# PREVALENCE OF THE GENETIC MUTATION *CYP2C8*5* IN SELECTED ETHNIC GROUPS IN SOUTHERN GHANA

**BY**

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## DECLARATION

I hereby declare that this submission is my own work towards the award of Master of Philosophy in Biochemistry and that to the best of my knowledge, it contains no material previously published by another person nor a material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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***"I am grateful for the doors of opportunity and those who oil the hinges."***

**- Author Unknown**

## ABSTRACT

Effects of genetic variability on drug efficacy and tolerability are important. Many pharmacologically-relevant genetic polymorphisms show variability in different populations. Information on allelic frequency is useful in identifying adverse drug reaction (ADRs) risk populations, understanding therapeutic failures and optimising doses for efficacy and efficiency. CYP2C8 are clinically important haem-containing group of enzymes which metabolize several drugs (e.g. anti-malarial, anti-diabetic, anti-cancer, non-steroidal anti-inflammatory) and endogenous substances (e.g. all-trans-retinoic acid, steroidal hormones and arachidonic acid). *CYP2C8*5* homozygous individuals are poor metabolizers with increased risk of drug toxicity. The study determined prevalence of clinically relevant cytochrome P450 (CYP) *2C8*5* polymorphism in 80 unrelated individuals, 10 each from the ethnic groups Akyem, Ashanti, Anlo, Ewe, Fanti, Ga, Krobo and Nzema in Southern Ghanaian population. 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. Allelic frequency for *CYP2C8*5* was 0.8375 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. The study population may be at risk of toxicity in using drugs metabolized by CYP2C8 because of the high prevalence determined since *CYP2C8*5* mutants have been reported to have a reduced enzymatic activity.

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## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| ACT | Artemisinin-Based Combination Therapy |
| AQ | Amodiaquine |
| AA | Artesunate Amodiaquine |
| AL | Artemether Lumefantrine |
| DP | Dihydroartemisinin Piperaquine |
| bp | Base Pair |
| BSA | Bovine Serum Albumin |
| CQ | Chloroquine |
| CYP | Cytochrome P450 |
| CYP2C8 | Cytochrome P450 2C8 Enzyme |
| *CYP2C8* | Cytochrome P450 2C8 Gene |
| *CYP2C8*5* | Cytochrome P450 *2C8*5* Variant Allele |
| CYP2C8.5 | Cytochrome P450 2C8 Variant Enzyme |
| dbSNP | Single Nucleotide Polymorphism Database |
| DEAQ | N-desethylamodiaquine |
| DNA | Deoxyribonucleic Acid |
| $dH_2O$ | Distilled Water |
| dNTPs | Deoxynucleotide Triphosphates |
| EDTA | Ethylene Diamine Tetra Acetic Acid |
| EtBr | Ethidium bromide |
| IUTLD | International Union against Tuberculosis and Lung Disease, |
| KATH | Komfo Anokye Teaching Hospital, Kumasi Ghana |
| KBTH | Korle Bu Teaching Hospital, Accra, Ghana |
| KNUST | Kwame Nkrumah University of Science and Technology |
| $MgCl_2$ | Magnesium Chloride |

| | |
|---|---|
| Milli Q | Milli Q double distilled water |
| MPAC | Malaria Policy Advisory Committee |
| NCDs | Non-Communicable Diseases |
| NMIMR | Noguchi Memorial Institute for Medical Research, UG, Legon, Ghana |
| PCR | Polymerase Chain Reaction |
| PBS | Phosphate Buffered Saline |
| pH | Hydrogen-ion exponent |
| RFLP | Restriction Fragment Length Polymorphism |
| sddw | Sterile double distilled water |
| SAHS | School of Allied Health Sciences |
| SNP | Single Nucleotide Polymorphism |
| SP | Sulfadoxine-Pyrimethamine |
| TAE | Tris – Acetate EDTA |
| Taq | *Thermus aquaticus* (DNA polymerase) |
| TE | Tris-EDTA |
| $T_m$ | Melting Temperature |
| UGMS | University of Ghana Medical School, Korle-Bu, Ghana |
| WHO | World Health Organization |

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of the Study

The socio-economic status of every country is reflective of the health status of its population since the health status of the population directly affects the productivity of that country. The major diseases that are of direct public health interest currently in sub-Saharan African countries include HIV, tuberculosis and malaria (Gow, 2002). Nonetheless, non-communicable diseases (NCD) such as cancer, diabetes and cardiovascular diseases (CVD) are also on the rise due to improved quality of life and increased life expectancy (WHO, 2012a). Of these, diabetes and malaria are causing more mortality and morbidity (Lopez *et al.*, 2006) which leads to a more debilitating effects on the productive work force of a developing country such as Ghana.

Malaria is a mosquito-borne disease caused by a parasite of the genus *Plasmodium.* Malaria, if not treated, may result in the development of severe complications and ultimately death. According to WHO (2013), there were about 207 million cases of malaria in 2012 (uncertainty range of 135 to 287 million) and an estimated 627,000 mortalities (uncertainty range of 473,000 to 789,000) mostly among African children. Ninety percent of all mortalities related to malaria occur in sub-Saharan Africa (WHO, 2013). Malaria mortality rates have fallen by 45% globally since 2000 and by 49% in the WHO African Region (WHO, 2013). Malaria is also generally linked with poverty, it is a cause of poverty and a major obstacle to economic development (Gollin, 2007).

Malaria is the fifth cause of death from infectious diseases worldwide following respiratory infections, HIV/AIDS, diarrhoeal diseases and tuberculosis (WHO, 2013). In Africa, malaria is the fourth leading cause of death from infectious diseases (WHO, 2013). In Ghana, malaria accounts for over 44% of reported outpatient visits and it is estimated that about 22% children below 5 year die due to malaria (Nordstrom and Dybul, 2013).

In order to reduce such high mortalities, factors that contribute to malaria management and treatment need to be identified and monitored. The transmission of malaria parasite can be reduced by preventing mosquito bites through the use of insecticide treated bed nets and insect repellents. Also mosquito-control strategies such as spraying insecticides inside houses and draining of standing water to prevent the breeding of mosquitoes have had similar results (Picone *et al.*, 2013). Although many are under development, the challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is yet to be achieved (Kilama and Ntoumi, 2009, von Seidlein and Bejon, 2013).

Chemotherapy is the most readily available treatment option. Although a range of antimalaria drugs exist, with time the parasites develop resistance to these drugs. This was the case with chloroquine (CQ) and it led to a change of it as the first-line choice of drug for the treatment of uncomplicated malaria to other alternatives such as the artemisimin-based combination therapy (ACT) as recommended by the WHO and the Ghana Health Services (WHO, 2012b, Dodoo *et al.*, 2009). Several drugs are also available as prophylaxis to prevent malaria in travellers to malaria-endemic countries.

The burden associated with non-communicable diseases (NCDs) is also on the increase with diabetes increasing by 88% from 1990 to 2010 in all of Africa (Murray and Lopez, 2013). A major reason for this is due to increased average life expectancy and increased quality of life leading to most populations growing older. Worldwide, mortalities associated with NCDs, especially those due to ischemic heart disease and diabetes, have grown by 30% since 1990 (Institute for Health Metrics and Evaluation *et al.*, 2013)**.** On a milder note, the overall population growth has also contributed to this increase in deaths from non-communicable diseases (Danaei *et al.*, 2011).

Cancers, glycaemia and diabetes are part of such NCDs rising globally. An increase in both glycaemia and diabetes is driven by population growth and ageing as well as by increasing age-specific prevalence. About 57 million deaths occurred worldwide in 2008. Of these, 36 million (63%) were attributed to NCDs, principally CVDs, diabetes, cancer and chronic respiratory diseases (Alwan *et al.*, 2010). NCD deaths are anticipated to increase by 15% worldwide between 2010 and 2020 to about 44 million deaths (WHO, 2008).

Whereas cancer treatment may involve different therapeutic options, the most effective choice of treatment for both malaria and diabetes is chemotherapy. Although progress has been made significantly in the development of strategies for management and treatment of these medical conditions, this is not the case for systems and enzymes in human that metabolize these drugs and make them tolerable to patients. One of such enzymes is cytochrome P450 (CYP) 2C8 which metabolizes the antimalarial drugs amodiaquine (AQ) and chloroquine (CQ) (Li *et al.*, 2002) as well as the antidiabetic drugs rosiglitazone and pioglitazone (Baldwin *et al.*, 1999, Yamazaki *et al.*, 2000). It also metabolizes the anti-cancer drug paclitaxel (Rahman *et*

*al.*, 1994) and the antiarrhythmic drug amiodarone (Ohyama *et al.*, 2000). Non-steroidal anti-inflammatory drugs (NSAIDs) (McGreavey *et al.*, 2005) and HMG-CoA reductase inhibitor cerivastatin (Muck, 2000) are also substrates of CYP2C8. Furthermore, CYP2C8 plays an important 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 CYP family is a diverse group of enzymes that function to catalyse the oxidation of organic substances. Lipids, steroidal hormones and drugs are the major substrates of CYP enzymes (Nebert and Russell, 2002). All drugs are detoxified, most of which require bioactivation to form active compounds of metabolites, and ultimately excreted from the body. CYP are the most important enzymes involved in detoxification and bioactivation of most drugs which accounts for about 75% of their total metabolism (Guengerich, 2008).

Of the CYP enzymes, CYP2C8 is very important in the treatment of malaria because it is the main enzyme that metabolizes AQ, which is now widely used as the first line choice of drug for the treatment of malaria. Inability of the enzyme to metabolize AQ leads to toxic side effects such as abdominal discomfort, nausea, vomiting, headache, dizziness, blurring of vision, mental and physical weakness, and fatigue (van Beek and Piette, 2001). These symptoms are generally mild and short-lived. The most severe undesirable reactions include itching, dyskinesia, neuromuscular disorders, cardiovascular malfunction, hearing loss, ocular damage, agranulocytosis and peripheral neuropathy (Ahmad, 2000, p.11).

Furthermore, some of the drugs used in treatment of diabetes such as thiazolidinediones, rosiglitazone and pioglitazone are metabolized by CYPs. These are peroxisome-proliferator-activated receptor-γ agonists that regulate the transcription of the many genes associated with the metabolism of glucose and lipids as well as the sensitivity of insulin and adipocyte differentiation (Yki-Jarvinen, 2004). Rosiglitazone and pioglitazone are completely metabolized by CYP2C8 in the liver (Shah and Mudaliar, 2010, Sahi *et al.*, 2003). Due to liver toxicity the antidiabetic agent troglitazone, which is also a substrate for CYP2C8, has been withdrawn from usage (Yamazaki *et al.*, 1999). Without a functional CYP2C8 enzyme, majority of the drugs which are substrate for the enzyme become toxic to users and thus cause more harm than good.

The loss of function of the CYP2C8 enzyme could be due to mutations in the gene that code for the protein. Individuals with such mutations will be unable to metabolize drugs that are substrates for the CYP2C8. Individuals with mutations in CYP2C8 who are poor metabolizers are unable to tolerate drug doses metabolized by the enzyme (Dai *et al.*, 2001). Studies have shown a high prevalence of certain clinically relevant mutant alleles of *CYP2C8* in southern and northern Ghana (Rower *et al.*, 2005, Kudzi *et al.*, 2009). It is thus imperative to identify and determine the prevalence of other clinically important mutant alleles of *CYP2C8* gene in the Ghanaian population to facilitate modifications of treatment for such individuals with respect to drug choice and dosing.

## 1.2 Problem Statement

Mutations in the gene for CYP2C8 results in a dysfunctional enzyme (Gerbal-Chaloin *et al.*, 2001, Goldstein, 2001). As a result, individuals with mutations in CYP2C8

may be unable to tolerate or metabolize substrates of the enzyme while others will clear the drug faster than expected from the body. One such medically important mutation is *CYP2C8*5*. The mutant allele *CYP2C8*5* is as a result of a deletion of adenine (471) on exon 3 resulting in a frameshift in the mRNA leading to the introduction of a stop codon at residue 177. This leads to encountering a stop codon earlier than will normally occur (Bahadur *et al.*, 2002). Thus the resulting product of the enzyme lacks 64% of the normal enzyme structure and this affects the active site (haem-binding) and 5 out of 6 substrate recognition sites of the enzyme (Nakajima *et al.*, 2003). Consequently, individuals homozygous for *CYP2C8*5* are poor metabolizers (PM) who may find it difficult to tolerate drugs metabolized by CYP2C8 and suffer adverse drug reactions (ADRs) (Dai *et al.*, 2001).

The mutations of the enzyme can result in slow activity of the enzyme leading to inability to comprehensively breakdown the substrates such as a drug and this could lead to adverse side effects of the drugs such as liver toxicity and kidney failure amongst others. Over time, the adverse effects of the drugs could lead to permanent damage such as glaucoma (blindness) in affected individuals (Resnikoff *et al.*, 2004). In the case of pregnant woman, this may endanger the expectant mother and the foetus since the development of the baby could be adversely affected or may lead to mortality (McGready *et al.*, 2012).

## 1.3 Aims and Objectives

### 1.3.1 General Objective

The aim of the study was to genotype *CYP2C8*5* variants in selected ethnic groups in Southern Ghana.

### 1.3.2 Specific Objectives

1. To determine the overall prevalence of *CYP2C8*5* genotype in the selected ethnic groups in Southern Ghana.
2. To determine the distribution of *CYP2C8*5* genotype within the selected different ethnic groups in Southern Ghana.
3. To determine the association between *CYP2C8*5* genotype and adverse effects of drugs metabolised by the enzyme.
4. To determine the extent to which individuals with *CYP2C8*5* genotype depend on drugs metabolized by CYP2C8.

## 1.4 Justification of Study

Different populations exhibit several clinically relevant pharmacological genetic polymorphisms with respect to drug metabolism. As such information on the allelic frequency distributions within specific populations are imperative in understanding and explaining therapeutic successes or failures, categorizing potential risk clusters of individuals for ADRs and the optimisation of doses for therapeutic efficacy within populations.

With the increase in the use of chemotherapy as a form of treatment due to the high prevalence of malaria (WHO, 2013) and the increase in NCDs such as diabetes and cancers (WHO, 2012a), information on the systems which facilitate the tolerability of such drugs needs to be available. This will enable good selection of drugs with maximum efficacy but minimum toxicity and its associated adverse effects for a given population. The efficient use of the principle of benefit to risk ratio in making well-informed decisions about the type of treatment most suitable for patients and drug dosing will thus be enhanced.

In northern Ghana, studies have determined the prevalence of *CYP2C8*2* mutation among individuals who are unable to metabolize the first line treatment for malaria (AQ) to be high (16.75%) (Rower *et al.*, 2005). Also studies on southern Ghanaians by Kudzi *et al.* (2009) reported the prevalence of *CYP2C8*2* (17%), *CYP2C8*3* (0%) and *CYP2C8*4* (0%). Although *CYP2C8*5* is one of the most clinically relevant mutant forms of *CYP2C8,* no work has reported the prevalence of *CYP2C8*5* in Ghana as at the time of this study.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

## 2.1 CYTOCHROME P450 ENZYMES

The cytochrome P450 superfamily is a large and diverse group of haem-containing monooxygenases (Wrighton and Stevens, 1992) which function to catalyse the oxidation of endogenous substances usually metabolic intermediates such as lipid and steroidal hormones as well as exogenous substances such as drugs and other toxic chemicals. About 57 human CYP450 isoenzymes (57 genes and over 59 pseudogenes) consisting of 18 CYP families (CYP1–CYP4) and 44 subfamily members have been identified and characterized (Cui *et al.*, 2012). Generally, members of the CYP1, CYP2 and CYP3 families are of clinical importance because they contribute significantly in the metabolism of major drugs (Bishop-Bailey *et al.*, 2014). There are however other CYP families associated with metabolic reactions which are linked mainly to endogenous function (Daly, 2004, Nerbert and Russell, 2002).

The important CYP2C subfamily enzymes are encoded as groups of polymorphic genes on chromosome 10q24.33 arranged from centromere to telomere as Cen-CYP2C18-CYP2C19-CYP2C9-CYP2C8-Tel (Gray *et al.*, 1995). Although the sequences of these four isoforms are about 80% identical, they can have unique substrate specificities, and collectively they are responsible for the metabolism of about 20-30% of all clinical medications in humans (Goldstein, 2001).

### 2.1.1 Cytochrome P450 Isoenzymes

Approximately 40% of cytochrome P450 enzymes especially CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A5 show genetic polymorphism (Zanger *et al.*, 2008). These genotypic polymorphisms generally give rise to four clinically important phenotypes: poor metabolisers, intermediate metabolisers, extensive metabolisers as well as ultrarapid metabolisers in certain cytochrome P450 enzymes such as CYP2D6 (Zhou, 2009).

Phenotypes classified as intermediate metabolisers have normal metabolic function by these enzymes. Poor metabolisers, metabolise drugs less extensively due to having a dysfunctional enzyme and this leads to increased plasma concentrations of drug substrates compared to the intermediate or normal metabolizers. By contrast, phenotypes classified as extensive metabolisers metabolise drugs more rapidly compared to the intermediate or normal metabolisers. This leads to lower plasma concentration of the drugs which consequently results in poor drug effects or therapeutic failures (Ingelman-Sundberg, 2005).

### 2.1.2 Nomenclature

The term CYTOCHROME P450 (CYP) was first used in 1961 (Omura and Sato, 1962). The name “CYTO” is derived from the fact that the enzymes are found in the cytoplasm of cells, the “CHROME” refers to the spectrophotometric properties of the enzyme and the letter “P450” stands for pigment which has a spectral peak at 450 nm when the iron in the haem part of the enzyme is reduced and bonded with carbon monoxide (Nerbert and Russell, 2002, Omura and Sato, 1962).

All CYPs are named using an assigned number to indicate the gene family having >40% amino acid sequence distinctiveness (example *CYP2* for the second gene), an English alphabet to indicate the subfamily having more than 55% amino acid sequence identity (example *CYP2C* for the subfamily C), and a number for the gene (example CYP2C8 for the 8 genes on the subfamily C on the gene family 2) (Nerbert and Russell, 2002). The names of CYP genes are written in italics (example *CYP2C8*), and the specific alleles are indicated with an asterisk followed by an Arabic numeral (example *CYP2C8*3*). The reference sequence is generally "*2" which is the second allele sequenced and thus not automatically the most prevalent allele in all ethnic populations (Ingelman-Sundberg, 2005). The name of the protein product encoded by the gene is not italized; rather a period is placed in between the gene product and the Arabic numeral (example CYP2C8.3).

### 2.1.3 Structure, Location and Function

Cytochrome P450 enzymes found in humans are mainly proteins associated with membranes and are situated at the inner membrane of mitochondria or on the endoplasmic reticulum (Werck-Reichhart and Feyereisen, 2000). Most CYPs can metabolize multiple substrates, and many can catalyse multiple reactions, which accounts for their central importance in metabolizing the extremely large number of endogenous and exogenous molecules. These substrates include drugs, toxic compounds and metabolic products (Capdevila *et al.*, 1981, Goldstein, 2001).

Cytochrome P450 enzymes can be found in many other tissues in the human body where they are involved in many essential processes such as hormone (oestrogen and

testosterone) synthesis and breakdown, cholesterol production and vitamin D metabolism (Nelson *et al.*, 1993).

### 2.1.4 Overview of Metabolic Pathways of Cytochrome P450 Enzymes

The main observed reaction catalysed by cytochrome P450 is a monooxygenase reaction that involves the insertion of an atom of oxygen into an organic substrate (RH) with the reduction of another oxygen atom to water (Figure. 2.1). Below is a simplified overview of the reaction catalysed by cytochrome P450 enzymes.

$$\mathbf{RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O}$$

The reactions catalysed by CYP enzymes include dealkyklation, dehalogenation, epoxidation, N-oxidation and S-oxidation as well as oxidative deamination (Gibson and Skett, 2001, p.25-31, Parkinson 2001). The first step in the catalytic cycle of CYP enzymes involves the substrate binding to the apoprotein moiety of the ferric ($Fe^{3+}$) haemo-protein. Next is the reduction of the ferric ($Fe^{3+}$) state to the ferrous ($Fe^{2+}$) state through the acceptance of an electron from nicotinamide adenine dinucleotide phosphate (NADPH) involving NADPH cytochrome P450 reductase (Sheweita, 2000, Werck-Reichhart and Feyereisen, 2000).

In the third step, an oxygen molecule binds to the ferrous ion of the complex, followed by a second one-electron reduction. By the end of the third step, one atom of the oxygen is reduced to water and the other oxygen atom inserted into the substrate of the enzyme. In the final step, the complex dissociates to the product, an oxidized metabolite and a free enzyme to allow the catalytic cycle to repeat (Sheweita, 2000,

Werck-Reichhart and Feyereisen, 2000). Below is the catalytic cycle for CYP450 reactions.

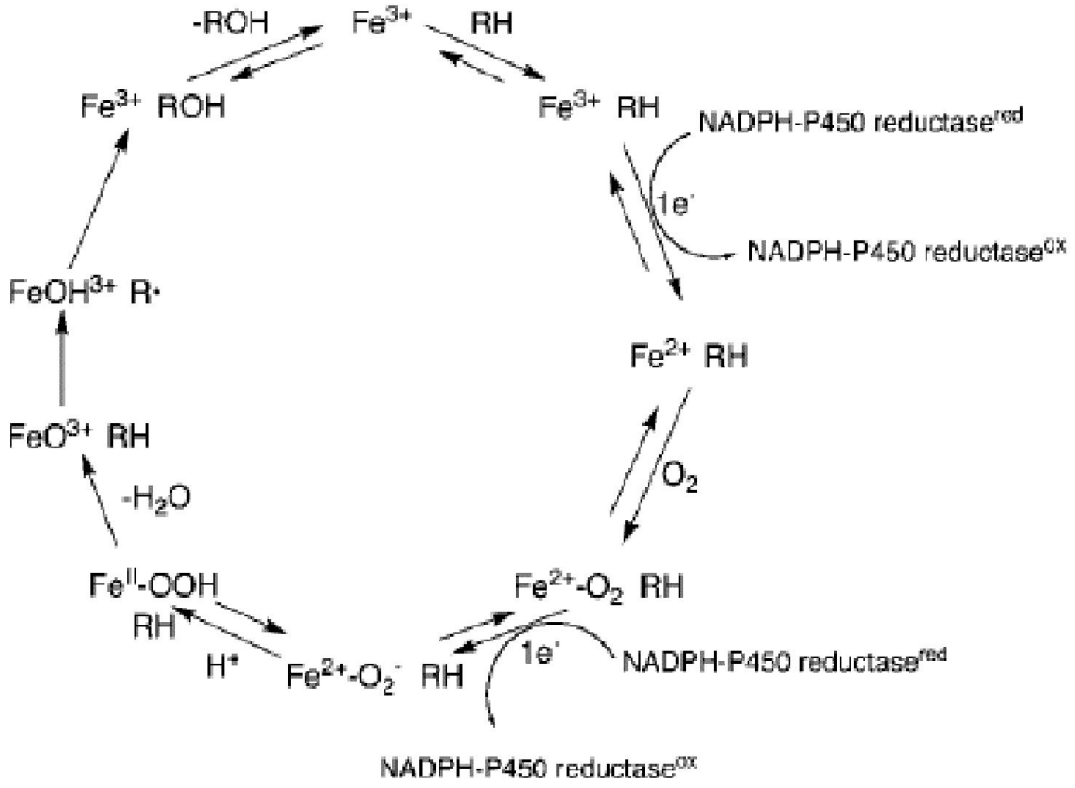



**Figure 2.1: Catalytic cycle for CYP450 reactions (Guengerich, 2008).**

### 2.1.5 Drug Interaction and Metabolism

Although drugs are detoxified and ultimately removed from the human body, most drugs require bioactivation of the parent ingredient to form active compounds of the drug. CYPs are the main enzymes involved in drug metabolism and bioactivation, accounting for about 75% of their total metabolism (Guengerich, 2008, Zanger and Schwab, 2013) as shown below in Figure 2.2.

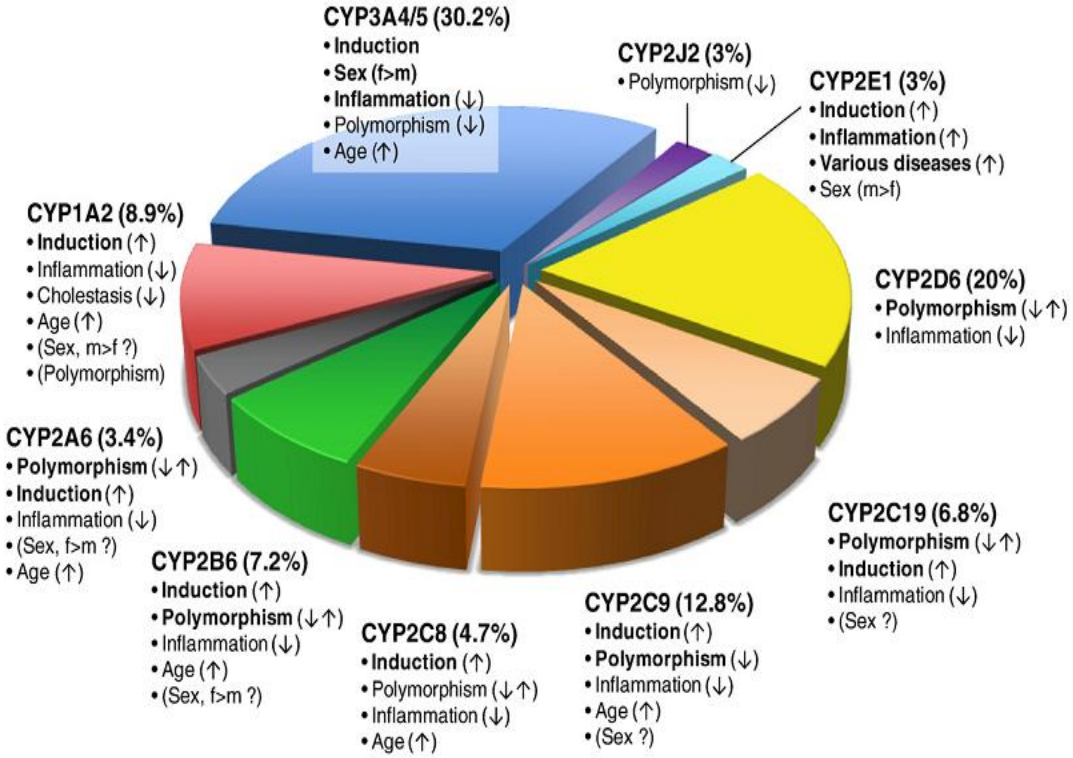



**Figure 2.2: Fraction of clinically important CYP P450 isoforms and factors influencing variability (Zanger and Schwab, 2013).**

A diverse group of drugs can increase or decrease the activity of many different CYP isoenzymes. Increase in enzyme activity can be achieved through enzyme induction where the drug induces the biosynthesis of the isoenzyme. Conversely, decrease in the activity of the enzyme can be achieved through the process of enzyme inhibition where the drug directly inhibits of the activity of the CYP enzyme (Obach, 2000). Whereas the excessive increase in the activity of the enzyme results in therapeutic failure, enzyme inhibition is the main cause of most adverse drug interactions, since changes in CYP enzyme activity may affect the metabolism and clearance of various drugs.

In situations where a drug (drug A) inhibits CYP-mediated metabolism of another drug (drug B), there may arise a situation where the clearance time of drug B in the body increase (build up in the body) which can lead to toxicity of the cell in the body with symptom of drug overdose. These types of situations of drug interactions may require dosage modification or the use of drugs that are non-interactive with respect to the functioning of CYPs. It is thus necessary to consider such drug interactions and their effects on expression levels when choosing: drugs of critical clinical consequences to patients, drugs with seriously vital side-effects and drugs with small effective therapeutic time frames (Martignoni *et al.*, 2006).

Nevertheless, some drugs can go through a change in their concentration in the plasma as a result of a changed level of the metabolism of such drugs. For example, research has shown that the anti-epileptic drug phenytoin when administered provokes the production and building up of the enzymes CYP1A2, CYP2C9, CYP2C19 and CYP3A4 in patients (Anderson, 2004). The resulting effect is that plasma concentration of drugs like amiodarone or carbamazepine which are substrates for these enzymes may decrease because of the enzyme induction and if critical dosages are involved, it may lead to therapeutic failure due to the high clearance rate of the drug from the plasma (Anderson, 2004).

### 2.1.6 Drug Elimination

All drugs are removed from the human body by the process of metabolism and excretion with water. Since majority of drugs are lipophilic compounds which facilitates their entry into cells through the lipid bilayer of cell membranes, they will have to be bio-transformed into more water soluble forms before they can be excreted

from the body (Meyer, 1996). The main organ involved in the metabolism and elimination of drugs from the body is the liver, nevertheless other organs especially the kidney and gastrointestinal tract as well as the lungs and the skin also play an important role to some extent (Krishna and Klotz, 1994).

The biotransformation of an orally administered drug begins upon its entry in the gastrointestinal tract by enterocytes after which it enters the liver through the portal vein. It is then moved into the systemic circulation. By this time the oral bioavailability of the drug has generally reduced extensively in the case of many drugs. This is known as first-pass metabolism (Shen *et al.*, 1997).

Conventionally the biotransformation of drugs are categorized into phase I and phase II reactions (Figure. 2.3). The phase I biotransformation of drugs involves reactions where there is an insertion of a functional group into substrate by enzymes known as phase I enzymes. Phase II biotransformation of drugs involve conjugation reactions where phase II enzymes conjugate their substrates with endogenous substances (Josephy *et al.*, 2005, Gibson and Skett, 2001, p.25-32).

In general CYP enzymes in the endoplasmic reticulum catalyze reactions involve in Phase I metabolism of drugs with the metabolites produced usually inactive or less active than the parent drug although there are many toxic metabolites as well as metabolism-activated drugs or pro-drugs (Meyer, 1996).

Phase II metabolism enzymes are usually located in both the cytoplasm and on the endoplasmic reticulum of the cell where they function to bio-transform their substrates into more water-soluble forms and thus facilitate their excretion. Examples

of phase II enzymes are sulfotransferases (SULT), UDP-glucuronosyltransferases (UGT) and glutathione transferases (GST) (Cribb *et al.*, 2005). Regardless of this classification, phase I enzymes have the capacity to catalyze conjugated drugs and phase II reactions can take place in several unaltered drug (Josephy *et al.*, 2005).

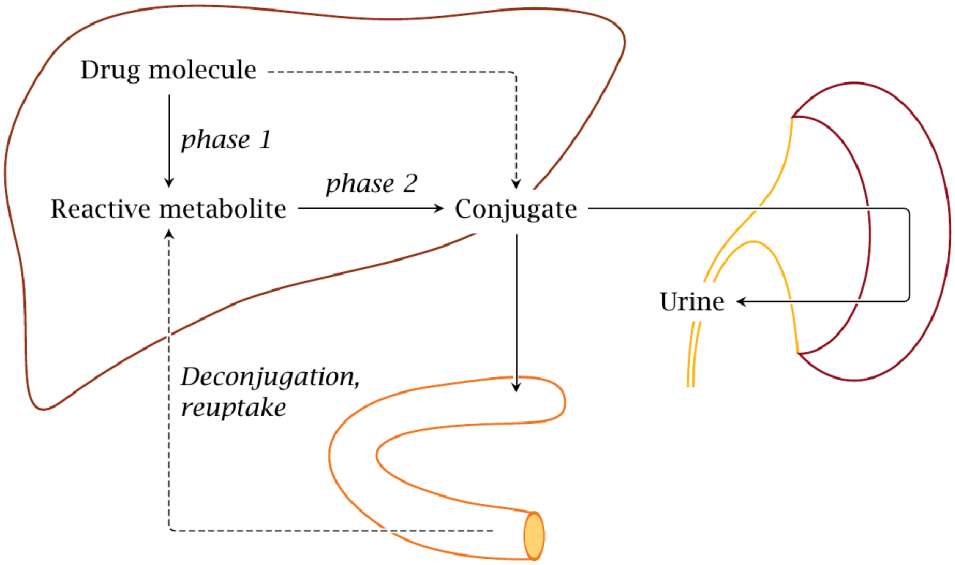



**Figure 2.3: Drug metabolism facilitates drug elimination (Palmer, 2003)**

Finally, inter individual variability with respect to drug disposition is high and may be modulated by the genetic make-up of an individual, the environmental, and disease state. A major part of the individual differences in response to drugs can be due to drug interactions (both inhibition and induction) and genetic variability in drug-metabolizing enzymes as well as the drug transporters and receptors (Ho and Kim, 2005, Wilkinson, 2005).

## 2.2 CYP2C8 Enzyme

### 2.2.1 CYP2C8 Enzyme Structure

The CYP2C8 enzyme has a crystal structure of 2.7Å resolution (Figure 2.4) with a comparatively bulky active site cavity (1438 Å3) (Schoch *et al.*, 2004). It has comparatively a structure and size similar to that of CYP3A4 (1386 Å3), although the shape of the cavities differ significantly (Yano *et al.*, 2004). The similarity in size can account for the observation of CYP2C8 and CYP3A4 having many similar substrates compared to other CYPs even though CYP2C8 normally catalyses reactions that results in the formation of different metabolites (Säll *et al.*, 2012).

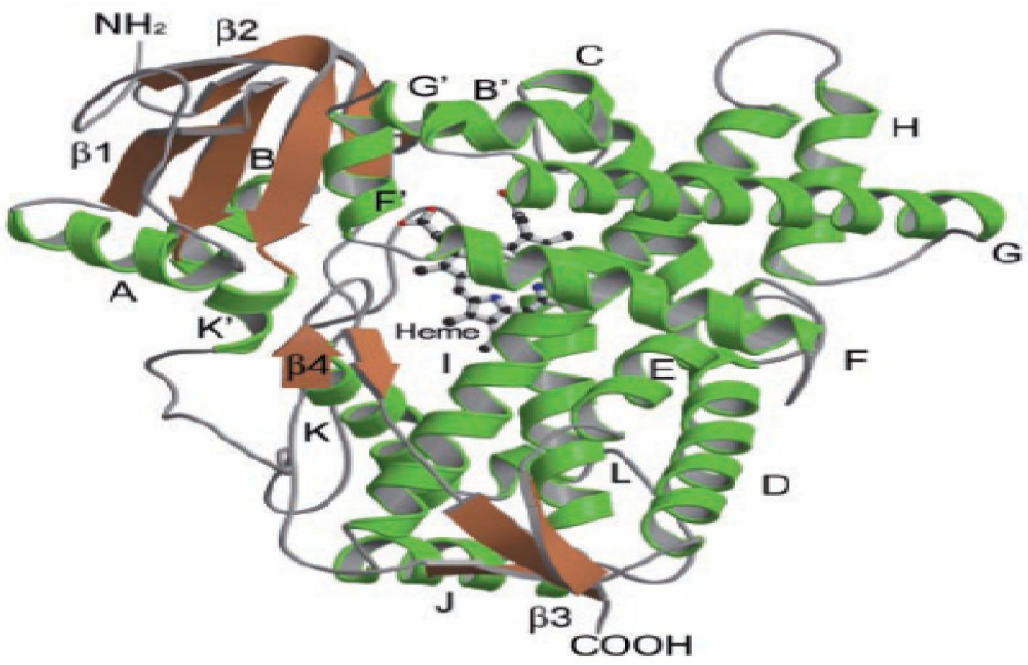



**Figure 2.4: Distal face of structure of CYP2C8 with beta sheets shown brown, loops coloured gray and helices are coloured green (Human Cytochrome P450 Allele Nomenclature Committee, [06-02-2014]).**

** The helices and beta sheets are labelled sequentially from the N terminus according to common usage for P450 structures.*

### 2.2.2 CYP2C8 Enzyme Location

According to work done by Rowland-Yeo K *et al.* (2004), CYP2C8 accounts for approximately 6% of the total CYP content in liver . Also CYP2C8 protein had been detected in the kidney, the salivary ducts, mammary gland, brain, ovary, uterus, intestine and adrenal cortical cells (Enayetallah *et al.*, 2004) although protein expression of CYP2C8 in the intestine is in low levels (Lapple *et al.*, 2003).

### 2.2.3. CYP2C8 Enzyme Function and Substrates

The significance of CYP2C8 in drug metabolism has been known since 2005 (Totah and Rettie, 2005). CYP2C8 is mainly responsible for the metabolism of the antidiabetic drugs rosiglitazone and pioglitazone, the antiarrhythmic amiodarone, the natural anticancer drug paclitaxel, and the antimalarials AQ, which is now commonly used as a selective marker activity. In addition to AQ, CYP2C8 has a major role in metabolizing other antimalarials such as CQ and dapsone (Kerb *et al.*, 2009). Additional drugs metabolized primarily by CYP2C8 include some retinoic acid drugs used in acne and cancer treatment. Some overlap in CYP2C8 substrate specificity occur with CYP2C9 and an example is the metabolism of ibuprofen (Goldstein, 2001, Lai *et al.*, 2009).

CYP2C8 also plays a major role in the metabolism of many common drugs such as cerivastatin, pioglitazone, repaglinide and rosiglitazone, aspirin, morphine, taxol and amoxicilin amongst others (Rahman *et al.*, 1994, Baldwin *et al.*, 1999, Li *et al.*, 2002, Wang *et al.*, 2002, Kajosaari *et al.*, 2005, Bidstrup *et al.*, 2006, AlKadi, 2007, a

review). Furthermore, CYP2C8 has been shown to metabolize certain glucuronide conjugates of drugs such as diclofenac acyl glucuronide (Kumar *et al.*, 2002).

In addition, there are certain endogenous substances such as arachidonic and retinoic acid which are also metabolized by CYP2C8 (Nadin and Murray, 2000) to biologically active metabolites which participate in important biochemical process in the modulations of the pathogenesis of cardiovascular diseases such as myocardial infarction and hypertension. The most striking finding about CYP2C8 is that it shares a number of substrates with CYP3A4, but less with other CYPs (Naraharisetti *et al.*, 2010).

#### *2.2.3.1 Anti Malaria Agents*

In 2013, a survey by WHO showed that there are 104 countries and territories in which malaria is presently considered endemic. Malaria transmission is ongoing in 97 countries and territories, with 7 countries involved in the prevention of reintroduction phase (WHO, 2013). There are an estimated 3.4 billion people at risk of malaria worldwide. WHO estimated about 207 million globally reported cases of malaria in 2012 (uncertainty range 135–287 million) and 627 000 deaths (uncertainty range 473 000–789 000) (WHO, 2012b). Most cases (80%) and deaths (90%) occurred in Africa and most deaths (77%) were in children under 5 years of age (WHO, 2013).

In view of the challenges associated with the use of some antimalarial drugs with respect to strain-resistance and effectiveness in the treatment of malaria, the recommended first-line drug for the treatment of malaria is currently a combination therapy using drugs that differ in terms of their mode of action as a global strategy for malaria control (Bjorkman, 2002). An antimalarial drug that has currently received

particular attention as a candidate for use in combination therapy is AQ. Artesunate-Amodiaquine is currently the first line drug choice for treatment of malaria (WHO, 2013). WHO recommends that, except in the first trimester of pregnancy, artemisinin-based combination therapy (ACT) should be used for the treatment of malaria caused by *P. falciparum* because animal studies have indicated that the drugs can be toxic to embryos.

CYP2C8 is the only enzyme responsible for the biotransformation of AQ (Adjei *et al.*, 2008a), and in the case of CQ it performs a key function. The secondary pathway for the metabolism of CQ is restricted to genetic and inhibitory factors in Africans (O'Connell *et al.*, 2011). The fact that CQ concentrations usually decreases exponentially and it is quickly eliminated compared to its initial concentrations in the blood implies that it generally exist in concentrations just adequate to pick out resistant compared to sensitive *P. falciparum* parasites. Phenotypes classified as poor metabolizers (PM) with respect to anti-malaria drugs have a slower metabolism of the drugs which results in an increased period of the interaction between the malaria parasite and sub-therapeutic molecules of the drug. This consequently becomes a co-factor which influences drug-resistance selection (Paganotti *et al.*, 2011).

The two major metabolites of AQ are desethylamodiaquine and 2-hydroxydesethylamodiaquine (Churchill *et al.*, 1985). Inability of the enzyme to metabolize AQ leads to toxic side effects of the drug such as abdominal discomfort, nausea, vomiting, headache, dizziness, blurring of vision, mental and physical weakness (AlKadi, 2007, a review, Ahmad, 2000, p.11). These symptoms are generally mild and short-lived. The main severe undesirable reactions of AQ toxicity include itching, cardiovascular abnormalities, dyskinesia, ocular damage,

neuromuscular disorders, hearing loss, agranulocytosis and peripheral neuropathy (Reynolds, 1989, p.1896). In a study conducted among Ghanaians in 2009 involving 401 participants, the commonest side-effects reported by the second day were headaches and body weakness (Asante *et al.*, 2009) as well as bradycardia (Adjei *et al.*, 2009). Nevertheless studies have shown that artesunate-amodiaquine (AA) and artemether-lumefantrine (AL) are effective in the treatment of uncomplicated malaria in children in Ghana with low drug-related adverse events although the efficacy of AA in Ghana necessitates constant monitoring and evaluation (Adjei *et al.*, 2008b).

#### *2.2.3.2 Anti-Diabetic Agents*

Due to the rise in life expectancy as a result of increased quality of life, type 2 diabetes mellitus, as a non-communicable disease, is on the rise. The consequence of diabetes is associated with a high burden of morbidity. Diabetes is one of the leading causes of visual impairment and blindness in developed countries (Resnikoff *et al.*, 2004). Furthermore, the risk of tuberculosis is three times higher in individuals with diabetes (WHO and IUTLD, 2011). Epidemiological studies in 2011 reported prevalence rate of diabetes as 7% in urban West African countries and 12% in urban East African countries (Hall *et al.*, 2011). In a study in 2002 involving 4733 study subjects with a participation rate of 75%, the crude prevalence of diabetes was 6.3% (Amoah *et al.*, 2002). In Ghana, diabetes prevalence ranges between 6% in urban Accra, (the country's capital) and 9% in Kumasi, the second largest city (de-Graft Aikins *et al.*, 2014).

The main mode of treatment of Type 2 diabetes is chemotherapy. It involves the use of oral anti-diabetic drugs which are characterised by significant inter-individual

variability clinically with respect to efficacy and adverse effects. This is due to genetic factors contributing to individual differences in drug bioavailability, transport, metabolism and action or response (Xie *et al.*, 2001, Wilkinson, 2005).

Pioglitazone and rosiglitazone are thiazolidinedione compounds used in the treatment of type 2 diabetes. These are peroxisome-proliferator-activated receptor-γ agonists which facilitate the transcription of many genes associated with glucose metabolism, lipid metabolism, adipocyte differentiation and insulin sensitivity (Yki-Jarvinen, 2004). Both are extensively metabolised by CYP2C8 through hydroxylation and oxidation reactions in the liver to form primary and secondary metabolites (Eckland, 2000).

Rosiglitazone metabolism is catalyzed by CYP2C8 where the drug undergoes the process of *N*-demethylation and hydroxylation, subsequently sulphate and glucuronic acid conjugation occurs. *N*-desmethylrosiglitazone and para-*0*-sulphate rosiglitazone are the main metabolites and both are considered less potent than the parent drug (Baldwin *et al.*, 1999). Pioglitazone metabolism is also a CYP2C8 catalysed reaction involving the process of hydroxylation and oxidation, followed by conjugation with sulphate and glucuronide. In general, M-IV (hydroxy-derivative of pioglitazone) and M-III (keto-derivative) are the chief metabolites found *in vivo* (Eckland, 2000).

Another substrate metabolized by CYP2C8 is repaglinide which is taken orally for the treatment of type 2 diabetes as an insulin secretagogue, a short-acting drug agent that causes the secretion of insulin (Bidstrup *et al.*, 2006). Repaglinide is almost completely metabolized by Cytochrome P450 enzymes with less than 2% of an oral dose excreted unchanged in humans (van Heiningen *et al.*, 1999). Both CYP2C8 and

CYP3A4 are associated with the metabolism of repaglinide although CYP2C8 contributes to a greater extent than CYP3A4 *in vivo* (Bidstrup *et al.*, 2006).

Given the important role of CYP2C8 in repaglinide metabolism, research studies have examined the influence of CYP2C8 polymorphisms on repaglinide pharmacokinetics. A research study in 2003 to determine the effect of CYP2C8 genotype on the pharmacokinetics of the repaglinide, mean area under the plasma concentration time curve (AUC) and maximum plasma concentration ($C_{max}$) were 45% and 39% lower respectively in *CYP2C8*1/*3* heterozygous genotype subjects compared to wild-type homozygous genotype (Niemi *et al.*, 2003). In addition, although it was not statistically significant, repaglinide AUC was about 13% lower in *CYP2C8*1/*4* genotype subjects compared to wild-type homozygous (Niemi *et al.*, 2003).

Furthermore, CYP2C8 and CYP3A4 are the major enzymes responsible for catalysing the biotransformation of the anti-diabetic agent thiazolidinediones troglitazone and pioglitazone (Glamočlija and Jevrić-Čaušević, 2010, Yamazaki *et al.*, 1999).

#### *2.2.3.3 Anti-Cancer Agents*

Paclitaxel (taxol) which is the main anti-cancer agent used in the treatment of tumours such as breast, lung and ovarian cancers, is metabolized by CYP2C8 enzyme to form the major active metabolite 6-α-hydroxypaclitaxel although CYP3A4 and CYP3A5 also metabolise paclitaxel to lesser extents (Rahman *et al.*, 1994).

The polymorphic nature of the *CYP2C8* gene has been associated with varying degrees of metabolite of the drug. Studies has linked considerable decrease in the biotransformation of paclitaxel and arachidonic acid metabolism with *CYP2C8*3* allele, and as well as a less degree of reduction with the *CYP2C8*4* alleles (Soyama *et al.*, 2001). In terms of toxicity, patients with the *CYP2C8*1/*3* genotype have an increased risk of hematological toxicity as well as motor neuropathy (Green *et al.*, 2009).

#### *2.2.3.4 Anti-Inflammatory and Non-Steroidal Agents*

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of drugs used as analgesic (painkillers), antipyretic (fever-reducers) and inflammations treatment. They are also known as non-steroidal anti-inflammatory medicines (NSAIMs) or non-steroidal anti-inflammatory agents (NSAIAs). These drugs are distinguished from steroids in having a related eicosanoid-depressing and anti-inflammatory action. Examples of NSAIDs are aspirin, diclofenac, naproxen and ibuprofen among others (Miedzybrodzki, 2003). Although numerous NSAIDs have been shown to be metabolized by CYP2C9, CYP2C8 is also involved in the metabolism NSAIDs such as naproxen, piroxicam and celecoxib (Rodrigues, 2005). Decreased ibuprofen clearance which occurs in considerable proportions of healthy individuals is strongly associated with *CYP2C8* and *CYP2C9* polymorphisms although not enantio-specific

(Garcia-Martin *et al.*, 2004). Also as compared with individuals with no mutations, individuals with the common genotypes *CYP2C8*1/*3* plus *CYP2C9*1/*2* show decreased ibuprofen clearance (Garcia-Martin *et al.*, 2004).

Much of studies conducted on the effects of NSAIDs and *CYP2C8* genotype have implicated NSAID as the cause of adverse effects such as acute gastrointestinal bleeding which is the most common (Ruder *et al.*, 2011). Furthermore, a multivariable regression analysis study, concluded that significant predictors of NSAID-induced bleeding were *CYP2C8*3* genotype and alcohol consumption (Blanco *et al.*, 2008). The same study linked highest bleeding risk with patients having both *CYP2C8*2* and *CYP2C8*3* mutant alleles (Blanco *et al.*, 2008).

Nonetheless NSAIDs have the ability to induce protective effects such as reduced colorectal cancer risk, with the most notable of such NSAIDs being ibuprofen and aspirin (Rostom *et al.*, 2007). Also patients with *CYP2C8* or *CYP2C9* mutant alleles exhibit a reduction in the metabolism of NSAIDS which implies the protective effect of the drugs (NSAIDs) will be prolonged (McGreavey *et al.*, 2005). However, *CYP2C8*3*, *CYP2C9*2* and *CYP2C9*3* mutant alleles did not modify the protective effects associated with NSAID usage, with a significant reduction in the risk of colorectal cancer, in the population (McGreavey *et al.*, 2005).

### 2.2.4 Inhibitors and Inducers of CYP2C8

#### *2.2.4.1 Inhibitors of CYP2C8*

Many xenobiotics with an inhibitory potential on CYP2C8 have been identified and characterized *in vitro*; the most widespread being ketoconazole and clotrimazole (Ong *et al.*, 2000), quercetin (Rahman *et al.*, 1994), and gemofibrozil (Wang *et al.*, 2002).

Quercetin was initially used as a diagnostic inhibitor *in vitro* regardless of its low selectivity. Trimetoprim and montelukast have also been shown to be good selective inhibitors of CYP2C8 *in vitro* (Wen *et al.*, 2002). Furthermore, it has been reported that trimethoprim is a moderate potent inhibitor of CYP2C8 *in vivo* (Niemi *et al.*, 2004) while montelukast has been reported to have no inhibitory effect on CYP2C8 substrates such as repaglinide and pioglitazone *in vivo,* possibly due to the wide-ranging plasma protein binding of montelukast (Kajosaari *et al.*, 2006).

#### *2.2.4.2 Inducers of* CYP2C8

The primarily known inducer for the expression of CYP2C8 *in vivo* is the drug rifampin which is also known to induce many other drug metabolism pathways (Kajosaari *et al.*, 2005). Thus there is the possibility of decreasing plasma concentration of CYP2C8 substrates even in situations where these substrates have other alternative elimination pathways. Other possible inducers of CYP2C8 are phenobarbital (Raucy *et al.*, 2002) and the herbal supplement St. John's wort which contains the compound hyperforin (PXR ligand). Since most enzyme inducers usually have a "broad spectrum effect," these inhibitors can modulate the expression of many CYP isozymes (Komoroski *et al.*, 2004).

## 2.3 CYP2C8 Gene

### 2.3.1 CYP Gene Structure

The gene that code for the CYP2C8 enzyme, known as the "*CYP2C8* gene", is 31kb in size and consists of 9 exons (Klose *et al.*, 1999). It is located on chromosome 10q24 as a multigene cluster with 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 whole *CYP2C* gene family spans about 400kb and has linkage between its genes (Yasar *et al.*, 2002).

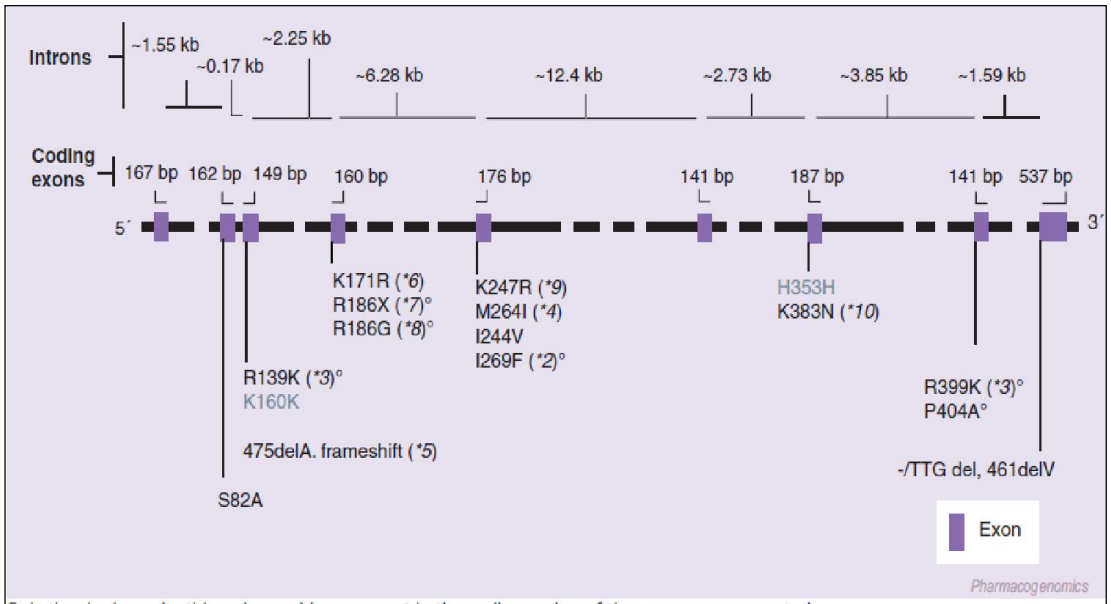
The nucleotide sequences of *CYP2C8* gene shares about 74% homology with the *CYP2C9* gene. From these highly homologous genes, CYP2C8, CYP2C9 and CYP2C19 are clinically important and it has been observed that functionally important genetic polymorphs confer differences in the metabolism of the substrates of these CYP2C subfamily enzymes. The regulation of the transcription of the *CYP2C8* gene is modulated through nuclear receptors such as hepatic nuclear factor-4α receptor, androstane receptor, pregnane X receptor and glucocorticoid receptor (Kojima *et al.*, 2007).

### 2.3.2 Genetic Polymorphism of *CYP2C8* Gene

The *CYP2C8* gene is polymorphic, and the distribution of variant alleles differs among different ethnic populations. The wild-type gene of CYP2C8 is referred to as *CYP2C8**1 (with sub variants **1A, *1B* and **1C*). Studies indicate that Indian populations have predominantly the wild type of *CYP2C8* gene with allele frequency of 98% for *CYP2C8*1*, 0.8% for *CYP2C8*2*, 1.2% for *CYP2C8*3* and no *CYP2C8*4* (Muthiah *et al.*, 2005).

Although there are about 15 identified variants of *CYP2C8*, studies indicate that *CYP2C8*2, CYP2C8*3, CYP2C8*4* and *CYP2C8*5* are of clinical importance because the resultant enzyme product from these polymorphisms have altered enzyme activity with respect to *CYP2C8*1* wild type. *CYP2C8*2* (805A>T, Ile269Phe) is the most common in blacks although it is very rare in Caucasian populations (Totah and Rettie, 2005). *CYP2C8*3* (416G>A, Arg139Lys and 1196A>G, Ly399Arg) results in

a 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* (430C>T, Arg144Cys) and *CYP2C8*3* variant alleles, where about 95% of phenotypes having the *CYP2C8*3* variant allele have also been reported to be having the *CYP2C9*2* variant allele (Yasar *et al.*, 2002). *CYP2C8*4* (792C>G, Ile264Met) is also present in Caucasians with a frequency of about 8% (Totah and Rettie, 2005). *CYP2C8*5* which was reported to be 0.025% (Nakajima *et al.*, 2003) and 0.09% (Soyama *et al.*, 2002) in Japanese results from a deletion of adenine (471) on exon 3 which leads to a frameshift mutation. This in turn leads to an early stop codon at residue 177 during protein synthesis (Bahadur *et al.*, 2002). Figure 2.5 below shows the major CYP2C8 polymorphisms with respect to the coding regions polymorphism on the CYP2C8 gene structure.




Only the single nucleotide polymorphisms present in the coding region of the gene are represented.
°Alleles coding for documented or predicted reduced enzyme activity;
*2: Two-fold higher $K_M$ for paclitaxel transformation [17];
*3: 15% of *1-associated turnover number [17];
*5: Expected to be an inactive gene [18];
*7: No *in vitro* detectable activity [21];
*8: *In vitro* enzyme activity 10% of the wild-type [21];
P404A: Reduced protein expression leading to decreased $V_{max}/K_M$ [32].

**Figure 2.5: CYP2C8 gene and known single nucleotide polymorphisms.**

(Human Cytochrome P450 Allele Nomenclature Committee, www.cypalleles.ki.se/ [06-02-2014]).

Moreover, information from the Human Cytochrome P450 Allele Nomenclature Committee and dbSNP indicates that many unusual nonsynonymous polymorphic CYP2C8 alleles exist, comprising of *CYP2C8*6* through to *CYP2C8*14*. Many others are yet to be classified and given a 'star' designation (Human Cytochrome P450 Allele Nomenclature Committee, [06-02-2014]). Although sequence variants in the *CYP2C8* gene are of most clinical relevance, it is worth noting that polymorphisms in the *CYP2C8* promoter region of the *CYP2C8* gene also exist, the most commonly known one being -271C>A and -370T>G (Rodriguez-Antona *et al.*, 2008).

### 2.3.3 Genetic Polymorphism of *CYP2C8* in Africans and in Ghanaians

Many polymorphic alleles of the human *CYP2C8* gene have been shown to exhibit inter-individual differences with respect to metabolism of CYP2C8 substrates. Despite these, few reports exist on the genetic polymorphism of *CYP2C8* in Africans.

The overall prevalence and distribution of *CYP2C8*2* allele frequency in most African population is currently not known. However, some studies have been conduct in some parts of Africa. Studies reported the prevalence of the *CYP2C8*2* allele to be about 13.9% in Zanzibar (Kim *et al.*, 2003). Also work done on malaria patients in Zanzibar established the prevalence of the CYP2C8 polymorphism frequencies among 165 unrelated patients, reporting *CYP2C8*2* (14%), *CYP2C8*3* (2.1%) and *CYP2C8*4* (0.6%) (Cavaco *et al.*, 2005). In southern Burkina Faso, the prevalence of *CYP2C8*2* is about 11.5% (Parikh *et al.*, 2007) while in central Burkina Faso the prevalence was shown to be 9.9% in the Fulani and 23.7% in the Mossi-Rimaibè (Paganotti *et al.*, 2011). Studies conducted in 2012, found the prevalence of *CYP2C8*2* allele to be 22% in the Senegalese populations, 10.5% in Ugandans and 15% in the Malagasy population of Madagascar (Paganotti *et al.*, 2012).

A study conducted in children from Northern Ghana reported a prevalence of 17% for the *CYP2C8*2* allele but observed the absence of *CYP2C8*3* and *CYP2C8*4* in the study population (Rower *et al.*, 2005). Further work conducted by Kudzi *et al.* (2009) in southern Ghanaian populations observed allelic frequencies: *CYP2C8*2* (17%), *CYP2C8*3* (0%) and *CYP2C8*4* (0%).

From these studies, the *CYP2C8*2* allele is likely the most predominant form of the *CYP2C8* variant in African populations. The prevalence of C*YP2C8*2* is generally much higher in black populations than in Caucasians populations whereas the prevalence of *CYP2C8*3 and CYP2C8*4* are higher in Caucasians, Asians and East Africans but absent in West African populations and very uncommon in African Americans (Bahadur *et al.*, 2002). Also *CYP2C8*2* frequency is relatively higher in West Africans and African Americans than in East Africans (Paganotti *et al.*, 2012). These point to the heterogeneous nature of polymorphism of *CYP2C8* in African populations as a whole.

Although the *CYP2C8*5* leads to an enzyme that lacks activity and hence is the most serious form of the mutation, no work has been done on the prevalence of *CYP2C8*5* in African populations. Although the clinical implications of *CYP2C8*5* are severe in genotypes homozygous for the mutant allele, as at the time of this work there was no report on prevalence of *CYP2C8*5* in Ghanaian population.

### 2.3.4 Clinical Implications of *CYP2C8* Polymorphism

The wild-type *CYP2C8* gene also known as *CYP2C8*1* codes for the normal CYP2C8 enzyme in humans. There are many nonsynonymous polymorphisms in the *CYP2C8* gene but the *CYP2C8*2*, *CYP2C8*3*, *CYP2C8*4*, and *CYP2C8*5* are the most clinically-relevant polymorphisms (Nebert and Russell, 2002, Nakajima *et al.*, 2003, Soyama *et al.*, 2002). Extensive studies on their clinical implications in affected individuals have been carried out because these polymorphisms lead to altered enzyme activities which subsequently affect the metabolism of their substrates especially xenobiotic substances such drugs (Nerbert and Russell, 2002). As such,

individuals with two deficient copies of the wild type allele are classified as poor metabolizers (PM), those with one deficient copy of the wild type allele are classified as intermediate metabolizers (IM), those with two functional wild type allele are classified as extensive metabolizers (EM) and those with more than two functional allele are classified as ultrarapid metabolizers (UM) (Ingelman-Sundberg, 2005).

The variant *CYP2C8*2* is most common in Africans and has been associated with phenotypes known as PM in subjects carrying two copies of the defective allele (Cavaco *et al.*, 2005). Clinically, such individuals have an increased drug half-life which increases the chance of them experiencing adverse drug reactions and toxicity (Parikh *et al.*, 2007). In most non-African populations especially Caucasian, poor metabolisers are individuals with the *CYP2C8*3* allele; the *CYP2C8*2* is rarely present. Nonetheless, Asians and Caucasians are not considered as a homogenous group with respect to *CYP2C8* allele frequencies. Thus in individuals with Asian or European ancestry, intra-ethnic differences increases the risk of experiencing adverse reactions when using drugs metabolized by the CYP2C8 enzyme (Garcia-Martin *et al.*, 2006).

Although there is no direct evidence about the role of *CYP2C8* genetic variance in CQ pharmacokinetics, research has linked indirectly lower CQ metabolism to *CYP2C8*2*-carriers through the association between the allele and rates of CQ-resistant *P. falciparum* parasites (Paganotti *et al.*, 2011). Research has also indicated that the *CYP2C8*2* variant shows a decrease in the intrinsic clearance of AQ about six times compared to the wild type (Parikh *et al.*, 2007). In northern Ghana, studies have reported a high prevalence of *CYP2C8*2* mutation (16.75%) in individuals who are unable to metabolize AQ the first-line treatment for malaria (Rower *et al.*, 2005).

The odds of CYP2C8 polymorphism is significant on the modulation of AQ metabolism and thus the build-up of the drug with its potential adverse effects (Churchill *et al.*, 1985).

On the contrary, in African-American populations, the *CYP2C8*3* allele is at a very low frequency but represented by *CYP2C8*2* allele with a prevalence of about 18% for the poor metabolizers (Dai *et al.*, 2001). Research conducted *in vivo*, on the *CYP2C8*3* sequence variant indicated that the polymorphism was associated with reduced concentrations of repaglinide and rosiglitazone in the plasma (Niemi *et al.*, 2003, Kirchheiner *et al.*, 2006). In other studies conducted *in vitro*, it was observed that there was a remarkable decrease in the activity of CYP2C8.3 variant enzyme in metabolising chemical compounds and drugs such as arachidonic acid, paclitaxel, and AQ (Soyama *et al.*, 2001, Bahadur *et al.*, 2002, Parikh *et al.*, 2007). Also, the clearance of ibuprofen was markedly decreased in carriers of the *CYP2C8*3* allele (Garcia-Martin *et al.*, 2004). Nevertheless, no effect was evident on the pharmacokinetics of paclitaxel in relation to the CYP2C8 genotype (Henningsson *et al.*, 2005). Thus, the *in vivo* effect of the *CYP2C8*3* allele in general is not clear based on the research findings available for different drug substrates. Also studies on the hepatic microsomes from individuals heterozygous for *CYP2C8*4* reported enzyme activities of CYP2C8.4 comparable to other individuals heterozygotes for *CYP2C8*3* (Bahadur *et al.*, 2002).

The *CYP2C8*5* mutation results in production of an enzyme that lacks 64% of normal protein structure (Nakajima *et al.*, 2003, Soyama *et al.*, 2002). Thus individuals homozygous for *CYP2C8*5* will be poor metabolizers (PM) and may find it difficult to tolerate drugs metabolized by CYP2C8 and suffer ADRs (Dai *et al.*,

2001, Bahadur *et al.*, 2002). Individuals heterozygous for *CYP2C8*5* but with other sequence variants of the *CYP2C8* gene such as *CYP2C8*2, CYP2C8*3 or CYP2C8*4* will still be at risk of having adverse drug reactions and increase risk of having cardiovascular diseases such as hypertension and acute myocardial infarction because the fully functional CYP2C8 enzyme may still be unavailable (Totah and Rettie, 2005). Even in individuals with the wild type *CYP2C8* gene, a case study conducted in Ghana by Adjei *et al.* (2009) reported the occurrence of bradycardia as an adverse drug reactions after a standard dose of AQ. This coincided with the time of expected peak concentrations of the active metabolite of AQ and suggested a direct drug effect. In the subjects, there were normal plasma concentrations of the focal metabolites of AQ (N-desethylamodiaquine and N-bis-desethylamodiaquine) (Adjei *et al.*, 2009).

The dysfunction of the enzyme CYP2C8 may be due to mutations although other factors such as the inhibitors of the enzyme may play a role. Thus, the frequency of the variants of the CYPs in subpopulation must be determined to allow for effective management and treatment through understanding and explaining therapeutic success or failures, categorizing potential risk group of individuals for adverse drug reactions and the optimisation of doses for therapeutic efficacy in populations.

Beside cancer therapeutics, pharmacogenetic tests have been introduced to identify the genotype of patients with the aim of conducting individualized treatments. An example is the use of the analysis of thiopurine S-methyltransferase genotypes to facilitate the prediction of toxicity in patients to be treated with 6-mercaptopurine or azathioprine. Also uridine 5'-diphosphoglucuronosyl-transferase 1A1 genotype can be used to predict the toxicity of the drug irinotecan. Although there is information on cytochrome P450 (CYP) polymorphisms and their relationship with drug toxicity and

response, there is currently limited use of CYP genotypes to individualize treatments (Daly, 2007) and almost non-existent in Ghana due to the unavailability of adequate information on CYPs. Much of the information on cytochrome P450 (CYP) polymorphisms and their relationship with drug toxicity and response are limited to the Western world. In subtropical region where chemotherapy is a problem owing to unavailability of drugs, cost and the existence of fake drugs, the importance of such information on system to facilitate the safe use of drugs cannot be overemphasized.

Africa is employing artemisinine-derivative-based combination therapy on a large scale with the selected combination for the first line treatment option being artesunate-amodiaquine. Furthermore, cardiovascular diseases, diabetes and cancers on the increase globally (WHO, 2012a). Information on the systems which facilitate the tolerability of such drugs needs to be available. It is therefore imperative to analyse the heterogeneity of the *CYP2C8* gene in the population especially where malaria, diabetes and cancers continue to be among the major public health problems. This will create a healthy nation through the development and improvement of health delivery and pharmacogenetics for better life quality of ultimately. One of such sequence variant of the *CYP2C8* gene worthy of consideration is *CYP2C8*5* which results in a dysfunctional enzyme.

This study was thus designed to determined the allelic frequency of *CYP2C8*5* in selected (major) ethnic groups namely: Akyem, Ashanti, Fanti, Anlo, Ewes, Ga, Krobo and Nzema in Southern Ghanaian populations. The study was also to assess the association between the *CYP2C8*5* genotype and reported adverse drug reactions in the study subjects as well as the extent of dependency on drugs metabolized by CYP2C8 enzyme by the study subjects.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 CHEMICALS, REAGENTS AND EQUIPMENT

The various buffers and solutions used in the study were prepared as described in Appendix I. The sources and manufacturers of the chemicals, reagents, and equipment used for the study are listed in Appendix II

### 3.2 STUDY DESIGN

The study was cross-sectional and involved the use of participants with biological ethnic backgrounds from selected ethnic groups in southern Ghana. The participants comprised equal proportion of males and females from each of the selected ethnic groups in southern Ghana.

### 3.3 ETHICAL CLEARANCE AND CONSENT FROM STUDY SUBJECTS

Ethical approval of the study protocols were obtained from the Committee on Human Research, Publications and Ethics of the Kwame Nkrumah University of Science and Technology, School of Medical Sciences and Komfo Anokye Teaching Hospital, Kumasi-Ghana with ethical clearance number CHRPE/AP/089/14. Prior to entering the study, all the subjects were given information (both oral and written) about the study. Subsequently informed written consents (appendix III) were obtained from all the study subjects.

### 3.4 STUDY SUBJECTS AND STUDY SITES

Study subjects were randomly recruited from individuals who had come to either donate blood (Accra Area Blood Unit, Korle-Bu Teaching Hospital, Accra and the

Komfo Anokye Teaching Hospital Transfusion Medicine Unit, Kumasi), or conduct laboratory tests (Hohoe and Winneba Municipal Hospitals). The study subjects were categorized into the various major ethnic groups found in the southern part of Ghana namely: Ashanti, Fanti, Akyem, Ewe, Anlo, Ga, Krobo and Nzema. Figure 3.1 shows the map of Ghana and the sample collection sites for the study.

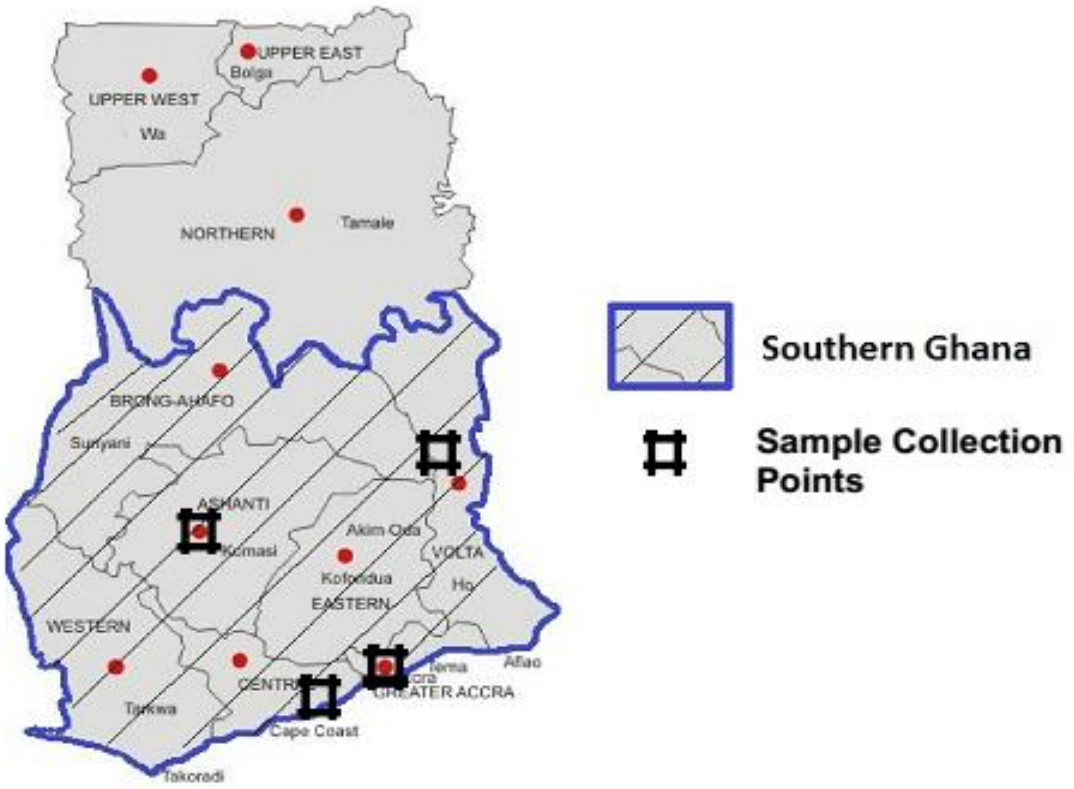



**Figure 3.1: Map of Ghana showing sample collection sites in the southern sector.**

## 3.5 STUDY INCLUSION AND EXCLUSION CRITERIA

### 3.5.1 Inclusion Criteria

The eligible participants of the study were adults from any of the selected (major) ethnic groups in southern Ghana who were willing to participate in the study. The age range was 18 years and above in order to recruit participants who could understand and answer questions related to their medical and genetic backgrounds. Also only

individuals whose grandparents were of the same ethnic background were recruited for the study to ensure a purer gene pool with respect to the biological ethnic origins.

### 3.5.2 Exclusion Criteria

Those who were excluded from the study included individuals who did not give consent, those whose work regularly exposed them to carcinogens of any form, and participants whose grandparents were of mixed biological ethnic backgrounds.

## 3.6 BLOOD SAMPLES AND MEDICAL HISTORY DATA COLLECTION

### 3.6.1 Blood Sample Collection

Dried blood spots (DBS) on labelled Whatman filter paper were prepared from peripheral blood samples from the study subjects. The DBSs were air dried and stored in biohazard plastic bags containing a desiccator until DNA was extracted.

### 3.6.2 Medical History Data Collection

In addition, questionnaires (appendix IV) were administered to the participants to obtain information about their (i) medical history on major diseases which require the use of drugs metabolized by the CYP2C8 enzyme (ii) general attitude toward chemotherapy as a treatment option and (iii) medical history on adverse drug reaction with them personally as well as that of their family.

## 3.7 MOLECULAR ANALYSES

### 3.7.1 DNA Extraction From Blood Blot Samples

Genomic DNA was extracted from the DBS collected using the Tris-EDTA (TE) buffer method (Plowe *et al.*, 1995).

About 3 $mm^3$ of DBS was punched using a clean sterilized perforator and the piece placed into a 1.5 ml Eppendorf tube. The perforator was wiped with 70% ethanol after each cut as a precaution against cross contaminating of the samples. The punched filter paper was soaked in 200 µl of 99.9% methanol. After incubation at room temperature (24 $^oC$) for 15 minutes, the methanol was removed by pipetting and the sample in the tube air-dried before adding 200 µl of TE buffer. The tube was then incubated at 50 °C for 15 minutes but it was uncapped briefly every 5 minutes to release pressure during the incubation. The punched filter paper at the bottom of the tube was then gently press down to the lower third of the tube several times using a pipette tip. A new pipette tip was used for each sample to avoid cross contamination. Next, the tube was heated at 97 °C for 15 minutes to elute the genomic DNA. The tube and its contents were vortex intermittently during the incubation step. After the incubation, the tube was centrifuged briefly at maximum speed for 2 minutes to remove any liquid content on the walls of the tube. Finally, the supernatant (TE buffer containing the extracted genomic DNA) in the tube was transferred into another labelled tube without picking the filter paper. Quantification of DNA extracted was carried out using an absorbance reader. The DNA extract was then stored at -40ºC.

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

Allele specific (AS) PCR was used for the analysis of A475 del (Frameshift, *CYP2C8*5*) with the primers 5'-AGG CAATTC CCC AAT ATC TC-3' (3S) and 5'-TCA CCC ACC CTTGGTTTT C-3' (mutant) (Nakajima *et al.*, 2003). Genomic DNA was amplified in a 20 µl reaction mix (Table 3.1) using the reaction conditions shown in Table 3.2. For each reaction, a no-DNA negative control and a DNA positive control were also used in the set up.

**Table 3.1: PCR Reaction Mixture**

| **Reagents** | **Volume (µl)** |
|---|---|
| Sterile double distilled $H_2O$ | 9.2 |
| 10x PCR buffer | 2.0 |
| 10 mM dNTP mix | 0.8 |
| 2.5 mM $MgCl_2$ | 1.2 |
| 10 µM Primer 1 (*5 Forward) | 0.8 |
| 10 µM Primer 2 (*5 Reverse Mutant) | 0.8 |
| DNA *Taq* Polymerase (5Units/µl) | 0.2 |
| DNA Template | 5 |
| **Total** | **20** |

**Table 3.2: PCR Cycling Conditions**

| **Cycle** | **Temperature** | **Time** | **No. of cycles** |
|---|---|---|---|
| Initial Denaturation | 94 ºC | 4 minutes | 1 |
| Denaturation | 94 ºC | 1 minute | |
| Annealing | 51 ºC | 1 minute | 40 |
| Extension | 72 ºC | 2 minutes | |
| Final extension | 72 ºC | 7 minutes | 1 |

### 3.7.3 Gel Electrophoresis

The PCR products were electrophoresed in 2% agarose gels stained with 0.5 µg/ml ethidium bromide. Eight microlitres (8 µl) of each sample was added to 1 µl of 10x

bromophenol blue gel loading dye and loaded in gels prepared with 1x TAE buffer. The setup was electrophoresed at 100 V for 45 minutes using a mini gel system, visualized by ultraviolet transillumination and photographed. The expected fragment size of the PCR product was 550 bp. These were estimated by comparing them with the mobility of a 100 bp molecular weight marker.

### 3.8 STATISTICAL ANALYSES

Chi-square ($\chi^2$) and Student's t-test analyses were performed with IBM SPSS version 20.0 using a confidence interval of 95% for a test of associations between presence of *CYP2C8*5* mutant allele and reported adverse drug reactions. Graphs were plotted using Microsoft Excel 2007.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Demographics of Study Subjects

#### 4.1.1 Ethnic Distribution of Study Subjects

A total of 80 study subjects comprising of 40 males and 40 females were recruited for the study. The 80 study subjects comprised of 10 subjects each from the ethnic groups Akyem, Ashanti, Fanti, Eve, Anlo, Ga, Krobo and Nzema all from southern Ghana. The study subjects provided information about their ethnic background which was used to categorize them.

#### 4.1.2 Age Distribution of Study Subjects

The age distribution of the study subjects ranged from 18 to 53 years with an average of 30.63 ± 8.75 years. Majority of the study subjects were between the ages of 21 to 35 years representing about 65.82% of the entire study subjects. The lowest age group among the study subjects was between 51-55 years representing 2.53% (Figure 4.1).

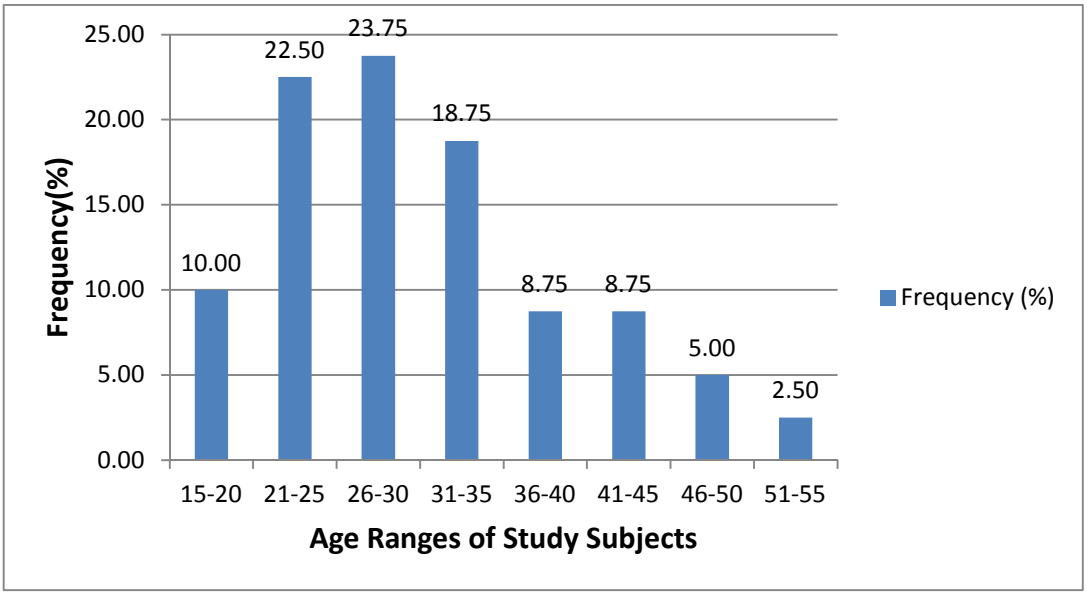



**Figure 4.1: Age distribution of study subjects.**

### 4.1.3 Educational Levels of Study Subjects

Table 4.1 shows the educational background ranging from no formal to tertiary education with some of the participants having informal education. Majority of the study subjects had access to secondary (45%) and tertiary education (42.5%).

**Table 4.1: Educational levels of study subjects**

| Educational Levels | Frequency (%) |
|---|---|
| None | 1 (1.25) |
| Basic | 7 (8.75) |
| Secondary | 36 (45.00) |
| Tertiary | 34 (42.50) |
| Informal | 2 (2.50) |
| **Total** | **80 (100)** |

### 4.1.4 Socio-Economic Status of Study Subjects

As shown in Table 4.2, majority of the study subjects (43.75%) were of middle level socioeconomic group and 32.5% were of low-level socioeconomic groups.

**Table 4.2: Socio-economic status distribution of study subjects**

| Socio-Economic Status | Frequency (%) |
|---|---|
| Upper | 19 (23.75) |
| Middle | 35 (43.75) |
| Low | 26 (32.50) |
| Poor | 0 (0.00) |
| **Total** | **80 100)** |

### 4.1.5 Body Mass Index (BMI) Distribution of Study Subjects

The Body Mass Index (BMI) distribution of the study subjects ranged from 15.78 kg/m$^2$ to 36.75 kg/m$^2$ with a mean of 22.36 ± 3.20 kg/m$^2$. Majority of the study subjects (77.5%) had a BMI within the range of 18.5 - 24.9 kg/m$^2$ (Figure 4.2). The second highest observed BMI was the overweight range of 25.0-29.9 kg/m$^2$ (13.75%). Only 2.5% of the study population were obese (BMI ≥ 30 kg/m$^2$).

Stratification of the BMI by age, gender, educational level and ethnicity (Table 4.3) showed the obese study subjects as males within the 26 - 35 years age group.

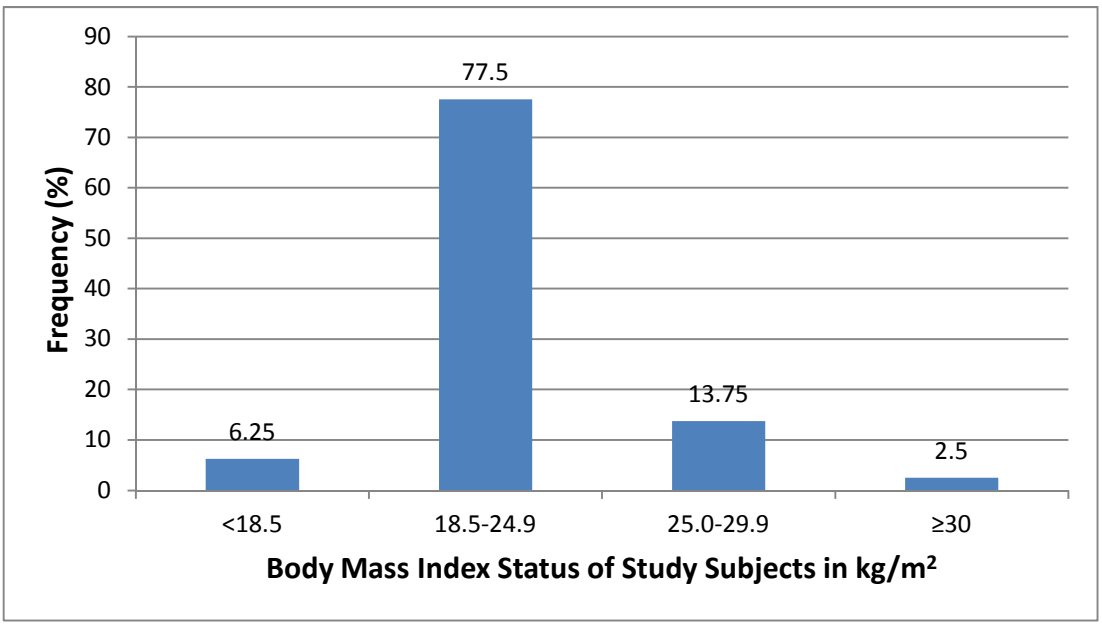



**Figure 4.2: Body Mass Index of the Study Subjects**

**Table 4.3: BMI distribution stratified by age, gender, education and ethnicity**

| Variables | | BMI in kg/m$^2$ (n (%)) | | | |
|---|---|---|---|---|---|
| | | **<18.5** | **18.5-24.9** | **25.0-29.9** | **≥30** |
| **Age Ranges** | 15-20 | 3(37.5) | 5(62.5) | 0(0) | 0(0) |
| | 21-25 | 0(0) | 18(100) | 0(0) | 0(0) |
| | 26-30 | 0(0) | 16(84.21) | 2(10.52) | 1(5.26) |
| | 31-35 | 2(13.33) | 7(46.67) | 5(33.33) | 1(6.67) |
| | 36-40 | 0(0) | 7(100) | 0(0) | 0(0) |
| | 41-45 | 0(0) | 5(71.43) | 2(28.57) | 0(0) |
| | 46-50 | 0(0) | 2(50) | 2(50) | 0(0) |
| | 51-55 | 0(0) | 2(100) | 0(0) | 0(0) |
| | **Total** | **5(6.25)** | **62(77.5)** | **11(13.75)** | **2(2.5)** |
| **Gender** | Female | 3(7.5) | 31(77.5) | 6(15.0) | 0(0) |
| | Male | 2(5.0) | 31(77.5) | 5(12.5) | 2(5.0) |
| | **Total** | **5(6.25)** | **62(77.5)** | **11(13.75)** | **2(2.5)** |
| **Educational Level** | None | 0(0) | 1(50) | 1(50) | 0(0) |
| | Basic | 2(28.57) | 4(57.14) | 1(14.28) | 0(0) |
| | Secondary | 2(5.56) | 31(86.11) | 3(8.33) | 0(0) |
| | Tertiary | 1(2.94) | 26(76.47) | 5(14.71) | 2(5.88) |
| | Informal | 0(0) | 0(0) | 1(100) | 0(0) |
| | **Total** | **5(6.25)** | **62(77.5)** | **11(13.75)** | **2(2.5)** |
| **Ethnicity** | Akyem | 1(10) | 8(80) | 1(10) | 0(0) |
| | Ashanti | 0(0) | 10(100) | 0(0) | 0(0) |
| | Fanti | 0(0) | 10(100) | 0(0) | 0(0) |
| | Anlo | 1(10) | 6(60) | 3(30) | 0(0) |
| | Ewe | 1(10) | 9(90) | 0(0) | 0(0) |
| | Ga | 0(0) | 6(60) | 4(40) | 0(0) |
| | Krobo | 2(20) | 6(60) | 1(10) | 1(10) |
| | Nzema | 0(0) | 7(70) | 2(20) | 1(10) |
| | **Total** | **5(6.25)** | **62(77.5)** | **11(13.75)** | **2(2.5)** |

## 4.2 AS-PCR Genotyping Analysis

Genomic DNA extraction followed by AS-PCR using primers specific for amplifying the mutant allele *CYP2C8*5* was carried out on all 80 samples. Figure 4.3 below is a representative of the ethidium bromide stained agarose gel electrophoregram of the PCR results.

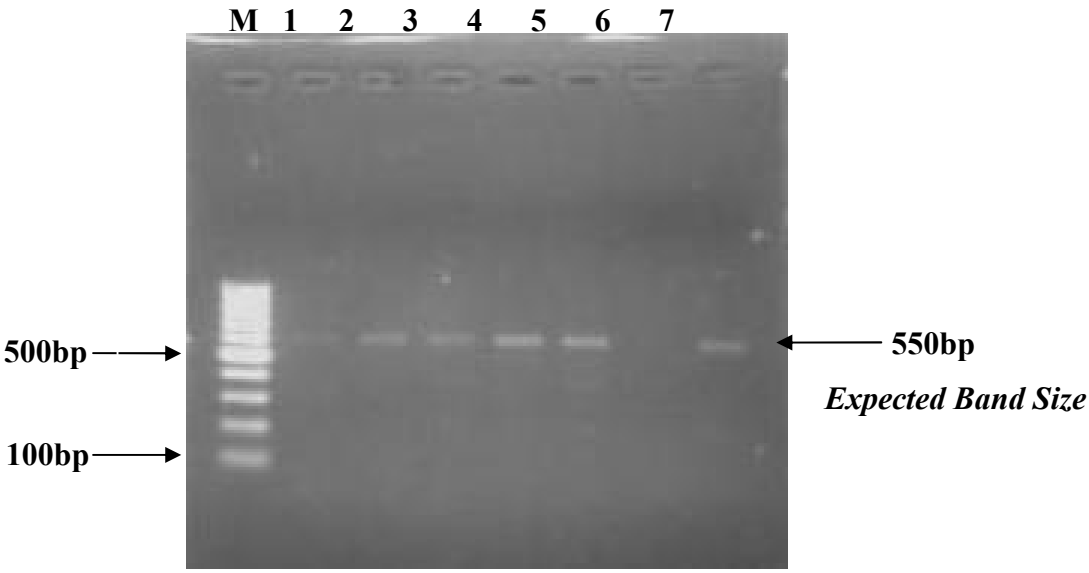



**Figure 4.3: Ethidium bromide stained 2.0% agarose gel electrophoregram of PCR amplified DNA fragments of *CYP2C8*5* for the Ashanti male subjects.**

M = 100 bp molecular weight marker; lanes 1 - 5 = PCR positive samples; lane 6 = negative control; lane 7 = positive control.

Table 4.4 below shows the frequencies of the *CYP2C8*5* mutant allele in the study population. The overall frequency was 83.75%. The highest frequency (100%) was recorded in the Ga and Krobo ethnic groups. The Fanti and Nzema ethnic groups had the lowest frequencies, 70% each.

Males had a higher frequency (92.5%) compared to females (75%). There was no significant difference within the ethnic groups with respect to the frequencies of the *CYP2C8*5* mutant allele (*p=0.636*).

**Table 4.4: Frequency of *CYP2C8*5* analysis in study population**

| | **Positive *CYP2C8*5* Mutants (%)** | | |
|---|---|---|---|
| | **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)** |

## 4.3 History and Attitude of Study Subjects in Accessing Health Care

### 4.3.1 Responses on General Information on Sickness and Medication

Table 4.5 shows the preferred treatment options for medical conditions which require chemotherapy. Majority of the study subjects (48.75%) indicated that they use only drugs while 38 (47.5%) indicated they use both drugs and herbal preparations. Interestingly one study subject indicated using only herbs whilst two indicated they do not medicate. With regards to the frequency of using approved drugs by the study subjects in the past five years, 73 (91.25%) indicated they use approved drugs at least 1-3x, 2 (2.5%) indicated 4-6x and 5 (6.25%) indicated having not used any drug. Also, 69 (86.25%) indicated they used drugs metabolized by CYP2C8 over a period

of 5 years about 1-3x, 2 (2.5%) indicated 4-6x and 9 (11.25%) indicated having not used any such drug.

**Table 4.5: Preferred treatment option and drug usage by study subjects**

| Variable | Category | Frequency (%) |
|---|---|---|
| **Preferred Treatment Options** | Drug Only | 38 (47.5) |
| | Herbs Only | 1 (1.25) |
| | Drugs and Herbs | 39 (48.75) |
| | None | 2 (2.5) |
| | **Total** | **80 (100)** |
| **Approved Drug Usage in a Year** | 0x | 5 (6.25) |
| | 1-3x | 73(91.25) |
| | 4-6x | 2(2.5) |
| | >6x | 0(0) |
| | **Total** | **80 (100)** |
| **Drugs Metabolized by CYP2C8 Usage in Five Years** | 0x | 9 (11.25) |
| | 1-3x | 69 (86.25) |
| | 4-6x | 2 (2.5) |
| | >6x | 0 (0) |
| | **Total** | **80 (100)** |

Table 4.6 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 4.6 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 4.6: 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 | 4 (5) | |
| **Study Subjects** | Chloroquine | 18 (22.5) | |
| | CQ and AQ | 5 (6.25) | |
| | Aspirin | 1 (1.25) | |
| | Other | 6 (7.5) | |
| | **Total** | **33 (41.25)** | |

### 4.3.2 Responses on Information on Malaria and Antimalarial Usage

Table 4.7 shows the preferred malaria treatment options and frequency of antimalarial usage in the past five (5) years by the study subjects. Majority, 70 (87.5%) of the study subjects use only drugs while 4 (5%) use both drugs and herbal preparations for managing malaria.

**Table 4.7: Preferred malaria treatment options**

| Variable | Category | Frequency (%) |
|---|---|---|
| **Preferred Treatment Options** | Drug Only | 70 (87.5) |
| | Herbs Only | 4 (5) |
| | Drugs and Herbs | 4 (5) |
| | None | 2 (2.5) |
| | **Total** | **80 (100)** |
| **Frequency of Antimalarial Drug Usage** | 0x | 9 (11.25) |
| | 1-3x | 53 (66.25) |
| | 4-6x | 16 (20) |
| | 7-9x | 0 (0) |
| | >10x | 2 (2.5) |
| | **Total** | **80(100)** |
| **Antimalaria Drug Used** | Artesunate Amodiaquine | 10 (12.5) |
| | Artemether Lumefantrine | 14 (17.5) |
| | Artemisinin Combination Therapy | 42 (52.5) |
| | Not Sure | 14 (17.5) |
| | **Total** | **80 (100)** |

With respect to the frequency of malaria drug usage, most (66.25%) of the respondents reported that they have used antimalarial drugs at least 1-3x in the past five years, while 2 (2.5%) indicated they have used antimalarial drugs >10x in the past five (5) years (Table 4.7).

Table 4.7 further shows the type of antimalarial drugs commonly used by the study subjects. Forty-two (52.5%) indicated they use Artemisinin Combination Therapy (ACT), 14 (17.5%) use Artemether Lumefantrine (AL) and 10 (12.5%) use Artesunate Amodiaquine (AA).

Table 4.8 shows reported history of antimalaria drug reaction by the study subjects. Fifty-seven (71.25%) of the respondents indicated that they have no history of antimalaria drug reaction, while 19 (23.75%) indicated that they have a history of antimalaria drug reaction.

**Table 4.8: History of adverse drug reaction to antimalarial drugs**

| Variable | Category | Frequency (%) |
|---|---|---|
| **Adverse Drugs Reactions to Antimalarials** | Yes | 19 (23.75) |
| | No | 57 (71.25) |
| | Not Sure | 4 (5) |
| | **Total** | **80 (100)** |
| **Antimalaria Drug Reacted To** | None | 61 (76.25) |
| | Artesunate Amodiaquine | 4 (5) |
| | Chloroquine | 9 (11.25) |
| | Artesunate Amodiaquine and Chloroquine | 6 (7.5) |
| | Other | 0 (0) |
| | Unknown | 0 (0) |
| | **Total** | **80 (100)** |

Concerning antimalaria drugs reacted to by the study subjects, 9 (11.25%) indicated they reacted to CQ, 6 (7.5%) reacted to both CQ and AQ, while 4 (5%) reacted to only AQ (Table 4.8).

### 4.3.3 Responses on Information on NSAIDs and Cancer Drugs

Table 4.9 shows the dependency of the study subjects on some common NSAIDs and cancer drugs metabolized by the CYP2C8 enzyme. Concerning any history of drug reaction to these common drugs by the study subjects, only one of the study subjects had a history of adverse drug reaction to aspirin.

**Table 4.9: Frequency of the usage of NSAIDS and cancer drugs**

| Drugs | Frequency of Use of Drug Metabolized By CYP2C8 (%) | | | |
|---|---|---|---|---|
| | 0x | 1-3x | 4-6x | 6-10x |
| Aspirin | 76 (95.0) | 4(5.0) | 0 (0) | 0 (0) |
| Ibuprofen | 51 (63.8) | 26 (32.5) | 3 (3.8) | 0 (0) |
| Amoxicillin | 37 (46.3) | 36 (45.0) | 7 (8.8) | 0 (0) |
| Taxol | 80 (100) | 0 (0) | 0 (0) | 0 (0) |

## 4.4 Association of BMI, adverse drug reaction and *CYP2C8*5* allele status

Table 4.10 shows the association of *CYP2C8*5* with reported history of adverse drug reaction. There was no significant association ($p>0.05$) between study subjects having *CYP2C8*5* mutant allele and history of adverse drug reactions.

**Table 4.10: History of adverse drug reaction and *CYP2C8*5* allele status**

| *CYP2C8*5* Allele Status | History of Adverse Drug Reaction | | | | |
|---|---|---|---|---|---|
| | Yes (%) | No (%) | Not Sure (%) | Total | *p-value* |
| Presence | 25 (37.87) | 37 (56.06) | 4(6.06) | 66 | ***0.323*** |
| Absence | 8 (57.1) | 6 (42.86) | 0(0) | 14 | |
| **Total** | **33 (41.25)** | **43(53.75)** | **4(5.0)** | **80**(100) | |

Also chi-square analysis showed no significant difference ($p>0.05$) between preferred treatment options with respect to having the *CYP2C8*5* mutant allele. Nevertheless

majority (95.45%) of the individuals with the mutant allele indicated that they use either drugs alone (31, 46.96%) or drugs with herbs (32, 48.48%) (Table 4.11).

**Table 4.11: Preferred treatment option and *CYP2C8*5* allele status**

| ***CYP2C8*5*** | **Preferred Treatment Options** | | | | | |
|---|---|---|---|---|---|---|
| **Allele Status** | **Drugs (%)** | **Herbs (%)** | **Mixed (%)** | **None (%)** | **Total** | ***p-value*** |
| Presence | 31(81.57) | 1(100) | 32(82.05) | 2(100) | 66(82.50) | ***0.466*** |
| Absence | 7(18.42) | 0(0) | 7(17.95) | 0(0) | 14(17.50) | |
| **Total** | **38** | **1** | **39** | **2** | **80** | |

There was no significant association (*p>0.05*) between *CYP2C8*5* mutant allele status and frequency of antimalarial drugs usage (Table 4.12). However, the study subjects who used antimalarial drugs at least 1-3x in the past 5 years were the highest.

**Table 4.12: Antimalarial usage in past 5 years and *CYP2C8*5* allele status**

| **CYP *2C8*5*** | **Frequency of Antimalarial Drug Usage** | | | | **Total** | ***p-value*** |
|---|---|---|---|---|---|---|
| **Allele Status** | **0x** | **1-3x** | **4-6x** | **>10** | | |
| Presence | 8(12.12) | 43(65.15) | 13(19.69) | 2(3.03) | 66 | ***0.556*** |
| Absence | 1(7.14) | 10(71.2) | 3(21.42) | 0(0) | 14 | |
| **Total** | **9** | **53** | **16** | **2** | **80** | |

In addition, when the history of drug reaction to antimalarial drugs and *CYP2C8*5* allele status was cross tabulated, no significant association (*p>0.05*) was observed (Table 4.13).

**Table 4.13: Adverse drug reactions to antimalarials and *CYP2C8*5* allele status**

| CYP *2C8*5* Allele Status | Reaction to Anti-malaria Drugs (%) | | | Total | *p-value* |
|---|---|---|---|---|---|
| | Yes | No | Not Sure | | |
| Presence | 19(28.78) | 45 (68.18) | 3 (4.54) | 66 | ***0.237*** |
| Absence | 2 (14.28) | 11(78.57) | 1 (7.14) | 14 | |
| **Total** | **20** | **56** | **4** | **80** | |

Furthermore, there was no significant association (*p>0.05*) between the *CYP2C8*5* status and frequency of taking full course prescriptions (Table 4.14).

**Table 4.14: Taking full course of medication and *CYP2C8*5* allele**

| CYP *2C8*5* Allele Status | Frequency of Taking Full Course of Medication | | | | *p-value* |
|---|---|---|---|---|---|
| | Always | Most Times | Occasionally | Never | |
| Presence | 12 (80) | 21 (80.76) | 13 (92.85) | 20(80) | ***0.485*** |
| Absence | 3 (20) | 5 (19.23) | 1 (7.15) | 5 (20) | |
| **Total** | **15 (100)** | **26 (100)** | **14 (100)** | **25 (100)** | |

# CHAPTER FIVE

## 5.0 DISCUSSION

Genetic variability on drug efficacy and tolerability are important. Many pharmacologically-relevant polymorphisms show variability in different populations. Information on allelic frequency is useful in identifying adverse drug reaction risk populations, understanding therapeutic failures and optimising doses for efficacy and efficiency. Many research studies have shown that mutation in the gene which code for the enzyme CYP2C8 leads to a dysfunctional enzyme (Gerbal-Chaloin *et al.*, 2001, Goldstein, 2001). The study determined prevalence of clinically relevant *CYP2C8*5* polymorphism in 80 unrelated individuals, from selected ethnic groups in Southern Ghana. It also evaluated the distribution of *CYP2C8*5* genotype within selected ethnic groups (Akyem, Ashanti, Fante, Anlo, Ewes, Ga, Krobo and Nzema) in Southern Ghana, the association between *CYP2C8*5* genotype and adverse effects of drugs metabolised by the enzyme and the extent individuals with *CYP2C8*5* genotype depend of drugs metabolized by CYP2C8.

From the study, the genotypic frequency of *CYP2C8*5* in selected ethnic groups in Southern Ghana was 83.75%. In general, much of the study subjects (88.75%) depend highly (>1-3x in a year) on drugs metabolized by the CYP2C8 enzyme. Despite this high prevalence, there was no statistically significant association ($p$>0.05) between the presence of the mutant allele *CYP2C8*5* and individual with reported history of adverse drug reactions.

Pharmacogenetic studies in Ghana have shown that the prevalence of genetic variabilities in *CYP2C8* is high especially that of *CYP2C8*2*, 16% (Rower *et al.*,

2005) and 17% (Kudzi *et al.*, 2009). The observations from this study supports and augments these findings through the observation that the frequency of the *CYP2C8*5* allele among selected ethnic groups in Southern Ghana was high (83.75%). Work conducted on Japanese subjects observed the prevalence of *CYP2C8*5* to be 0.25% (Nakajima *et al.*, 2003) and Soyama *et al.* (2002) also reported the heterozygote of *CYP2C8*5* in Japanese population with a frequency of 0.9%. Comparatively the prevalence observed in this study is very high. There was no significant difference in the frequency of the mutation within the selected ethnic groups. That notwithstanding, the information from some of the tribes can be used on other tribes that share a close relation with those in Ghana such as the Ewes who extend from Ghana through Togo to Benin and the Akans who extend into Ivory Coast as well as individuals abroad.

Clinically, the high prevalence of the mutant allele observed in the study implies the sample population may be at risk of using medications metabolized by CYP2C8. CYP2C8 enzyme metabolizes many drugs of clinical importance and endogenous substrates such as arachidonic acid to biologically active metabolites some of which play important physiological roles in the pathogenesis of cardiovascular diseases, such as hypertension and acute myocardial infarction (Totah and Rettie, 2005). This implies that individuals homozygous for *CYP2C8*5* may find it very difficult to tolerate most drugs solely metabolized and degraded by CYP2C8 and hence suffer severe adverse drug reactions. On the contrary, individual homozygous for the *CYP2C8*5* using drugs that require the action of the CYP2C8 enzyme to convert the prodrug such as AQ into its active metabolite DEAQ will experience therapeutic failure although they will experience the risks or side effects associated with the drug.

Some of the drug substrates might not even bind to the enzyme since *CYP2C8*5* mutation affects not just the active but also 5 out of 6 substrate recognition site on the enzyme. Evidently, it was reported in the media in Ghana that individuals using artesunate amodiaquine were having ADRs when it was first introduced. Interestingly these were also observed in studies conducted in Ghana (Adjei *et al.*, 2009).

The BMI distribution of the subjects showed that most of the study subjects (77.5%) had normal weight. Underweight individuals are more likely to experience adverse drug reactions, while overweight individuals are more at risk of therapeutic failure and will require more dosage compared to the normal weight individuals. The normal BMI observed in the study implies that most of the study subjects should be able to tolerate standard drug dose under normal circumstances.

Ghana attaining a Middle Income Country (MIC) status since November 2010 (Aiyar *et al.*, 2013, p.5) implies that the socioeconomic status of the Ghanaian population may have improved over the years. A person's social class has a significant impact on their physical health, their ability to receive adequate medical care and nutrition, as well as their life expectancy (Budrys, 2010, p.183-184). Usually people of lower socioeconomic class experience a range of health problems (higher rates of infant mortality, cancer, cardiovascular disease, and disabling physical injuries) compared to those of the higher class (Chen *et al.*, 2010). Although Ghana currently runs a National Health Insurance Scheme, certain important medications are not included on the scheme. With none of the study subjects classified in a poor socio-economic class (Table 4.2) implies majority of them could afford drugs covered or not covered under the NHIS of Ghana. This corresponded to responses by the study subjects that they frequently practiced self-medication (Table 4.14). The problem of fake drugs on the

counter coupled with the ease to access drugs over the counter increases the risk of having drugs that lead to toxicity with respect to the high level of the *CYP2C8*5* mutant allele prevalent in the study population.

Considering the product of *CYP2C8*5* is an enzyme that has an altered or reduced enzyme activity, although the prevalence of the *CYP2C8*5* allele in the study was very high, statistically there was no significant difference *(p>0.05)* between study subjects having the *CYP2C8*5* allele and reported history of ADRs. This observation could stem from the fact that most Ghanaians lack adequate knowledge on drug reactions. Although majority of the study subjects were educated (Table 4.1), there is no in-depth formal medical education encompassing adverse drug reactions to most Ghanaians (Goodman *et al.*, 2007). Consequently, they probably were unable to recognize the symptoms and signs associated with adverse drug reactions. Nonetheless, these individuals may relate well with health professionals and make well-informed choice with respect to treatment options although the risk of having a CYP*2C8*5* allele will be less severe if the population is well informed about ADRs. On the contrary, those from the rural areas are at lesser risk of having the *CYP2C8*5* since fewer drugs are likely to be available in the rural areas, although some of the herbal preparation they use may require the action of CYP2C8 to metabolize them (Thomford *et al.*, 2015).

In addition, the study subjects could be heterozygous genotypically inheriting a wild-type and a mutant allele (*CYP2C8*1* and *CYP2C8*5*) or even have two different mutant alleles (*CYP2C8*2* and *CYP2C8*5)* such that the activity of the CYP2C*.2 enzyme is just decreased but not absent. This can be the case since previous works conducted indicated that the *CYP2C8*2* is the most predominant allele (16-17%) in

the Ghanaian population and in blacks (Kudzi *et al.*, 2009, Rower *et al.*, 2005, Bahadur *et al.*, 2002).

Also, there could be the possibility of multiple enzymes acting on the same drug. CYP2C8 and CYP3A4 are the major enzymes responsible for catalysing the biotransformation of both troglitazone and pioglitazone. Also Rosiglitazone is metabolised by CYP2C9 and CYP2C8 (nucleotide sequences of CYP2C8 gene shares about 74% homology with CYP2C9 gene) underlining the fact that pharmacogenetic variability influences the pharmacokinetics of oral antidiabetic drugs (Glamočlija and Jevrić-Čaušević, 2010). The similarity in size also accounts partly for the observation of CYP2C8 and CYP3A4 having many related substrates compared to other CYPs although CYP2C8 normally catalyses reactions that results in the formation of different metabolites (Säll *et al.*, 2012). If the wild type of a different enzyme that metabolizes the same drug substrate is present, the effect of the active enzyme will mask the inactivity of the dysfunctional CYP2C8.5 enzyme and thus reduce the risk of experiencing a severe adverse drug effect as would have been expected.

In general, increased use of drugs is associated with increased risk of having adverse drug reactions. Many studies have linked increased dependency of drug use to many side effects, the most serious of them being glaucoma. St Lucia, a small country with a population of less than 190, 000 as at 2015 is the leading country in the world with the highest incidence of glaucoma. Ghana has the second highest incidence of glaucoma in the world and the highest in Africa (Melamed *et al.*, 2010). Interestingly, there is a genetic link between the Ghanaians and the people St Lucia (Abu-Amero *et al.*, 2012).

Many (88.75%) of the study subjects depended highly (>1-3x in five years) on drugs metabolized by the CYP2C8 enzyme (Table 4.5). It was also observed that majority of the study subjects over (91.25%) use approved drugs at least 1-3x in a year for treatment of various medical conditions (Table 4.5). Considering the high prevalence of the *CYP2C8*5* mutant allele in the study population, they may be at risk of adverse drug reactions in using drugs metabolized by the CYP2C8 enzyme especially in individuals homozygous for this mutant allele. This corresponded with 25 (37.87%) study subjects indicating they have a history of adverse reactions particularly to antimalarial drugs such as chloroquine and amodiaquine. Although majority of the study subjects were educated (Table 4.1), there is inadequate knowledge in the general population on the dangers association with drug overuse Ghanaians (Goodman *et al.*, 2007).

Furthermore, majority of study subjects (87.5%) indicated that they treat malaria with drugs. This could be due to the high educational level of the study subjects, which were about 97.5% as well as the socio-economic level although some of the study subject (5%) indicated the use of herbal preparation in the treatment of malaria. These individuals may not have any adverse drug reactions from the use of anti malaria drugs, but could experience toxicity from the herbal preparations especially herbs containing quinine-based extracts and other unknown compounds. It may even be more detrimental in those who use both drugs and herbal preparation because drug-drug interactions may lead to more stress on the liver or unavailability of the essential compounds or active metabolites in the system because they are bound to each other (Gurley *et al.*, 2002). Interestingly, 2% of the study subjects indicate that they do not treat malaria. These could be individuals who have developed a strong immune system against malaria such that they are able to fight the malaria parasite efficiently

without the use of chemical agents. They could also be sickle cell carriers with an abnormal red blood cells which are uninhabitable by the *Plasmodium* parasites hence are immune to malaria (Williams *et al.*, 2005).

Moreover, most patient trust health professionals and will follow their prescriptions without question. Although most health professionals will do their best for patients, certain information about patients such as history of adverse drug reaction or genotype of patients are not readily available to facilitate selecting the most appropriate treatment options. With majority of the study subjects (68.75%) indicating that they finish their full course of medications means they could have prolonged ADRs as a result of long use on such drugs. Considering the increase risk associated with having *CYP2C8*5,* taking the full course of medication frequently implies an increase in the risk having ADRs from a particular drug if any is experienced. The situation could be worse in individuals homozygous for the *CYP2C8*5* variant allele especially the 18% of the study subjects who indicated that they always finish their course of medication.

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

The observed frequencies of *CYP2C8*5* allele in the selected ethnic groups in Southern Ghana was high (83.75%). There was no significant difference ($p>0.05$) in the frequency of *CYP2C8*5* allele between the selected ethnic groups. There was also no significant association ($p>0.05$) between having *CYP2C8*5* mutant allele and reported history of adverse drug reactions. Many (88.75%) of the study subjects depended highly (>1-3x in a year) on drugs metabolized by CYP2C8 enzyme.

### 6.2 Recommendations

More research needs to be conducted on a larger population of the ethnic groups to obtain information on all clinically relevant CYPs for possible mutations of clinical significance.

Also, sequencing of the whole *CYP2C8* gene including the promoter and enhancer regions for the selected ethnic groups will be imperative in obtaining the comprehensive information on the frequency of the CYP2C8 mutant alleles and making a full assessment of the genetic variability in the general population.

Finally, the extents to which transcriptional factors modulate the expression of the *CYP2C8* gene must also be evaluate.

### 6.3. Limitations of the Research Study

The small sample size might limit the applications of the findings. In addition, the gene pool of the study subjects is likely to be heterogeneous through interbreeding. Although an attempt was made to address this by recruiting only individuals whose maternal and parental grandparents (four grandparents) were all of the same biological ethnic background. Also, the information about ethnicity was provided by the participants. There was no way to cross-check this information. Thus, there may have been some level of interbreeding in the study population which could not be accounted for in the study.

Some level of bias in data from the questionnaire due to under-reporting or over-reporting bias in subject's responses is also possible. For instance, it is possible some of the study subjects might have forgotten about some of the experiences they might have encountered with respect to their history of drug usage and ADRs.

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# APPENDICES

## APPENDIX I

## PREPARATION OF STANDARD SOLUTIONS USED IN STUDY

The following solutions were prepared using sterile distilled water and whenever appropriate the solutions were autoclaved at 121 lb/ for 15 minutes using Sakura autoclave (Tiyoda manufacturing Co. Ltd, Japan). Below are procedures used in the preparation of solutions for the molecular work aspect of the study.

### 0.5M EDTA (pH 8.0)

46.53 g of EDTA was dissolved in 200 ml distilled water. The pH was adjusted with NaOH to 8.0 and the volume made up to 250 ml. It was autoclaved at 121 $^{o}$C for 15 minutes and stored at room temperature.

### 0.5 M Tris

15.15 g of Tris was dissolved in 150 ml of sterile distilled water, the pH adjusted to 8.0 with concentrated HCl and final volume made to 250 ml. This was autoclaved at 121 $^{o}$C for 15 minutes and kept at room temperature.

### 2 M Tris

60.55 g of Trizma base was weighed, 150 ml of sterile distilled water added and the pH adjusted to 8 with concentrated HCl and the volume made up to 1000 ml. This was autoclaved at 121 $^{o}$C for 15 minutes and kept at room temperature.

### Tris-EDTA (TE) pH 8.0

This was prepared using 1 M Tris-HCl and 0.5 M EDTA both pH 8.0. A final solution of 10 mM Tris and 0.1 mM EDTA was prepared. This was autoclaved at 121 $^{o}$C for 15 minutes and kept at room temperature.

**Ethidium Bromide (10 mg/ml)**

1 g of ethidium bromide was completely dissolved in 100 ml of sterilized distilled water and stored in the dark at room temperature.

**50x TAE Buffer**

242 g of Tris base was weighed, 57.1 ml glacial acetic acid and 100 ml of 0.5 M EDTA was added. The pH was adjusted to 7.7 and the volume made to 100ml with sterilized distilled water.

**Deoxynucleotide Triphosphates**

Each deoxynucleotide triphosphate (dATP, dCTP, dGTP and dTTP) had an initial concentration of 100 mM. To make a mix of 10 mM concentration, 10 µl of each deoxynucleotide triphosphate was taken into a sterile eppendorf tube with sterile pipettes. The mix was then made to 100 µl with sterile distilled water.

**DNA Molecular Weight Marker**

100 bp ladder from Roche Diagnostics Gmbh was used with a concentration of 0.25 µg/µl. This was then diluted in sterile distilled water and the gel loading buffer in the proportion 5:5:2 in the order: ladder, distilled water and loading buffer respectively.

## APPENDIX II

## CHEMICALS REAGENTS AND EQUIPMENT

### 1. Chemicals and reagents used for DNA extraction and PCR

#### *Sample Collection and DNA Extraction*

Whitman Filter paper Grade 9.Absolute ethanol, absolute methanol, Trizma Base (Tris –hydroxymethyl-amino methane), ethylene diamine tetra acetic acid (EDTA), phosphate buffered saline (PBS) (Sigma, USA),

#### *PCR*

10x PCR Buffer (Invitrogen, USA), Magnesium Chloride ($MgCl_2$) (Promega, USA), *Taq* DNA polymerase (Promega, USA), deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP) (Promega, USA), Oligonucleotide Primers.

#### *Gel Electrophoresis*

Ethidium bromide bromophenol blue, agarose (molecular biology grade), 100bp molecular weight marker (Roche Diagnostics Gmbh).

### 2. Equipment

#### *DNA Extraction*

Perforator (AGM 6 mm Single Hole Punch Plier, USA), Sakura Autoclave (Tiyoda Manufacturing Co. Ltd, Japan), Centrifuge 5415D (Eppendorf, Germany), Standard Mini Vortexer (VWR Scientific Products, UK),12" Laboratory Thermometer (Midwest Homebrewing and Winemaking Supplies, UK), Heat Block (Grant instruments, Cambridge, England), Magnetic Stirrer (Fisher Scientific, USA),

***PCR***

GeneAmp PCR system 2700 Thermocycler (Applied Biosystems, Courtaboeuf, France),PTC 100 thermo-cycler (MJ Research Inc., USA).

***Gel Electrophoresis***

Adventurer Pro Weighing Balance (Ohaus Corporation, NJ USA), Microwave oven (LG, USA),TOYOBO Transilluminator Model TM-20 (Japan), TOYOBO FAS-III monitor system (Japan), Mini gel system (BIORAD, USA).

## APPENDIX III

## ETHICAL CLEARNCEAND CONSENT FROM STUDY SUBJECTS

## PARTICIPANT INFORMATION LEAFLET

**Title of Research:** Prevalence of the Genetic Mutation*CYP2C8*5* in Selected Ethnic Groups in Southern Ghana.

**Name(s) and Affiliation(s) of Researcher(s):** This study is being conducted by Dr. Christopher Larbie of Dept of Biochemistry and Biotechnology, KNUST- Kumasi, Dr. Charles Brown of Dept of Medical Laboratories, UG – Accra and Mr. Dominic Selorm Yao Amuzu of Dept of Biochemistry and Biotechnology, KNUST – Kumasi.

**Background (Please explain simply and briefly what the study is about):**

The socio-economic status of every country in reflective of the health status of its population as the health status of the population affects directly the productivity level of that country. The major diseases that are of direct public health interest currently in Ghana as a sub-Saharan African country include Malaria, Diabetes and Bilharzias. The death and morbidity which leads to a less available work force of the country.

According to the WHO report 2012, the prevalence of Malaria is still high and that of diabetes is also on the increase. To reduce such high deaths, factors that contribute to management and treatment such diseases need to be identified and monitored. The most effective choice of treatment is chemotherapy for both malaria and diabetes. Though significant progress has been made in the country concerning the development of strategies for the management and treatment of these medical

conditions the same cannot be said of the enzymes that make the drugs to be used safely. One of such enzymes worth considering is an enzyme called Cytochrome P450 (CYP) 2C8 which breaks down the malarial drugs Amodiaquine and Chloroquine, as well as drugs used for the treatment of diabetes (thiazolidinedione, rosiglitazone and pioglitazone) Cancer (paclitaxel or taxol) and inflammation or pain killers (ibuprofen and diclofenac).

Inability of the enzyme to break down these drugs leads to toxics level of the drugs which increases the side effects of the drug. On the contrary is the enzyme works to much the drug will be removed from the system to quickly and hence make it ineffective. Amodiaquine for examples leads to toxic side effects such as abdominal discomfort, nausea, vomiting, headache, dizziness, blurring of vision, mental and physical weakness, and fatigue. Others are itching, heart abnormalities, neurological disorder, neuromuscular disorders, blurred vision, and hearing loss. Furthermore, troglitazone, a thiazolidinedione drugs used in the treatment of diabetes have been withdrawn owing to these drugs being toxic to the liver. Also, CYP also acts on other drugs used in the treatment of cancer such as paclitaxel of which studies have shown a significant reduction their breakdown was due gene mutations. Research has shown in increase in the side effect ibuprofen and diclofenac such as bleeding in small and large intestines in individual with two genetic mutations of the enzyme.

The loss of function of the CYP2C8 enzyme could be due to mutations in the gene. Individuals with such mutations will be unable to metabolize drugs that are substrates for the CYP2C8. Recent studies have shown the mutations in CYP2C8 in Northern Ghana (Rower *et al.,* 2005) thus it will be imperative for studies to be done in Southern Ghana. This project is designed to determine the prevalence of genetic

mutations in the gene for the enzyme CYP2C8. As such it is imperative for such mutations to be known to facilitate modifications of treatment for such individuals with respect to drug choice and dosing.

**Purpose(s) of Research:** The aim of this research is to find out the prevalence of genetic mutations in Cytochrome p450 2C8 in Southern Ghana. By the completion of this research study, it is expected that data will be available to establish any mutations associated with Cytochrome P450 2C8 in individuals in Southern Ghana. The findings of this work will influence education, management and treatment of Malaria, Diabetes and Cancer with respect to chemotherapy options.

We will randomly selected individuals who come to the Blood Bank Unit of Komfo Anokye and Korle-Bu Teaching Hospital to donate blood and are willing to participate in the study. If you volunteer to participate in this study, we would take approximately 4 drops of blood during your blood donation process onto a filter which will be taken to the laboratory for analysis paper. In addition you will be required to answer a questionnaire concerning your medical history with respect to the study which will help us have make a meaningful conclusion of the study. In total we expect to recruit 100 subjects within southern Ghana.

**Risk(s):** The drawing of blood during the blooding donation process will cause some pain and discomfort with bruising at the site of the insertion of the syringe. Also the questions contained in the questionnaire will demand personal medical information about the history of your health status and your family health history, which might be discomforting but will be used only for the purpose of the study. In the event that you

feel you do not wish to continue the study, you can request to be withdrawn from the study at anytime.

**Benefit(s):** There may not be any direct benefit for partaking in the study, but you may benefit through counselling from experts. The study will however help we the investigators determine the prevalence of Cytochrome P450 2C8 genetic mutations in southern Ghana there by making information about the enzymes available for effective management and treatment of malaria and other drugs which require Cytochrome P450 2C8 for its metabolism. Any abnormal test results will be explained to you and you will be referred for medical care, if necessary and available.

**Confidentiality:** We assure you that all information collected during the study will be given code numbers which will be reserved in confidence and undisclosed. No name will be recorded. Data collected cannot be linked to you in anyway. No name or identifier will be used in any publication or reports from this study. Moreover, all scientific findings of the study will be presented without the disclosure or identification of your name. However, as part of our responsibility to conduct this research properly, we may allow officials from ethics committees to have access to your records. Furthermore, you should feel at will to contact us at a later date should you later have questions or concerns that you wish to be addressed.

**Voluntariness:** Being a participant should be out of your own free will. You are not obliged to partake in the study. Enrolling in this research is entirely voluntary.

**Alternatives to Participation:** If you choose not to participate, this will not affect your treatment or blood donation exercise in this hospital or unit in any way.

**Withdrawal from the Research:** You can choose to be in this study or not. If you volunteer to be part, you may withdraw without having to explain yourself at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also choose not to answer any question you find uncomfortable or privateand still remain in the study.

**Consequence of Withdrawal:** There will be no consequence, loss of benefit or care to you if you choose to withdraw from this study. Please note however, that some of the information that may have been obtained from you without identifiers (name etc), before you chose to withdraw, may have been modified or used in analysis reports and publications. These cannot be removed anymore. We do promise to make good faith effort to comply with your wishes as much as practicable.

**Costs/Compensation:** There will be no compensation or direct benefit of the study for you apart from the conclusions that will be drawn at the end of the study.

**Contacts:**

If you have any question concerning this study, please do not hesitate to contact the researchers Dominic S. Y. Amuzu on 0243601178 (dsyamuzu.edu@gmail.com) or Dr Christopher Larbie on 0243445961 (ekowlarbie@gmail.com). If you have any questions or concerns and would like to talk to someone other than the researcher(s), you are encouraged to contact: **The Head of Dept, Dept of Biochemistry and Biotechnology, College of Sciences, KNUST, Kumasi, (Tel: (+233-3022060298).**

CONSENT FORM FOR THE STUDY

STATEMENT OF PERSON OBTAINING INFORMED CONSENT:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

**DATE:**___/_____/_____ **NAME:**_____

STATEMENT OF PERSON GIVING CONSENT:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____

DATE: ___/___/____ SIGNATURE/THUMB PRINT: _____

**APPENDIX IV**

**QUESTIONNAIRE FOR THE RESEACH STUDY**

**QUESTIONNAIRE FOR EVALUATING THE PREVALENCE OF THE GENETIC MUTATION *CYP2C8*5* IN SOUTHERN GHANA**

Date: ___/_____/_____

**SECTION A: BIODATA OR PERSONAL INFORMATION**

STUDY SUBJECT CODE:…… SAMPLE CODE:……..…..

Age (yrs):….. Weight (kg):…..... Height (m):…..... BMI ($kg/m^2$):…….

Gender: ….. Marital Status: …… Religion: ……..…….

Home Town:……..... Place of Birth:……..…….....

Residence: …….....Physical Address:……

Tel:..... Email:…….....….....……

Educational Level: ☐None ☐Basic ☐Secondary ☐Tertiary ☐Informal

Income Level: ☐ Low ☐ Middle ☐ High ☐Very High

Occupation:…….....

**SECTION B: GENERAL BIOLOGICAL AND ETHNIC BACKGROUND**

**Self**

1. What is your Biological or Ethnic Background? If other specify:……

| Asanti | Akyem | Fanti | Ewe | Anlo | Ga | Krobo | Nzema |
|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

**Mother**

2. What is the Biological or Ethnic Background of your mother? If other:……...……

| Asanti | Akyem | Fanti | Ewe | Anlo | Ga | Krobo | Nzema |
|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

3. What is the Biological or Ethnic Background of your mother's father? If other:…….

| Asanti | Akyem | Fanti | Ewe | Anlo | Ga | Krobo | Nzema |
|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

4. What is the Biological or Ethnic Background of your mother's mother? If other:……..

| Asanti | Akyem | Fanti | Ewe | Anlo | Ga | Krobo | Nzema |
|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

**Father**

5. What is the Biological or Ethnic Background of your father? If other:…….……

| Asanti | Akyem | Fanti | Ewe | Anlo | Ga | Krobo | Nzema |
|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

6. What is the Biological and Ethnic Background of your father's father? If other:…….

| Asanti | Akyem | Fanti | Ewe | Anlo | Ga | Krobo | Nzema |
|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

7. What is the Biological and Ethnic Background of your father's mother? If other:……...

| Asanti | Akyem | Fanti | Ewe | Anlo | Ga | Krobo | Nzema |
|---|---|---|---|---|---|---|---|
| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

**SECTION C: MEDICAL HISTORY ON DRUG USAGE**

1. Have you ever reacted to any drug in your life time? If yes specify:……

☐ Yes ☐ No ☐ Not Sure

2. Have any of **your parents** ever reacted to **any drug**? If yes specify:……

☐ Yes ☐ No ☐ Not Sure

3. Have any of **your siblings ever** reacted to **a drug**? If yes specify:…….

☐ Yes ☐ No ☐ Not Sure

4. How do you generally treat infections which require chemotherapy?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

5. How do you treat other medical conditions that require chemotherapy?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

**SECTION D: HISTORY ON MALARIA AND ANT-MALARIA DRUGS**

1. How frequent do you get malaria in 5 years?

☐ 0x ☐ 1-3x ☐ 4-6x ☐ 7-9x ☐ >10x

2. How do you treat malaria infections?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

3. How frequent do you treat malaria infections in a 5 years?

☐ 0x ☐ 1-3x ☐ 4-6x ☐ 7-9x ☐ >10x

4. How often do you use antimalaria drugs to treat malaria in 5 years?

☐ 0x ☐ 1-3x ☐ 4-6x ☐ 7-9x ☐ >10x

5. If you use antimalaria drugs, which of these do you use? If other specify:……

☐ ArtesunateAmodiaquine ☐ Artemether-Lumefantrine ☐ Dihydroartemisinin Piperaquine ☐ Not Sure

6. Have you ever reacted to any anti-malaria drug? If yes specify:……..

☐ Yes ☐ No ☐ Sure

7. Are you currently on any of these antimalarial drugs ?If other specify:…….

☐ ArtesunateAmodiaquine ☐ Artemether-Lumefantrine ☐ Dihydroartemisinin Piperaquine ☐ Not Sure

8. Have any of your family members react to any of the following anti-malaria drugs?If other specify:…….....

☐ ArtesunateAmodiaquine ☐ Artemether-Lumefantrine ☐ Dihydroartemisinin Piperaquine ☐ Not Sure

**SECTION E: HISTORY ON DIABETES AND ANTIDIABETIC DRUGS**

1. Have you been diagnosed by any health professional as being diabetic?

☐ Yes ☐ No

2. If yes from the question above, how do you treat your diabetes?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

3. If you use drugs to treat diabetes how often do you take the medication in a month?

☐ 0x ☐ 1-3x ☐ 4-6x ☐ >6x

4. Which anti-diabetic drugs do you often use? If other specify……..

☐ Metformin ☐ Sulfonylureas ☐ Glitazones ☐ Insulins ☐ None

5. Have you reacted to any anti-diabetic drugs before? If other specify……..

☐ Metformin ☐ Sulfonylureas ☐ Glitazones ☐ Insulins ☐ None

6. Has any family member ever reacted to any of the following anti-diabetics drugs?

☐ Metformin ☐ Sulfonylureas ☐ Glitazones ☐ Insulins ☐ None

## SECTION F: INFORMATION ONDRUGS METABOLISED BY CYP2C8

1. How frequent do you get sick in a year?

☐ 0x ☐ 1-3x ☐ 4-6x ☐ >6x

2. How do you treat your sicknesses in general?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

3. How frequent do you take in approved, standard or orthodox drugs in a year?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

4. How often do you use drugs such as Aspirin in a year?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

5. How often do you use drugs such as Taxol or Paclitaxel in a year?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

6. How often do you use drugs such as Ibuprofen in a year?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

7. How often do you use drugs such as Amoxicillin in a year?

☐ Drugs ☐ Herbs ☐ Mixed ☐ None

8. Have you ever reacted to any of the following drugs?

☐ Aspirin ☐ Ibuprofen ☐ Amoxicillin ☐ None ☐ Not Sure

9. Has any family member ever reacted to any of the following drugs?

☐ Aspirin ☐ Ibuprofen ☐ Amoxicillin ☐ None ☐ Not Sure

## SECTION G: ATTITUDE TOWARD MEDICATION IN GENERAL

1. Do you take the full course of a medication prescribed by a medical professional?

☐ Always ☐ Most Times ☐ Occasionally ☐ Never

2. Do you practice self-medication (Using drugs without the prescription by a doctor)?

☐ Always ☐ Most Times ☐ Occasionally ☐ Never

3. Do you read the information about drugs provided by the manufacturers of the drug?

☐ Always ☐ Most Times ☐ Occasionally ☐ Never

4. Do you read the expiry date information on drugs provided by manufacturers?

☐ Always ☐ Most Times ☐ Occasionally ☐ Never

5. Do you take seriously the information about drugs provided by manufacturers?

☐ Always ☐ Most Times ☐ Occasionally ☐ Never

6. Do you seek help for clarification about the information provided by manufacturers?

☐ Always ☐ Most Times ☐ Occasionally ☐ Never