

***Characterization of variants of CYP2D6 and
CYP3A4 drug metabolizing enzymes among Ewe
ethnic group in Ghana***



AN MPhil PROJECT REPORT SUBMITTED BY:

KPODO, GERVIN DELALI.

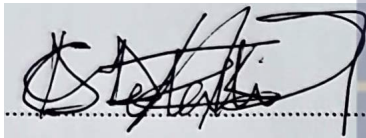
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TO THE DEPARTMENT OF BIOCHEMISTRY, CELL AND
MOLECULAR BIOLOGY OF THE UNIVERSITY OF GHANA
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AWARD OF A MASTER OF PHILOSOPHY DEGREE IN
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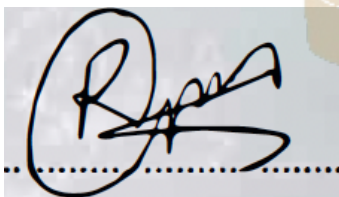
DECLARATION

I, Gervin Delali Kpodo do hereby declare that the work in this dissertation that was supervised by Professor Regina Appiah-Opong and Rev. Dr. W.S.K Gbewonyo is my own quotation. Summaries and methodology which were required from other sources have been duly referenced. This dissertation has also not been accepted for any degree and not concurrently submitted for award of other degrees.

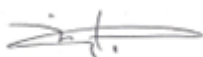


GERVIN DELALI KPODO
(STUDENT)

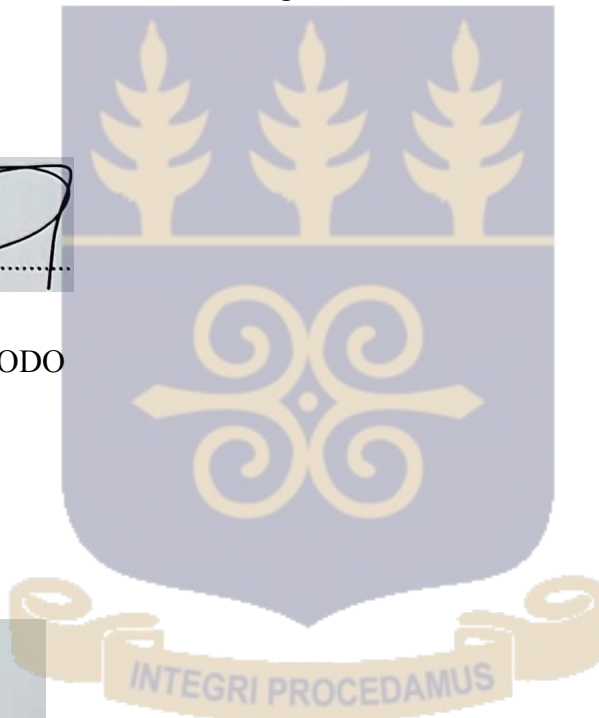
21st January, 2024



PROFESSOR REGINA APPIAH-OPONG
(SUPERVISOR)



DR. W. S. K. GBEWONYO
(CO-SUPERVISOR)



DEDICATION

I dedicate this project to The Almighty God, My family, my supervisors, my classmates and friends who have been supportive throughout for their constant love, support and inspiration.

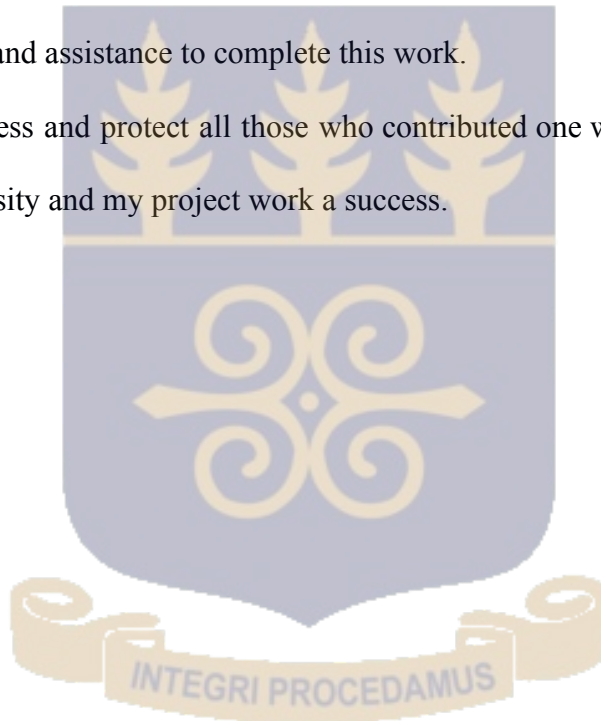


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I am very thankful to the Lord almighty for His goodness and mercies and sustenance through this period.

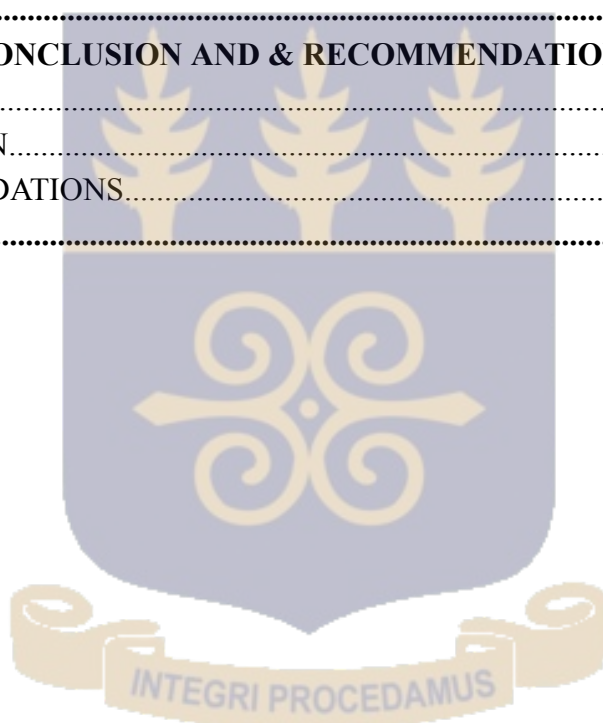
I would also like to express my heartfelt gratitude to my supervisor Professor Regina Appiah-Opong and Dr. W.S.K Gbewonyo for their valuable suggestions and critiques that helped shape this thesis. I would also like to thank Dr. Yaw Aniweh of the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) for his assistance during my molecular techniques, Dr. Samuel Mawuli Adadey and Mr. Edu Nelson for their support in providing mentorship and assistance to complete this work.

May the good Lord bless and protect all those who contributed one way or the other to make my study in the university and my project work a success.



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ABSTRACT

Studies have highlighted specific genetic traits linked to certain ethnic populations like the sickle cell anaemia being a genetic defect associated with African and African American populations. Allelic variants of drug metabolizing enzymes are no exception to this fundamental concept. Cytochrome P450 enzymes (CYPs) are responsible for metabolizing a major fraction of drugs with specific enzymes like CYP2D6 and 3A4 playing significant roles. Knowledge of individual/ethnic allelic variants of drug metabolizing enzymes as well as their resulting phenotypes can aid drug developers in providing individual/ethnic specific optimal dosages to improve therapeutic efficacy in Ghana. This study was aimed at determining the allelic type and frequency among the Ewe ethnic group in Ghana and their expected enzyme activity. The study entailed collection and genomic sequencing of whole blood samples from Ewe individuals in the Adidome (Southern part) and Hohoe (Northern) townships and genotypic analysis by Restrict digest of PCR amplified gene targets. From this study, the percentage of the Ewes in Ghana with the CYP2D6*3 and *4 variants were found to be 0.8% and 8.8% respectively which is consistent with studies done on other sub-Saharan populations. The frequency for the CYP3A4*1B, a variant responsible for decreased enzyme activity was found to be 2.42% of the sampled population of Ewes in Ghana, which varied from other previous studies done on varying Ghanaian populations with estimated ratios of 51%. Further studies on a larger sample size and different ethnic groups could pave the way for proper individualized drug therapy in Ghana.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Alterations in the protein activity and expression can frequently be attributed to genetic variations, of which genetic polymorphisms have been known to be one of the more common causes of genetic variations in humans (Levy *et al.*, 2015). These polymorphisms are known to result in interindividual variations in drug metabolism. Among the genes encoding transporters, metabolizing enzymes, and drug targets, polymorphisms in drug-metabolizing enzymes have the greatest impact on inter-individual differences in drug response (Ingelman-Sundberg *et al.*, 2018).

Studies on mutations associated with many drug metabolising enzymes have helped to provide some explanations for adverse drug reactions (ADRs) as well as therapeutic failures. Cytochrome P450 enzymes (CYPs) are a large group of drug metabolizing enzymes. In humans, CYP2D6 and 3A4 are known to metabolize over 70% of orally administered drugs on the market (Rendic & Guengerich, 2015). Many of these enzymes exhibit polymorphic variations that affect drug metabolism and efficacy. (Crews *et al.*, 2021) This has led to regulatory guidelines emphasizing the importance of characterizing metabolic pathways during drug development (EMA, 2020).

Quite a number of CYPs are polymorphically expressed, and these can be characterized into four polymorphic phenotypes; the poor metabolizers (PM) that lack the functional enzyme, intermediate metabolizers (IM) being heterozygous for a defective allele, extensive metabolizers

(EM) that carry two functional gene copies and ultra rapid metabolizers (UM) that have more than two functional gene copies (Gaedigk *et al.*, 2017).

The study of polymorphisms in drug-metabolizing enzymes serves as one of the foundations for pharmacogenomics, a cornerstone of personalized medicine. Modifying drug dosages and therapeutic combinations based on an individual's genetic makeup is critical for drugs with a narrow therapeutic window (Rostami-Hodjegan & Tucker, 2007; Ingelman-Sundberg *et al.*, 2018). Pharmacogenetics and pharmacovigilance in Africa, despite its large gene pool, is still at its zygotic stage, hence, the need for more influx of data (Matimba *et al.*, 2022).

CYP enzymes, including CYP2D6 and CYP3A4, exhibit extensive polymorphisms. CYP2D6 genetic polymorphisms can lead to variable metabolism of drugs such as antidepressants, opioids, and beta-blockers, impacting their therapeutic efficacy and risk of ADRs (Wang *et al.*, 2022). Similarly, CYP3A4 polymorphisms influence the metabolism of immunosuppressants, anticancer agents, and other widely used drugs (Elens *et al.*, 2020).

Ethnic variations have been shown to play key roles in the distribution of these polymorphisms. The CYP2D6 gene duplications that result in the UM phenotype have been found to be more common and established in African populations, which results in a faster drug metabolism and potential therapeutic failures. (Gaedigk *et al.*, 2017).

Since most African populations have not been systematically characterized for *CYP2D6* allelic variation, many more CYP2D6 alleles are likely to be discovered in these under-represented populations (Wang *et al.*, 2022). This study sought to characterize the genetic polymorphisms occurring in CYPs 2D6 and 3A4 among the Ewe ethnic group in Ghana and predict their corresponding phenotypes and the effects on drug metabolism. This will contribute to the growing body of pharmacogenomic data, ultimately supporting precision medicine initiatives for

underserved populations.

1.2 RATIONALE

The differential expression of these allelic variants of CYP450s vary from one ethnic group to another, hence the need to understand and characterize these allelic variants based on populations (ethnicity) to aid in the provision of optimal drug dosage.

Incidence of a myriad of variant alleles of CYPs lead to different phenotypes which in turn either decrease or increase enzymatic activity. This may result in therapeutic failure, toxicities or other adverse drug effects. Knowledge of individual or ethnic group allelic variants of drug metabolizing enzymes as well as their resulting phenotypes can aid drug developers in providing individual or ethnic group specific optimal dosages to improve therapeutic efficacy in Ghana, since there is very little knowledge about the distribution of CYP alleles in Ghana and the African population.

1.3 HYPOTHESIS

Significant allelic variants of Cytochrome P450s (CYP2D6 and CYP3A4) enzymes do exist among the Ewe population in Ghana.

1.4 AIMS AND OBJECTIVES

1.4.1 AIM

This study aimed at determining the allelic type and frequency of CYP2D6 and 3A4 among the Ewe ethnic group in Ghana and their predicted phenotype.

1.4.2 SPECIFIC OBJECTIVES

- A. To amplify and characterize the *3 and *4 variants of CYP2D6 and the *1B variant of CYP3A4 gene.
- B. To predict the phenotypes of these allelic variants observed in the study.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 STRUCTURE AND REACTION MECHANISM OF CYTOCHROME P450 ENZYMES

Cytochrome P450s (CYPs) involved in xenobiotic transformation are iron-containing microsomal (sometimes mitochondrial) monooxygenases, most of which are found in the liver though they are quite ubiquitous and can be found in virtually all parts of the body. It is worth noting that CYPs have been discovered in numerous organisms including plants, fungi, bacteria and even viruses (Rasmussen *et al.*, 2008; Lamb *et al.*, 2003). The name cytochrome P450s are so named because of their absorptive ability at 450nm when bound to carbon monoxide as reviewed by Esteves *et al.*, (2021).

The heme iron is proximally co-ordinated by a cysteine residue in its thiolate (deprotonated) form, and this ligation state is considered to be an active form of the enzyme. The cysteine thiolate-co-ordinated ferric P450 typically has its major absorbance maximum at 420 nm. They are the largest class of Phase I enzymes and are responsible for metabolism of almost 90% of clinically administered drugs (Esteves *et al.*, 2021).

By 2016, about Fifty-seven (57) CYP genes which involved three main clinically relevant CYP families, had been found in humans. These three families are responsible for metabolizing and detoxifying xenobiotics which includes about 30-40% of clinically administered drugs. These families are CYP1, CYP2 and CYP3 (Arici & Ozhan, 2017). The metabolic activity of these CYP enzymes is influenced by several intrinsic and extrinsic factors such as diet and genetics.

Cytochrome P450s are involved in mostly phase I metabolism of drugs and other xenobiotics through reactions such as hydrolysis, reduction and oxidation. These reactions essentially convert the lipophilic substrates of these CYP enzymes into more polar molecules to allow for easy transport and absorption across the phospholipid bilayer of cell membranes. Action of Phase I enzymes like CYP450s also facilitate easier conjugation from Phase II enzymes which increases the molecular weight of the metabolites, detoxify and increase the hydrophilicity of these reactive metabolites for easier elimination (Stanley, 2017; Rendic and Guengerich, 2015). As shown in figure 2.1, CYPs convert APAP or Acetaminophen (N-acetyl-p-aminophenol) into its metabolite; N-acetyl-p-benzoquinone imine (NAPQI), making a much more suitable substrate for conjugation by Phase II enzymes like UDP-glucuronosyltransferase (UGT), and consequent excretion.

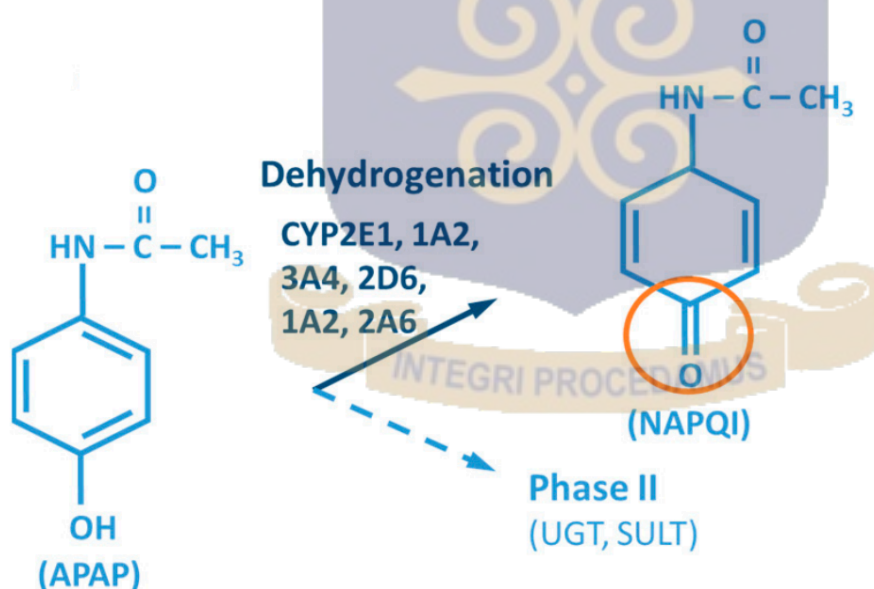
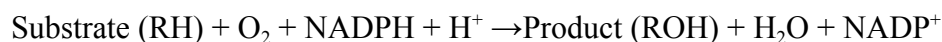


Figure 2.1.1 An example of a CYP mediated biotransformation of a Xenobiotic. This image depicts the dehydrogenation reaction of Acetaminophen that can be metabolised by a large class of CYPs including CYP2E1, 2D6, 3A4, 1A2 and 2A6. These enzymes convert APAP or Acetaminophen (N-acetyl-p-aminophenol) into its metabolite; N-acetyl-p-benzoquinone imine (NAPQI). Adapted from Esteves, Rueff, & Kranendonk (2021).

The basic reaction catalyzed by CYP enzymes as described by Schlichting *et al* (2008) is monooxygenation in which one atom of oxygen is incorporated into a substrate, designated RH, and the other is reduced to water with reducing equivalents derived from NADPH, as follows:



More specifically, Guengerich (2008) reviewed that the metabolic activity of CYP substrates occurs with the help of Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) as a coenzyme for the reaction. The basic reaction catalyzed by cytochrome P450 is monooxygenation in which one atom of oxygen is incorporated into a substrate, designated RH, and the other is reduced to water while the coenzyme NADPH is oxidized to NADP⁺ (Nicotinamide Adenine Dinucleotide Phosphate).

The substrate initially binds to the CYP450 enzyme after which there is a reduction of the CYP heme protein from its Fe³⁺ state into the reduced Fe²⁺. This reduction reaction is facilitated by a single electron transfer from NADPH-CYP reductase. O₂ binds to Fe²⁺ and the Fe²⁺O₂ complex is converted to Fe²⁺OOH complex by the addition of H⁺ and a second electron from NADPH CYP-reductase or cytochrome b₅. A second proton (H⁺) is added to the Fe²⁺OOH complex to form H₂O and (FeO)³⁺. The (FeO)³⁺ complex then transfers its oxygen atom to the substrate thereby returning the CYP enzyme to its original conformation as depicted in figure 2.1.2. If the catalytic cycle is interrupted (uncoupled) following introduction of the first electron, oxygen is released as superoxide anion (see “Superoxide anion production”) (Parkinson & Ogilvie, 2013).

However, there are a small number of P450s that evolved to naturally utilize Hydrogen peroxide (H₂O₂) as the sole oxygen and electron donor to catalyze oxidative reactions through the peroxide shunt pathway. These CYPs are called peroxygenases (Matsunaga *et al*, 2000).

Peroxygenase P450s use the peroxide shunt pathway (B to F) that bypasses the need for external electrons, and interaction with H_2O_2 facilitates direct conversion of the substrate-bound, high-spin P450 to the ferric-hydroperoxo species (Grant *et al*, 2016).

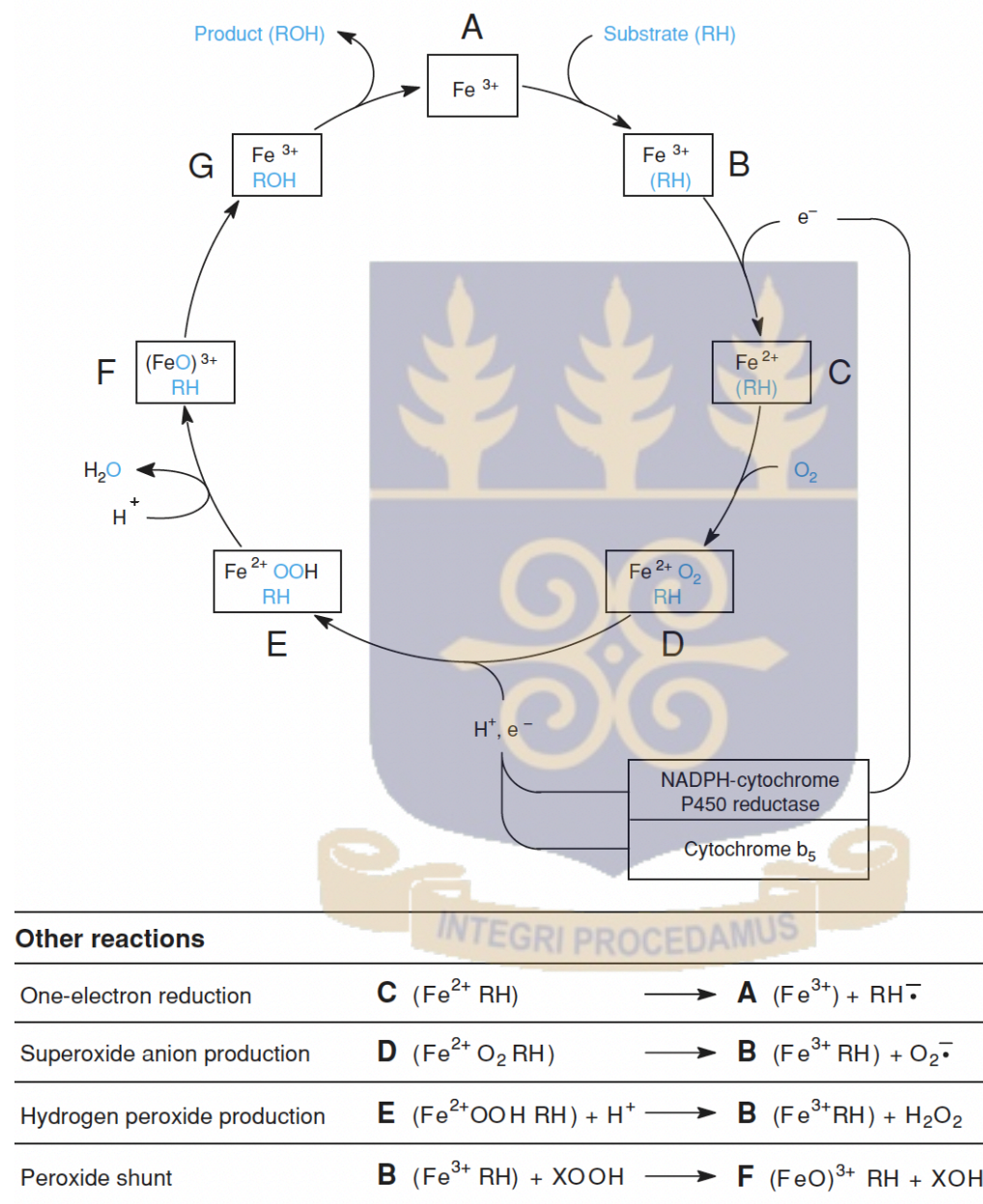


Figure 2.1.2 A detailed depiction of the CYP450 catalytic cycle adapted from Casarett (2008).

2.2 NOMENCLATURE OF CYTOCHROME P450s

The modern nomenclature associated with CYP450s began to take shape in the later part of the 1980s pioneered by Nebert *et al* (1987), where the naming of CYPs is largely based on the amino acid sequence similarity associated with these enzymes. Initially, these families of CYPs were labelled with the roman numerals. In recent times, CYPs are classified into the same family which is denoted by the Arabic numeral (i.e., CYP1, CYP2, CYP3, CYP18, etc...) when they possess more than 40% amino acid sequence similarity. The next sub-family which is denoted by a letter of the English alphabet are enzymes of the same family with $\geq 55\%$ in amino acid sequence similarity e.g., CYP3A. The gene encoding the isoenzyme is then designated by an Arabic numeral (e.g., CYP3A4) while the allelic variant of the isoenzyme is designated by an asterisk (*) followed by an Arabic numeral. By consensus, all wild-types are given the designation *1, hence the wildtype of the CYP3A4 isoenzyme is designated CYP3A4*1 with any other discovered polymorphism (Esteves *et al.*, 2020).

2.3 FACTORS AFFECTING CYP450 METABOLISM

The cytochrome P450 (CYP450) enzyme system is essential for the metabolism of numerous xenobiotics and endogenous compounds. CYP2D6 and CYP3A4 are among the most clinically significant isoenzymes, as they metabolize a broad range of xenobiotics. The activity of these enzymes is altered by a number of biological and chemical factors, which contribute to variability in drug metabolism among individuals and populations.

Drug metabolism is affected by a variety of factors which include genetic polymorphisms, epigenetics and other demographic factors such as age, diet, ethnicity, the presence of gut

microflora and gender. The administered concentrations of orally administered drugs can be reduced by microbes within the alimentary canal by a phenomenon known as first pass elimination. (Wang *et al.*, 2017).

Age plays a crucial role in determining CYP450 activity. Generally, drug sensitivity is heightened among infants and people of old age. Neonates exhibit immature enzyme systems, with enzymes like CYP3A4 reaching functional maturity only after the first year of life. Conversely, elderly individuals often show reduced CYP450 activity due to age-related physiological decline, impacting drug metabolism, induction and excretion. This has led to the formulation of the Beer's list of drugs for old age groups based on their risk benefit-ratio. For instance, the clearance of midazolam, a CYP3A4 substrate, is reduced in older adults (Lin *et al.*, 2016).

Species differences significantly impact CYP450 metabolism, complicating the extrapolation of animal data to humans. Human CYP3A4, for example, shares partial functional similarity with rodent CYP3A isoforms, yet differences in substrate specificity and metabolic capacity persist. These discrepancies necessitate careful consideration in preclinical studies and highlight the limitations of animal models (Khidkhan *et al.*, 2016).

Gender-related variations in CYP450 activity are often influenced by hormonal fluctuations. Studies have shown that females tend to have higher CYP3A4 activity than males, possibly due to the modulatory effects of estrogen. This difference can result in altered pharmacokinetics for drugs like erythromycin and verapamil, both of which are CYP3A4 substrates as reviewed by Zanger & Schwab (2013). Pregnancy further exemplifies the impact of hormonal changes, with increased CYP3A4 activity observed during this physiological state, leading to accelerated

clearance of drugs such as nifedipine and indinavir (Tanino *et al.*, 2019).

Genetic polymorphisms are a critical determinant of CYP450 function. CYP2D6 is highly polymorphic, with over 145 allelic variants identified. These genetic differences categorize individuals into poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), or ultra-rapid metabolizers (UM), profoundly affecting drug response. For instance, poor metabolizers may experience reduced efficacy of prodrugs like codeine, while ultra-rapid metabolizers risk toxicity from standard doses (Gaedigk *et al.*, 2017).

Dietary components can modulate CYP450 activity, leading to drug-diet interactions. Grapefruit juice, a potent inhibitor of CYP3A4, can significantly elevate plasma concentrations of drugs such as simvastatin, increasing the risk of adverse effects. Similarly, St. John's Wort induces CYP3A4, reducing the efficacy of oral contraceptives and immunosuppressants (Tapaninen *et al.*, 2010). The role of gut microbiota in modulating drug metabolism is increasingly recognized, as microbial enzymes can interact with and modulate CYP450 activity. For example, antibiotics that disrupt gut microbiota have been shown to alter the metabolism of drugs like digoxin, a CYP3A4 substrate. Foods like green tea, whose prominence has transcended a host of ethnicities has been implicated in the inhibition of CYP 3A and CYP2D6 enzymes found in the liver and intestinal microsomes among Japanese (Sasaki *et al.*, 2017).

CYPs, like other xenobiotic metabolising enzymes can be induced and inhibited equally. Binding of these inducers to the regulatory elements of these enzymes can upregulate CYP synthesis and activity downstream. The CYP 1A2 is inducible by the anti-protozoan drug, omeprazole as well as certain calciferous vegetables. Omeprazole is co-administered with anti-cancer drugs to reduce the side effect of nausea that is attributed to certain anti-cancer drugs. However, induction

of CYP 1A2 which also metabolizes these anticancer agents by omeprazole has been linked to anti-cancer drug resistance (Chen *et al*, 2017).

Altered physiological states, such as liver diseases like hepatitis or hormonal imbalances, can profoundly affect CYP450 function. In hepatic impairment, reduced enzyme activity often leads to prolonged drug half-life and increased systemic exposure. Similarly, hyperthyroidism has been associated with increased CYP3A4 activity, while hypothyroidism tends to reduce it (Maheshwari *et al.*, 2018).

Induction and inhibition of CYP450 enzymes by xenobiotics are well-documented. Rifampin, a potent inducer of CYP3A4, enhances the metabolism of co-administered drugs, necessitating dose adjustments. Conversely, inhibitors like ketoconazole reduce CYP3A4 activity, increasing the potential for toxicity. Similarly, environmental chemicals such as organophosphates and heavy metals can modulate enzyme activity, either through direct inhibition or by altering gene expression (Abdou *et al.*, 2020).

Chemical properties of substrates also play a role in determining their interaction with CYP450 enzymes. Molecular size and shape influence substrate binding, as seen with CYP2D6, which favours compounds with a basic nitrogen atom and specific spatial arrangements. Lipophilicity enhances the likelihood of interaction with enzymes like CYP3A4, which metabolizes highly lipophilic drugs such as cyclosporine and midazolam (Marsh *et al.*, 1999). The ionization state of a compound, dictated by its pKa, further affects its affinity for enzyme binding sites, with weakly basic drugs often demonstrating higher metabolic rates.

CYP450-mediated drug metabolism, particularly by CYP2D6 and CYP3A4, is a complex interplay of biological and chemical factors. Comprehensive understanding of these variables is

essential for optimizing therapeutic strategies and mitigating adverse effects. Figure 2.3 summarizes the abundance and how the activity of CYP isoforms are either upregulated or downregulated by varying factors where polymorphism has been identified to both upregulate and downregulate CYP2D6 activity.

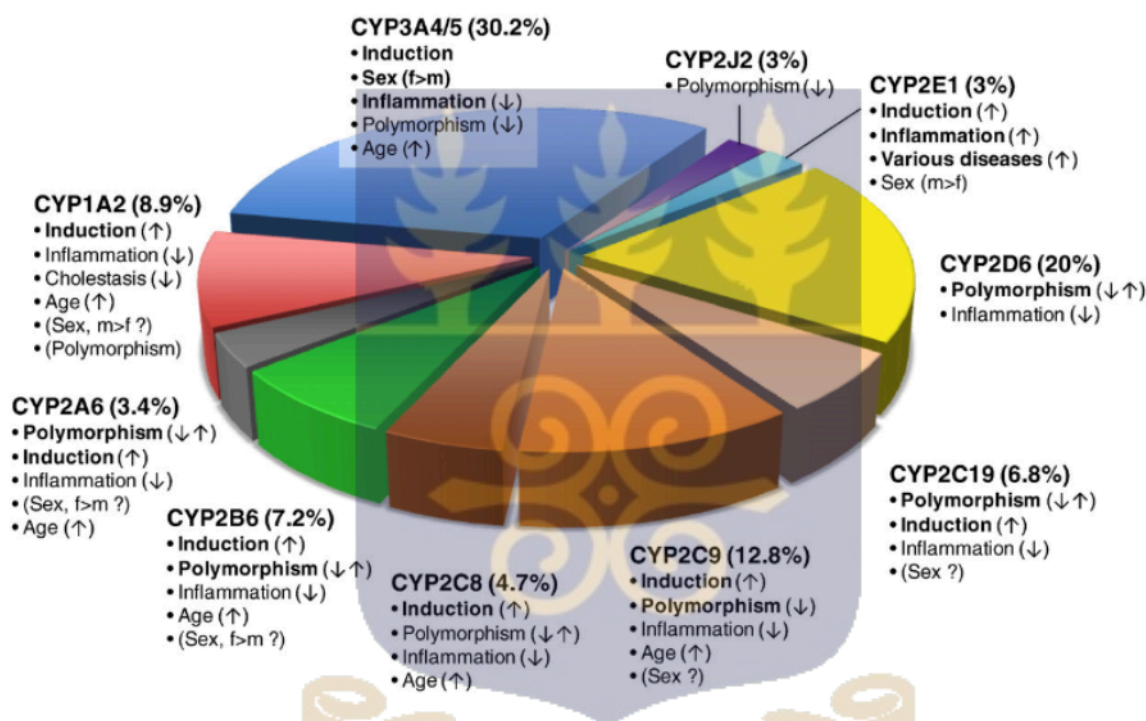


Figure 2.3 Fraction of Clinically used drugs metabolised by CYP isoforms and factors influencing their variability. Adapted from Zanger and Schwab (2013)

2.4 CYP2D6

CYP2D6, is known to play important roles in the metabolism of codeine and about 25% of clinically important drugs such as antidepressant, beta-blockers, opioid analgesics and antihistamines with no known inducer yet its activity is highly variable, primarily due to genetic polymorphisms as reviewed by He *et al.* (2015). The gene which is located on chromosome 22, close to the CYP2D7 and CYP2D8 non-functional pseudogenes spans 4.4 kilobase (kb) pairs and

is known to have more than 145 identified allelic variants and sub-variants. (Alvarado *et al*, 2021; Kimura *et al*, 1989) Among these, the CYP2D6*2, CYP2D6*3 and CYP2D6*4 alleles stand out for their clinical significance and population-specific distributions. CYP2D6*1, CYP2D6*2 and CYP2D6*35 variants lead to normal or increased enzymatic activity while other alleles cause decreased enzyme activity (*9, *10, *17, *21 and *41). The variants *3, *4, *5 and *6 have been known to result in complete loss of function phenotypically (Numanagić *et al.*, 2018). The *3 allele is characterized by a frameshift mutation resulting in a premature stop codon that leads to complete loss of enzymatic activity.

These variants contribute to the four phenotypic categories of CYP2D6 metabolism: poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultra-rapid metabolizers (UMs). Poor metabolizers, who often carry loss-of-function alleles such as CYP2D6*3, experience impaired drug metabolism, resulting in reduced therapeutic efficacy or increased toxicity for certain medications. For instance, CYP2D6 PMs derive little benefit from codeine due to its inadequate conversion to morphine (Gaedigk *et al.*, 2017). Conversely, ultra-rapid metabolizers, frequently associated with CYP2D6 gene duplications of active alleles like CYP2D6*2, are at heightened risk of opioid toxicity (Crews *et al.*, 2021).

The global distribution of CYP2D6 alleles varies significantly across populations, influenced by evolutionary, genetic, and environmental factors. Among Caucasians, the frequency of CYP2D6 PMs is approximately 5-10%, predominantly due to the prevalence of non-functional alleles such as CYP2D6*3 and CYP2D6*4. In contrast, these alleles are relatively rare in Asian populations, where reduced-function alleles like CYP2D6*10 predominate, resulting in a higher prevalence of intermediate metabolizers (Ingelman-Sundberg *et al.*, 2017).

Sub-Saharan African populations display unique CYP2D6 allele distributions, reflecting their rich genetic diversity. Studies highlight a high prevalence of functional alleles such as CYP2D6*1 and CYP2D6*2, alongside notable frequencies of reduced-function alleles like CYP2D6*17. In Ghana, the prevalence of the *2 variant have been reported to occur between 10-30% with similar frequencies found in populations from Uganda and Ethiopia (Miura *et al.*, 2022; Ahmed *et al.*, 2019). The prevalence observed in Southern African and Malagasi populations identified were 4.5% and 6.4% respectively (Dodgen *et al.*, 2013; Mehlotra *et al.*, 2021) while the *3 which translates to the PM phenotype has been reported to exist in less than 1% of study populations. (Twesigomwe *et al.*, 2023; Griese *et al.*, 1999)

The incidence of the CYP2D6 PM genotype varies considerably from one ethnic group to the next: It is 5–7% in Caucasians, 2–4% in Africans and African Americans, and 1–2% in Turkish and Asian (Thai, Chinese, and Japanese) subjects. Similarly, the incidence of the CYP2D6 gene duplication, which gives rise to the CYP2D6 UM genotype, varies among ethnic groups: It is up to 29% in Ethiopians, 20% in Saudi Arabians, 5–10% in Southern European Caucasians, only 1–2% of Northern European Caucasians, 5–10% of Turkish subjects, and up to 2% in African, African American, and Asian subjects. (Koren *et al.*, 2006). In Ghana, Griese *et al.* (1999) highlighted in their study, the high incidence of the CYP2D6*1, *2 and *17 variants with these alleles occurring at frequencies of 43.7, 10.6 and 27.7%, respectively. The prevalence of the no-function CYP2D6*4 allele has been determined to be highest in Ashkenazi Jewish and European subjects and lowest in East Asian subjects, averaging 22%, 18%, and 0.6%, respectively. (Gaedigk *et al.*, 2017).

CYP2D6 PMs are often at increased risk of exaggerated pharmacological responses. Some drugs, such as the analgesic codeine, which is demethylated to morphine and the anticancer drug

tamoxifen (which is hydroxylated and N-demethylated to endoxifen), are converted to pharmacologically active metabolites by CYP2D6. Consequently, CYP2D6 PMs derive less therapeutic benefit than CYP2D6 EMs. In contrast, in the case of codeine, CYP2D6 UMs are at increased risk from morphine toxicity (Gasche *et al.*, 2004). It has also been hypothesized that CYP2D6PMs may have a lower risk of developing lung cancers due to their inability to activate certain carcinogens with the UMs standing a greater risk (Parkinson & Ogilvie, 2013). Quinidine is a known inhibitor of CYP2D6.

The *3 variant occurs because of a Frameshift mutation at position 2549, that leads to a deletion of the Adenine base while the *4 variant (also known as the splicing variant) occurs at position 1846 of exon 4 of the CYP2D6 gene, where a Guanine base is substituted for an Adenine base with both variants leading to reduced enzyme activity as shown in figure 2.4.

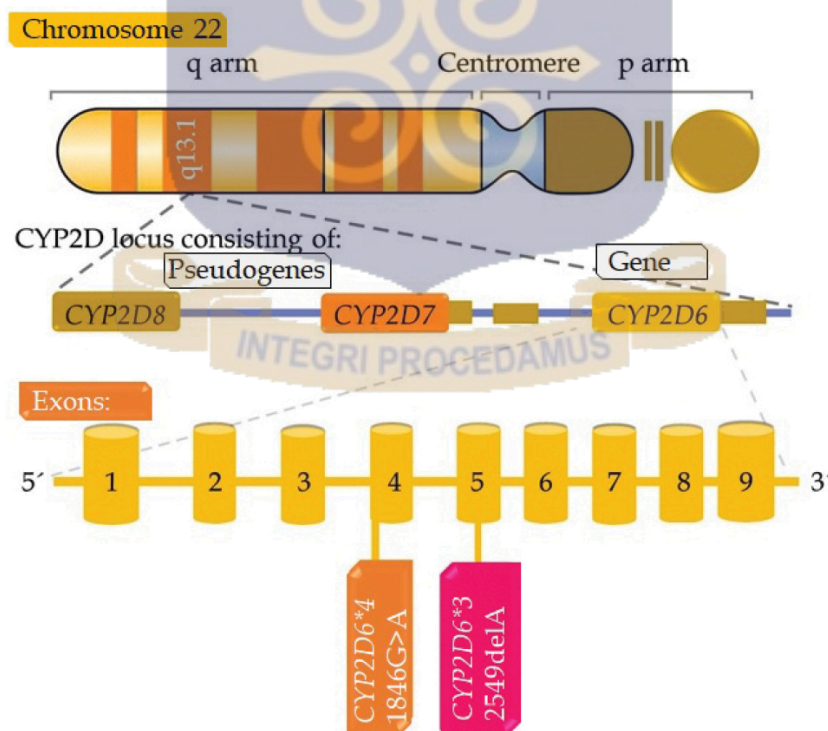


Figure. 2.4 Location of the *3 and *4 polymorphism of the CYP 2D6 genes Adapted from Alvarado *et al* (2021)

2.5 CYP3A4

CYP3A4 is abundantly expressed in the liver with trace amounts in the intestines, prostate and breast. (Parkinson and Oglivie, 2013). It plays a critical role in the metabolism, detoxification, and bioactivation of numerous xenobiotics and endogenous substrates. As one of the most important enzymes in drug metabolism, CYP3A4 handles approximately 50% of clinically used drugs, including acetaminophen, fluconazole, aflatoxin B₁ and tacrolimus. Its ability to metabolize structurally diverse substrates highlights its importance in pharmacokinetics, influencing drug efficacy, safety, and potential interactions (Wilkinson, 2005). For drugs like paracetamol, CYP3A4 contributes to the formation of N-acetyl-p-benzoquinone imine (NAPQI), a reactive and potentially toxic intermediate, particularly when glutathione stores are depleted. This underscores the enzyme's dual role in therapeutic activation and drug-induced toxicity, making it a pivotal factor in the pharmacological outcomes of widely used medications. Inducers like St. John's Wort increase the enzyme's transcription via activation of the pregnane X receptor (PXR), leading to enhanced metabolism of its substrates. This induction has significant clinical implications; for instance, co-administration of St. John's Wort with oral contraceptives, anti-HIV drugs, or cyclosporine has been shown to reduce plasma drug concentrations, often resulting in therapeutic failure (Moore *et al.*, 2000). On the other hand, inhibitors such as ketoconazole and ritonavir competitively or non-competitively block the enzyme's active site, leading to decreased drug clearance and increased systemic drug levels. Furanocoumarins in grapefruit juice, a well-known dietary inhibitor of intestinal CYP3A4, have been implicated in adverse drug reactions due to elevated drug concentrations of substrates like simvastatin, increasing the risk of myopathy and rhabdomyolysis (Guo and Yamazoe, 2004; Siwek, Krupa

and Worón, 2024). Environmental and lifestyle factors further modulate CYP3A4 activity, compounding the variability seen with genetic differences. Diet, exposure to environmental chemicals, and concurrent use of herbal medicines or dietary supplements are known to alter CYP3A4-mediated metabolism. In regions like sub-Saharan Africa, the widespread use of herbal remedies may significantly affect drug metabolism, as many traditional medicines act as inducers or inhibitors of CYP enzymes. Additionally, the burden of comorbidities such as malaria, tuberculosis, and HIV, which are prevalent in these populations, often necessitates polypharmacy. This increases the likelihood of drug-drug interactions mediated by CYP3A4, leading to altered therapeutic outcomes (Appiah-Opong *et al.*, 2018).

The enzyme plays a key role in activating prodrugs like cyclophosphamide and detoxifying potentially harmful compounds, emphasizing its diagnostic significance. As reviewed by Kim *et al.*, (2024), polymorphisms or environmental factors that reduce CYP3A4 activity can lead to drug accumulation, heightening the risk of toxicity, as seen with drugs like tacrolimus and midazolam. Conversely, enhanced CYP3A4 activity may result in subtherapeutic drug levels, contributing to therapeutic failure, particularly in oncology and immunosuppressive regimens (Robles Piadres). The enzyme's relevance extends to adverse drug reactions, where variability in CYP3A4 function influences the risk and severity of side effects, making it a focus for precision medicine approaches.

Drug-drug interactions mediated by CYP3A4 have profound clinical implications. For instance, the co-administration of CYP3A4 substrates with strong inhibitors or inducers often necessitates dosage adjustments to mitigate the risk of toxicity or therapeutic failure. Tacrolimus, a calcineurin inhibitor metabolized by CYP3A4, illustrates the delicate balance required in immunosuppressive therapy; small changes in enzyme activity can lead to graft rejection or

nephrotoxicity. (Robles-Piedras & González-López, 2009). Similarly, interactions between CYP3A4 substrates like midazolam and inhibitors such as itraconazole underscore the importance of understanding enzyme modulation in clinical practice.

The Human CYP3A4 gene is located on chromosome 7 and is about 25 kbp long with 13 exons and 12 introns. CYP3A4*1B variant, associated with a decrease in enzymatic activity of CYP3A4 gene, has been reported between 69% and 82% among Ghanaians (Kudzi *et al.*, 2010). In other populations, the variants that have shown marked decrease in enzymatic activity are *8, *11, *13, *16A, *16B, and *22 (Eiselt *et al.*, 2001).

The CYP3A4*1B variant (a single nucleotide polymorphism, A/G) exists in the nifedipine-specific element (NFSE) in the 5'-flanking region of the CYP3A4 gene, specifically at position -392 in the promoter region as displayed in figure 2.5. Allelic frequencies of 9% have been recorded in Caucasians, 53% in Africans and 0% in Taiwanese. (Rebbeck *et al.*, 1998; Westlind *et al.*, 1999). Sata *et al.*, (2000) as recorded prevalence of 66.7% and 4.2% of the *1B variants in black and white test participants respectively. There have been epidemiological correlations between individuals with the *1B variant and the incidence of prostate cancer (Plummer *et al.* 2003). The allelic frequency of CYP3A4*1B has been reported to fall from 48% in African Americans to non-existent in Asians, Ashkenazi Jewish and Mexican Indians. Despite the high frequency of this allele in African Americans, it was interestingly absent in Africans from North Sahara (Lamba *et al.*, 2002).

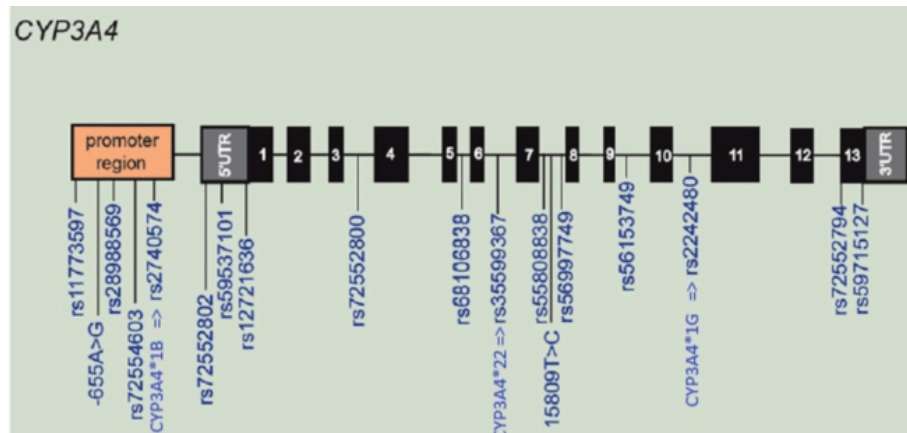


Figure.2.5 Gene structure of CYP3A4. The *1B variant occurs in the promoter region of the gene. Adapted from El-Shair *et al* (2019)

2.6 THE EWE ETHNIC GROUP

The Ewe population is the third-largest ethnic group in Ghana, making up approximately 14% of the country's total population as reported in the 2021 National Census (<https://statsghana.gov.gh/ghfactsheet.php>). They are also the largest ethnic group in Togo, comprising about 30% of the population, with historical and cultural linkages to populations in Benin and Nigeria (<https://www.britannica.com/topic/Ewe-people>). In Ghana, the Ewe people predominantly reside in the central to southern parts of the Volta Region, an area incorporated into Ghana in 1956.

Previous studies have established genetic and phenotypic variations among Ghanaian ethnic groups. For instance, Robertson *et al.* (2024) observed that the Ewe population exhibited significantly greater nasal length and nasal tip protrusion compared to the Akan ethnic group. Similarly, Kudzi *et al.* (2009) characterized polymorphisms in CYP2C8, CYP2C9, and CYP2C19 genes in a Ghanaian population. Their findings revealed that the CYP2C9*11 variant was uniquely detected in the Ewe and Ga ethnic groups, with frequencies of 5% and 3%,

respectively, suggesting subtle genetic differences among these groups. Nevertheless, the study concluded that Ghanaian ethnic groups are largely genetically homogenous. The frequency of the CYP2C9*11 variant was also detected to be 3% in a study by Allabi *et al.* (2003) establishing a genetic similarity with the Ewe population in Ghana.



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

DNA extraction kit and PCR kit were obtained from QIAGEN (QIAGEN Sciences Inc., MD, USA). EDTA Tubes and Safety Lok blood collection kit was obtained from BD Vacutainer®, USA. All restriction endonucleases and primers used in the study were obtained from New England Biolabs, USA.

3.2 STUDY DESIGN

A randomized cross-sectional study was carried out involving two communities in the Volta region: Adidome and Hohoe. The screening for target groups and controls involved using a semi-structured screening questionnaire. Subsequently, those who qualified as participants for the study were retained in the study after they had provided written informed consent. Controls were recruited from the University of Ghana campus and Suhum located in the Greater Accra and Eastern Regions of Ghana respectively. Blood samples (5ml) were collected from each participant by qualified technicians for DNA isolation, sequencing, cloning and characterization of the enzymes. The samples were then transported to the Department of Biochemistry, Cell and Molecular Biology in the University of Ghana, Legon for further studies.

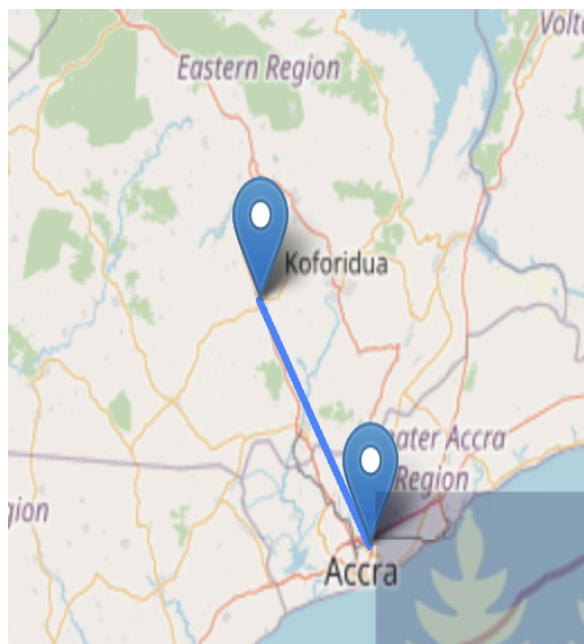


Figure 3.2a. Study sites where control samples were collected

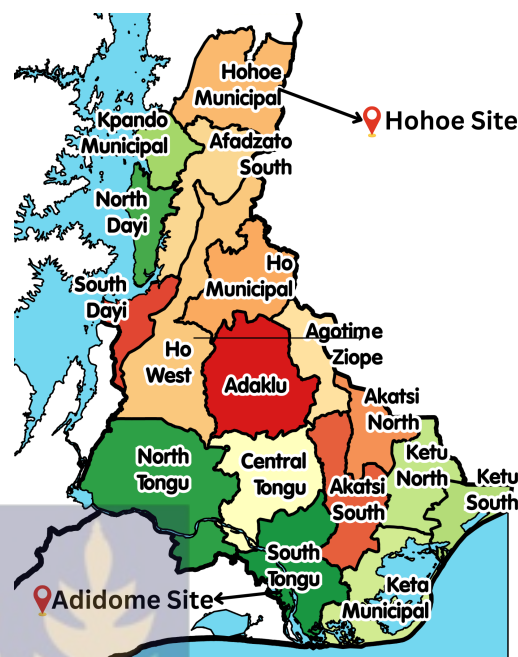


Figure 3.2b Hohoe and Adidome sites for test samples

Figure 3.2 Study sites in the Volta region of Ghana

3.3 COMMUNITY ENTRY

Before commencement of the study the district health directorates as well as principal hospitals in the two study sites namely; Hohoe and Adidome Government Hospitals and Health directorates were visited to discuss the study and seek approval. Upon receipt of approval, a meeting with opinion leaders (on health especially) in the area was sought and the relevance of the study explained to them.

3.4 ETHICAL APPROVAL

Ethical clearance was obtained from the Institutional Review Board (IRB) of Noguchi Memorial Institute for Medical Research at the University of Ghana with clearance number NMIMR-IRB CPN 074/20-21.

3.5 SAMPLE SIZE

Using the formula, $n = [z^2 \cdot p(1-p)] / E^2$, with a z score of 1.96, a prevalence rate of 69% (Kudzi *et al.*, 2010) and a margin of error of 0.05, an expected sample size of 329 was to be screened for the study. However, financial challenges meant that a sample size of one hundred and twenty-four (124) test participants were recruited for this preliminary study, coupled with a control population of thirty (30) participants.

3.6 INCLUSION AND EXCLUSION CRITERIA

3.6.1 INCLUSION CRITERIA

For the persons who provided written consent to participate in the study, only healthy individuals between ages 18-65 years and whose parents and grandparents are Ewes were included in the study. This was determined by oral interviews with participants ascertaining the ethnicity of their parents and grandparents. Participants were considered as Ewes if they had up to two generations being Ewes, that is both paternal and maternal grandparents should be known Ewes. Healthy individuals, aged between 18-65 years, who did not meet the above criteria and did not have any of their previous lineages being Ewes were recruited into the control group. The criteria for selection was pictured in figure 3.6.

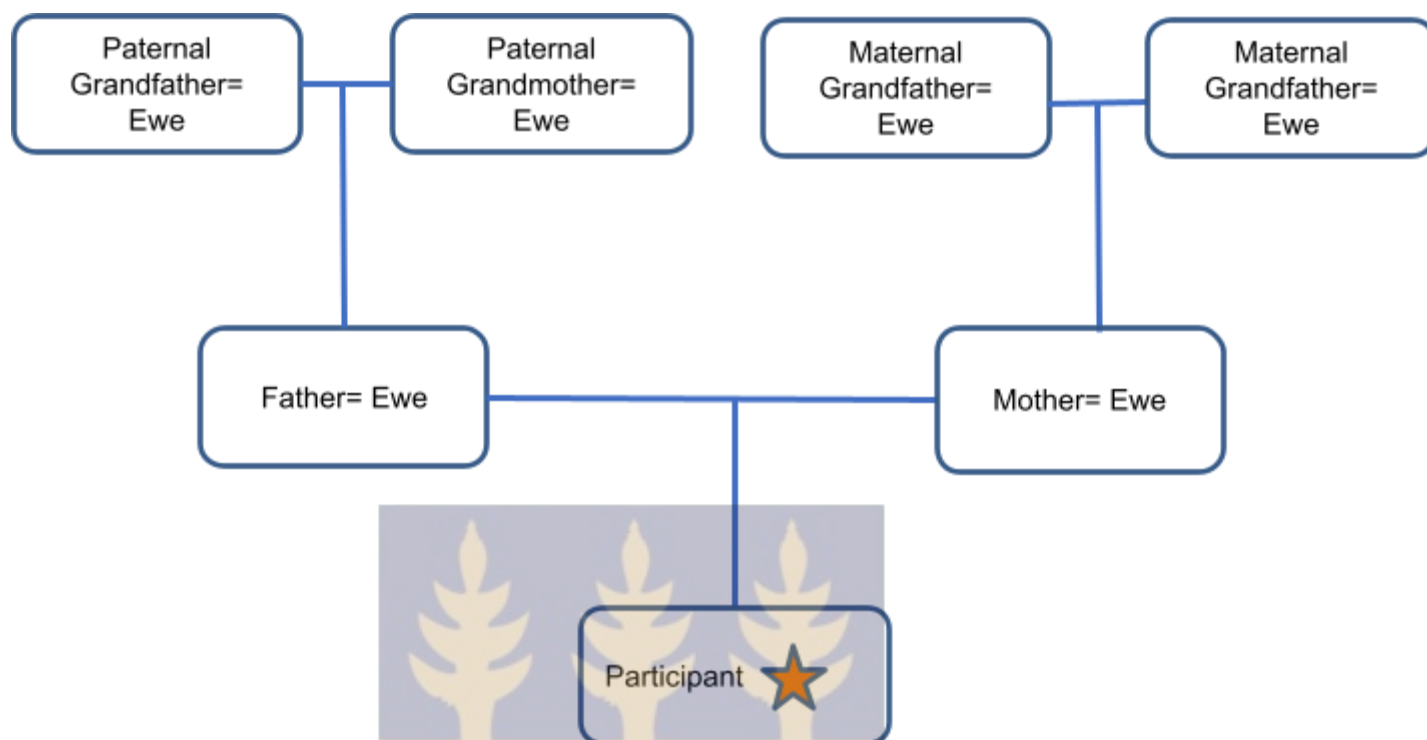


Figure 3.6 Schematic view of Inclusion Criteria for test participants recruited for the study

3.6.2 EXCLUSION CRITERIA

Non-Ewes (except the controls) and sick persons were not included in the study. Individuals below age 18 years and above 65 years were also not included in the study because they comprise a vulnerable group and they may be well represented by their parents or older siblings due to the nature of the study.

3.7 DATA COLLECTION QUESTIONNAIRE

A semi-quantitative questionnaire was administered to the study participants to collect data on their medical history, drugs they regularly use and any form of adverse drug effects. It also contained the gender and ethnic background.

3.8 SAMPLE COLLECTION

Five millilitres (5ml) of venous whole blood samples were obtained from both the control and study participants. The study samples originated from members of the Ewe ethnic group in the

selected areas of Hohoe, Adidome and their surroundings, while the control samples were obtained from the University of Ghana in Accra and Suhum. The blood was collected into EDTA tubes and later spotted on labelled Whatman No. 3 filter papers for archiving.

3.9 EXTRACTION OF DNA FROM WHOLE BLOOD

Prior to genotyping, DNA from all individual participants; both control and test participants were extracted from venous blood samples collected. This was done using the QIAGEN QIAamp® DNA mini and Blood Mini kit according to the manufacturer's protocol. ([DNA extraction protocol from venous blood](#)) The extracted DNA product was then stored at -20°C for further studies. The description of the protocol is as follows:

An aliquot of 20µl of QIAGEN protease was placed into a 1.5ml microcentrifuge tube, this was followed by adding 200µl of the whole blood sample. A volume of 200µl of Buffer AL was added and the mixture vortexed, for 15 seconds followed by incubation on a heating block at 56°C for 10 minutes. The mixture was briefly centrifuged after which 200µl of 96% ethanol was added and further vortexing of the reaction mix done. The mixture was then transferred into a clean 2ml QIAamp mini spin column and centrifuged at 6000 x g for 1 minute. The filtrate was discarded. The spin column was placed on a fresh collection tube and 500µl of Buffer AW1 was added. The new mixture was also spun at 6000 x g for 1 minute and the filtrate was discarded. A further addition of 500µl of buffer AW2 was done and the mixture spun at the speed of 20,000 x g for 3 minutes and the filtrate was discarded. The spin process was repeated for a further 1 minute to discard any remaining volume of buffer AW2. The spin column was then placed in a clean 1.5 ml microcentrifuge tube and 100µl of Buffer AE was added as an elution step. The mixture was incubated at 25°C for 5 minutes and centrifuged at 6000 x g for 1 minute. The eluate contained purified DNA which was then quantified using a Nanodrop technique. The

purified DNA was stored in a -20°C freezer to be used for further studies.

3.10 AMPLIFICATION OF CYP 2D6 AND 3A4 GENES

Primers obtained were reconstituted by adding the respective amounts of distilled water as stated by the manufacturer. A working concentration of 10 μ M primer solution was then prepared by adding 90 μ l of distilled water to 10 μ l of the 100 μ M stock solution. For the Polymerase Chain Reaction (PCR) amplification of the CYP2D6*3 and *4 targets as well as the CYP 3A4*1B target, a modified form of the manufacturer's protocol (Table 1) was adopted (QIAGEN PCR Plus Kit). The reaction was performed in a total reaction volume of 15 μ l with the respective constituents displayed in the table below. A reaction mixture for the negative control was developed with water added to template DNA in the reaction mix.

Table 1. Volumes for PCR amplification of target genes

Reagent	Unit Volume/ μ l
2X Reaction Master Mix	7.5
Forward Primer (10 μ M)	0.3
Reverse Primer (10 μ M)	0.3
Nuclease Free Water	4.4
Template DNA	2.5
TOTAL	15

The amplification process consisted of an initial denaturation step at 95°C for 5 minutes followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 60 seconds, and extension at 72°C for 30 seconds. The final extension step was performed at 68°C for 10 minutes. Details of primer sequences, amplicon sizes and restriction enzymes for the Restriction Digest reaction are shown in Table 2. The resulting amplicons from the conventional PCR

reaction were subjected to 2% agarose gel electrophoresis with 0.3µl of Ethidium bromide and a 1% Tris-Acetate-EDTA (TAE) running buffer at 100 volts for 40 minutes. The resultant separation was then viewed using an A600 Imager.

Table 2. Primers and restriction enzymes used in PCR and Restriction Fragment Length Polymorphism of samples

Enzyme	Forward Primer(s)	Reverse Primer(s)	Restriction Enzyme	Expected PCR Amplicon Size	References
CYP2D6*3	5'-GGC TGG GTC CCA GGT CAT AC 3'	5'-GCT GGG GCC TGA GAC TT 3'	<i>BsaI-HF</i>	201bp	Singh <i>et al</i> (2011)
CYP2D6*4	5'-TGC CGC CTT CGC CAA CCA CT 3'	5' TCG CCC TGC AGA GAC TCC TC 3'	<i>BstNI</i>	309bp	
CYP3A4*1B	5'-GGAAT GAGGACA GCCATAG AGACAAG GGGA-3'	5'CCTTTC AGCTCTG TGT TGCTCTT TGCTG-3'	<i>MboII</i>	385bp	Uckun <i>et al</i> (2018)

3.11 RESTRICTION DIGEST OF PCR AMPLICONS

The amplicons CYP2D6*3 and *4, and CYP3A4*1B, variants were further digested with restriction endonucleases to identify individuals with single nucleotide polymorphisms (SNPs) in these targets and how they translated into their predicted enzyme activity. The digestion was

performed by adding 2µl of the PCR amplicon to a reaction mix of 8µl in volume to form a total volume of 10µl. The reaction mixture contained varying volumes of the restriction enzyme, distilled water and a buffer as displayed in the table below (Table 3).

Table 3. Constituent for Restriction Digestion

Enzyme	Enzyme Volume (µl)	Buffer Volume (µl)	Distilled water (µl)	Amplicon volume (µl)
CYP 2D6*3	0.01	1	6.99	2
CYP 2D6*4	0.03	1	6.97	2
CYP 3A4*1B	0.5	1	6.5	2

3.12 DATA ANALYSIS

Data obtained from the questionnaires were coded and analysed using Microsoft Excel. Descriptive statistical analyses, including frequencies and percentages, were used to summarise demographic characteristics and responses from participants. The restriction digestion results were analysed by comparing the observed variant patterns with established reference variants and the control group. Genotype and allele frequencies were calculated for the identified variants. The distribution of genotypes in the study population was evaluated for compliance with the Hardy-Weinberg equilibrium using the chi-square goodness-of-fit test. The Hardy-Weinberg equation was applied as follows : $p^2 + 2pq + q^2 = 1$. Where **p** = frequency of wildtype allele **q** = frequency of mutant allele. Deviation from equilibrium was assessed using the *Chi-square test* with degree of freedom (df) = 1 and critical value = 3.84 (where p = 0.05). Thus, test critical values of less than 3.84 conform to the Hardy-Weinburg equilibrium.

CHAPTER FOUR

4.0 RESULTS

Cytochrome P450 (CYP) enzymes, particularly CYP2D6 and CYP3A4, are integral to the metabolism of a wide array of drugs, influencing therapeutic efficacy and adverse drug reactions. Genetic polymorphisms in these enzymes significantly affect their enzymatic activity, contributing to interindividual and interethnic variability in drug metabolism. In the Ghanaian population, polymorphisms such as CYP2D6*3, CYP2D6*4, and CYP3A4*1 variants hold clinical relevance due to their high prevalence and functional implications. The CYP2D6*3 variant, resulting from a frameshift mutation that leads to a truncated and nonfunctional enzyme, is associated with poor metabolizer phenotypes, thereby reducing the efficacy of prodrugs like codeine that require bioactivation. Similarly, the CYP2D6*4 allele, a splicing defect caused by a single nucleotide polymorphism, also leads to a loss of enzyme activity. These variants are critical in understanding the therapeutic responses and drug toxicity profiles in the Ghanaian population, where access to pharmacogenetic testing remains limited.

On the other hand, allelic variations of CYP3A4 such as CYP3A41B, which is highly prevalent in sub-Saharan Africa, including Ghana, have been linked to altered enzyme expression and activity. These polymorphisms influence the metabolism of commonly used drugs, including antiretrovirals, antimalarials, and anticancer agents, underscoring the need for population-specific pharmacogenomic research to optimize therapeutic outcomes. The distribution and clinical impact of these variants highlight the genetic diversity within the Ghanaian population and emphasize the necessity of integrating pharmacogenomic data into

clinical decision-making. By tailoring drug therapies based on CYP2D6 and CYP3A4 polymorphisms, there is potential to enhance drug efficacy, minimize adverse drug reactions, and improve overall healthcare outcomes in Ghana and the broader sub-Saharan region.

In this study, we explored the polymorphic variations of CYPs 2D6 and 3A4 among the 124 members of the Ewe ethnic group in Ghana.

4.1 DEMOGRAPHIC DATA OF PARTICIPANTS

For the control group, there was even gender distribution. Out of a total of 30 participants from the control group, 15 participants each were recruited for the male and female population. Among the test participants, 68 females were recruited which constituted 54.4% of the test population, while the male population was 57, which constituted 45.6% of the test population which can be visualized in Table 4.1. Most of the participants who availed themselves for sampling fell between the age ranges of 18-24 years with percentages of 45.2% and 73.3% among the test and control groups, respectively (Table 4).. Eighteen out of the 30 participants for the control group belonged to the Akuapem ethnic group while 20% of the participants belonged to the Ga-Dangme ethnic group (Table 4).. Among the control group, more than 50% of the individuals surveyed were at a tertiary education level which is unsurprising as the University of Ghana served as one of the study sites. For the classification of highest level attained by the test participants, there was an even distribution among those that completed Junior High School (33), those that completed Senior High School (36) and those who had attained tertiary education (37) as shown in Table 4.1. These figures are figuratively higher than the average literacy rate in Ghana which stands at 76.6% according to the Ghana Statistical Service (<https://statsghana.gov.gh/ghfactsheet.php>).

Table 4. Demographic Data of Control and Participant groups in the study

Variable	Control Group(n, %) [Total= 30]	Participant Group (n,%) [Total= 124]
Gender		
Male	15(50%)	68 (54.4%)
Female	15(50%)	57(45.6%)
Age Range		
18-24	22 (73.3%)	56(45.2%)
25-29	3(10.0%)	17(13.7%)
30-39	1 (3.3%)	20 (16.1%)
40-49	2 (6.7%)	19 (15.3%)
50-59	1(3.3%)	5 (4.0%)
60-65	1(3.3%)	3 (2.4%)
Ethnicity		
Akuapem	18 (60%)	0
Ashanti	1 (3.3%)	0
Bono	1 (3.3%)	0
Dagaare	1 (3.3%)	0
Fante	1 (3.3%)	0
Ga-Adangme	6 (20%)	0
Guan	1 (3.3%)	0
Ewe	0	124
Highest Level of Education		
None	3(10.0%)	14 (11.3%)
Primary/Basic Education	2(6.7%)	4 (3.2%)
Junior High	3(10.0%)	33 (26.6%)
Senior High	4 (13.3%)	36 (29.0%)
Tertiary	18 (60.0%)	37 (29.9%)

4.2 PCR AMPLIFICATION OF CYP2D6*3, CYP2D6*4 AND CYP3A4*1B TARGET SITES.

After extracting and purifying DNA from venous blood, specific amplicons for the three CYP isoenzymes were amplified using Polymerase Chain Reaction (PCR) to enable effective genotyping with restriction endonucleases. The resulting amplicons were separated on a 2% agarose gel stained with ethidium bromide. Figure 4.2.1 shows a representative gel illustrating the band patterns of the amplicons generated after amplifying the CYP2D6*3 target site. The expected band size, with a molecular weight of 201 bp, aligns with findings by Singh *et al.* (2011), confirming the effectiveness of the PCR and the selected primers.

Similarly, Figures 4.2.2 and 4.2.3 present representative gels from the amplification of CYP2D6*4 and CYP3A4*1B target sites, respectively. For CYP2D6*4, the amplicons displayed the expected molecular weight of 309 bp, while the amplification of CYP3A4*1B yielded bands of the expected molecular weight of 385 bp, as shown in Figure 4.2.3. These results confirm the successful amplification of the target sites in each case with similar results observed in studies by Singh *et al.* (2011) and Uçkun *et al.* (2018).

For all amplifications, a 100 bp molecular weight marker (MW) was used as a reference. Distilled water was included in place of template DNA as a negative control (NC) in the reaction mix. As expected, no bands were observed in the NC lanes, as demonstrated in Figures 4.2.1 and 4.2.3, further validating the reliability of the amplification process.

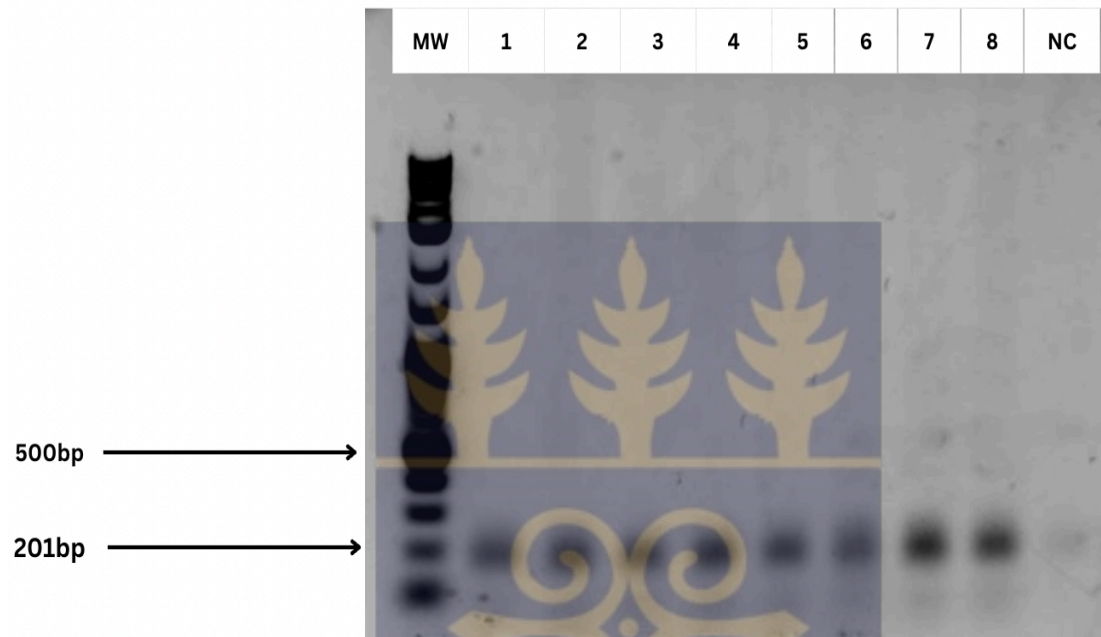


*Figure 4.2.1 Amplification of CYP2D6*3 target site. The resultant amplicon produced has a molecular weight of 201bp which correlates with studies done by Singh et al. (2011).*

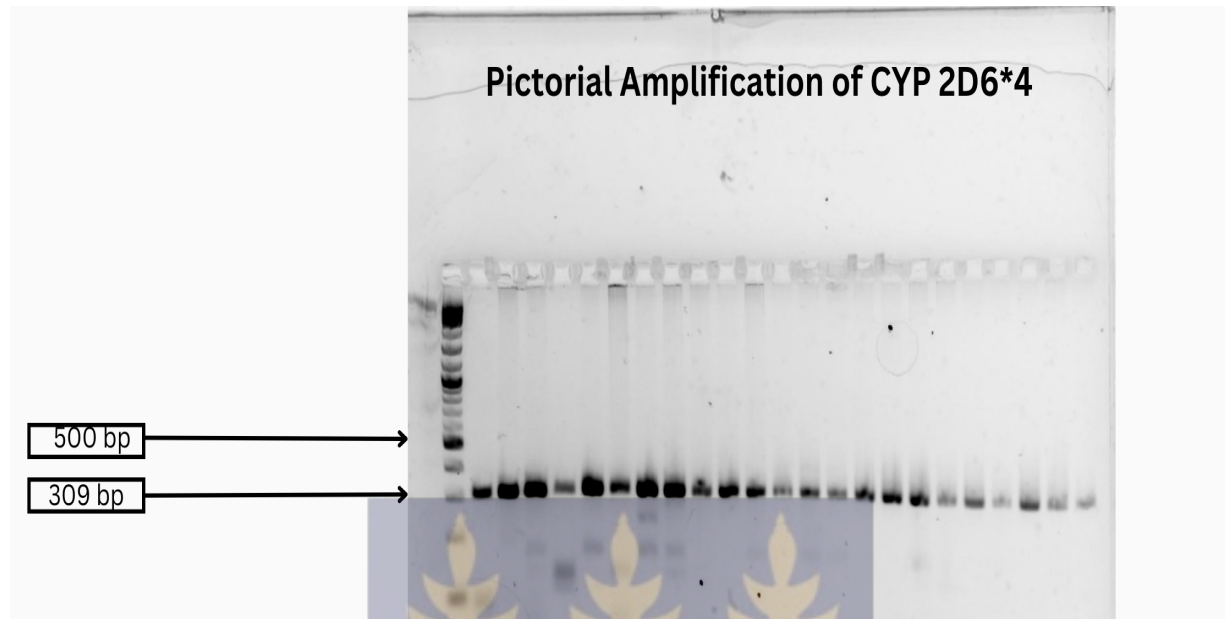


*Figure 4.2.2 Amplification of CYP2D6*4 target site. The resultant amplicon produced has a molecular weight of 309bp which correlates with studies done by Singh et al. (2011).*

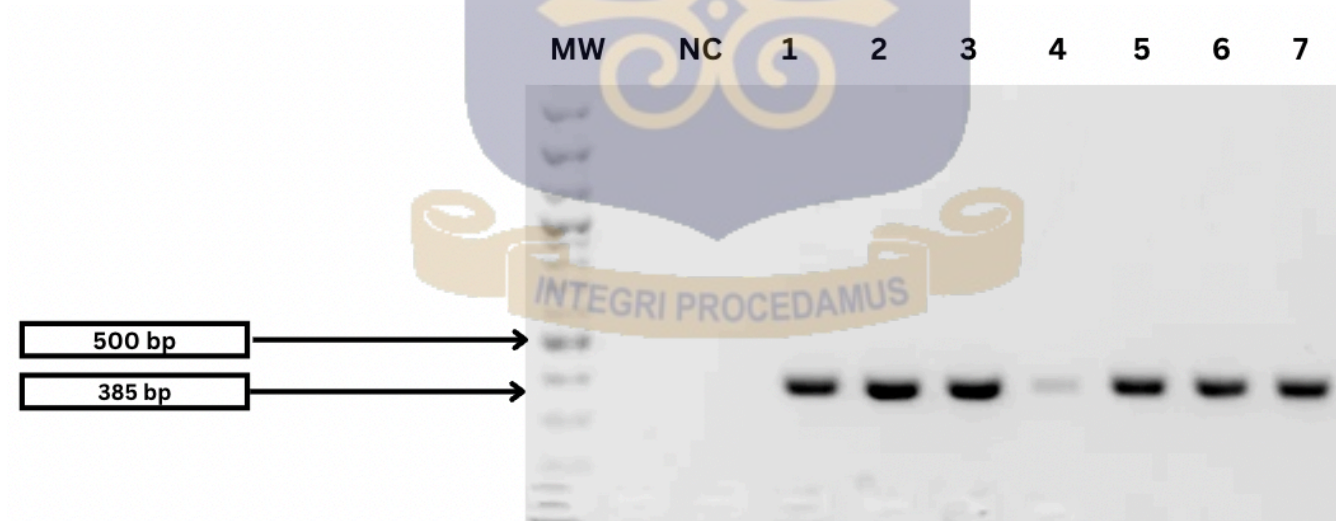


*Figure 4.2.3 Amplification of CYP3A4*1B target site. The resultant amplicon produced has a molecular weight of 385bp.*

4.3 RESTRICTION DIGEST OF AMPLICONS OF CYP2D6*3, CYP2D6*4 AND CYP3A4*1B TARGET SITES.

The CYP2D6*3 mutation involves the deletion of an adenine base at position 2549 of the gene. The amplicon for this target site has a molecular weight of 201 bp. Upon digestion with the *BsaI-HF* endonuclease, distinct banding patterns are observed based on an agarose gel after electrophoresis. In the case of the homozygous wildtype allele (wt/wt), no fragmentation occurs, and the amplicon retains its size of 201 bp. For the heterozygous allele (wt/mt), digestion produces three fragments with molecular weights of 201 bp, 180 bp, and 21 bp. The homozygous mutant allele (mt/mt) produces two fragments with molecular weights of 180 bp and 21 bp. However, these smaller fragments are not clearly visible under UV visualization on an agarose gel, as shown in Figure 4.3.1. The well labelled M, represents the molecular weight marker.

The CYP2D6*4 mutation results from a single nucleotide substitution of adenine for guanine at position 1846 (G1846A). The amplicon for this target site has a molecular weight of 309 bp. Upon digestion with the *BstNI* endonuclease, the genotype-specific patterns are as follows. For the homozygous wildtype allele (wt/wt), digestion produces two fragments with molecular weights of 201 bp and 108 bp, which are visible in lanes 1-4, 6, 8 and 10 in Figure 4.3.2. The heterozygous allele (wt/mt) produces three fragments with molecular weights of 309 bp, 201 bp, and 108 bp. In contrast, the homozygous mutant allele (mt/mt) undergoes no fragmentation, and the amplicon retains its size of 309 bp, as seen in lane 9 of Figure 4.3.2.

The CYP3A4*1B mutation involves the deletion of an adenine base at position -259 of the gene. The amplicon for this target site has a molecular weight of 385 bp. Upon digestion with the *MboII* endonuclease, different genotypes show distinct patterns. The homozygous wildtype allele

(wt/wt) exhibits fragments of 175, 169, and 41 bp in lanes 3-6. The heterozygous allele (wt/mt) produces three fragments with molecular weights of 210 bp, 175 bp, and 169 bp (lane 2). For the homozygous mutant allele (mt/mt), digestion generates two fragments with molecular weights of 210 bp and 175 bp (lane 1). The smallest fragment of 41bp is not visible under UV light, however this is not relevant for in genotyping the CYP3A4 isoenzyme. This is illustrated in Figure 4.3.3.

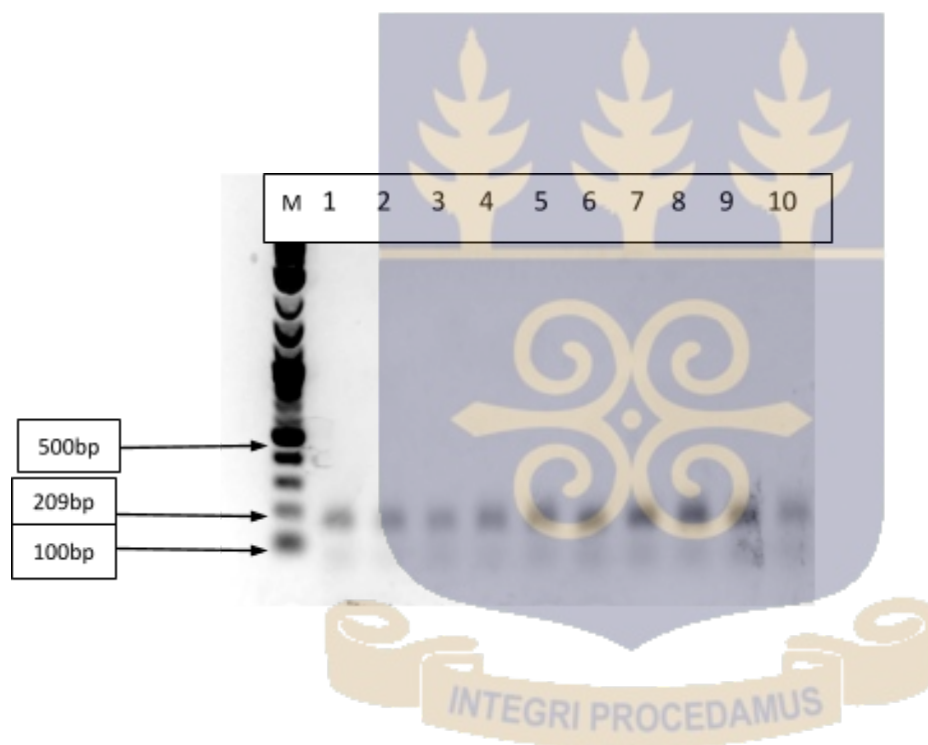


Figure. 4.3.1 Digestion of 201bp amplicon generated from the amplification of the CYP2D6*3 target.

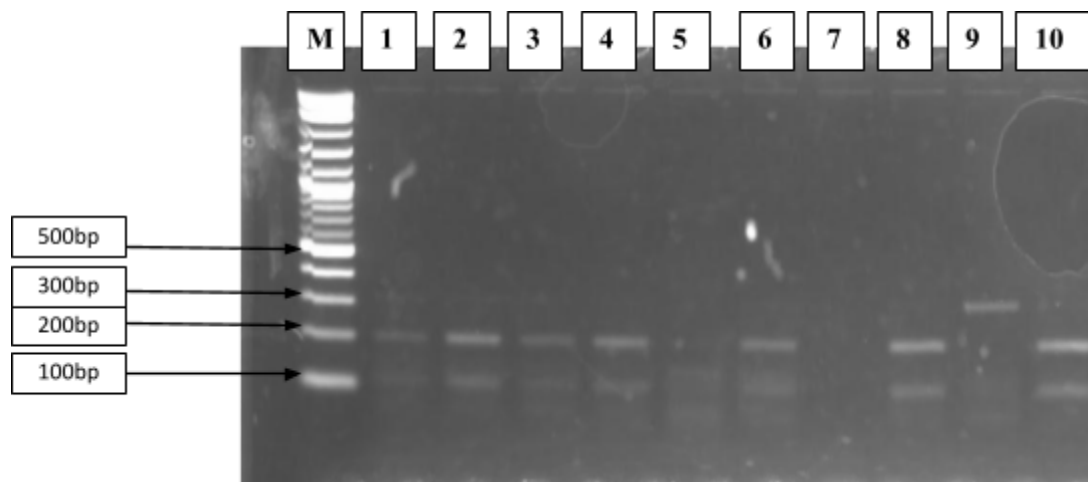


*Figure. 4.3.2 Digestion of 309 bp amplicon generated from the amplification of the CYP2D6*4 target.*

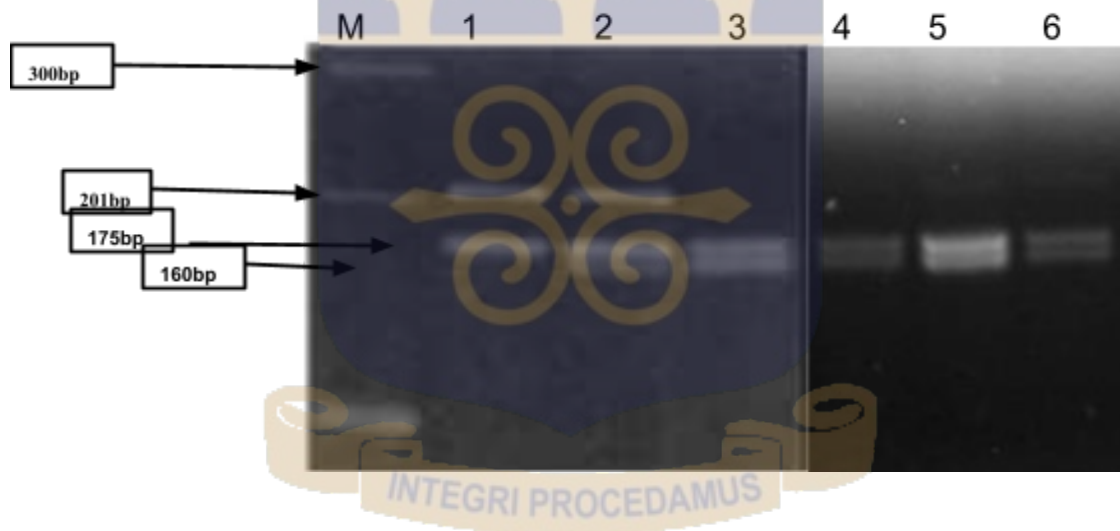


*Figure. 4.3.3 Digestion of 385bp amplicon generated from the amplification of the CYP3A4*1B target.*

4.4 DISTRIBUTION OF CYP2D6*3, CYP2D6*4 AND CYP3A4*1B ALLELIC VARIANTS AMONG STUDY AND CONTROL PARTICIPANTS

This data was curated from the qualitative analysis of banding patterns formed from the Agarose gel electrophoresis run after digesting amplicons of the target area. For the CYP2D6*3 variant, analysis of the 30 control samples revealed that all of them (100%) presented the homozygous wildtype allele, with no heterozygous or homozygous mutant alleles observed. Among the 124 samples from the Ewe group, only 1 individual (0.81%) was identified as possessing the homozygous mutant allele, while no heterozygous alleles were observed (Figure 4.4.1).

For the CYP2D6*4 variant, analysis of the 30 control samples indicated that 18 individuals (60%) possessed the homozygous wildtype allele, 9 individuals (30%) exhibited the heterozygous allele, and 3 individuals (10%) presented the homozygous mutant allele. Among the 124 test participants, 98 individuals (79%) were identified as having the homozygous wildtype allele. Additionally, 10 individuals (8%) presented the heterozygous allele, and 11 individuals (9%) were found to have the homozygous mutant allele. The identification of the alleles for 5 participants (4%) was inconclusive, as observed in Figure 4.4.2.

For the CYP3A4*1B variant, the analysis of the 30 control samples showed that 19 individuals (63.3%) presented the homozygous wildtype allele, 3 individuals (10%) exhibited the heterozygous allele, and 8 individuals (26.7%) were identified as having the homozygous mutant allele. Among the 124 samples from the Ewe group, 3 individuals (2.42%) were found to possess the homozygous mutant allele, while the remaining 121 individuals (97.58%) were identified as having the homozygous wildtype allele. No heterozygous alleles were observed in the Ewe group for this variant, as shown in Figure 4.3.3.

The genotype distributions of the studied variants were evaluated for conformity with the Hardy-Weinberg equilibrium using the Chi-square test. This analysis is commonly applied in population genetics studies to determine whether observed genotype frequencies correspond to those expected under conditions of random mating and absence of evolutionary pressures.

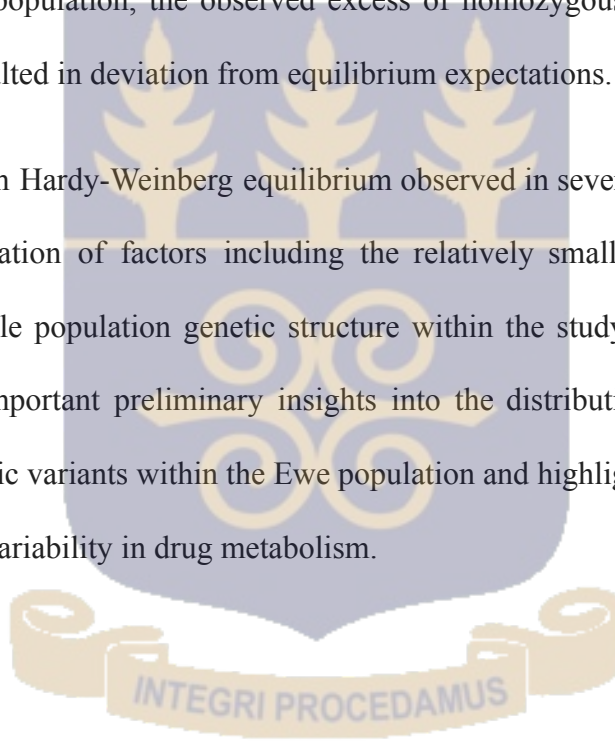
For the CYP2D6*3 variant, the genotype distribution among the Ewe population deviated significantly from Hardy-Weinberg equilibrium. This deviation was primarily attributable to the extremely low frequency of the mutant allele and the absence of heterozygous genotypes within the study population. The presence of only a single homozygous mutant individual and no heterozygous carriers suggests that the variant occurs at a very low frequency within this population. Similar observations have been reported in several African populations, where the CYP2D6*3 allele has been shown to be relatively rare compared with other CYP2D6 variants. In contrast, the control population showed no variation in genotype distribution, as all individuals possessed the homozygous wildtype allele. Although this technically conforms to equilibrium expectations, meaningful statistical evaluation is limited in situations where no allelic variation exists.

For the CYP2D6*4 variant, the control population was found to conform to Hardy-Weinberg equilibrium, suggesting that the observed genotype distribution in this group is consistent with expected allele frequencies under random mating conditions. However, the genotype distribution among the Ewe population deviated significantly from equilibrium expectations. This deviation may reflect the relatively small sample size used in the present study compared with the initially calculated sample size of 329 individuals. Reduced sample size can increase sampling error and may lead to distortion of genotype frequencies. Additionally, the observed deficit of heterozygous individuals relative to the expected frequency could indicate possible population

substructure or selective pressures affecting this allele within the population.

Similarly, the CYP3A4*1B variant demonstrated significant deviation from Hardy-Weinberg equilibrium in both the control and Ewe populations. In the Ewe population, the absence of heterozygous individuals despite the presence of homozygous mutant alleles contributed substantially to this deviation. Such patterns may arise from sampling limitations, population stratification, or potential genotyping inaccuracies associated with restriction digestion-based assays. In the control population, the observed excess of homozygous genotypes and reduced heterozygosity also resulted in deviation from equilibrium expectations.

Overall, deviations from Hardy-Weinberg equilibrium observed in several of the variants may be attributed to a combination of factors including the relatively small sample size, low allele frequencies, and possible population genetic structure within the study groups. These findings nevertheless provide important preliminary insights into the distribution of clinically relevant cytochrome P450 genetic variants within the Ewe population and highlight potential implications for pharmacogenomic variability in drug metabolism.



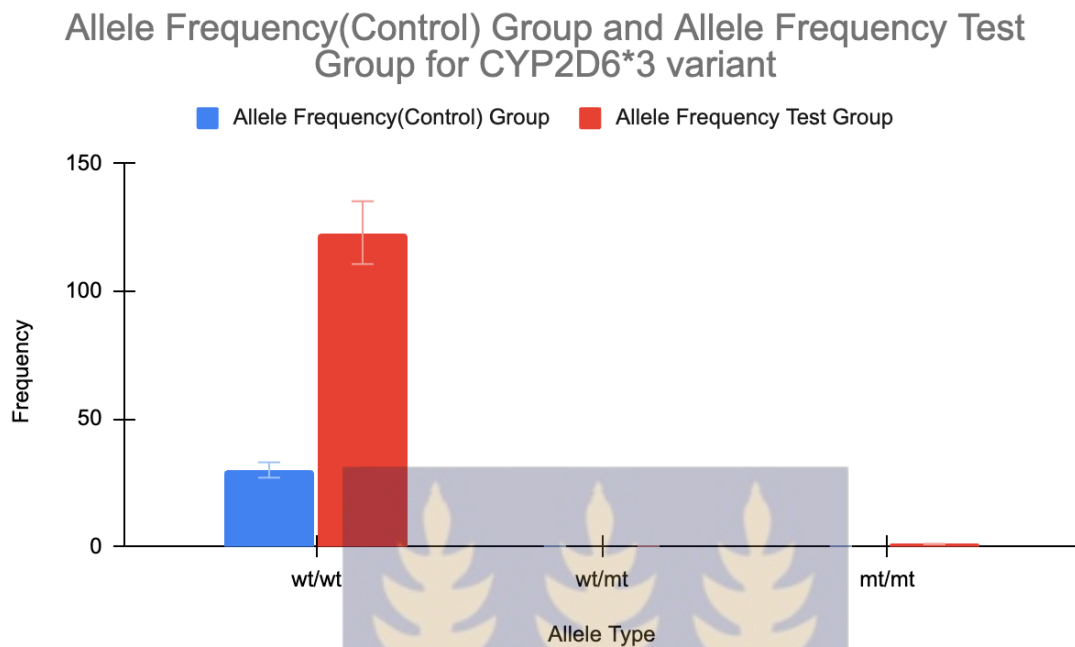


Figure 4.4.1 Allelic Frequency of CYP2D6*3 variant among the Ewe population in Ghana as compared to the non-Ewe population.

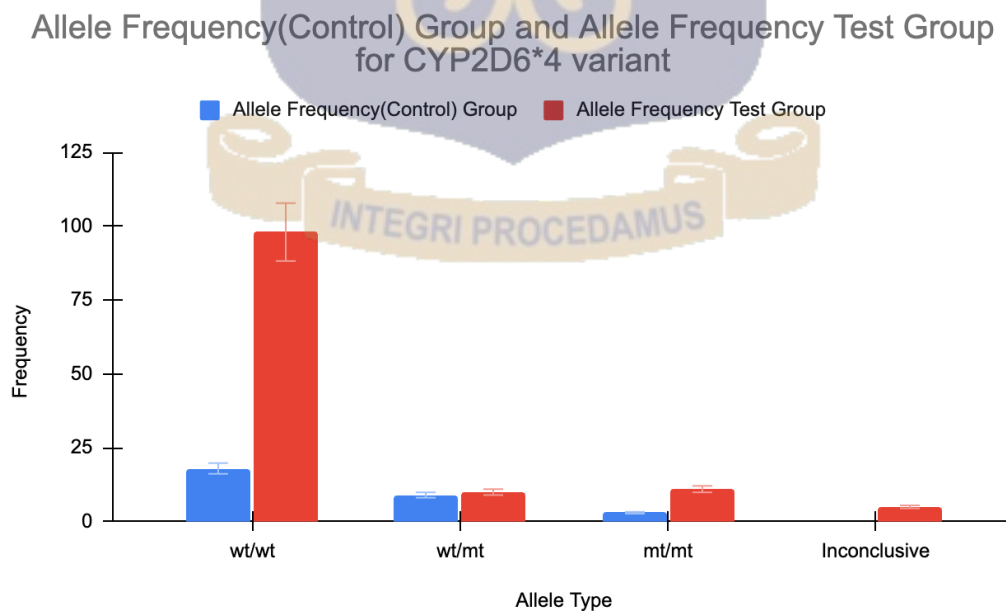


Figure 4.4.2 Allelic frequency of CYP2D6*4 variant of Ewe population against non-Ewes.

Allele Frequency(Control) Group and Allele Frequency Test Group
for CYP 3A4*1B variant

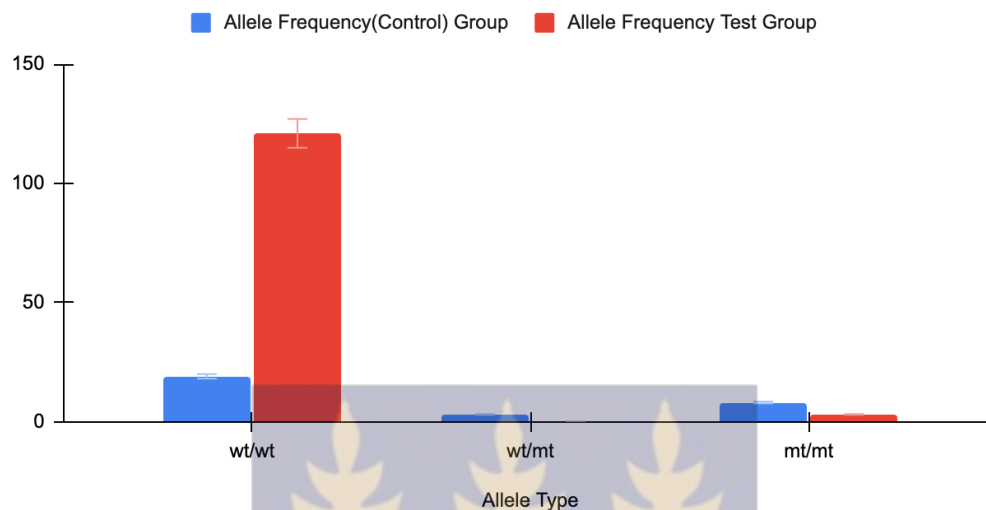


*Figure 4.4.3 Allelic Frequency of CYP3A4*1B variant among the Ewe population in Ghana as compared to the non-Ewe population.*

Table 5. Hardy-Weinburg Equilibrium status and distribution of alleles in participants

Variant	Population	(Chi square Test χ^2)	Hardy-Weinburg Equilibrium Status
CYP2D6*3	Ewe	125	Not in equilibrium
CYP2D6*3	Control	0	Not applicable
CYP2D6*4	Ewe	48.72	Not in equilibrium
CYP2D6*4	Control	1.2	In equilibrium
CYP3A4*1B	Ewe	123.3	Not in equilibrium
CYP3A4*1B	Control	17.8	Not in equilibrium

4.5 TRANSLATED CYP PHENOTYPES OF OBSERVED GENOTYPES AMONG THE EWE ETHNIC GROUP IN GHANA AND CONTROLS

There are four main phenotypic classifications of cytochrome P450 (CYP) enzymes. The first

group, poor metabolizers (PM), arises from individuals carrying two defective (homozygous mutant) alleles or deletions, leading to significantly decreased enzymatic activity. The second group, intermediate metabolizers (IM), includes individuals with one functional allele and one defective or deleted allele. Genotypically, these individuals are heterozygous. The third group, extensive metabolizers (EM), represents the normal and most common phenotype, characterized by individuals with two functional alleles (homozygous wildtype). Finally, the ultrarapid metabolizers (UM) phenotype is observed in individuals with duplicated or multiple copies of the functional allele, resulting in increased enzymatic activity.

As shown in Figure 4.5.1, most of the participants sampled were classified as extensive metabolizers (EM), with prevalence rates of 99%, 79%, and 98% among the CYP2D6*3, CYP2D6*4, and CYP3A4*1B isoenzymes, respectively. The poor metabolizer (PM) phenotype was observed in approximately 1% of the test participants for the CYP2D6*3 and CYP3A4*1B variants. In contrast, the PM phenotype was more frequent in the CYP2D6*4 variant, accounting for 8.8% of the test population. Additionally, 8% of participants with the CYP2D6*4 variant were classified as intermediate metabolizers (IM).

In comparison to the test population, the control group displayed some variations in phenotypic distributions. Among the control samples, 60% and 63% of individuals were classified as EMs for the CYP2D6 and CYP3A4 isoenzymes, respectively. The PM phenotype was identified in 10% of the control group for the CYP2D6*3 variant and 26.7% for the CYP3A4*1B variant. Intermediate metabolizers were observed in 30% of the control group for the CYP2D6*4 variant and 10% for the CYP3A4*1B variant. Notably, no IMs or PMs were observed in the control group for the CYP2D6*3 variant, as illustrated in Figure 4.5.2.

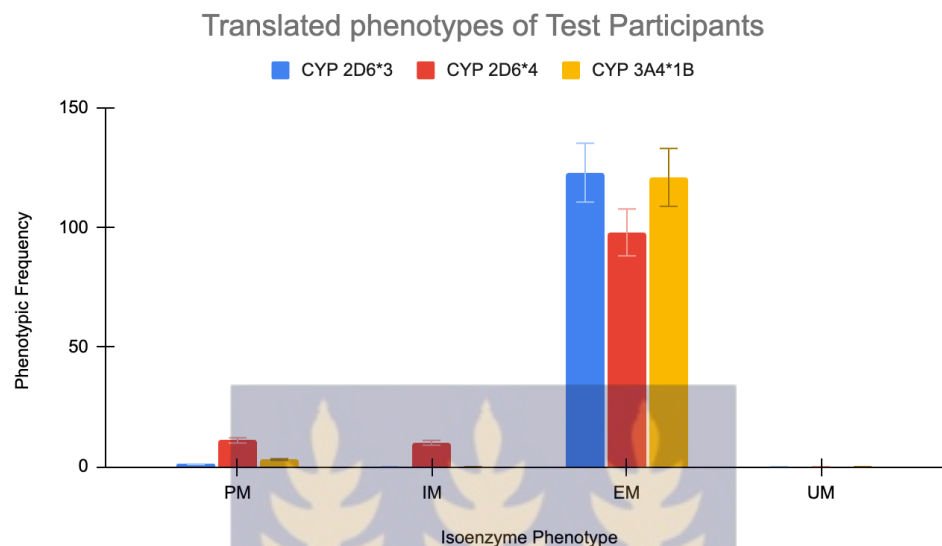


Figure 4.5.1 Phenotypic frequency of CYP2D6*3, CYP2D6*4 and CYP3A4*1B among the Ewe ethnic group

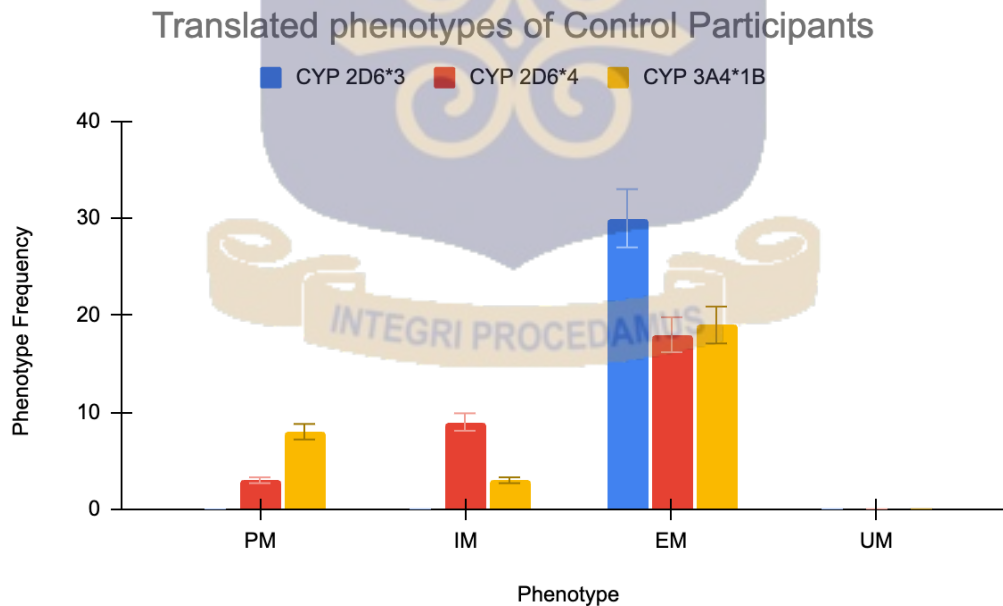


Figure 4.5.2 Phenotypic Frequency of CYP2D6*3, CYP2D6*4 and CYP3A4*1B among the Ewe ethnic group

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND & RECOMMENDATIONS

5.1 DISCUSSION

Cytochrome P450 (CYP) enzymes are responsible for approximately 90% of phase I metabolism of clinically used drugs. Among these, a significant fraction of reactions is catalyzed by five key enzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Notably, CYP3A enzymes account for the largest proportion of metabolic reactions, participating in 13% of general chemical metabolism and 27% of drug metabolism (Rendic and Guengerich, 2015). CYP3A4 metabolizes 22% of marketed drugs and 29% of those under development, whereas CYP2D6 is involved in 14% and 12% of these drugs, respectively (Rendic and Guengerich, 2015).

Individual differences in drug metabolism are largely influenced by variations in CYP enzyme expression, particularly in the liver and intestines (Puszek *et al.*, 2021). External factors such as diet, prior drug exposure, and the use of tobacco or alcohol also affect CYP expression and activity (Sathyanarayanan, Haapala, & Sikanen, 2020). Understanding these enzymes is vital for advancing pharmacogenomics and achieving personalized medicine. This study focused on investigating the genetic polymorphisms of CYP2D6 and CYP3A4 among the Ewe ethnic group in Ghana, specifically the CYP2D6*3, CYP2D6*4, and CYP3A4*1B variants.

Recruiting participants presented challenges, as the inclusion criteria required individuals with Ewe ancestry over three generations. This proved difficult due to interethnic marriages and migration within Ghana. Participants were predominantly aged 18–24, consistent with the

demographic of voluntary blood donors in Ghana (Doku *et al.*, 2022). Most participants (84.5%) had formal education beyond basic education (primary) level, while only 10% reported no formal education.

The advent of the COVID-19 pandemic and the misinformation surrounding vaccination against it served as a major public health obstacle when it came to the area of community penetration and sample collection in the study sites. Obtaining samples from the control population on the other hand did not prove much of an issue as most of the participants were students at the University of Ghana and hence, it was easier to engage them and properly explain the long-term impact this foundational study would have on personalized medicine and Global healthcare delivery. This same reason could account for the equal balance in gender between the samples collected from the control group (15:15). The gender distribution in the test population had the proportion of female to male being 54:46 percentage wise. Factors that could account for the possible variation is the reluctance of the male population to willingly submit themselves to any form of health screening with any major incentives (Figure 4.1.1)

Polymerase chain reaction (PCR) successfully amplified target regions containing the polymorphisms. Amplicons of 201 bp (CYP2D6*3), 309 bp (CYP2D6*4), and 385 bp (CYP3A4*1B) were observed, consistent with previous studies (Singh *et al.*, 2011; Uçkun *et al.*, 2018). Restriction digestion of these PCR products revealed genetic diversity in the Ewe population, contributing valuable data to pharmacogenomics research.

CYP2D6, a crucial enzyme, metabolizes approximately 25% of clinically used drugs, including antidepressants, antipsychotics, beta-blockers, and tamoxifen. Between 40–70% of drug recipients exhibit atypical responses, often due to polymorphisms in genes encoding

drug-metabolizing enzymes (Lauschke & Ingelman-Sundberg, 2016; Verbeurgt, Mamiya, & Oesterheld, 2014). CYP2D6, the most polymorphic of the CYP enzymes, includes non-functional variants such as CYP2D6*3 (2549delA) and CYP2D6*4 (1846G>A), which contributes to the poor metabolizer (PM) phenotype (<https://www.pharmvar.org/gene/CYP2D6>; Byeon et al., 2018).

The CYP2D6*3 variant accounts for almost 2% of PM phenotypes globally (Aklillu *et al.*, 2003). Among Asians, CYP2D6*3 is almost absent, and CYP2D6*4 occurs at frequencies below 1%, resulting in a PM phenotype in less than 1% of individuals (Saghafi *et al.*, 2018). The relatively low PM phenotype in Asians has been identified to play a role in the reduced efficacy of tamoxifen therapy for breast cancer patients (Zembutsu *et al.*, 2017). In this study, CYP2D6*3 was absent in controls, while only 0.8% of the Ewe participants carried the homozygous mutant allele, aligning with previous findings in Sub-Saharan Africa (Aklillu *et al.*, 2003; Dandara *et al.*, 2001). The extremely low frequency of the CYP2D6*3 variant observed in the present study is consistent with previous reports indicating that this allele occurs at relatively low frequencies in African populations. Studies have reported CYP2D6*3 frequencies ranging from 0–9% across African populations, suggesting that the variant is generally rare but may exhibit regional variability across the continent. The distribution of the CYP2D6*4 variant observed in this study is comparable to previously reported data showing moderate frequencies of this allele in several populations worldwide. Global pharmacogenetic studies indicate that the CYP2D6*4 allele occurs at frequencies of approximately 11–15% in many populations, although lower frequencies have been reported in some African populations compared with European populations where the allele is more common. The mutant CYP2D6*4 allele was observed in 10% of controls and 8.8% of the Ewe participants, consistent with data indicating higher frequencies of CYP2D6*4 than

CYP2D6*3 in African populations (Gaedigk *et al.*, 2002; Thomford *et al.*, 2024).

CYP2D6 activity influences pain management by converting drugs such as codeine and tramadol into their active forms, morphine and O-desmethyltramadol, respectively (Reizine *et al.*, 2021). Variations in CYP2D6, particularly the PM phenotype, significantly affect therapeutic outcomes, often necessitating higher dosages for pain relief (Matic *et al.*, 2022). PM individuals, more prevalent among Europeans, are at an increased risk of tramadol neurotoxicity when abused due to their reliance on higher doses for efficacy (Gaedigk *et al.*, 2017).

Additionally, CYP2D6 metabolizes antimalarial drugs such as primaquine and tafenoquine into their active form, 5-hydroxy-primaquine, which targets *Plasmodium vivax* hypnozoites (Bennett *et al.*, 2013; Huang *et al.*, 2021). CYP2D6*3 and *4 variants reduce drug efficacy, underscoring the need for further research into CYP variants as a potential cause of antimalarial treatment failures.

Cytochrome P450 3A4 (CYP3A4) is a clinically significant enzyme in drug metabolism, processing approximately 50–75% of all drugs in use, including key medications like acetaminophen, statins, and anticancer agents (Parkinson & Ogilvie, 2013). Its broad substrate range and presence in vital metabolic organs, such as the liver and intestines, make it a crucial factor in determining drug bioavailability, therapeutic efficacy, and safety. Genetic polymorphisms, environmental factors, and drug-drug interactions significantly influence CYP3A4 activity, explaining individual differences in drug response. Additionally, CYP3A4 contributes to the activation and detoxification of endogenous and exogenous compounds, including testosterone and various painkillers and anticancer drugs (van Eijk *et al.*, 2019).

The CYP3A4*1B variant, a polymorphism occurring in the 5' promoter region of the gene,

results from an adenine-to-guanine (A>G) substitution and has been associated with reduced enzyme activity and the poor metabolizer (PM) phenotype (Kadlubar *et al.*, 2003; Kudzi *et al.*, 2010).

In this study, only 2.42% of participants from the Ewe ethnic group in Ghana carried the CYP3A4*1B mutant allele, while the majority (97.58%) were extensive metabolizers as shown in Figures. This distribution contrasted sharply with the control group, which included other indigenous Ghanaian ethnicities, where 27% carried the homozygous mutant allele (Figure 4.4.3 and Figure 4.5.1-4.5.2). These findings deviate from earlier studies, such as those by Kudzi *et al.* (2010) and Tayeb *et al.* (2000), which reported a 51% frequency of the CYP3A4*1B mutant allele in other Ghanaian populations. However, small sample sizes in this study limit statistical interpretations. Notably, the CYP3A4*1B variant is rare among Caucasians and has not been reported in Asians (Świechowski *et al.*, 2019).

Rebbeck *et al.* (2007) observed that CYP3A4*1B is more common in African-Americans than in other ethnic groups and may lead to reduced CYP3A4 protein activity. Increased expression of CYP3A enzymes is associated with accelerated metabolism of many drugs and may also play a role in cancer progression. Fujimura *et al.* (2007) detected CYP3A4 in both healthy and cancerous prostate tissues, observing significantly lower immunoreactivity scores in prostate cancer cells. This finding underscores the importance of CYP3A4 in disease incidence and highlights its potential role in personalized medicine.

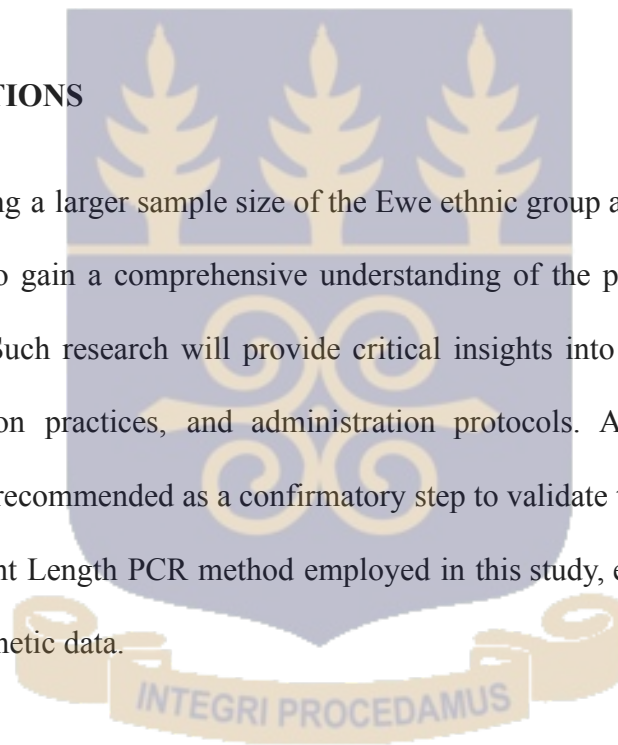
5.2 CONCLUSION

This study confirmed the presence of allelic variants of the CYP2D6 and CYP3A4 enzymes among the Ewe population in Ghana, as well as among other indigenous groups used as controls.

The observed frequencies align broadly with previous studies conducted on Ghanaian and Sub-Saharan African populations. However, notable differences were found in the frequency of the heterozygous mutant allele of the CYP3A4*1B variant. These variations may have important pharmacological implications, particularly in the diagnosis and treatment of prostate cancer among the Ewe ethnic group, given the association of the CYP3A4*1B variant with the onset of prostate cancer.

5.3 RECOMMENDATIONS

Further studies involving a larger sample size of the Ewe ethnic group and other ethnic groups in Ghana are necessary to gain a comprehensive understanding of the pharmacogenomics of the Ghanaian population. Such research will provide critical insights into its implications for drug importation, prescription practices, and administration protocols. Additionally, full-genome sequencing is strongly recommended as a confirmatory step to validate the findings derived from the Restriction Fragment Length PCR method employed in this study, ensuring greater accuracy and reliability of the genetic data.



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