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Cytochrome P450 2D6 (CYP2D6) Genotype and Phenotype Determination in the Nigerian Populations

***Benjamin U. Ebeshi[1,2], Oluseye O. Bolaji[1], Collen M. Masimirembwa[3]**

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria
[2]Department of Pharmaceutical & Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
[3]African Institute of Biomedical Science and Technology (AIBST), Harare, Zimbabwe

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***Corresponding author:**

E-Mail: ben.beshi@gmail.com

Telephone: +234-8059817538

ABSTRACT

This study sets to determine the genotype and phenotype frequencies of CYP2D6 in the Nigerian populations with a view to evaluate the inter-individual and inter-ethnic differences in drug response of clinically useful drugs, which are substrate of CYP2D6. Genotyping of CYP2D6 mutant alleles by polymerase chain reaction (PCR) and sequencing techniques were performed on 300 Nigerians comprising of (Hausa=98), (Ibo=101) and (Yoruba=101). Eighty six (86) subjects were phenotyped with dextromethorphan as the probe drug. The frequency of the defective CYP2D6*4 allele was found to be 2% in Hausa, 8% in Ibo and 3% in Yoruba subjects. The frequency of CYP2D6*17 allele was not significantly different (P>0.05) occurring at 18%, 14% and 22% in the Hausa, Ibo and Yoruba respectively. Also, the frequency of CYP2D6*29 allele was found to be 10%, 20% and 10% in the Hausa, Ibo and Yoruba, respectively. Of the 86 phenotyped subjects, 3 (3.5%) were identified as poor metabolizers (PM), 68 (79%) were extensive metabolizers (EM) and 15 (17.4%) were intermediate metabolizers (IM). The mean and median log10 dextromethorphan/dextrorphan ratios were 0.72 and 0.79 (SD=0.26) for PMs, −1.81 and −1.83 (SD=0.4) for EMs, and -0.51 and -0.62 (SD=0.37) for IMs, respectively. The overall allelic frequencies of CYP2D6*3, *4, *9, *10, *17, and *29 found are unique to the Nigerian populations analyzed. The phenotype data show a close correlation with the genotype data. These population–specific differences are consistent with findings in other African populations.

INTRODUCTION

The human cytochrome P450 2D6 (CYP2D6) is a member of the super gene family of the cytochrome P450s. It is involved in the oxidative metabolism of about 20-25% of all clinically used drugs, which include, antiarrhythmics (e.g. sparteine), antidepressants (e.g. desipramine), antipsychotics (e.g. haloperidol), β-blockers (e.g. metoprolol) and antitussives (e.g. dextromethorphan) [1, 2]. CYP2D6 is mainly expressed in the hepatic tissues but it is also found in the extra-hepatic tissues such as lung and heart [3]. The enzyme is characterized by a high range of interindividual and interethnic variability in activity due mainly to genetic polymorphism. These polymorphism can result in pronounced differences in substrate drugs clearance, resulting in concentrations higher or lower than the therapeutic range in a fraction of treated patients [4, 5]. As a consequence, such differences in CYP2D6 activity may lead not only to severe adverse reactions in clinical therapy, e.g., in antidepressant therapy [6], but also to non-response to medications, such as no observable analgesic effect after low doses of codeine in poor metabolizers (PM)[7] or lack of anti-depressant effect to notriptyline in ultrarapid metabolisers (UM) if given at standard doses[8]. Consequently, many pharmaceutical companies are interested in designing drug candidates that are not substrate of CYP2D6.

The CYP2D6 gene is localized on chromosome 22 of which approximately, 75 variant alleles have been identified with resulting inactive enzyme [9]. However, some have reduced catalytic activity of the enzyme while gene duplication (ranging from 3 to 13 copies), resulting in increased CYP2D6 activity, also occurs [10]. Four phenotypic sub-populations exist that define the rate of drug metabolism by CYP2D6: individuals with a poor (PM), intermediate (IM), extensive (EM), and ultrarapid metabolizers (UM)[11]. Individuals with a PM genotype lack any functional allele as in the Caucasian populations the most common nonfunctional alleles are CYP2D6*3, CYP2D6*4, and CYP2D6*5, with the former two alleles being rare among most Asian and Black African populations [12]. Whereas EM subjects have one or two and UM subjects have more than two functional alleles, which include CYP2D6*1 (wild-type) and CYP2D6*2

(~80% of the wild-type) in Caucasians[13], which can result in a strong effect on the metabolic phenotype when present in combination with a null allele. The IM genotype may result from heterozygosity for a nonfunctional allele and/or the presence of alleles with reduced activity such as CYP2D6*10, CYP2D6*17, and CYP2D6*29; which are prevalent in Asian and Black African populations but rare in the Caucasians[14]. Mutations causing nonfunctional alleles may be a complete deletion of the CYP2D6 gene, single-nucleotide deletions, crossover gene conversions, or point mutations[5]. Population heterogeneity is known to exist with regard to the frequency of the different mutation types causing nonfunctional alleles of CYP2D6[10]. CYP2D6 phenotyping studies have shown a frequency distributions in Caucasians of 7–10% for PM, 10-15% for IM, 70–80% for EM, and 3–5% for UM[15]. In Asians, PM frequency is only about 1-2%[16] while among black Africans there was great variation in prevalence of PMs ranging from 0 to 19% [17]. Some variants are more common in certain populations. For example, CYP2D6*17, which has reduced activity, is found predominantly in blacks[18] whereas CYP2D6*10 (which also confers reduced activity) is common among Southeast Asians but not among other populations [19].

CYP2D6 genotyping has a major impact in understanding the CYP2D6 polymorphism but this account only for a fraction of the overall variation as CYP2D6 activity maybe modified by certain environmental factors, e.g., pathophysiology and nutritional habits[19]. Thus, genotyping alone may not exactly predict an individual's phenotype. Consequently, phenotyping is necessary to obtain a precise picture of a person's actual enzymatic activity via administration of an appropriate probe drug followed by successive measurement of its concentrations and those of CYP2D6-dependent metabolites in body fluids.

CYP2D6 enzymatic activity and genetic polymorphism, which is of tremendous importance in drug metabolism due to its significant ethnic variability, have rarely been studied in Nigerian populations despite being a multi-ethnic society with the largest population in the African continent. It is for this reason that this study sets to evaluate the CYP2D6 allele frequencies and phenotype in the three major ethnic populations of Nigeria comprising Hausa, Ibo and Yoruba.

MATERIALS AND METHODS

Study Population

Three hundred healthy, unrelated subjects consisting of 215 males and 85 females, aged 18 to 45 years, who met the study inclusion criteria, were randomly selected, from the three major Nigerian ethnic groups of Hausa, Northern region (N=98), Ibo, Eastern region (N=101) and Yoruba, Western region (N=101) of Nigeria. Details of the study procedures were explained to the potential subjects after which, they were given an opportunity to make an independent decision to participate in the study. Eligible subjects were enrolled after signing the consent form and were classified as belonging to a particular ethnic group based on family history up to two previous generations. Eighty six (86) subjects were phenotyped with dextromethorphan as probe drug for CYP2D6. The ethics committee of Obafemi Awolowo University Teaching Hospital, Ile-Ife, approved the study.

Genomic DNA preparation

In all subjects genomic DNA was isolated from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, KJ Venlo, Netherlands) in accordance with the manufacturer's protocol. DNA samples were quantified and checked for purity using UV spectrophotometer (Shimadzu, Osaka, Japan). The absorbance (A) of the DNA and protein were simultaneously measured at the λmax of 260nm and 280nm, respectively. The purity of the DNA was estimated by calculating the A ratio of the DNA versus Protein (i.e., A260nm/A280nm) with pure DNA expected to have a ratio of at least 1.6. The concentration of DNA was estimated by multiplying the Absorbance at 260 nm by 50µg/ml and the dilution factor (i.e. A260nm x 50µg/ml x dilution factor). An aliquot of the DNA samples were stored at 4°C until further use and the remaining DNA samples were stored at -80°C for future use.

Genotyping analysis

The genotyping for CYP2D6*2, *3, *4, *10, *17 and *29 alleles were performed by polymerase chain reaction (PCR) and sequencing techniques. Allele-specific PCR was used for the amplification of CYP2D6*2 in accordance with the method of Johansson et al. (1993)[20] while CYP2D6*3 and CYP2D6*4 were according to Hein and Meyer (1990)[21]. The exon 6 mutation (2938C>T) was used to identify the presence of the CYP2D6*2 allele by discounting all the samples with an additional mutation in exon 2 (T1111>C1111) which is indicative of CYP2D6*17. The PCR analysis of the exon 2 mutation, CYP2D6*17 was according to Masimirembwa et al. (1996)[18] while genotyping CYP2D6*29 was according to Wennerholm et al. (2001)[22]. Similar primers were used for the PCR and sequencing analyses.

The PCR reactions were performed in a total of 20 µL mixture containing 5 ng/ml genomic DNA template, 250 µM of deoxynucleoside triphosphate mixture (i.e. dNTPs: dATP, dCTP, dGTP, dTTP) (Roche Biochemicals), 0.5 mM of each primer, 1x TiTaq buffer, and 0.25 units of TiTaq. The PCR cycles for CYP2D6*2, *3, *4, *10, *17 and *29 consisted of an initial denaturation at 94°C for 3 mins, followed by 35 cycles at 94°C for 30 sec, 59°C for 30 sec and 72°C for 60 sec. The Primers sequences are as shown in Table No.1. Sequencing of PCR product was performed using an ABI Prism® 3730 DNA analyzer with DNA sequencing analysis softwareTM, version 3.6.1 (Applied Biosystems, Brussels, Belgium). The sequencing reaction mixture consisted of a total volume of 12 µL, which was made up of 5 µL of purified PCR products added to a strip tube containing 2 µL of Big DyeTM terminator version 3.0, 1 µL of 5 x sequencing buffer, 1 µL of sequencing primer (2 µM) and 3 µL of double distilled water. The sequencing cycles consisted of initial denaturation of the DNA by incubating the reaction mixture at 96 °C for 1 min, followed by 25 cycles of denaturation at 96°C for 10 sec, primer annealing at 50°C for 5 sec and primer extension at 60°C for 4 min. Identification of SNPs was carried out using the novoSNP v2.1.9 software package[23]. Reference sequence was M33388 for CYP2D6. The identified SNPs were compared with the NCBI Single Nucleotide Polymorphism database (dbSNP) (http://www.ncbi.nlm.nih.gov/SNP)[24]. As SNPs can cause the introduction of pre-microRNA (miRNA) sites, this was included as part of the annotation in the novoSNP analysis procedure. Frequencies of SNPs were calculated using Genepop.

Phenotyping analysis

Study population preparation and selection

The pre-study screening was done by interviewing and physical examination. A thorough screening process was

Table No.1: Primer sequences for CYP2D6 alleles used in PCR and sequencing analyses

Gene (allele)	Primer sequences
CYP2D6 (whole gene)	F 5'-CCAGAAGGCTTTGCAGGCTTCAG-3' R 5'-ACTGAGCCCTGGGAGGTAGGTAG
CYP2D6*2	F 5'-AGGTGAGAGTGGCTGCCACGGTGG-3' R 5'-GATGGGCTCACGCTGCACATC-3'
CYP2D6*3	5'-ATTTCCCAGCTGGAATCC-3' 5'-GAGACTCCTCGGTCTCTC-3'
CYP2D6*4	F 5'-AGAGGCGCTTCTCCGTGTCCA-3' R 5'-CAGAGACTCCTCGGTCTCTCG-3'
CYP2D6*9	5'-GGATCCTGTAAGCCTGACCTC-3' 5'-ACTGAGCCCTGGGAGGTAGGTAG-3'
CYP2D6*17	F 5'-GTCGTGCTCAATGGGCTGGCGGCCGTGCGCGAGGCG-3' F 5'-GGCGAGGACACCGCCGACCGCCCGCCTGTGCCCAGTA3'
CYP2D6*29	F 5'-TATGGGCCAGCGTGGAGCGAGCAGAGGCGCTTCCGC-3' R 5'-AGATGCGGGTAAGGGGTCGCCTTCC-3'

undertaken to ensure that subjects were healthy and to exclude presence of infections and use of the possible inhibitors of CYP2D6 and CYP3A4. None of the subjects had a history of alcohol addiction or drug abuse or dependence, and they did not have any medical condition that required treatment. The subjects were advised not to take any medication (e.g. natural remedy or over-the-counter drugs) one week before the phenotype test. They were also asked to refrain from alcohol intake three days prior to the intake of the study drug and were asked to refrain from ingesting products containing grapefruit throughout the study. All subjects were asked to report any intake of medication throughout the study. At the time of entering the study all subjects were assessed as healthy.

Drug administration and sample collection

Prior to drug administration, all the subjects fasted overnight and were not allowed food for a period of 3 hours following drug administration. Food intake was not regulated thereafter. The study was performed using a parallel single dose design. The eighty six (86) subjects were each administered with 10ml dextromethorphan hydrobromide syrup (30mg/10ml) after, which they were encouraged to drink enough water. Urine samples were collected from all the volunteers just before drug administration and thereafter all urine voided between 0-8 hr were collected after drug intake. The total volume of urine was measured immediately after collection and the pH determined using a pH meter. Aliquot of urine sample was taken into fresh tubes and stored frozen at 20ºC before analysis.

Analytical methods

The total urinary concentrations of dextromethorphan (DMP) and its O–demethylated metabolite, dextrorphan (DP) were determined by using the high-performance liquid chromatographic (HPLC) method with UV detection described by Minoo et al. (2004)[25] with slight modifications. The liquid chromatographic system used was an Agilent 1100 series instrument (Agilent Technologies, USA) made up of quaternary pumps, a gradient mixer (Agilent, Germany) with a system purge and a variable wavelength (200-800nm) uv-vis detector model CE1100 (Agilent, Japan) with an 18µL flow cell and detection was done at the maximum wavelength of 278nm. Injection was by a Rheodyne model 7725 valve (Cotati, California, U.S.A.) fitted with a 20µL loop and an on-line vacuum degasser (Agilent, Japan). The column used was a reversed-phase (C-18) ultra sphere silica with 5µm particle size and 250 x 4.6 mm I.D (Beckman, USA). The mobile phase consisting of methanol: acetonitrile: 0.5%w/v ammonium acetate (10:10:80) was pumped through the column at a flow rate of 1ml/min. The pH of the mobile phase was adjusted to 2.8 with orthophosphoric acid and the analytical run was performed at ambient temperature.

Calibration curve for DMP and DP in urine

Blank urine (1ml) sample was each placed in six different extraction tubes and varying amounts of the stock solutions (100µg/ml) of DMP and DP were added to give concentrations of 0.2µg/ml, 0.5µg/ml, 1.0µg/ml, 2.0µg/ml, 4.0µg/ml and 5µg/ml for both DMP and DP. 20µL of the stock solution of the internal standard, morphine sulphate (1mg/ml), was added to each tube. The samples were rendered alkaline with 1M NaOH (0.5 ml) and whirlmixed for 1 min. 3ml of chloroform: isopropanol (70:30) mixture was added to each of the tubes and whirlmixed for 1min after, which the tubes were centrifuged at 2500 rpm for 10 min. The organic layer was transferred into another extraction tube. The extraction process was repeated and the pooled extract was evaporated to dryness in a water bath at 40°C. The residue was reconstituted in 100 µL of 0.1N HCl and whirlmixed before injecting 20 µL onto the HPLC. The peak area ratio was plotted against the concentration of each of the compounds injected. The regression analysis was carried out using Microsoft excel version 2007.

Precision studies for DMP and DP in urine

Intra-day run precision: Two sets, each set consisting of four centrifuge tubes, were used. Each tube in the first set contained 1 ml of blank urine sample spiked with the stock solution of the DMP and DP to give a concentration of 1 µg/ml. The second set also contained 1 ml of blank urine sample spiked with stock solution of the two compounds to give a concentration of 4 µg/ml of each. All the samples were then spiked with 20 µL of the internal standard solution. Extraction was done under alkaline conditions as earlier described and the residue was reconstituted in 100 µL 0.1N HCl, whirl-mixed before 20 µL was injected onto the HPLC. The coefficient of variation of each set was computed.

Inter-day run precision: The procedure above was followed but a sample for each set was analyzed daily for 4 days.

Recovery studies for DMP and DP from urine

Two sets, each set consisting of four centrifuge, tubes were used. Each tube in the first set contained 1 ml of blank urine sample spiked with the stock solution of the two compounds to give a concentration of 1µg/ml. The second set contained the same amount of blank sample spiked to give a concentration of 4µg/ml for DMP and DP. All the samples were then spiked with 20 µL of the internal standard solution. Extraction followed under alkaline conditions as described earlier. The residues were reconstituted in 100 µL 0.1N HCl and whirlmixed before 20 µL was injected onto the HPLC. In other tubes, the stock solutions were diluted in such a way as to give 1µg/ml and 4µg/ml for DMP and DP. The two tubes were spiked with 20 µL of the internal standard solution. To determine the recovery, the peak area ratio of the extraction method and the direct injection method were compared.

Determination of drug and metabolites in urine

Analysis of test urine samples: To 1 ml of urine sample in a centrifuge tube, 20 µL of the internal standard was added and the mixture was incubated for 18 hr with 0.5ml of 10, 000 IU β-glucuronidase. After the incubation, DMP and its metabolite, DP were extracted under alkaline conditions as previously described. The residue was reconstituted in 100 µL 0.1N HCl and whirlmixed before 20 µL was injected onto the HPLC. The metabolic ratio (MR) was calculated as the molar concentration ratio of dextromethorphan and its O-demethylated metabolite, dextrorphan in a 0-8 hour urine collection and was used to estimate CYP2D6 activity.

Statistical analysis

Allele frequencies of the CYP2D6 gene were obtained by direct counting. The log10 MR values between individuals were obtained by probit analysis (Stata Intercooled statistical program version 9). Shapiro-Wilk W test was used to test the normality of the log MR distribution with $P < 0.05$ as the level of significance.

RESULTS

Genotyping

Following PCR and sequencing performed for CYP2D6*3, *4, *9, *10, *17 and *29; the frequencies of CYP2D6 alleles in the three populations studied are summarized in Table No.2. The frequency of CYP2D6*4 allele was found to be 2%, 8%, and 3% in the Hausa, Ibo and Yoruba subjects respectively. The average frequency of 4% for CYP2D6*4 implies that there is a very low likelihood of PMs due to this allele in the Nigerian populations. The most common alleles were CYP2D6*17 and CYP2D6*29. The frequency of CYP2D6*17 allele was not significantly different in the three population ($P>0.05$) occurring at 18%, 14% and 22% in the Hausa, Ibo and Yoruba, respectively. Also, the frequency of CYP2D6*29 allele was found to be 10%, 20% and 10% in the Hausa, Ibo and Yoruba, respectively.

CYP2D6 phenotyping

Calibration curves for DMP and DP in urine

The calibration plot was linear over the concentrations of 0.2, 0.5, 1.0, 2.0, 4.0 and 5.0 µg/ml for DMP and DP, in urine. The correlation coefficients (R) for the mean standard curve following regression analysis were 0.995 and 0.998 for DMP and DP, respectively. Typical equations for the calibration curves for DMP and DP were $y=1.537x-0.115$ and $y=0.681x-0.265$, respectively.

Table No.2: CYP2D6 allele frequencies in the Hausa, Ibo and Yoruba populations

CYP2D6 Alleles	Hausa (n=98) %	Ibo (n=101) %	Yoruba (n=101) %
*CYP2D6*3*	0	0	0
*CYP2D6*4*	2	8	3
*CYP2D6*9*	0	0	0
*CYP2D6*10*	13	10	7
*CYP2D6*17*	18	14	22
*CYP2D6*29*	10	20	10

Table No.3: Results of precision and recovery studies for DMP and DP in Urine (n=5)

		Expected Conc. (µg/ml)	Observed mean DMP Conc. ± SD (µg/ml)	Observed mean DP Conc. ± SD (µg/ml)	Coefficient of variation (%)	
					DMP	DP
Precision	Intra-day run	0.5	0.53 ± 0.031	0.499 ± 0.01	5.81	2.5
		2.0	1.78 ± 0.082	2.11 ± 0.03	4.6	1.43
	Inter-day run	0.5	0.55 ± 0.02	0.51 ± 0.043	4.05	8.4
		2.0	1.84 ± 0.035	2.06 ± 0.038	1.90	1.85
Recovery (%)		1.0	0.71 ± 3.6	0.81 ± 3.4	**71.1**	80.8
		4.0	2.99 ± 4.2	4.19 ± 5.3	74.8	104.8

Precision and recovery of the analytical Method for DMP and DP in urine

The results of the intra-day and inter-day precision as well as the recovery from urine for DMP and DP are shown in Table No.3.

Frequency distribution of the metabolic ratios of the phenotyped subjects

The phenotyping study showed that the DMP/DP MR of the 86 subjects ranged from 0.04 to 2.54 with a median of 0.195 and a mean of 0.32 as shown in the frequency histogram (Fig. 1). Visual inspection and the probit transformation of the MR gave 0.32 as the antimode value (cut-off point) in the Nigerian subjects as shown in Fig. 2.

DISCUSSION

The clinical significance of CYP2D6 polymorphism in the metabolism of routinely used drugs (e.g. cardiovascular substances, neuroleptics and antidepressants) and the established interethnic variations in this enzyme among Caucasians, Orientals and a few other African populations made it necessary to study the distribution of the common CYP2D6 allelic variants in the three major ethnic groups of Nigeria.

In the Hausa, Ibo and Yoruba populations, the frequencies of CYP2D6*4 allele, which is responsible for genotypic PMs in Caucasians, was found to be 2%, 8%, and 3%, respectively. The result shows significant difference ($P<0.05$) in the frequency of CYP2D6*4 especially, the high frequency of 8% in the Ibos. The mean frequency of approximately 4% in this study was close to the 1-3% reported for the East and Southern African populations [17,18] and 2-5% reported for other Africans and African-Americans: 2.5% in Ghanaians[26]; 2% in Gabonese[27]; 1.8% in Ethiopians[28]; 3% in African-Americans[29]. This value is higher than the 0-1% reported in Asians[16] and lower than the 5-10% observed in Caucasians[30].

Results of previous studies in black African populations showed interesting similarities to those obtained in the three major Nigerian ethnic groups with respect to the CYP2D6 alleles. CYP2D6*4 was found in low frequency while CYP2D6*17 and CYP2D6*29 were found at high frequencies. The frequencies of CYP2D6*17 in the Hausa, Ibo and Yoruba populations were 18%, 14% and 22%, respectively. This is comparable to the frequencies of 20%, 26% and 34% reported among Tanzanians, South African Venda, and Zimbabweans,

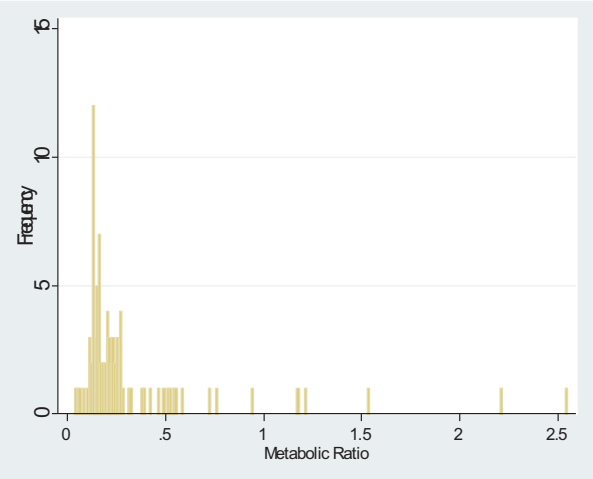

Figure 1: Frequency histogram of CYP2D6 activity as measured by DMP/DP metabolic ratios in the Nigerian

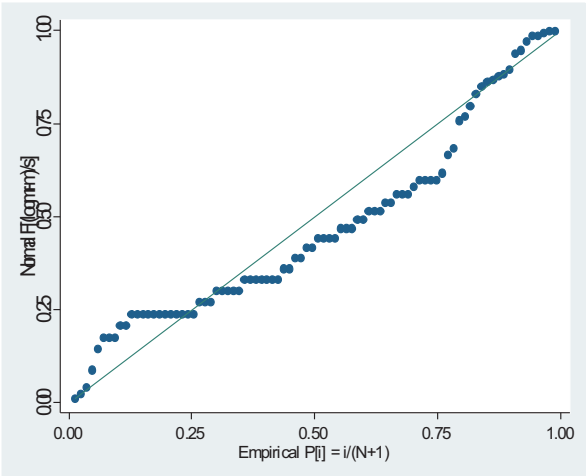

Figure 2: Probit plot analysis of CYP2D6 activity as measured by DMP/DP MRs in the Nigerian subjects

respectively [17, 22]. The result also showed similarities with the frequencies of 15-28% reported in Ghanaians [26], 15-26% in African-Americans[29] but different from the 0-0.01% in Caucasians and oriental populations[14,31]. These inherent similarities amongst the African populations and the obvious differences from the Caucasian and Oriental populations further confirms the impact of the African-specific alleles (i.e. CYP2D6*17 and CYP2D6*29) on the activity of CYP2D6.

Furthermore, the frequency of CYP2D6*29 allele was found to be 10%, 20% and 10% in the Hausa, Ibo and Yoruba, respectively. It can be seen from the results that the CYP2D6*4 and CYP2D6*29 alleles, which are responsible for PMs and IMs, respectively were significantly ($P<0.05$) higher in the Ibo than in the Hausa and Yoruba. The 20% frequency of CYP2D6*29 allele obtained in the Nigerian Ibo was similar to the 20% and 17% reported for the Tanzanians and Shona[18, 22]. In contrast CYP2D6*4 allele is the most frequent CYP2D6 allele in Caucasians with frequencies ranging from 20-25% [1]. Whereas CYP2D6*4 and CYP2D6*17 show some striking differences in frequency between Africans and Caucasian populations, the frequency of the CYP2D6*5 (gene deletion) reported among African populations is comparable to those reported for the Caucasians and Asians. Although, CYP2D6*5 allele was not characterized in this study, there are reports of similarities in the frequency of CYP2D6*5 in East Asians, Caucasians and other African populations[14]. The above therefore may be in line with the school of thought that CYP2D6*5 represents' an older allele whose existence preceded the divergence of human populations from pre-historical era.

The frequency of CYP2D6*4 is very low or nearly absent in Orientals, accounting for low PMs in the population as compared to the Caucasians. Moreover, CYP2D6 activity is lower in Oriental EMs, Nigerians in this study and other African EMs because of the dominance of low activity CYP2D6*10, CYP2D6*17 and CYP2D6*29 alleles compared to the Caucasian EMs. This invariably means that the Caucasian populations could experience two extremes in the activity of CYP2D6 enzyme as either PMs or EMs, whereas the Orientals and Africans may have more of IMs in their populations. Similarly, the absence of CYP2D6*3 and CYP2D6*9 alleles (predominantly found in Caucasians) in the Nigerian populations is in agreement with previous report of the absence of these alleles in the African populations[4].

An attempt was made to evaluate the correlation between genotyping and phenotyping data in the Nigerian populations using the innocuous dextromethorphan as a CYP2D6 probe drug. In a laboratory setting with limited facilities, an attempt to redevelop a simple and cost effective liquid-liquid extraction method, which employs HPLC with ultra-violet (UV) detection, was made. This method emphasizes off-column pretreatment as a necessary panacea for effective separation and quantitation of DMP and DP in urine. In this study, the choice of chloroform/isopropanol as the extracting solvents resulted in some improved recovery of DMP compared to previous reports in literature[25]. The analytical method employed in this study is one of the few methods that can detect DMP and its metabolite, DP despite the relatively low recovery of DMP. The lower limit of quantitation for DMP in urine, which was 0.2µg/ml made it possible to monitor the unchanged drug in the samples.

The HPLC method used for the analysis gave a good resolution of DMP from its metabolite, DP and the internal standard (Morphine). There were little or no interference from endogenous compounds in most of the biological samples used in the study, thus facilitating accurate determination of the drug and its metabolite. The method also gave good recoveries for DMP (>70%) and DP (>80%) in urine. A number of methods have been described in literature for the determination of DMP and DP in urine including, HPLC with fluorescence detection[32], HPLC with ultraviolet detection[33] and gas chromatography with flame ionization[34] and mass spectrometric detection[35]. In most of these methods, the limit of quantification of DMP was high for the accurate determination of the DMP. Numerous endogenous peaks were present under the chromatographic conditions specified in several of these methods, complicating the quantification of DMP or DP. Besides, the sample preparation procedures described in these methods generally suffered from low analyte recoveries. Finally, the selectivity of these methods in the presence of a number of other metabolites of DMP was not established.

The phenotyping study showed that the log10 DMP/DP MR of the 86 subjects ranged from −3.2 to 0.93 with a median of −1.64 and a mean of −1.50. The probit plot analysis in Fig. 2 shows a skewed trimodal distribution of CYP2D6 activity in this population. This trimodal distribution shows apparent distinct population subgroups, which allows for classification of the Nigerian subjects as EM, IM and PM, and a possibility of a few UM in this population. The probit transformation of the log MR gave 0.32 (log10 0.32= -0.495) as the antimode value in the Nigerian subjects. When compared to the established antimode value of 0.3 (log10, −0.5229) described for Caucasians and African-Americans[30,36], it was found that of the 86 phenotyped Nigerian subjects, 3 (3.5%) were determined to be PM, 68 (79%) were EM and 15 (17.4%) were IM. The mean and median log10 DMP/DP ratios were 0.72 and 0.79 (SD=0.26) for PM, −1.81 and −1.83 (SD=0.4) for EM, and -0.51 and -0.62 (SD=0.37) for IM, respectively. These data show a trend toward a larger mean log MR (reduced enzyme activity) as the number of active alleles decreases. However, a substantial scatter exists within the specific phenotypes in most of the population. The statistical analysis using Shapiro-Wilk W test for normal distribution, showed that the log MR data significantly deviated from normality ($P = 0.00004$). This was also reflected in the large standard deviation within the sub-groups, been highest in the EM phenotype. Thus, log10 MR values did not allow a clear distinction between the EM and UM on one hand but gave a clear distinction between IM, and PM phenotypes on the other hand. This could probably be due to the small sample size and the large interindividual variability observed in the study. The PM phenotype frequency of 3.5% obtained in this study is in agreement with the reported poor metabolizer frequency of 0 to 5% in Nigerians[37, 38].

CONCLUSION

The overall allelic frequencies of CYP2D6*3, *4, *9, *10, *17, and *29 found are unique to the Nigerian populations analyzed. These population–specific differences are consistent with the other African populations. The phenotype data show a close correlation with the genotype data and in agreement with previous phenotyping studies in Nigerian populations.

REFERENCES

1. Mizutani T. PM frequencies of major CYPs in Asians and Caucasians. Drug Metab. Rev. 2003:35:99-106.

2. Ingelman–Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J. 2005:5:6–13.

3. Zanger UM, Fischer J, Raimundo S, Stuven T, Evert BO, Schwab M, Eichelbaum M. Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. Pharmacogenetics 2001:11:573–585.

4. Solus JF, Arietta BJ, Harris JR, Sexton DP, Steward JQ, McMunn C. Genetic variation in 11 phase I drug metabolism genes in an ethnically diverse population. Pharmacogenomics 2004:5:895–931.

5. Grant RW. Drug metabolism and variability among patients in drug response. N. Engl. J. Med. 2005:352:2211-2221.

6. Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, Brockmoller J. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. Mol. Psychiatry 2004:9:442–473.

7. 7.Gasche Y, Daali Y, Fathi M, Chiappe A, Cottini S, Dayer P, Desmeules J. Codeine intoxication associated with ultrarapid CYP2D6 metabolism. N. Engl. J. Med. 2004:351:2827-2831.

8. Bertilsson L, Dahl ML, Dalen P, Al-Shurbaji A. Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. Br. J. Clin. Pharmacol. 2002:53:111–122.

9. Nelson DR. Cytochrome P-450 nomenclatures home page (http://www.imm.ki.se/CYPalleles/cyp2d6.htm) [cited 12th September, 2010].

10. Gaedigk A, Coetsee C. The CYP2D6 gene locus in South African Coloureds: unique allele distributions, novel alleles and gene arrangements. Eur. J. Clin. Pharmacol. 2005:64(5):465-475.

11. Matsunaga M, Yamazaki H, Kiyotani K, Iwano S, Saruwatari J, Nakagawa K, Soyama A. Two novel CYP2D6*10 haplotypes as possible causes of a poor metabolic phenotype in Japanese. Drug Metab. Dispos. 2009:37(4):699-701.

12. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics 2002:3:229-243.

13. Griese EU, Zanger UM, Brudermanns U, Gaedigk A, Mikus G, Morike K, Stuven T, Eichelbaum M. Assessment of the predictive power of genotypes for the in-vivo catalytic function of CYP2D6 in a German population. Pharmacogenetics 1998:8:15–26.

14. Kim K, Johnson JA, Derendorf H. Differences in drug pharmacokinetics between East Asians and Caucasians and the role of genetic polymorphisms. J. Clin. Pharmacol. 2004:44:1083-1105.

15. Fux R, Morike, K., Prohmer AM., Delabar U, Schwab M, Schaeffeler E, Lorenz G, Gleiter CH, Eichelbaum M, Kivisto KT. Impact of CYP2D6 genotype on adverse effects during treatment with metoprolol: a prospective clinical study. Clin. Pharmacol. Ther. 2005:78:378–387.

16. Xie H-G, Kim RB, Wood AJJ, Stein CM. Molecular basis of ethnic differences in drug disposition and response. Annu. Rev. Pharmacol. Toxicol. 2001:41:815-850.

17. Dandara C, Masimirembwa CM, Magimba A, Kaaya S, Sayi J, Sommers DK, Synman JR and Hasler JA. Genetic polymorphism of CYP2D6 and CYP2C19 in East and Southern African populations including psychiatric patients. Eur. J. Clin. Pharmacol. 2002:56:653-657.

18. Masimirembwa C, Persson I, Bertilsson L, Hasler J, Ingelman–Sundberg MA. A novel mutant variant of the CYP2D6 gene (CYP2D6*17) common in a black African population: association with diminished debrisoquine hydroxylase activity. Br. J. Clin. Pharmacol. 1996:42:713–719.

19. Sakuyama K, Sasaki T, Ujiie S, Obata K, Mizugaki M, Ishikawa M, Hiratsuka M. Functional characterization of 17 CYP2D6 allelic variants (CYP2D6.2, 10, 14A-B, 18, 27, 36, 39, 47-51, 53-55 and 57). Drug Metab. Dispos. 2008:36(12):2460-2467.

20. Johansson I, Lundqvist E, Bertilsson L, Dahl ML, Sjoqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. Proc. Natl. Acad. Sci. USA. 1993:90:11825-11829.

21. Hein M and Meyer UA. Genotyping of poor metaboliser of debrisoquine by allele-specific PCR amplification. Lancet 1990:336 (8714):529-532.

22. Wennerholm A, Johansson I, Hidestrand M, Bertilsson L, Gustafsson LL, Ingelman-Sundberg, M. Characterization of the CYP2D6*29 allele commonly present in a black Tanzanian population causing reduced catalytic activity. Pharmacogenetics 2001:11:417-427.

23. Weckx S, Del Favero J, Rademakers R, Claes L, Cruts M, De Jonghe P. novoSNP, a novel computational tool for sequence variation discovery. Genome Res. 2005:15:436–442.

24. The National Center for Biotechnology Information (NCBI) databases, (http://blast.ncbi.nlm.nih.gov/Blast.cgi): The National Library of Medicine (NLM) [cited 13th June, 2009].

25. Minoo A, Mohammad-Reza R, Mohsen A. Simple chromatography method for simultaneous determination of dextromethorphan and its main metabolites in human plasma with fluorimetric detection. J. Chromatogr. B. 2004:802:317–322.

26. Griese E-U, Asante-Poku S, Ofori-Adjei D, Mikus G, Eichelbaum M. Analysis of the CYP2D6 gene mutations and their consequences for enzyme function in West African population. Pharmacogenetics 1999:9:715-723.

27. Panserat S, Sica L, Gerard N, Mathieu H, Jacqz-Aigrain E, Krishnamoorthy E. CYP2D6 polymorphism in a Gabonese population: contribution of the CYP2D6*2 and CYP2D6*17 alleles to the high prevalence of the intermediate metabolic phenotype. Br. J. Clin. Pharmacol. 1999: 47:121-124.

28. Aklillu E, Persson I, Bertilsson L, Johansson I, Rodrigues F, Ingelman-Sundberg M. Frequent distribution of ultrarapid metabolizers of debrisoquine in an ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. J. Pharmacol. Exp. Ther. 1996:278:441-446.

29. Wan Y-JY, Poland RE, Han G, Konishi T, Zheng Y-P, Berman N, Lin K-M. Analysis of the CYP2D6 gene polymorphism and enzyme activity in African- Americans in Southern California. Pharmacogenetics 2001:11:489-499.

30. Sachse C, Brockmoller J, Bauer S, Roots I. CYP2D6

variants in a Caucasian population: allele frequencies and phenotypic consequences. Amer. J. Hum. Genet. 1997:60:284–295.

31. Droll K, Bruce-Menssah K, Otton S, Gaedigk A, Sellers E, Tyndale R. Comparison of three CYP2D6 probe substrates and genotypes in Ghanaians, Chinese and Caucasians. Pharmacogenetics 1998:8:325-333.

32. .Bendriss EK, Markoglou N, Wainer IW. High-performance liquid chromatography assay for simultaneous determination of dextromethorphan and its main metabolites in urine and in microsomal preparations. J. Chromatogr. B. 2001:754:209–215.

33. Park YH, Kullberg MP, Hinsvark ON. Quantitative determination of dextromethorphan and three metabolites in urine by reversed-phase high performance liquid chromatography. J. Pharm. Sci. 1984:73:24-29.

34. Wu YJ, Cheng YY, Zeng S, Ma MM. Determination of dextromethorphan and its metabolite dextrorphan in human urine by capillary gas chromatography without derivatization. J. Chromatogr. B. 2003:784:219.

35. Baumann P, Jonzier-Perey M. GC and GC-MS procedures for simultaneous phenotyping with dextromethorphan and mephenytoin. Clin. Chim. Acta. 1988:171:211-222.

36. He N, Daniel HI, Hajiloo L, Shockley D. Dextromethorphan O-demethylation polymorphism in an African-American population. Eur. J. Clin. Pharmacol. 1999:55:457-459.

37. Iyun AO, Lennard MS, Tucker GT, Woods HF. Metoprolol and debrisoquine metabolism in Nigerians: lack of evidence for polymorphic oxidation. Clin. Pharmacol. Ther. 1986:40:387-394.

38. Lennard MS, Iyun AO, Jackson PR, Tucker GT, Woods HF. Evidence for dissociation in the control of sparteine, debrisoquine and metoprolol metabolism in Nigerians. Pharmacogenetics 1992:2:89-92.