

Prevalence of the Genetic Mutation CYP2C8*5 in Selected Ethnic Groups in Southern Ghana

Abstract

Aim: The study determined prevalence of clinically relevant CYP2C8*5 polymorphism in 80 unrelated individuals, from selected ethnic groups in Southern Ghana. Medical history on adverse drug reactions of the subjects and level of dependency on drugs metabolized by CYP2C8 enzyme was obtained by questionnaire. Allele Specific-PCR analyses were used to genotype CYP2C8*5 alleles in the study subjects.

Results

Allelic frequency for CYP2C8*5 was 83.75% which was statistically significant ($p < 0.05$). There was no significant difference ($p > 0.05$) in the prevalence of CYP2C8*5 allele within the ethnic groups. Also, there was no significant association ($p > 0.05$) between CYP2C8*5 allele and reported ADRs. Many (88.75%) of the study subjects depended highly ($>1-3x$ in a year) on drugs metabolized by CYP2C8.

Conclusions

The high prevalence of CYP2C8*5 determined in the study population may indicate a high risk of toxicity in using drugs metabolize by CYP2C8 since CYP2C8*5 mutants have been reported to have a reduced enzymatic activity. This is the first reported study on prevalence of CYP2C8*5 in Ghanaians.

Key words: CYP2C8 mutation, drug toxicity, Artesunate, Amodiaquine, Chloroquine, malaria

Background

Cytochrome P450 (CYP) family enzymes are diverse group of haemoproteins bound to membranes of the endoplasmic reticulum and mitochondria where they function in catalyzing the oxidative metabolism of many endogenous and exogenous compounds such as drugs and carcinogens (Redlich et al., 2008). Based on sequence homology, 57 isoforms of CYPs have been identified and classified in humans. CYPs are found in abundance in the liver and, to a lesser extent, in extra hepatic tissues as such the kidney, salivary ducts, intestine and adrenal cortical cells (Enayetallah et al., 2004), with low expression of CYP2C8 protein in the intestines (Lapple et al., 2003). The CYP2C subfamily is responsible for catalyzing the metabolism of about 20% of clinically prescribed drugs. Cytochrome P450 2C8 (CYP2C8) is

a member of the CYP2C subfamily and account for approximately 6% of the total CYP content in liver (Rowland-Yeo et al., 2004). CYP2C8 plays important roles in the metabolism of about 5% of therapeutic drugs in phase I processes such as anti-malaria drugs (amodiaquine, chloroquine and dapsone); anti-cancer drug (paclitaxel) (Rahman et al., 1994), anti-diabetes drugs (rosiglitazone, pioglitazone and repaglinide) (Yamazaki et al., 2000, Baldwin et al., 1999); antiarrhythmic drug (amiodarone) (Ohyama et al., 2000), and HMG-CoA reductase inhibitor (cerivastatin) (Muck, 2000). Furthermore, CYP2C8 play a role in the metabolism of endogenous compounds such as lipids, steroidal hormones, retinoids and arachidonic acid (Zeldin et al., 1996, Nadin and Murray, 1999, Capdevila et al., 1981). The gene that code for the CYP2C8 enzyme is known as the *CYP2C8* gene which is 31KB in size consisting of 9 exons (Klose et al., 1999). It is located at chromosome 10q24 in a multigene cluster containing the other CYP2C subfamily members CYP2C9, CYP2C18 and CYP2C19 (Finta and Zaphiropoulos, 2000) prearranged as Cent–CYP2C18–CYP2C19–CYP2C9–CYP2C8-Tel (Gray et al., 1995). The nucleotide sequences of CYP2C8 gene shares about 74% homology with CYP2C9 gene. The whole CYP2C gene family spans about 400kb and has linkage between its genes (Yasar et al., 2002). The CYP2C8gene is polymorphic, and the distribution of variant alleles differs among ethnic populations. The wild-type gene of CYP2C8 is referred to as *CYP2C8**1 (**1A*, **1B* and **1C*) and although there are about 15 identified variants of CYP2C8, studies have shown only *CYP2C8**2, *CYP2C8**3, *CYP2C8**4 and *CYP2C8**5 to have altered enzyme activity with respect to *CYP2C8**1. *CYP2C8**2 (c.805A>T, p.Ile269Phe) is the most common in blacks although it is very rare in Caucasian populations (Totah and Rettie, 2005). *CYP2C8**3 (c.416G>A, p.Arg139Lys and c.1196A>G, p.Lys399Arg) results in the change in the amino acid sequence of CYP2C8 and is mostly found in Caucasians, with an allele frequency of approximately 10-20% (Totah and Rettie, 2005). There is a link between *CYP2C9**2 (c.430C>T, p.Arg144Cys) and *CYP2C8**3 variant

alleles, where about 95% of phenotypes having the CYP2C8*3 variant allele have also been reported to be with the CYP2C9*2 variant allele (Yasar et al., 2002). CYP2C8*4 (c.792C>G, p.Ile264Met) allele is also present in Caucasians with a frequency of about 8% (Totah and Rettie, 2005). CYP2C8*5 results from a deletion of adenine (471) on exon 3 which lead to a frame shift in the mRNA which in turn leads to an early stop codon at residue 177 of during protein synthesis (Bahdur, 2002). The resulting product of the enzyme lacks 64% of normal enzymes protein structure affecting the substrate recognition (haem-binding) site and 5 of 6 substrate recognition sites of the enzyme (Nakajima et al., 2003). This implies that individuals homozygous for CYP2C8*5 who are poor metabolizers (PM) may find it difficult to tolerate drugs metabolized by CYP2C8 and suffer Adverse Drug Reactions (Dai et al., 2001).

Report indicates that the prevalence of CYP2C8*2 mutation was determined to be 16.75% in northern Ghanaian populations (Rower et al., 2005) and 17% in Southern Ghanaians (Kudzi et al., 2009).

Aims

This study determined the allele frequency of CYP2C8*5 variant in selected ethnic groups in southern part of the Ghanaian population by estimating the distribution of CYP2C8*5 genotype frequencies in the Ashanti, Fanti, Anlo, Ewes and Ga ethnic groups in Southern Ghana. The study also determined association between the CYP2C8*5 genotype and reported adverse drug reactions in the study subjects with respect to drugs metabolised by the enzyme. Finally the study determined the extent to which individuals with genotypes of the mutant allele depended of drugs metabolized by CYP2C8 enzyme.

81 **Materials and Methods**

82 **Ethical Issues**

83 The research work was approved by the Committee on Human Research, Publications and
84 Ethics, Kwame Nkrumah University of Science and Technology, School of Medical Sciences
85 and Komfo Anokye Teaching Hospital, Kumasi-Ghana (CHRPE/AP/089/14). The
86 participants were all fully briefed on the importance of the study and written informed
87 consent was obtained from all the study subjects who participated in the research.

88

89 **Study Design and Subjects**

90 The study was cross-sectional involving 80 study subjects comprising of 40 males and 40
91 females with 10 subjects from each ethnic group being Akyem, Ashanti, Fanti, Eve, Anlo,
92 Ga, Krobo and Nzema in Southern Ghana. They were healthy individual blood donors at
93 Blood Units of Korle-Bu Teaching Hospital (Accra), Komfo Anokye Teaching Hospital
94 Transfusion Medicine Unit (Kumasi), Hohoe Municipal Hospital (Hohoe) and Winneba
95 Municipal Hospital (Winneba), who were willing to participate in the study. Only individuals
96 whose grandparents are of the same ethnic background were considered for the study.
97 Participation was entirely voluntarily.

98

99 **Blood Sample Collection**

100 Peripheral blood samples were obtained from the study subjects and preserved on labelled
101 Whatman № 3 filter papers which were air dried and stored in a plastic bag until DNA was
102 extracted.

103

Medical History Data Collection Questionnaire

A questionnaire was also administered for information on medical history of the study subjects. They were asked if they have any history of adverse drug reactions and if yes then they indicate which drugs were involved. The study subjects were also given a list of common drugs metabolised by CYP2C8 enzyme and asked to indicate how often they use any of these drugs. The Body Mass Index of the study subjects was also obtained to reflect the health status of the study subjects.

Molecular Analysis

DNA Extraction from Blood Blot Samples

The genomic DNA was extracted from the filter paper blood blots collected using the TE buffer extraction method (Plowe et al., 1995). The extract DNA was stored at -40 °C in the DNA microtubes in a Deep freezer until use after quantification using spectrophotometric analyses.

AS-PCR A475 del (Frameshift, CYP2C8*5)

Allele specific (AS) PCR analysis was used for the analysis of A475 del (Frameshift, CYP2C8*5) with the primers 5'-AGG CAA TTC CCC AAT ATC TC-3' (3S) and 5'-TCA CCC ACC CTT GGT TTT C-3' (mutant) (Nakajima et al., 2003). The DNA was amplified in 20µl reaction mix containing 1X PCR buffer (Invitrogen, USA), 25mM MgCl₂ (Promega, USA), 10mM DNTP mix containing each of the four different forms of deoxyribonucleotide triphosphates (DNTPs), 10µM of the primers 5'-AGG CAA TTC CCC AAT ATC TC-3' (3S), 5'-TCA CCC ACC CTT GGT TTT C-3' (mutant) (Nakajima et al., 2003) and 5U/µl of DNA Taq Polymerase. An amount of 5µl extracted genomic DNA was used as template for the PCR reaction. Sterile double distilled water (ddH₂O) was added to the mix to make the volume up to 20µl. For each reaction, a non-DNA negative control and positive control

containing the expected DNA was also used in the set up. The PCR was run in Gene Amp PCR System 9700 thermo-cycler using cycling conditions as follows: initial denaturation (94°C for 4 minutes), followed by 40 cycles of denaturing (94°C for 1 minute), Annealing (51°C at 1 minute), Extension (72°C for 2 minutes) and Final Extension of cycle (72°C for 7 minutes). The final reaction volume of 20µl contained 8-10 µl of the amplified products. The PCR machine was set keep the PCR products at 4°C until they were removed from thermo-cycler for storage and further analysis.

Gel Electrophoresis

The PCR products were electrophoresed in 2% agarose gels prepared with 1x TAE buffer and stained with 0.5µg/ml ethidium bromide dye. About 8µl of each sample of DNA was added to 1µl of 10x bromothymol blue gel loading dye. The gels were run in a 1x TAE buffer at 100V for 45 minutes using mini gel system (BIORAD, USA), visualized by ultraviolet transillumination and photographed using a Sony Toyobo FASIII gel documentation system. The sizes of the PCR products were calculated using the mobility of a 100bp molecular weight ladder of DNA (Promega, USA) as the reference.

Statistical Analysis

For the analysis of the data Chi-square (χ^2) and Student t-test analyses were performed with SPSS version 20.0 using a confidence interval of 5% and graph plotted using Microsoft Excel version 2007.

Results

Demographics of Study Subjects

The age distribution of the study subjects ranged from 18 to 53 years with an average age of 30.63 ± 8.75 years. Majority of the study subjects had access to secondary (45%) and tertiary

education (42.5%). The Body Mass Index (BMI) distribution of the subjects ranged from of 15.78kg/m² to 36.75kg/m² with a mean of 22.36 ± 3.20kg/m².

Distribution of BMI of Study Subjects

Statistically, there was no significant difference between the study subjects when their BMI was cross tabulated against presence or absence of the *CYP2C8*5* mutant allele ($p>0.05$).

Table 1: Body Mass Index (BMI) and *CYP2C8*5* Status of Study Subjects.

BMI Status	CYP 2C8*5 Mutant Allele Status		<i>P-value</i>
	Presence (%)	Absence (%)	
<18.50	3 (4.54)	2 (14.28)	
18.50 - 24.90	54 (81.81)	8 (57.1)	
25.00 - 29.90	9 (13.63)	2 (14.28)	
>=30.00	0 (0)	2 (14.28)	
Total	66 (83.75)	14 (16.25)	0.007

Genotyping of *CYP2C8*5* and Ethnic Distribution

DNA was extracted from all the samples (n = 80) and PCR was performed on the DNA samples using primers specific for amplifying mutant allele of *CYP2C8*5*. Figure 1 is an ethidium bromide stained agarose gel electrophoregram showing the positive and negative samples.

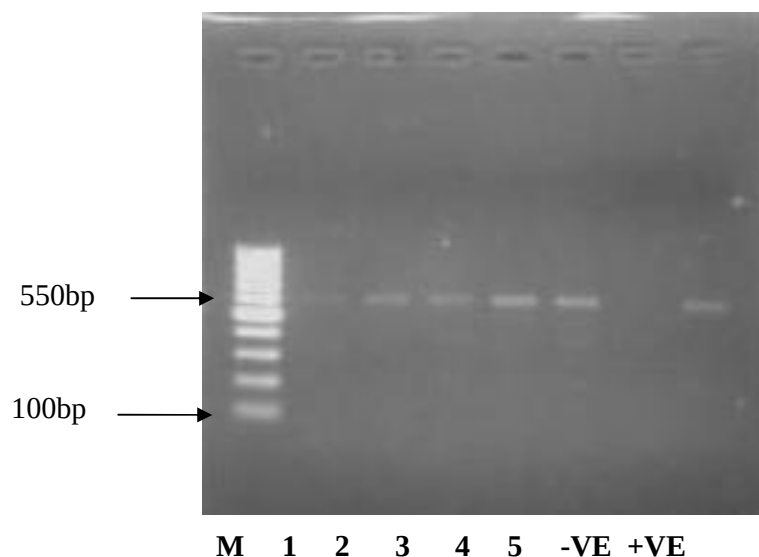


Figure 1: Ethidium bromide stained 2.0% agarose gel electrophoregram showing PCR amplified DNA sequences of CYP2C8*5 for the Ashanti Male Subjects.

The study determined the prevalence of *CYP2C8*5* allele as 83.75% (Table 2). There was no significant difference between the selected ethnic groups with respect to the observed prevalence of *CYP2C8*5* in the study population ($p>0.05$) (Table 2).

Table 2: Frequency of CYP2C8*5 Distribution in Study Population.

Ethnicity	Positive <i>CYP2C8*5</i> Mutants (%)			<i>P-value</i>
	Females (5)	Males (5)	Overall (10)	
Akyem	3 (60)	5 (100)	8 (80)	
Ashanti	4 (80)	4 (80)	8 (80)	
Fanti	3 (60)	4 (80)	7 (70)	
Anlo	4 (80)	4 (80)	8 (80)	
Eve	4 (80)	5 (100)	9 (90)	
Ga	5 (100)	5 (100)	10 (100)	
Krobo	5 (100)	5 (100)	10 (100)	
Nzema	2 (40)	5 (100)	7 (70)	

Total (80)	30 (75)	37 (92.5)	67 (83.75)	0.636
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Adverse Drug Reactions and CYP2C8*5 Mutant Allele Status

Table 3a shows reported history of adverse drug reactions by the study subjects. Thirty-three (41.25%) of the respondents indicated they had a history of adverse drug reaction, while 4 (5%) were not sure whether they had a history of adverse drug reaction. Table 3a further shows the drugs involved in the adverse drug reactions among the study subjects. Eighteen (22.5%) of the study subjects reported that they have reacted to Chloroquine CQ, while 4 (5%) reacted to only Amodiaquine (AQ) and 5 (6.25%) reacted to both CQ and AQ.

Table 3a: History of adverse drugs reactions and drugs involved

Variable	Category	Frequency (%)	p-value
History of Adverse	Yes	33(41.25)	0.000
Drug Reaction by	No	43(53.75)	
Study Subjects	Not Sure	4(5)	
	Total	80 (100)	
Drugs Reacted to by	Amodiaquine (AQ)	4 (5)	
Study Subjects	Chloroquine (CQ)	18 (22.5)	
	CQ and AQ	5 (6.25)	
	Aspirin	1 (1.25)	
	Other	6 (7.5)	
	Total	33 (41.25)	

Table 3b shows the cross tabulation of CYP2C8*5 against reported history of adverse drug reaction. The study subjects reported that they react to the drugs Chloroquine and Amodiaquine (both antimalarial drugs) as well as Aspirin. Statistically, there was no

significant association between having CYP2C8*5 allele and reported history of adverse drug reactions ($p>0.05$).

Table 3b: CYP2C8*5 Mutant Allele and History of Adverse Drug Reactions

CYP 2C8*5 Allele Status	History of Adverse Drug Reaction			Total	P-value
	Yes (%)	No (%)	Not Sure (%)		
Presence	25 (37.87)	37 (56.06)	4(6.06)	66	
Absence	8 (57.1)	6 (42.86)	0(0)	14	
Total	33 (41.25)	43(53.75)	4(5.0)	80(100)	0.323

Extent of Dependency on Drugs Metabolized by CYP2C8 Enzymes

In generally many of the study subjects (88.75%) depend highly (1-3x in the past 5 years) on drugs metabolized by the CYP2C8 enzyme (Table 4). There was no significant association between the presence of the CYP2C8*5 mutant allele and reported history of adverse drug reactions ($p >0.323$).

Table 4: Extent of Dependency on Drugs Metabolized by CYP2C8 in Past 5 Years

Extent of Dependency on CYP2C8 Drugs Substrates in 5 years (%)		
Category	Frequency (%)	P-value
0x	9 (11.25)	
1-3x	69 (86.25)	
4-6x	2 (2.50)	
7-9x	0 (0.00)	
>10x	0 (0.00)	
Total	80 (100)	0.000

Discussion

Distribution of CYP2C8*5 Genotype

The overall prevalence of CYP2C8*5 observed in the study population was 83.75%. This was higher compared to that observed in the Japanese population as 2.5% involving 200 healthy subjects (Nakajima et al., 2003) and Soyama et al. (2002) who also reported the heterozygote of CYP2C8*5 in Japanese population to be 0.9% involving 54 subjects. Apart from the Japanese population, there is not much information on the allele frequency of CYP2C8*5 in other populations. The prevalence of CYP2C8*5 observed is also higher than the reported prevalence of CYP2C8*2 (17.0%) in the same Ghanaian population (Rower et al., 2005, Kudzi et al., 2009). The result of this study supports and augments the findings that CYP2C8 gene mutation is high in the Ghanaian population. In addition, the observed prevalence (83.75%) of CYP2C8*5 in the study is higher than other reported CYP2C8 gene mutations in the African population such CYP2C8*2 (12.0%) in Burkinabes (Parikh et al., 2007). It is also higher than CYP2C8*2 (21.0%), CYP2C8*3 (2.1%) and CYP2C8*4 (0.6%) reported by Cavaco et al. (2005) in 165 paediatric malaria patients in Zanzibar Islands which has a mixed race population of both Arabs and Asians.

There was no significant difference between the selected ethnic groups with respect to the observed prevalence of CYP2C8*5 in the study population ($p>0.05$) (Table 2). As such, the information from some of these ethnic populations may be useful on populations or individuals that share a close blood relation with those in the selected study populations but are outside Ghana such as the Ewes whose population extend from Ghana through Togo and Benin to Nigerian and Cameroun. These also include the Nzemas, Fantis and Ashantis whose population extends into Ivory Coast as well as individuals abroad.

Distribution of BMI and CYP2C8*5

Most the participants having healthy BMI (Table 1) is a reflection of their health status as well the ability to dose drugs correctly. There are medications which accumulate in adipose tissue and will necessitate an adjusted weight for a better accurate dosing for such drugs since not all the drugs are bioavailable. An adjusted, or dosing weight, is calculated to account for obese patients exceeding >125% of their ideal body weight (Wurtz et al., 1997, Blouin and Warren, 1999, Cheymol, 2000). Individuals who are underweight are less likely to tolerate drug while individuals who are overweight are prone to experiencing therapeutic failure and will require more dosage compared to the normal weight individuals. Despite the healthy BMI in the study population, individuals may still be at risk of having adverse reactions from normal dosing of drugs since the calculation for the dosing does not take into consideration the high prevalence of the CYP2C8*5 mutation observed. Rather dosing is based on the assumption that the population has normal individuals having the correct CYP2C8 wild type allele.

Adverse Drug Reaction and CYP2C8*5

The study population resides in an area where malaria is endemic with 3,415,912 confirmed cases in 2013 (WHO., 2015b, p. 119). As such most of the study subjects who have them use antimalarial drugs such as Amodiaquine and Chloroquine which they have a history of adverse reaction. Antimalarial treatment courses distributed as a proportion of estimated malaria cases in the public sector in 2014 was 100% for Ghana (WHO., 2015b, p. 69). The WHO recommended treatment for uncomplicated malaria is the artemisinin-based combination therapy (ACT) which most of the study subjects have been using to combat malaria (WHO., 2015a, p. 9).

Considering the structure of protein product of CYP2C8*5 and the lack of enzyme activity, it would have been expected that majority of the study subjects would report a history of adverse drug reactions but this was not observed in the study. This implies that the study subjects could be heterozygous individuals with one correct allele (CYP2C8*1) and one mutant allele (CYP2C8*5) or they could even have two different mutant alleles (CYP2C8*2

and *CYP2C8*5*) such that the activity of the enzyme is still present but at reduced levels. With previous works conducted indicating that *CYP2C8*2* is the most predominant (17%) of *CYP2C8* mutation in the Ghanaian population this is more likely to be the case.

Also some drug substrate have multiple enzymes involved in their metabolism (Glamočlija and Jevrić-Čaušević, 2010). The similarity in size accounts partly for the observation of *CYP2C8* and *CYP3A4* having many related substrates compared to other CYPs (Jaakkola et al., 2006). Hence although the frequency of *CYP2C8*5* was observed to be high in the population, if the wild type of another enzyme which metabolizes the same drug is present, the effect of the active enzyme will mask the absence of activity of the other enzyme to some extent. Studies conducted in Ghana however observed that individuals using Artesunate Amodiaquine, when it was first introduced were having Adverse drug reactions (Adjei et al., 2009). Nevertheless, due to the heterozygosity of *CYP2C8* gene, individuals having the *CYP2C8*5* may still produce a functional enzyme from the non-mutant *CYP2C8*5* allele and tolerate certain drugs. Such individuals are still at risk of drug toxicity since most of those heterozygous for the mutation will be considered as partial metabolizers. Such individual will have both a functional and non-functional enzymes.

Extent of Dependency on Drugs Metabolized by *CYP2C8* Enzymes

Although there was no significant association between the presence of the *CYP2C8*5* mutant allele and reported history of adverse drug reactions ($p > 0.323$), the study population may be at risk of using drugs metabolised by *CYP2C8* enzyme. This may be true considering the high presence of the *CYP2C8*5* allele in the study population and the high level of dependency on drugs metabolized by *CYP2C8* by the study subjects.

Conclusions

The prevalence of *CYP2C8**5 mutant allele in selected ethnic groups in Southern Ghana is high (83.75%). There was no significant difference in the prevalence of *CYP2C8**5 allele between the selected ethnic groups being Ashanti, Akyem, Fanti, Ewe, Anlo, Ga, Krobo and Nzema. Also there was no significant association between individuals with *CYP2C8**5 mutant allele and reported history of adverse drug reactions. Much of the study subjects (88.75%) depend highly (>1-3x in year) on drugs metabolized by *CYP2C8*. The study population may be at risk of using drugs metabolised by *CYP2C8* although no statistically significant association between presence of the *CYP2C8**5 allele and reported history of ADRs ($p > 0.323$) was observed in the study.

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