholic fatty liver disease and variously implicated to increase susceptibility to the gastric, nasopharyngeal, colorectal, urinary bladder and esophageal malignancies among a host of others.[9] Increased vulnerability to breast cancer has also been reported to be linked to the polymorphism of this enzyme (*CYP 2E1*6*) as well as coronary artery lesions in patients with Kawasaki disease. [10]

Ethnicity, apart from age, has been demonstrated to significantly determine cytochrome P450 2E1 gene expression. Induction of this enzyme has been shown to be the first step in the development of chemically mediated cancers.[11] It also metabolises acetaminophen, isonicotinic acid hydrazide (INH), chlorzoxazone, acetone, alcohol, aniline, vinyl chloride, benzene, N-nitrosodimethylamines, and styrene. A clear understanding of the role played by cytochrome P450 enzymes may help in preventing potentially harmful drugs co-administration and enable adjusting individual patients' drug regimen early in the course of therapy to provide an optimal response with minimal adverse drug reactions. This study aimed at determining SNP in Fulani in Northwest in Nigeria which is one of the most populous ethnic group in West Africa

MATERIALS AND METHODS

Description of Study Population, design and eligibility criteria

Volunteers from Fulani extractions were recruited from Sokoto suburb in Sokoto state northwest Nigeria through their expressed consents. The study was exploratory and only participants that were healthy as confirmed by physical examination, vital signs and tested negative for Hepatitis B surface antigen were enrolled.

Sampling Techniques, Study Site, Ethical Consideration:

The participants were selected by criterion sampling technique. Only volunteers who satisfied the inclusion criteria were selected. Blood sampling, DNA extraction, Polymerase Chain Reaction (PCR) and gel electrophoresis were done at the Centre for Advanced Medical Research of the Usmanu Danfodiyo University, Sokoto as well as Direct Sequencing. The study was carried out according to the Declaration of Helsinki. All participants read and signed a study-specific informed consent form before participating in study procedures. The study approved by the Ethics Committee of Sokoto State Ministry of Health.

Cytochrome P450 2E1 Genotyping

Two alleles of cytochrome P450 *2E1*5 rs 3813867* and *rs2031920* were sequenced for all the participants and the results were validated using Bio Edit software to determine the site of polymorphisms.

The 5mls of blood sample was collected in EDTA container and frozen and was later used for 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 addition of 200µl of blood sample and vortex for 15 seconds. 200µl of lysis buffer (AL buffer) was added and vortexed for another 15 seconds and incubated at 56^{0}C for 10 minutes. After brief centrifuge, 200µl ethanol (96-100%) was added and pulse vortex for 15seconds.

The mixture was loaded to QIAamp spin column in a 2ml collection tube and centrifuged at 8000rpm for 1 minute. The spun column was later opened and 500µl of buffer (AW1) 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 was added and incubated at room temperature for 5 minutes and subsequently centrifuged at 8000rpm for 1 minute.

Polymerase chain reaction

PCR condition was set up according to manufacturer's instruction followed by optimization to select the optimum conditions using forward 5-CCAGTCGAGTCTACATTGTCA-3' and reverse 5'-TTCATTCTGTCTTCTAACTGG-3' primers.

Table 1: The Amplification Cycling Conditions

Cycle	Time	Operation	Temperature
1	20-40 seconds	Denaturation	95℃

30	30-60 seconds	Annealing	**55**℃
	1-minute	Elongation	**72**℃
	5 minutes	Final extension	**72**℃

PCR product was divided into two aliquots. One for gel electrophoresis, the other for sequencing

DATAANALYSIS

The sequencing data was validated with Bio Edit software.

RESULTS

Table 2: Demographic Characteristics of Participants (n=20)

Variable	Range	Median	Standard Deviation
Age (years)	19-46	23.0	8.4
BMI (Kg/m^2)	13.7-37.2	20.9	6.3

Key: BMI; body mass index

Gel electrophoresis:

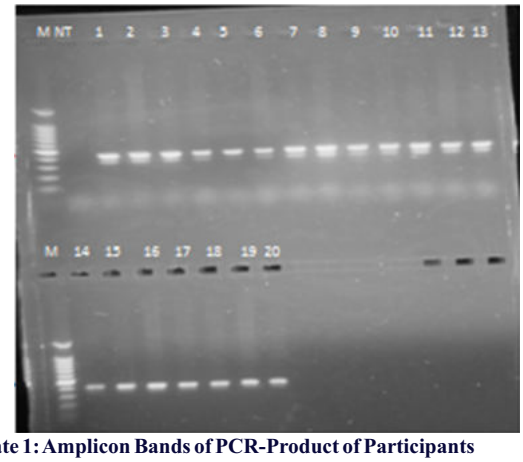

Plate 1: Amplicon Bands of PCR-Product of Participants

M-100bp Molecular marker (Promega), NT=no template control. Samples 1-20 413bp (amplicons) bands of PCR products of DNA samples targeting cytochrome 2E1 gene, using SX Firepol PCR Mastermix on Kyratech Super cycler Trinity PCR Machine.

Direct sequencing at Promoter Region of Cytochrome 2E1 Gene:

The direct sequence chromatograms were of higher quality with little background noise (Figures 1 and 2). The consensus sequence generated from individual participants were located to the Cyp 2E1 gene and spanned both target *rs 2031920* to *rs 3813867* polymorphism region. The sequence also spanned other known non-coding polymorphisms. All the twenty volunteers returned wild type *rs 2031920* and *rs 3813867* regions (Figure 3)

Three (3) volunteers (15%) revealed new *rs 147346389* A>C polymorphism at nucleotide position 4029. Also, three (3) other volunteers (15%) revealed *rs 35806299* A>G polymorphism at position 3661. One (5%) new (unlabelled) A>C polymorphism was revealed at position 3906 (Figure 3).

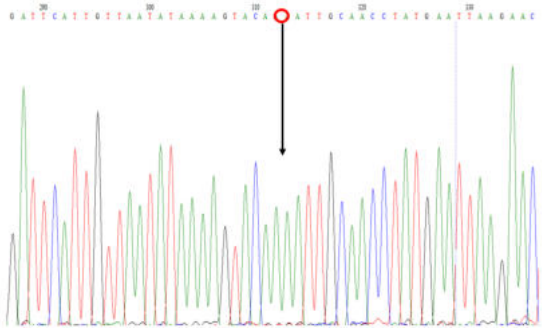

Figure 1: Direct Sequence Chromatograms Representative (rs 2031920)

Allele showing a participant with wild type. The highlighted *"C"* is cytosine, indicating the presence of wild type *CYP2E1*5* in this subject.

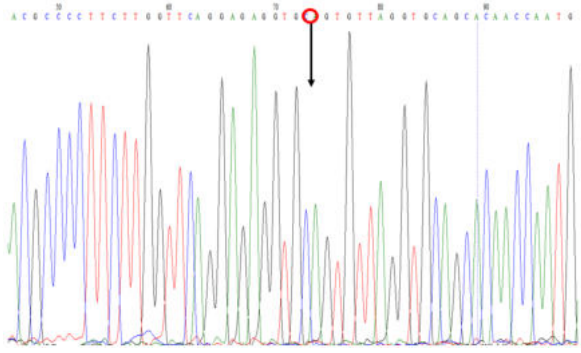

Figure 2: Direct Sequence Chromatogram Representative *(rs 381 3867)*

Allele showing a chromatogram from a participant with wild type. The highlighted "G" is guanine, indicating the presence of wild type *CYP2E1*5* in this subject.

(*)= Non-coding transcripts Figure 3: Chequer-card display of cyp 2E1 promoter region polymorphism in the study sample.

SUBJECT ID	POLYMORPHISM accession number /LOCATION/relative to Exons-Introns					
	rs3813867 **(Target 1)**	***rs2031920*** **(Target 2)**	**rs147346339/4029/upstream A>G (*)**	**rs35806299/3661/upstream.A>G (*)**	**rs……./3906/upstream A>C(*)**	**New**
1						
2						
3						
4						
5					rs……./3906/ A>C	rs……./3906/ A>C
6						
7			rs147346339 A>C			rs147346339 A>C
8			rs147346339 A>C			rs147346339 A>C
9						
10						
11						
12						
13				rs34808295 'S'= C or G		rs34808295 'S'= C =A>C
14				rs35806299 'R'= A or G.		
15				rs35806299 'R'= A or G.		
16						
17			rs147346339 A>C			rs147346339 A>C
18						

19						
20						
Cumulative percentage	**100%**	**100%**	**15%**	**15%**	**5%**	**25%**
Remark	Wild type	Wild type	Polymorphism	Polymorphism	Polymorphism	polymorphism

Key: Grey area= wild type

DISCUSSION

To the best of our knowledge, this study was the first to examine single nucleotide polymorphism of cytochrome P450 *2E1*5* among Nigeria's Fulani ethnic group. It was targeted at SNPs in genes *CYP2E1*5 C-1053* (*rs 2031920*) and *CYP2E1*5 G-1293C* (*rs3813867*). These two genetic polymorphisms in the 5'-flank region identified respectively have been reported to alter transcriptional activity of the enzyme especially on paracetamol metabolism [12]

All amplicon bands from the PCR of participants' DNA were demonstrated, signifying the expression of the cytochrome P450 2E1 enzyme in respective participants. This does not, however, determine the enzyme's activity in vivo. Numerous single nucleotide polymorphisms have been documented in previous studies to alter the activities of xenobiotic metabolizing enzymes yet very few have shown a direct relationship with acetaminophen-induced liver toxicity.[13].

The findings in this study on the two targeted genes of cytochrome *2E1*5* allele studied that are concerned with drugs metabolism revealed no polymorphism as only wild types were demonstrated among the participants. This observation has failed to support what was reported among the Chinese population with 16.1% frequency.[12] This, however, is not surprising taking cognizance of the ethnic and racial diversities between the two populations. It is noteworthy however that, seven polymorphisms were detected upstream, of which five were new and never described previously.

For the gene *CYP2E1*5 C-1053*, (*rs2031920*) the finding in this study differed remarkably from prior work documented but in agreement with the findings reported among East-Asian population.[14] Individuals that are homozygous carriers for T variant for the gene tend to have a two-fold increase in the elimination rate of paracetamol when compared with CC and CT and hence lead to increase N-acetyl p-amino benzoquinoimine (NAPQI) turnover and ultimately liver injury.[15]

Studies involving single nucleotide polymorphisms (SNPs) in cytochrome P450 2E1*5 enzyme might have been able to explain the role genetics plays in both circumstances, between individuals and interethnic variability in xenobiotics metabolism and outcomes of therapy. Previous studies have reported polymorphism of this enzyme with significantly varying frequencies even among the same population.[12] This genotype study has provided new insight regarding cytochrome 2E1 genetic information on Nigerian Fulani ethnic group though the sample size was obviously small.

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