

**The relationship between *CYP2D6* polymorphisms and Tardive Dyskinesia
in black Zimbabwean psychotic patients on typical antipsychotics**

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Masters of Clinical Pharmacology Degree
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Declaration

I, Josiah Tatenda Masuka, certify that this dissertation is my original work and has been prepared in accordance with guidelines of the Master of Clinical Pharmacology Program, University of Zimbabwe. I further attest that this work has not been submitted, in part or in full, for any other degree at any university and/or any publication.

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I, having supervised and read this dissertation, I am satisfied that this is the original work of the author in whose name it is being presented. I confirm that the work has been completed satisfactorily for presentation in the examination.

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Dedication

To my loving mother, Leonora (may your soul rest in peace), my girlfriend Karen, my classmates, staff and lecturers MSc Clinical Pharmacology 2013 and AiBST students and staff, I thank you for your unwavering support.

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Abstract

CYP2D6 polymorphisms have been associated with different drug efficacies and adverse effect profiles including Tardive Dyskinesia (TD). The occurrence of these polymorphisms has also been noted to be different amongst different racial or ethnic groups. In Asians and Caucasians *CYP2D6**10 and *CYP2D6**3, *4 and *5 have been positively associated with TD respectively. In Africans no clear relationships with the prevalent reduced function *CYP2D6* genotypes has been shown.

The objective of this study was to determine whether occurrence of TD is associated with the most prevalent reduced function *CYP2D6* genotypes in black Zimbabweans – *CYP2D6**17 and *29. AIMS scoring and *CYP2D6* genotyping was carried out on patients exposed to first generation or typical antipsychotic medications at Parirenyatwa Annexe and Harare Psychiatric units in an unmatched case control study.

The main outcome measures were *CYP2D6**17 and *29 genotypes. The relationship between TD and mutant *CYP2D6**17 homozygote and heterozygote genotypes was not statistically significant with p values of 0.740 and 0.442 in the haloperidol exposed and 0.587 and 0.150 in those exposed to haloperidol, FD and CPZ respectively. No *CYP2D6**29 result could be determined due to a failure in genotyping for this SNP. The results presented suggest no association between the major reduced function *CYP2D6* allele *17 and TD in black psychotic Zimbabwean patients.

Abbreviations and Acronyms

| | |
|-------------------|---|
| TD..... | Tardive dyskinesia |
| EPS..... | Extrapyramidal symptoms |
| AIMS..... | Abnormal Involuntary Movements Scale |
| FD..... | Fluphenazine decanoate |
| CYP2D6..... | Cytochrome P450, family 2, subfamily D, polypeptide 6 |
| GABA..... | Gamma Amino Butyric acid |
| GLI2..... | GLI family zinc finger 2 |
| HSPG2..... | Heparan sulphate proteoglycan 2 |
| CPZ..... | Chlorpromazine |
| RT - DNA PCR..... | Real Time – Deoxyribonucleic Acid Polymerase Chain Reaction |
| HPLC..... | High Performance Liquid Chromatography |
| MRCZ..... | Medical Research Council of Zimbabwe |
| JREC..... | Joint Research and Ethics Committee – UZCHS & Parirenyatwa |
| PM..... | Poor Metaboliser |
| EM..... | Extensive Metaboliser |
| IM..... | Intermediate Metaboliser |
| UM..... | Ultrarapid Metaboliser |
| NMDA..... | N-methyl-D-aspartate receptor |
| DRBD..... | Dopamine Receptor Blocking Drugs |
| DRD..... | Dopamine Receptor D |
| MnSOD..... | Manganese superoxide dismutase |
| HTR2A..... | 5-Hydroxytryptamine (serotonin) Receptor 2A (G protein-coupled) |
| BDNF..... | Brain-Derived Neurotrophic Factor |
| EPS..... | Extrapyramidal Symptoms |
| WHO..... | World Health Organisation |
| HIV..... | Human Immunodeficiency Virus |

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CHAPTER 1

1.0 INTRODUCTION AND LITERATURE REVIEW

Tardive dyskinesia (TD) is one of the major side effects of typical or first generation antipsychotic drug management. In a study conducted in Harare, in 1998, the prevalence of TD was observed to be 19.2% among patients taking typical antipsychotics.¹ The annual incidence of TD in a cohort of mostly Afro-Caribbean patients with a mean antipsychotic exposure of 18 years was noted to be 10.2% (95% CI = 7.7 to 13.5).² It has been noted that the risk of developing TD in African-Americans is two-fold that of whites.³

Several risk factors have been explored to explain the occurrence of TD. Tardive dyskinesia is positively correlated with age, being non-white, duration of neuroleptic exposure and dose.⁴ No effects were noted with the type of antipsychotic, psychiatric diagnosis and age at first antipsychotic exposure.⁴ However, in another study, drug type was noted to have a positive association with development of TD. Haloperidol and fluphenazine decanoate (FD) are positively related to TD development whereas clozapine and sulpiride are not.⁴ Other factors such as smoking, alcohol use and diabetes mellitus may increase the incidence of TD.^{5,6}

The enzyme *CYP2D6* metabolises up to 25% of current prescribed drugs.⁷ About 50% of psychiatric patients and 56% of psycho-geriatric patients use at least one drug that is metabolised by *CYP2D6*.⁸ Its polymorphism has been associated with some adverse effects in psychiatric patients.⁷ The polymorphs are distributed differently amongst racial or ethnic groupings.^{9,10} These polymorphs can be divided into functional, non-functional and reduced function alleles.¹⁰ The polymorphism of this enzyme leads to poor, intermediate, extensive and ultra-rapid metaboliser phenotypes.¹⁰ The wild type allele is *CYP2D6*1*. The most prevalent alleles in all races are *CYP2D6*1* and *CYP2D*2* alleles.⁹ These are functional

alleles encoding for extensive metabolism. The major non-functional allele in Caucasians is *CYP2D6*4* (17.2%) whereas the major reduced function alleles in East Asians and Sub-Saharan Africans are *CYP2D6*10* (39.4%) and *CYP2D6*17* (12.2%) respectively.^{9,10} In Zimbabweans *CYP2D6*1* & *CYP2D6*2* have a frequency of 54%, whereas *CYP2D6*10* and *CYP2D6*17* have frequencies of 6% and 34%¹¹ respectively.

The above noted polymorphic differentiations lead to adverse drug reactions. In addition to affecting the safety and efficacy,^{10,12} they also affect the cost of drug treatment.¹⁰ Growing evidence is pointing to the fact that different reduced or non-function *CYP2D6* polymorphic alleles are associated with TD in different ethnic groups.¹³ In patients homozygous for non-function alleles, the odds of developing TD have been observed to be 1.64 greater than in patients without this combination.⁸

In Asians, *CYP2D6*10* has repeatedly been shown to increase the risk of TD.¹⁴⁻¹⁶ However, in Caucasians *CYP2D6*3*, *CYP2D6*4* and *CYP2D6*5* have been observed to be linked with TD.^{17,18} Hitzeroth attempted to investigate the relationship between the development of TD and the *CYP2D6*4/*10* and **17* alleles in Xhosa schizophrenic patients, but failed due to difficulty in genotyping.¹⁹ In Hitzeroth's study, failure was only noted in cases as opposed to controls who were genotyped as expected. The researchers postulated that *CYP2D6* partial deletions or presence of specific genetic variants prevented primer binding.¹⁹ From the literature reviewed, no other paper addresses this relationship in Africans. However, the relationship between haloperidol and *CYP2D6* polymorphism was evident in a study in which the other major haloperidol metabolising enzyme (*CYP3A4*) was inhibited by itraconazole.²⁰ Other genes have also been associated with TD apart from *CYP2D6*. These include GABA pathways genes, *GLI2* gene and *HSPG2* gene.²¹

The difference between this study and previous studies is that it is investigating this relationship in black psychotic patients. Previous studies have focused on Caucasians and Asians. The study proposed here will attempt to correlate the major CYP2D6 reduced function alleles to the development of TD in black Zimbabweans exposed to first generation antipsychotics. Results from the study may help in delineating which patients need to avoid these drugs and which ones can safely use them. This might prove of significance in rational prescribing as the current practice is to try and prescribe atypical or second generation antipsychotics to all patients, to avoid the high prevalence of TD. This is due to a lower risk of TD attributable to second generation antipsychotics. However, haloperidol is more cost effective compared to the atypical alternative drugs eg olanzapine.²² Furthermore, atypical antipsychotics are fraught with important clinical adverse effects including significant weight gain,²³ type II diabetes mellitus, the metabolic syndrome and cerebrovascular complications.²⁴ Due to these limitations of the atypical antipsychotics, more efforts should be made to utilise the older drugs in those less susceptible to developing TD.

1.1 Tardive dyskinesia

Tardive dyskinesia is a persistent hyperkinetic movement disorder.²⁵ The condition is characterised by involuntary, repetitive, purposeless choreoathetotic movements which differ in form and localisation.²⁶ TD is a serious iatrogenic adverse drug reaction of dopamine receptor blocking drugs, DRBDs such as metoclopramide and antipsychotics.^{25,27} It is considered a late-developing extrapyramidal side effect of medications.^{27,28} For the neuroleptic induced TD, the diagnostic consideration involves a patient who has taken a neuroleptic for 3 months (1 month if older than 60 years) and develops the abnormal involuntary movements. The involuntary movements are further described based on their intensity, discontinuance and preparation of drug.²⁶

About 15 – 30 % of patients receiving antipsychotic/neuroleptic drugs develop tardive dyskinesia in the United States of America. This is noted to be a similar trend worldwide.²⁹ Orofacial, buccolingual and masticatory tardive dyskinesias are the most common presentations.²⁶ The other presentations are: tardive akathisia, tardive dystonia, tardive blepharospasm, tardive myoclonus and tardive tics.^{26,30}

Etiological factors in the development of TD have been studied widely. Chief among these factors is the use of dopamine receptor blocking drugs such as antipsychotics and antiemetics (metoclopramide and prochlorpromazine).²⁶ The incidence of TD with atypical antipsychotics was observed to be a fifth of that attributable to typical antipsychotics.³¹ However, this notion has been challenged in recent literature.³² Although previously it had been thought that the above statement holds true, Woods et al (2010) has highlighted a different observation. The incidence rates appear to be comparable in the two eras of use of the typical and atypical antipsychotics.³² The rate may be lower for the atypical antipsychotics, but care needs to be heed in their use.^{32,33}

Advanced age, total antipsychotic dosage and the duration of exposure have all been shown to have a positive correlation with TD development.^{34,35} The female sex increases vulnerability and severity of TD in antipsychotic exposed patients^{26,35}. The risk for TD in African or African-American races is up to twice that in white Caucasians.^{4,26} Smoking, alcohol use and diabetes mellitus have also been shown to increase the risk of TD.^{4,35} It has been shown that smoking induces enzymes which in turn shunt antipsychotic metabolism from *CYP2D6**3 and *4 to alternative pathways. This subsequently leads to neurotoxic metabolites which then cause deranged movements in patients with these genotypes.³⁶

Genetically inherited differences in antipsychotic metabolism through *CYP2D6* appear to predict the development of TD.¹³ Extrapyramidal side effects, TD and drug non-compliance

are increased in *CYP2D6* poor metaboliser (PM) patients compared to the extensive metaboliser (EM) and intermediate metaboliser (IM) phenotypes.¹³ The PM phenotype is thought to have higher serum antipsychotic levels compared to the other *CYP2D6* metabolism phenotypes.³⁷ This probably provides an explanation for the observed TD frequencies noted above.

The stress-diathesis model attempts to unify the postulated causative factors of TD. The antipsychotic type, duration of exposure and dosage are taken to be the stressors, with the diathesis being any condition increasing susceptibility to TD. The later includes disease related vulnerability, genetic predisposition to TD and decreased functional reserve of motor control systems.³⁸ Disease vulnerability may be accounted for by the fact that 4 to 40% of schizophrenics develop spontaneous TD without prior exposure to neuroleptics.³⁹

The leading pathophysiologic hypotheses underlying TD include post-synaptic dopamine receptor sensitisation, GABA insufficiency and structural abnormalities.⁴⁰ The later is marked by reduced basal ganglia volume on neuroimaging findings.²⁷ The other major hypothesis is that of neurotoxicity to the basal ganglia secondary to free radical by-products of catecholamine metabolism^{38,40} – the oxidative stress mechanism.^{41,42} Another hypothesis states that maladaptive plasticity leads to abnormal motor programming.⁴³ This is postulated to be due to the dopamine receptor hypersensitivity and altered NMDA receptor function.⁴³

Further evidence on the mechanisms of TD induction include prolonged blockade of postsynaptic dopamine (D₂)⁴⁴ receptors and damage to striatal GABA and cholinergic interneurons.³⁸ However it seems this is all mediated by the activity of dopamine on the post-synaptic dopamine D₂ receptors. Positron emission tomography (PET) studies have shown increased in-vivo dopamine D₂ receptor binding in humans after chronic antipsychotic therapy. This presents evidence of their up-regulation and possible role in TD pathogenesis.⁴⁵

Neuroleptic – induced TD is considered in a patient who presents with at least a 4 week history of persistent dyskinetic movements.²⁶ These movements should have begun during neuroleptic treatment or 4 weeks after stopping therapy. In the history, the patient should have been on a neuroleptic for at least 3 months or 1 month if older than 60 years.²⁶ It is a diagnosis of exclusion if the patient’s condition cannot be attributed to another drug or another medical/neurological diagnosis.

On examination, the patient will have purposeless, involuntary, snake – like movements.⁴⁶ Choreoathetotic limb and trunk movements, chewing, tongue protrusion, lip pursing, smacking and puckering and rapid eye blinking are among some of the typical observations in TD⁴⁶. These movements are present at rest, but can subside if the patient actively moves the affected body part.^{26,47} Relaxation and sleep also decrease symptoms of TD.⁴⁷

Table 1.1 Summary of TD presentations (created from data by Brasic , Waln O and Jankovic J)²⁶

| Specific Tardive Presentations | Description |
|--------------------------------|--|
| Classic tardive dsykinesia | isolated or predominant oro-buccolingual dsykinesia |
| Tardive stereotypy | seemingly purposeful, repetitive and coordinated movements in the limbs or trunk |
| Tardive akathisia | inner restlessness and inability to stay still |
| Tardive tics /tourettism | multiple motor or vocal tics |
| Tardive myoclonus | brief muscle jerks |
| Tardive chorea | random jerk-like movements |
| Tardive parkinsonism | parkinsonian symptoms/signs |
| Tardive dystonia | fixed posturing of neck, face, trunk or limbs eg retrocolis |
| Tardive pain | painful oral and genital sensations |
| Tardive blepharospasm | repetitive obicularis oculi muscle contractions |

In the management of TD, a number of differential diagnoses should be considered. These include: Sydenham's chorea, psychogenic movement disorders, epileptic disorders and (in the Zimbabwean setting) HIV CNS related complications amongst other neurological and medical diseases.^{26,44} Guided by the history and physical examination, laboratory and radiological investigations may be needed to help exclude such diseases as Wilson's disease and Systemic lupus erythematosus (SLE).⁴⁴

In patients with TD, stopping the offending antipsychotic drug and starting an atypical antipsychotic could be beneficial. Further benefit can be derived from stopping anticholinergic drugs, considering clozapine and suppressive therapy.³⁸ The latter includes the use of a typical antipsychotic 4 times daily or reserpine or tetrabenazine 25 mg to 150 mg daily (in 2 divided doses).³⁸ Experimental drugs such as clonazepam, amantadine, donepezil, melatonin and branched chain amino-acids have a beneficial role in TD management.³⁸ Vitamins E⁴⁶ and B₆^{46,48} also have an effect on TD; possibly due to their antioxidant properties.^{38,44} Propanolol and zolpidem are effective for managing tardive akathisia.⁴⁴

Surgical procedures can be used in the management of severe or treatment resistant TD.^{44,48} The techniques used include pallidotomy and pallidal deep brain stimulation.⁴⁸ This form of therapy has been shown to be effective and safe in a few studies. This might need replication in large clinical trials to fully assess the validity of this conclusion.⁴⁸ A combination of clonazepam, tetrabenazine and clozapine has been shown to alleviate all symptomatology within a month of starting therapy in treatment resistant TD.⁴⁹ This may be a better alternative to the surgical options just mentioned. However, this work was done on a small cohort of patients and thus the results need caution in their interpretation.

The most prudent and sound medical practice is to consider TD prevention in patients receiving DRBDs.⁴⁴ If an alternative therapy is available, it should be utilised instead of the

DRBDs.²⁵ In the event that this is not possible: the lowest effective dose of a single dopamine receptor antagonist drug, for the shortest duration of therapy should be used.²⁶ This should be for a compelling indication. Avoidance of off – label use should be the norm.³³ It is also imperative to get informed consent to start this therapy as the patient should know the possible outcomes of therapy.²⁶ The informed consent fosters a therapeutic alliance and possibly early patient report of drug adverse effects.

As a preventative approach for patients requiring an antipsychotic, consider the atypical antipsychotic drugs first. Atypical antipsychotic drugs have a lower incidence of TD compared to the typical antipsychotics.³¹ Even with these drugs, regular monitoring of early signs and symptoms of TD should be done.^{26,33} This allows for early discontinuation of the offending DRBD, which in turn is associated with resolution of TD.⁴⁴ However, the withdrawal of a DRBD should be gradual as rapid cessation may precipitate the Withdrawal Emergent Syndrome⁴⁸ and/or florid psychosis.^{26,44}

1.2 Pharmacogenetics and tardive dyskinesia

Several pharmacogenetic studies have been carried out to delineate the genetic susceptibility to developing neuroleptic-induced TD. The results have been inconsistent to date. These studies have shown positive associations in the following genes: *CYP2D6*, *CYP1A2*1F*, *DRD3 Ser9Gly*, *DRD2 Taq1A*, *HTR2A T102C* and *MnSOD Ala9Val*^{21,46}. These genes encode proteins for drug metabolism (DME), the dopaminergic and oxidative pathways respectively.⁴⁶

Interaction evidence has been shown between *BDNF* and *DRD3* polymorphs;⁵⁰ *MnSOD Ala9Val* and *DRD3 Ser9Val* polymorphs and *DRD3 Ser9Gly* and *CYP1A2 polymorphs* in the development of TD.^{46,50,51} *Many other synergistic interactions may be involved to explain the*

genetic susceptibility to TD in patients treated with antipsychotics. This may explain the inconsistency in the association studies on single SNPs or genotypes.

The genetic polymorphisms described above do provide a biologically plausible explanation for the presumed hypothetical mechanisms. Going back to the pathophysiology of TD, the hypotheses included neuronal degeneration, dopamine supersensitivity, oxidative stress and free radical damage. An exploration of these lead to the following notions:⁴⁶

1. DME polymorphs: - *CYP2D6* or *CYP1A2* PM phenotypes lead to reduced antipsychotic clearance and thus increased antipsychotic serum levels
2. Dopamine supersensitivity: - compensatory increase in dopamine receptors secondary to chronic D₂ blockade (possibly due to increased binding by some dopamine receptor variants)
3. Oxidative stress and free radical damage: - *reduced antioxidant activity by MnSOD polymorphs lead to poor free radical scavenging and neurodegeneration*

Several studies have been done to establish the relationship between TD and *CYP2D6* polymorphs or genotypes. The results have been conflicting with some showing a significant statistical relationship whereas others did not. Most of the studies reviewed in this article were done in Caucasians^{13,17,18,52,53} and Asians¹⁴⁻¹⁶. One such study in a Xhosa (African) population was attempted, but no result was obtained as the genotyping proved difficult.¹⁹

These studies used different methodologies and genotyping tools. Thus, a systematic review on the association or lack of associations from the observed data becomes difficult. However, it can be seen that genetic factors appear to play a role in the occurrence of extrapyramidal side effects (EPS) and also TD. Below is a summary of these studies:

Table 1.2 Studies on CYP2D6 relationship to TD and acute EPS

| Authors | CYP2D6 alleles or phenotype investigated | Conclusion | Ethnic group | Adverse effect |
|-------------------------------------|--|--|--------------|----------------|
| Ellingrod, V. L et al ¹⁸ | *3 and *4 | Significant association | Caucasians | TD |
| Lohmann, P, L et al ⁵² | *3, *4, *5 and *6 | No association | Caucasians | TD |
| Hitzeroth ¹⁹ | *4, *10 and *17 | No result obtained | Africans | TD |
| Kapitany, T et al ¹⁷ | *3, *4 and *5 | Significant association | Caucasians | TD |
| Inada, T et al ¹⁶ | *2, *3, *4, *10, and *12 | Significant association with *2 and *10 | Asians | Acute EPS |
| | | No association *2 and *10 | Asians | TD |
| Liou, Y., J et al ¹⁴ | *10 | Significant association | Asians | TD |
| Nikoloff, D ¹⁵ | 19 alleles | Significant association | Asians | TD |
| Crescent, A et al ⁵³ | *3, *4, *5 and *6 | Significant association with *4 (homozygous) and *6 (heterozygous) | Caucasians | EPS |
| Koblecki, C et al ¹³ | PM, IM and EM | EPS/TD in PM > in IM or EM | Caucasians | EPS or TD |
| Arthur, H et al ³⁷ | Enzyme status | No association | Caucasians | TD |

From table 2, it can be shown that different *CYP2D6* polymorphs are associated with TD in different ethnic groups. This relationship to race/ethnic group is consistent with the distribution of major non/reduced function alleles as explored in the next section.

A study to define the diversity in global *CYP2D6* allelic variation concluded that *CYP2D6* varies more within than between populations.⁹ They also found that, null or low-activity alleles occur at high frequencies in various areas of the world.⁹ The *CYP2D6* allele frequency has been shown to vary amongst ethnic groups. Functional allele frequencies in

Africans/African Americans, Asians and Caucasians are 50%, 50% and 71% respectively.¹⁰

The major reduced or non-function alleles in these groups are *CYP2D6*4*, *CYP2D6*10* and *CYP2D6*17* for Caucasians, Asians and Blacks respectively.^{9,10}

CYP2D6 polymorphic variation entails different phenotypic metabolic activity and since it differs between races, studies are needed to delineate the risk profiles of certain drugs in these populations individually. This assures optimal dosing recommendations based on empirical pharmacogenetic evidence.¹⁰

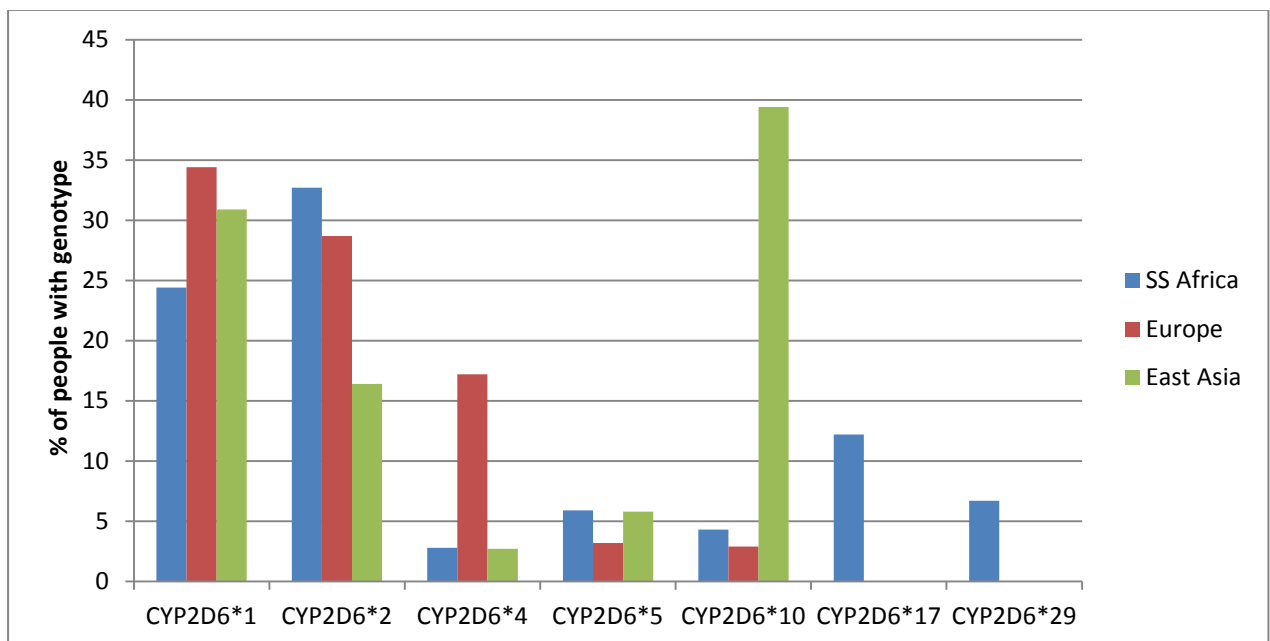


Figure 1.1 Graphical presentation of worldwide distribution of some *CYP2D6* alleles (created from data by Sistonen et al)⁹

The graph shows the graphical, global interracial representation of *CYP2D6* diversity comparing Southern Africa to Europe and East Asia.

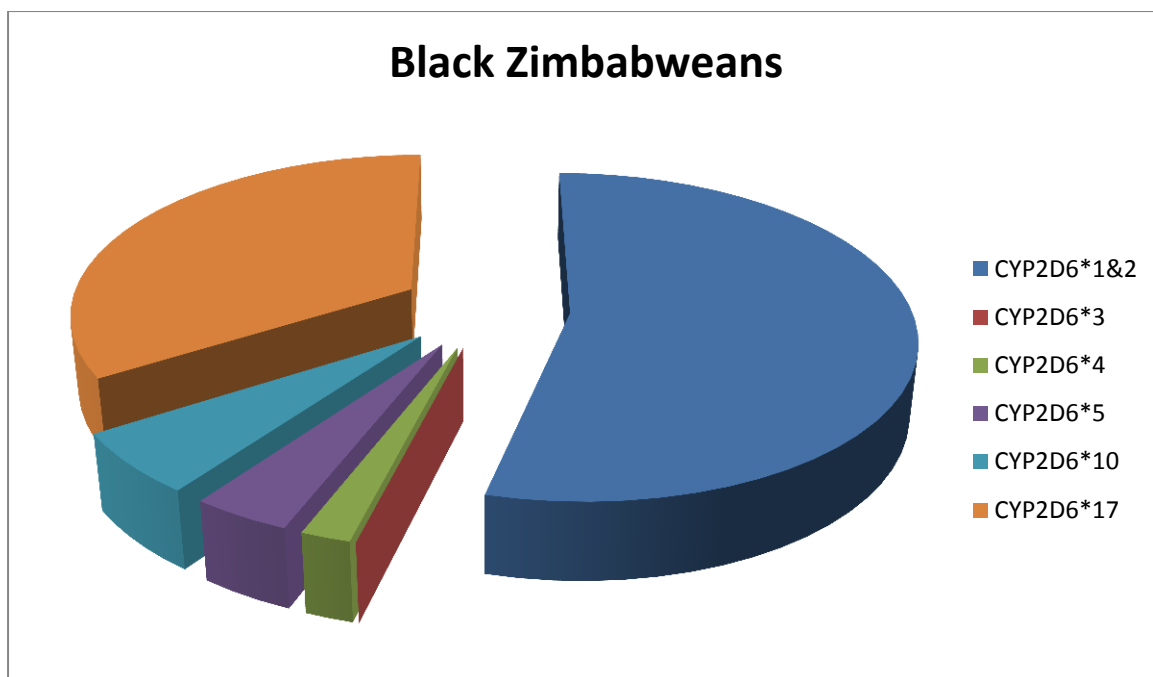


Figure 1.2 Frequency of normal activity and non/reduced function CYP2D6 alleles in Zimbabweans (created from data by Masimirembwa et al)¹¹

For the Zimbabwean context, Fig 2 shows the frequencies of normal activity alleles and the non/reduced function alleles. From this graphical presentation it can be deduced that if *CYP2D6* has a sizeable impact on antipsychotic pharmacokinetics, this study's focus should be on *CYP2D6*17*. It is the most frequently encountered reduced function *CYP2D6* variant in this population together with *CYP2D6*29*.⁹

There are as many as 74 *CYP2D6* polymorphic variants.⁷ These variants have different activity levels measured in vitro. Among these variants, *1 and *2 encode fully functional polymorphs whereas alleles *10, *17, *36 and *41 encode reduced function enzymes.^{7,9} Non functional proteins result from the following alleles: *3 to *8, *11 to *16, *18 to *21, *38, *40, *42, *44, *56 and *62.⁷ Substrate dependent activity levels have also been noted in other genotypic variants. *CYP2D6*10* and *CYP2D6*17* have activity levels that are 1.32 to 27.9% and 7.33 to 80.4% of the wild type variant's efficiency for different probe drugs.⁵⁴

Four different phenotypic variants have also been described in literature. These are namely PM, IM, EM and UM in order of increasing activity levels.⁷ These depend on the combinations of allelic variants inherited by a person. Extensive metabolisers (EM) have at least one functional allele and normal metabolic function. Intermediate metabolisers (IM) have a lower metabolic rate. Their genotype contains one non functional allele together with a low activity variant.⁵⁵ The poor metaboliser (PM) status occurs with alleles that do not code a functional enzyme, whereas the ultra-rapid metaboliser (UM) status occurs with duplication/amplification of fully functional alleles.^{55,56}

The wild type (*CYP2D6*1*) variant encodes an enzyme with normal activity. Some polymorphic variants have single nucleotide substitution conferring different enzyme activity levels for the *CYP2D6* product protein, an oxido-reductase. An example is the different substrate specificity of *CYP2D6*17* compared to *CYP2D6*1/*2*. This confers different enzyme kinetics for this allele to the wild type allele.⁵⁷ The *CYP2D6*17* allele contains C1023T, C2850T, G1661C and G4180C mutations.⁵⁸ This is associated with reduced debrisoquine hydroxylation.⁵⁸ The possible explanation is probably due to decreased affinity,⁵⁹ which in turn is secondary to altered *CYP2D6*17* active site amino acids.⁶⁰

The *CYP2D6*29* allele contains G1659A, G1661C, C2850T, G3183A and G4180C SNPs.⁵⁸ All these SNPs confer reduced enzyme function.⁵⁸ Table 3 shows the major *CYP2D6* alleles and their characterising mutations.

Table 1.3 CYP2D6 alleles and their characteristic mutations (modified from Bertilson et al)⁵⁶

| CYP2D6 allele | mutation | metabolic consequence |
|---------------|---------------|--------------------------------|
| *1/2 | N/A | normal activity |
| *4 | G1934A | splicing defect |
| *5 | gene deletion | no enzyme activity |
| *10 | C188T | unstable enzyme |
| *17 | C1111T | reduced activity |
| *29 | G3183A | reduced activity ⁵⁸ |

1.3 Pharmacokinetics and CYP2D6

Many typical and atypical antipsychotic drugs are predominantly metabolised by *CYP2D6*.⁶¹ Their steady state concentrations have also been noted to correlate with *CYP2D6* allele activity. Some of the drugs in this category include: typical antipsychotics – chlorpromazine, haloperidol, thioridazine and the atypical antipsychotics – risperidone and sertindole.⁶²

The PM status of *CYP2D6* leads to a longer half-life and higher steady state levels of Haloperidol and reduced haloperidol.⁶³ The effect on haloperidol is noted at doses less than 20 mg /day.⁶⁴ This is due to delayed clearance by the enzymatic *CYP2D6* processes at these doses. A similar relationship (except dose association) has also been reported with perphenazine and zuclopenthixol.⁵⁶

There is selective in vitro inhibition of *CYP2D6* by antipsychotic drugs. This is not observed in other cytochrome P450 isoforms. Commonly used antipsychotics in the Zimbabwean setting may have a huge potential for drug – drug interactions if co - administered. This is secondary to their ability to inhibit *CYP2D6*. In order of decreasing ability for inhibiting

CYP2D6 the drugs are: thioridazine, chlorpromazine, haloperidol, fluphenazine and risperidone.⁶⁵

The haloperidol pyridinium (a haloperidol metabolite) to haloperidol ratio positively correlates with the severity of TD and Parkinsonian EPS.⁶⁶ It is also known that the conversion of haloperidol and its tetrahydropyridine dehydrate metabolite happens through *CYP3A4* to the neurotoxic pyridinium metabolite.⁶⁷ In addition, *CYP3A4* becomes more important in haloperidol metabolism at doses greater than 20 mg/day.⁶⁴ This may explain the association of TD with high haloperidol doses. “The high affinity-low capacity *CYP2D6* is important at low doses of haloperidol, while the low affinity-high capacity *CYP3A4* becomes more important at higher doses.”⁵⁶ Thus, at doses > 20 mg/ day, *CYP3A4* metabolises haloperidol leading to production of the neurotoxic haloperidol pyridinium product which in turn causes TD.

From the reviewed literature, the steady state concentrations of fluphenazine decanoate do not appear to correlate with the occurrence of TD.⁶⁸ Could drug – drug interactions between FD and haloperidol be the reason for its apparent association with TD? This question may need to be addressed to aid in the understanding of neuroleptic – induced TD with FD.

Atypical antipsychotics have been in favour in the past couple of years due to their perceived favourable adverse effect profile. Unfortunately it has been observed that they also have serious side effects. These include serious weight gain, diabetes mellitus, dyslipidemias, the metabolic syndrome and consequent cardiac and neurological problems.^{23,24} In addition, these drugs appear to have only a slight advantage over the typical antipsychotics in preventing TD.³² The question then arises whether this class of drugs is more cost-effective compared to the typical antipsychotics. The reduction of TD with atypical antipsychotics might not be as cost-effective as would be acceptable. Rosenhack estimated at least 3 fold increase in the cost

of atypical antipsychotics to “conventional policy threshold” acceptable expenditure for medication.²² Thus considering the above stated arguments, pharmacogenetic testing may help in cost saving and providing safe medication.

1.5 Study justification

The WHO defines Pharmacovigilance as “the science and activities incorporating the detection, assessment, understanding, and prevention of adverse effects” (WHO).⁶⁹ To aid in achieving this objective, this study aims to assess and understand the occurrence of TD in patients taking typical antipsychotics in relation to *CYP2D6* polymorphisms. Few studies have focused on the African population, but similar investigations have been conducted in other ethnic groupings.^{9,19} With evidence suggesting a two-fold incidence in blacks compared to whites and the *CYP2D6* polymorphic variation amongst ethnic groupings, it is imperative to assess how *CYP2D6* variants correlate with TD in Africans.³ This will lead to the delineation of susceptible *CYP2D6* alleles and subsequent prevention of this debilitating serious ADR in these patients. Furthermore, it might be possible to avoid or reduce occurrence of TD (and EPS) if the principles of personalised therapy can be instituted.

1.6 Aims and objectives

Study Aim

To determine whether there is an association between the *CYP2D6*17* and *CYP2D6*29* genotypes and TD in patients exposed to haloperidol and/or fluphenazine decanoate, FD.

Research Question

Are *CYP2D6* genotypes associated with Tardive Dyskinesia?

Specific Objectives

1. To determine whether occurrence of TD is associated with particular *CYP2D6* genotypes in black patients taking haloperidol
2. To determine whether occurrence of TD is associated with particular *CYP2D6* genotypes in black patients taking haloperidol and fluphenazine decanoate

Study Hypothesis

There is a relationship between the *CYP2D6**17 or *CYP2D6**29 genotypes and increased risk of developing TD among patients taking haloperidol alone or patients prescribed haloperidol and FD

Statistical Hypothesis: $H_0: \beta_1 = 0$

$H_A: \beta_1 \neq 0$

CHAPTER 2

2.0 METHODOLOGY

Study period: 14 August 2013 to 4 November 2013

Study design: unmatched case - control study

Setting: patients from the outpatients departments of Parirenyatwa Annexe mental hospital and Harare hospital psychiatric unit.

Sampling: the cases and controls were taken from the psychiatric patients on antipsychotic therapy presenting to the respective outpatient departments of the 2 hospitals. All cases presenting to the outpatients clinic were included in the study. Controls were selected by systematic random sampling with every 10th patient in a consultation queue enrolled into the study.

Case definition: TD was determined by the AIMS score using the Schooler & Kane criteria (AIMS score ≥ 2 in 2 body areas OR ≥ 3 in 1 body area defining TD).⁷⁰

Inclusion criteria:

1. stable, psychiatric patients on antipsychotic therapy for 3 months
2. exposed to haloperidol and/or FD
3. patients above 18 years old – they can legally consent

Exclusion criteria:

1. Patients exposed to fluphenazine decanoate or haloperidol for less than 3 months. The diagnosis of neuroleptic induced TD can only be made after a 3 month exposure to these drugs.

2.1 Instruments and materials

Instruments: a) Applied Biosystems 7500 (standard) RT - PCR

b) Taqman assay

c) AIMS scoring test

d) AUDIT scoring test

Materials and equipment: HPLC and TaqMan® Drug Metabolism Genotyping panels (Roche Molecular Systems, Inc.).

2.2 Procedures

Clinical: Qualified medical doctors undergoing their Psychiatry internship rotation assisted the researcher in conducting the interviews. These interviewers were trained on conducting the AIMS and AUDIT scoring tests. In addition the procedures to be carried were demonstrated to ensure uniformity and reduce threats to the study's internal validity.

Every participant was taken through the informed consent process. Those who agreed to enter the study were then taken through the piloted demographic, AUDIT and AIMS questionnaires. Thereafter, samples of blood were withdrawn, labelled and send for storage at the laboratory.

The patient's study reference number was entered into a study log book. This was in the form **A/X**, where: **A** is the first letter of the hospital's name, **X** is the participant's hospital study entry number eg H/01. These same details were used for labelling the sample tubes and questionnaires. No other identifying details were entered onto these study tools. The log book was kept by the researcher.

Laboratory: Every effort was made to avoid contamination with extraneous DNA. In keeping with the PCR Good Laboratory Practices, strict protocols and procedures were used in carrying out the laboratory work. The work setup was thoroughly cleaned, with purification, amplification and analysis areas kept separate.

DNA extraction was done using the QIAamp DNA Mini kit (Qiagen, CA) according to the manufacturer's manual. Taqman[®] genotyping assays were used for allelic discrimination of *CYP2D6*17* (assay identification number C_2222771_40) and *CYP2D6*29* (assay identification number C_34816113_20) for the SNPs 1023C>T and 3183G>A, respectively. The total reaction volume was 25 ml comprising 2x Taqman[®] PCR mix Applied Biosystems), 20x drug metabolising genotyping assay mix and genomic DNA. The PCR reaction was carried out using the Applied Biosystems 7500 Real Time machine.⁷¹ The reaction consisted of 50°C denaturation for 2 minutes and 50 cycles with 95°C for 10 minutes and a final step of 92°C for 15 seconds.

Table 2.1 The Applied Biosystems reaction volume determination

| Component | Volume for 1 reaction/ μ l | Volume for 55 reactions/ μ l |
|-----------------|--------------------------------|----------------------------------|
| Master mix (2x) | 12.50 | 756.25 |
| Assay mix (20x) | 1.25 | 75.62 |
| Sample (10x) | 2.50 | 151.25 |
| Water | 8.75 | 529.38 |
| Total volume | 25.00 | 1512.50 |

NB: When all the required samples had been collected, the laboratory and data analysis was then carried out. This was all done in accordance with the protocol outline of this

methodology section. After the genotyping, the coding was then unblinded to further analyse the raw data.

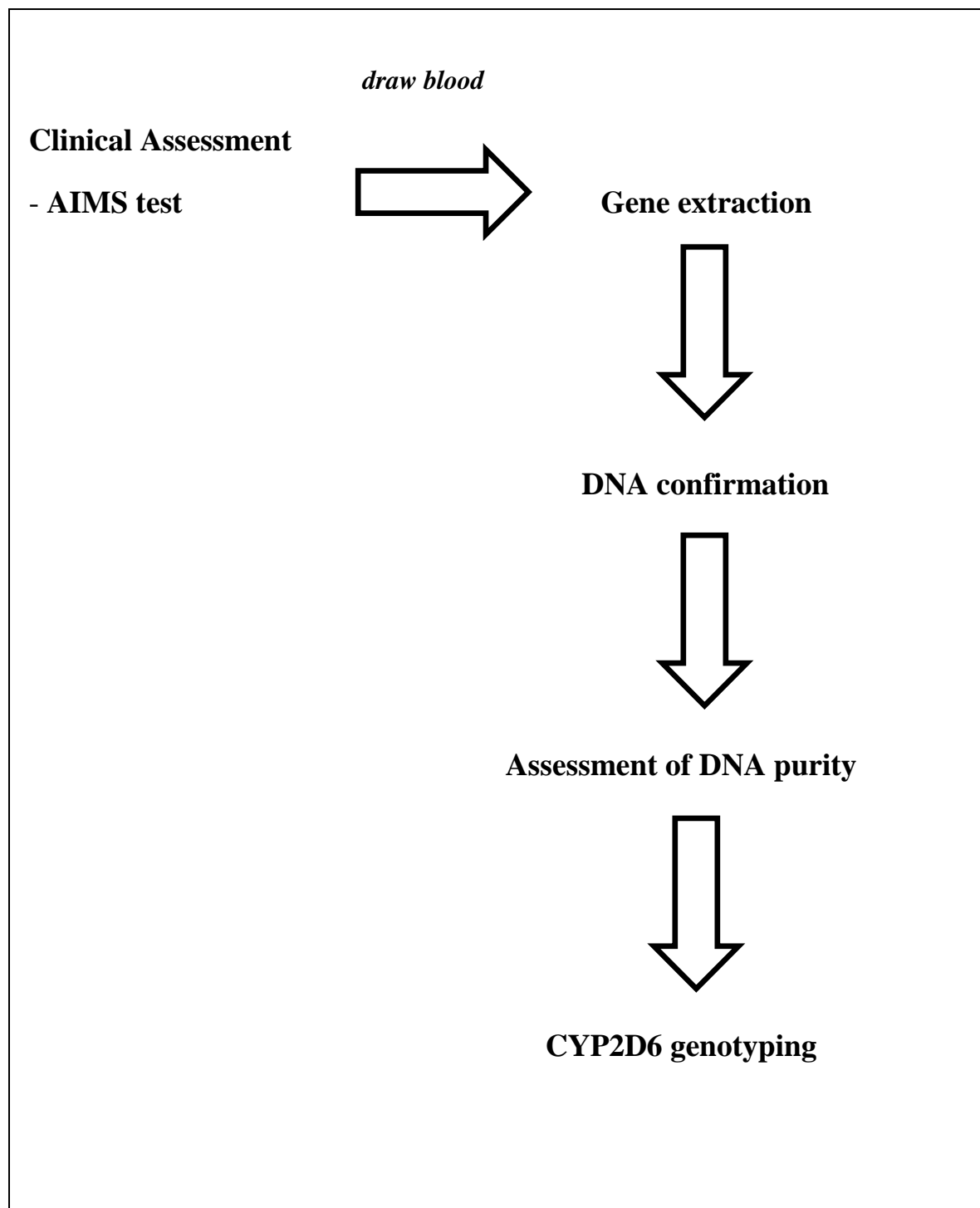


Fig 2.1 Project flowchart

The diagram shows the flow process of the project from blood/ sample collection to the RT pcr genotyping step.

2.3 Analysis

Sample size calculation:

The sample size was calculated using EPI Info 7 for an unmatched case control study with a 1 as to 2 ratio of cases to controls i.e 25 cases to 50 controls by the Fleiss method. This was done using the formula:

$$N = \frac{4(Z_{1-\alpha} + Z_{1-\beta})^2 \cdot \pi(1-\pi)}{(\pi_1 - \pi_0)^2}$$

where:

$$\pi_1 = \frac{\theta \pi_0}{1 + (\theta - 1) \pi_0}$$

and: $\pi = w_1 \cdot \pi_1 + w_0 \cdot \pi_0$; with exposure of 50% for controls and $\alpha = 0.10$, $\beta = 80\%$, $Z = z$ scores and an expected odds ratio of $\theta = 3$.

and:

Association determination:

Logistic regression and the calculation of odds ratios were done using an SPSS v. 17 (IBM systems) statistical package. The variables were as noted below. The relationship between the variables was modelled using the regression equation:

$$Y = \log (p/1-p) = \beta_0 + \sum_{j=1}^k \beta_j x_{ji} ;^{72}$$

where: $Y = \text{TD}$; AIMS score $\{\geq 2$ in 2 body areas OR ≥ 3 in 1 body area = 1} and any

$$\text{other score} = 0^{25}$$

$x_1 = \text{CYP2D6}^*17$ genotype; WW, WM and MM all dummy coded using SPSS

$x_2 = \text{CPZ}$ exposure status; exposed = 1, not exposed = 0

x_3 = Gender; M = 1 and F = 0

x_4 = Age at antipsychotic onset

x_5 = Duration on typical antipsychotics

*dummy variables created by SPSS software with the wild type, WW genotype as the reference level and 1.00 value for genotypes 1 and 2 for MM and WM respectively.

Hardy – Weinberg calculation:

Before genetic analysis of this sample is done, a Pearson's χ^2 “goodness of fit” test to detect departures from the Hardy-Weinberg equilibrium (HWE) for marker-genotype frequencies was done. Let H_A be: $p^2 + 2pq + q^2 = 1$, H_O : $p^2 + 2pq + q^2 \neq 1$, $\alpha = 0.05$, with 1 d.f. for the calculation and the decision rule: reject H_O if computed $\chi^2 \geq 3.8$.^{73,74}

2.4 Ethical considerations

Ethical approval was sought from MRCZ, JREC and Harare hospital ethics committees. Verbal and written consent was obtained from the patient or primary caregiver or managing consultant medical officer. The filled-in questionnaires and consent forms were kept in a secure locked cabinet in the department of Clinical Pharmacology for 3 years. 2 X 5ml samples of blood were drawn for genotyping and serum drugs level determination. No other tests were performed. Sample analysis was done at AiBST laboratories in Harare. The samples were kept in the AiBSt Biobank for the duration of the study and disposed of within 30 days of completion of the study.

Participants did not incur any out-of-pocket costs and they were not compensated for participating in the study. Information learned about *CYP2D6* genotypes and their effects on TD will help in its prevention in other psychotic patients prescribed typical antipsychotics in

future. Any concerning findings observed during the study, would be communicated to the patient and their managing psychiatrist. And any patient who got triggered by a question administered in the study would be referred to their managing psychiatrist for further treatment.

CHAPTER 3

3.0 RESULTS

A total of 52 participants consented to enter the study. Of these participants genotyping was successful in 50 for *CYP2D6*17* and 25 for *CYP2D6*29*. Analysis of results was only done for the *CYP2D6*17* genotype on the 50 participants with known genotype. There were no statistically significant differences in gender, CPZ exposure and duration of antipsychotic treatment. Current age, age at antipsychotic onset and exposure to haloperidol, fluphenazine decanoate and benzhexol were statistically significant. Table 4 below shows the participants' demographic characteristics.

Table 3.1 Demographic characteristics

| Patient characteristic | Case | Control | Intergroup comparison | |
|--|---------------|--------------|-----------------------|--------------------|
| | | | test statistic | p value |
| Gender | | | 0.720 | 0.396 [‡] |
| Male | 9 (50.0%) | 13 (40.6%) | | |
| Female | 9 (50.0%) | 19 (59.4%) | | |
| Current age/ years (mean ± sd) | 44.94 ± 13.47 | 32.34 ± 8.40 | 4.081 | 0.000 [†] |
| Duration/years (mean ± sd) | 12.78 ± 10.75 | 7.86 ± 7.69 | 0.138 | 0.073 [†] |
| Age at drug initiation /years (mean ± sd) | 31.61 ± 8.05 | 24.41 ± 8.51 | 2.929 | 0.005 [†] |
| Benzhexol exposure status | | | 15.680 | 0.000 [‡] |
| Exposed | 18 (100%) | 21(65.6%) | | |
| Not exposed | 0 | 11(34.4%) | | |
| CPZ exposure status | | | 0.080 | 0.777 [‡] |
| Exposed | 15 (83.3%) | 9 (71.9%) | | |
| Not exposed | 3 (17.7%) | 23(28.1%) | | |
| FD exposure status | | | 8.000 | 0.005 [‡] |
| Exposed | 14 (77.8%) | 21 (65.6%) | | |
| Not exposed | 4 (22.2%) | 11 (34.3%) | | |
| Haloperidol exposure status | | | 15.680 | 0.000 [‡] |
| Exposed | 15 (83.3%) | 24 (75.0%) | | |
| Not exposed | 3 (16.7%) | 8 (25.0%) | | |

† test statistic and p value values derived from a 2 tailed t test ; ‡ test statistic and p value derived from Chi-Square test

3.1 Laboratory results

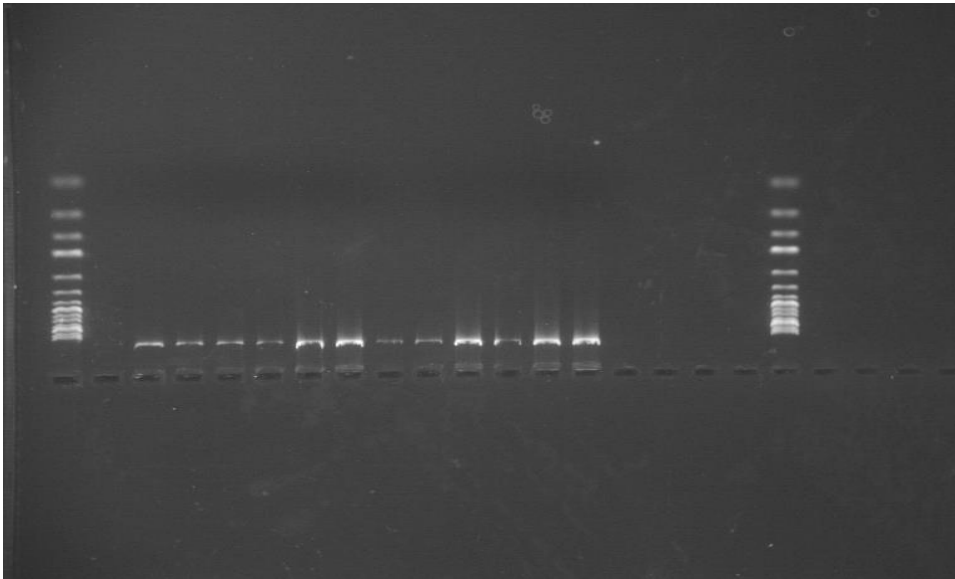


Fig 3.1 DNA confirmation image

Image shows successful genomic DNA bands (in between marker DNA ladders), after gene extraction for 1 of 4 batches done.

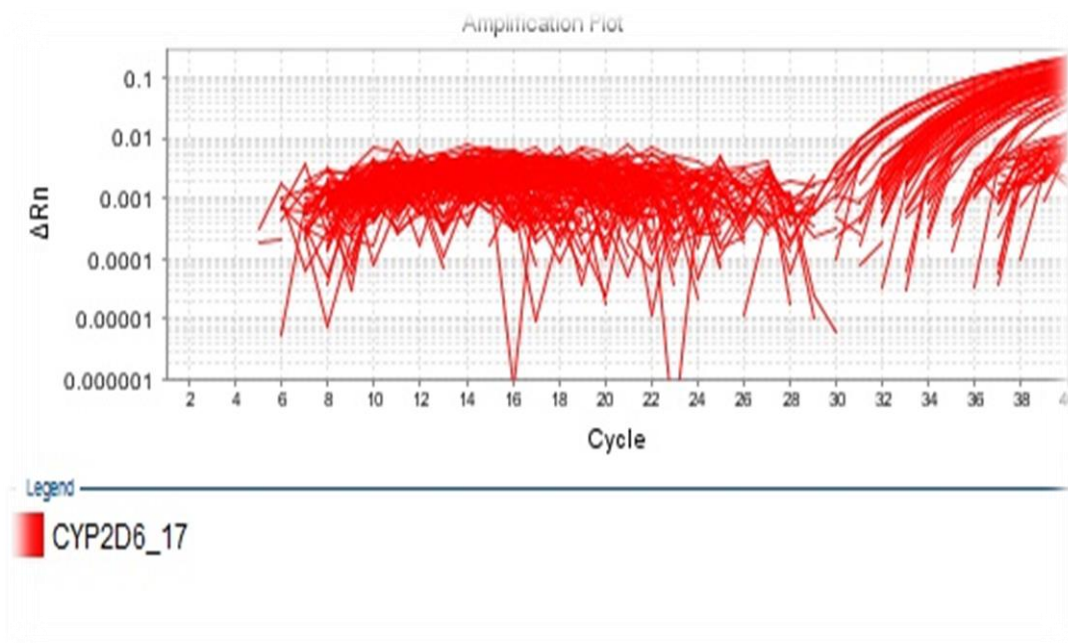


Figure 3.2 Applied Biosystems 7500 RT-PCR amplification plot curve for *CYP2D6*17*

The above figure shows the amplification plot for the *CYP2D6*17*, Taqman RT-PCR reaction process. From the 4th cycle to the 30th, it depicts background noise and from cycle 30, the graph shows target amplification of the desired SNP.

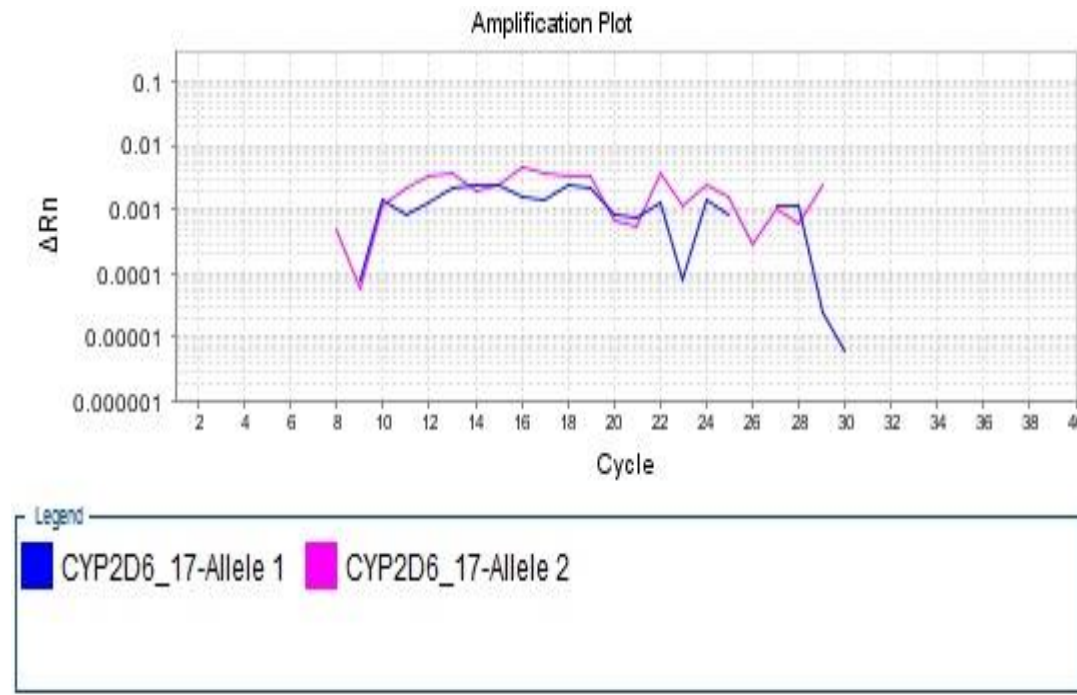


Figure 3.3 Applied Biosystems 7500 RT-PCR amplification plot curve for *CYP2D6*17*

This figure shows the amplification plot for a negative control for *CYP2D6*17*, Taqman RT-PCR reaction process from the 4th cycle to the 30th, which is only background noise.

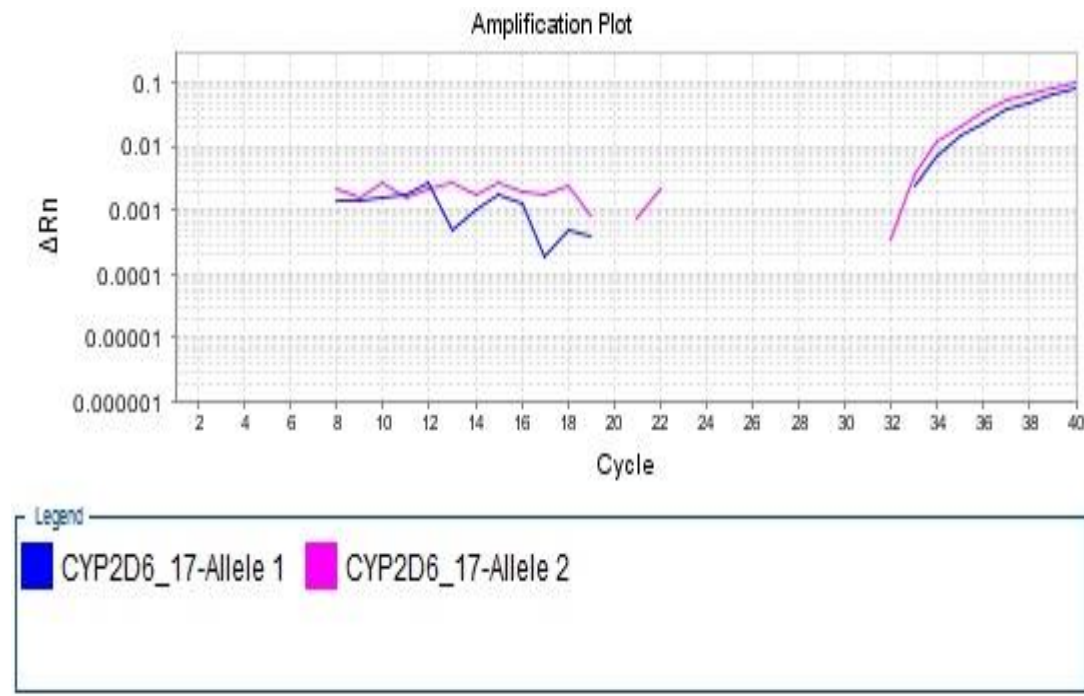


Figure 3.4 Applied Biosystems 7500 RT-PCR amplification plot curve for CYP2D6*17

The graph above shows the amplification plot for a single participant's sample who is heterozygous for the studied *CYP2D6*17* in the Taqman RT-pcr reaction process. There is background noise from cycle number 8 to 22. PCR amplification of the 2 alleles starts on cycle 32, with the different colours differentiating the 2 alleles.

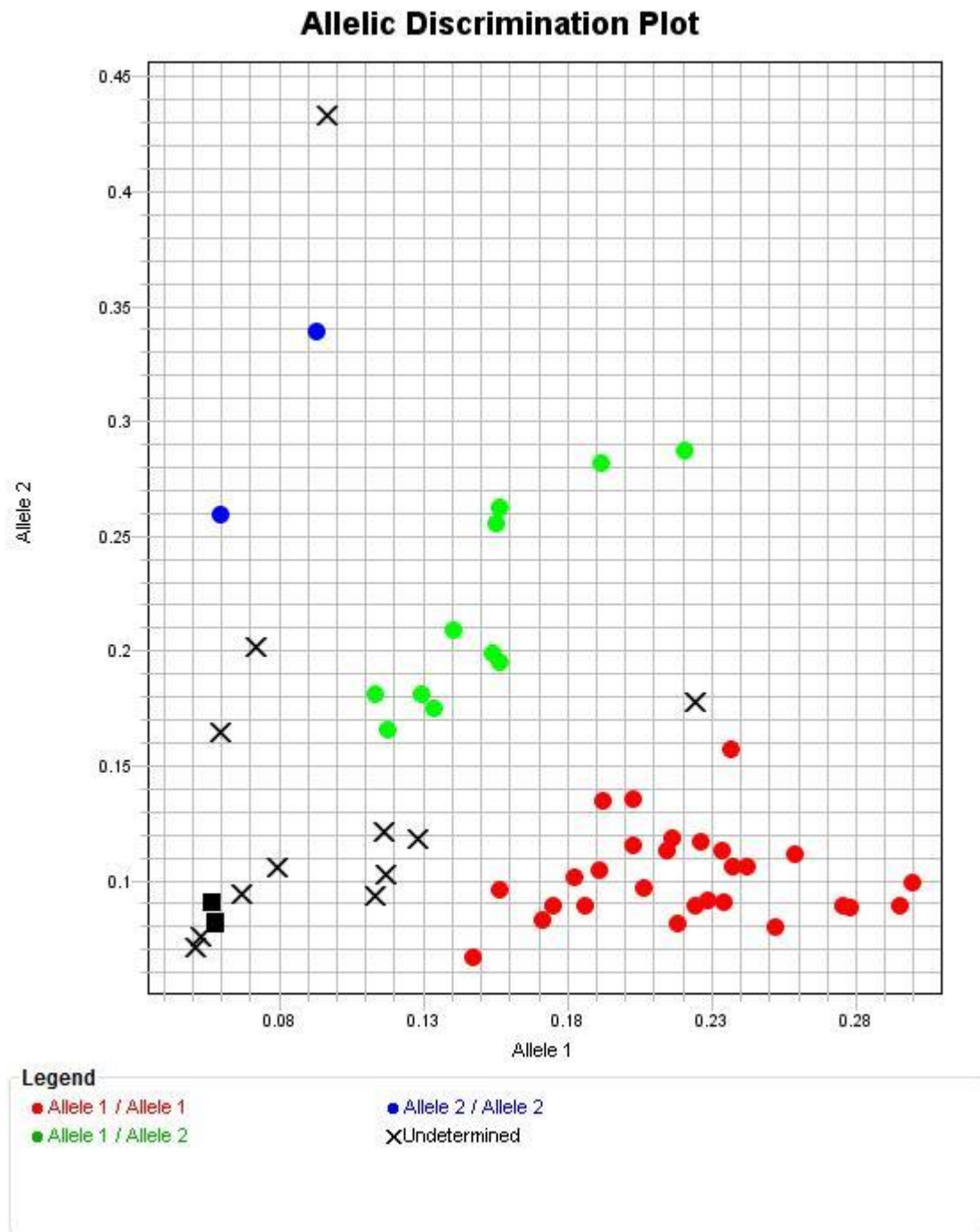


Figure 3.5 Applied Biosystems 7500 RT-PCR CYP2D6*17 genotyping printout

The graph shows the machine/automatically called results; for some, manual calling was done and only 7 needed to be repeated from the gene extraction stage. We managed to define 5 of these on the second attempt. Alleles 1 and 2 represent the wild type and mutant type alleles respectively.

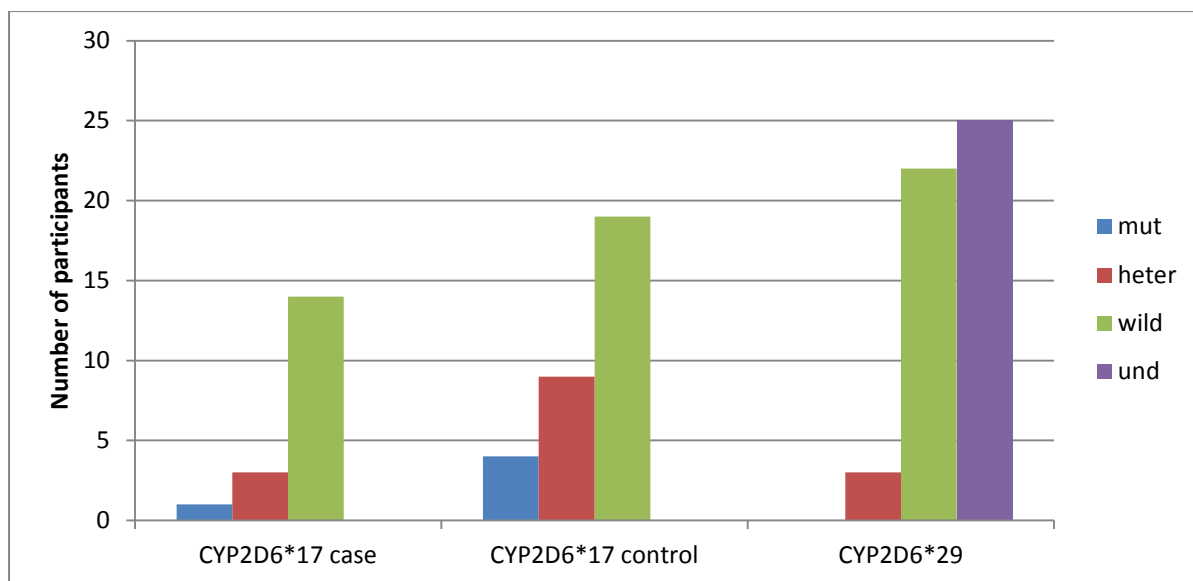


Figure 3.6 Column graph of genotype results/distribution in relation to TD status

The graph shows the distribution of *CYP2D6*17* variants in the cases against those in the controls. Results were only obtained for 25 participants for the *CYP2D6*29* variant and they are not plotted.

3.2 Hardy- Weinberg results

The genotype and allele frequencies are shown in Table 4.2. The overall sample was not in Hardy – Weinberg equilibrium, $\chi^2 = 4.52$ and $p = 0.033$. No significant difference was noted in cases and controls with p values of 0.198 and 0.114 respectively.

Table 3.2 Comparison of CYP2D6*17 genotype profiles between cases and controls

| | | Participant | | Frequency total observed | *H-W freq |
|----------------------------|-----------|-------------|------------|-----------------------------|-----------|
| | | case | control | | |
| Genotypes | WW | 14(13.35) | 19 (17.26) | 33 (30.42) | 60.84% |
| | MW | 3 (4.31) | 9 (12.48) | 12 (17.16) | 34.32% |
| | MM | 1 (0.35) | 4 (2.26) | 5 (2.42) | 4.84% |
| Total | | 18 (13.01) | 32 (29.99) | 50 (44.95) | |
| *HWE calculation: χ^2 | | 1.66 | 2.49 | 4.52 | |
| *P value: | | 0.198 | 0.114 | 0.033 | |
| *Allele freq: W | | | | 78.00% ⁸¹ | |
| M | | | | 22.00% ⁸¹ | |

The numbers in brackets are the expected frequencies under the Hardy – Weinberg Equilibrium law.* denotes the Pearson χ^2 test derived values. Let the hypotheses be: H_0 : the genotypic trait is distributed according to the Hardy - Weinberg Law and H_A : the genotypic trait is not distributed according to the Hardy - Weinberg Law. Calculation done using an HWE calculator.^{74,75}

3.3 TD association logistic regression results

For participants who were only exposed to haloperidol, a concurrent history of exposure to CPZ had an OR (odds ratio) of 0.054 (95% CI: 0.004 – 0.762), $p = 0.031$ and age at drug initiation had an OR of 1.138 (95% CI: 1.003 – 1.289), ($p = 0.045$) were statistically significant. Sex ($p = 0.458$), duration ($p = 0.138$), genotype MM ($p = 0.740$) and genotype MW ($p = 0.442$) were not significant predictors.

Table 3.3 Logistic regression results for participants receiving haloperidol ± neuroleptics

| Predictor variable | Regression coefficient | Odds Ratio | 95% C.I. for Odds Ratio | | P value |
|------------------------|------------------------|------------|-------------------------|--------|---------|
| | | | Lower | Upper | |
| CPZ | -2.913 | 0.054 | 0.004 | 0.762 | 0.031 |
| Sex | 0.846 | 2.330 | 0.250 | 21.754 | 0.458 |
| Duration | 0.129 | 1.138 | 0.984 | 1.315 | 0.081 |
| Age at drug initiation | 0.128 | 1.138 | 1.003 | 1.289 | 0.045 |
| Genotype (MM) | 0.620 | 1.859 | 0.048 | 71.979 | 0.740 |
| Genotype (MW) | 1.096 | 2.992 | 0.183 | 48.858 | 0.442 |
| Constant | -6.262 | 0.002 | | | 0.121 |

In participants exposed to haloperidol and/or FD, a concurrent history of exposure to CPZ had an OR of 0.162 (95% CI: 0.032 – 0.833), $p = 0.029$ and age at drug initiation had an odds OR of 1.134 (95% CI: 1.019 – 1.261), $p = 0.021$ were statistically significant. Sex ($p = 0.614$), duration ($p = 0.073$), genotype MM ($p = 0.587$) and genotype MW ($p = 0.150$) were not statistically significant.

Table 3.4 Logistic regression results for participants receiving haloperidol or FD or CPZ

| Predictor variable | Regression coefficient | Odds Ratio | 95% C.I. for Odds Ratio | | P value |
|------------------------|------------------------|------------|-------------------------|--------|---------|
| | | | Lower | Upper | |
| Sex | -0.423 | 0.655 | 0.127 | 3.391 | 0.614 |
| Genotype (MM) | 0.800 | 2.227 | 0.124 | 40.041 | 0.587 |
| Genotype (MW) | 1.579 | 4.852 | 0.566 | 41.563 | 0.150 |
| Age at drug initiation | 0.128 | 1.134 | 1.019 | 1.261 | 0.021 |
| Duration | 0.097 | 1.102 | 0.991 | 1.226 | 0.073 |
| CPZ | -1.817 | 0.162 | 0.032 | 0.833 | 0.029 |
| Constant | -5.953 | 0.003 | | | 0.046 |

Chapter 4

4.0 DISCUSSION

The aim of this study was to determine whether the major reduced function *CYP2D6*17* and *CYP2D6*29* alleles in black Zimbabweans correlate with the occurrence of Tardive Dyskinesia. Other studies on the topic have been done mostly in Asians and Caucasians, but information was lacking on the black African population. In addition, null or reduced activity *CYP2D6* alleles vary in their distribution amongst the races.^{9,10} *CYP2D6*4* is the major non – function allele in Caucasians whereas *CYP2D6*10* is the major reduced function allele in Asians⁹. In Africans and black Zimbabweans, *CYP2D6*17* and *CYP2D6*29* are the major reduced function alleles.¹¹ It was postulated that the prevalent reduced function alleles are related to TD occurrence.

The study managed to recruit 52 participants against a calculated sample size of 75. This was due to time constraints. This lowered the statistical power of the study. However, this is a pilot study and the results for this project give insight into the possible predictive factors to include in further studies. It also enables determination of allele frequencies and expected odds ratios needed in designing follow up studies.

In the present study, no association between *CYP2D6*17* and the occurrence of tardive dyskinesia was found. Previous studies done on the most prevalent reduced or non-function *CYP2D6* alleles in Asians and Caucasians had shown a statistically significant association with TD occurrence.^{14,15,17,18} This was shown for *CYP2D6*10* and *CYP2D6*4*. However, it must be noted that other studies found no significant association between these two variables.^{16,52} One other study by Hitzeroth et al in black, Xhosa speaking individuals failed to get any results due to genotyping failure.¹⁹ In these studies, there were notable differences in study design and the *CYP2D6* alleles investigated.

The possible reasons for lack of association are that other factors may be involved in the aetiology of this adverse drug reaction. The factors which were not included in the present study are HIV status, cumulative antipsychotic dose and psychiatric diagnosis. Cumulative antipsychotic dose is positively related with occurrence of TD.⁷⁶ On the other hand, schizophrenia is associated with spontaneous occurrence of TD. Increased risk of TD is also noted in siblings than in controls.³⁹ These variables may need to be controlled for in a similar study to obtain a holistic result for the genotype relationship to TD.

From the literature reviewed, risk factors for neuroleptic-induced TD development include age at antipsychotic treatment initiation, duration of treatment, the female sex, advanced age, smoking, typical antipsychotics and other dopamine receptor blocking drugs.⁴ In the present study the only factors which were noted to have a statistically significant role in the development of TD were age at drug initiation and exposure to chlorpromazine. No statistically significant results were found with the genotype and gender. Current age was not included in analysis as it was noted to be collinearly correlated to duration and age at drug onset.

The results on genotype are not very surprising as other studies, though in other races and with other *CYP2D6* alleles have shown no association before. Lohman et al showed no association between TD and *CYP2D6**3, *4, *5 and *6 in Caucasians.⁵² Inada et al also found no correlation between TD and *CYP2D6**2 and *10 in Asians with TD.¹⁶ However, it is rather surprising that gender did not have a statistically significant relationship with TD development. Even though it is commonly held that females are more at risk, the elderly females are the ones at increased risk. In young patients, TD risk is higher in men than in women.⁷⁷

According to the Hardy - Weinberg principle, allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary

influences.^{73,78} It is an important principle in population genetics allowing determination of whether evolution is occurring between generations or not. It gives a base from which to measure allele frequencies in a population.⁷⁸ In this study, the genotypes were not in Hardy Weinberg Equilibrium. This departure may be explained by natural selection, mutations, migration, finite population or non - random mating.⁷³ Since the studied sample was not in equilibrium, one or more of these factors could explain the shift. However the sample size was also small and it cannot be ruled out from explaining the departure from the Hardy – Weinberg equilibrium.

The major limitation in the current study was failure to reach an adequate sample size as previously calculated. This lowered the statistical power of the study. It was also assumed that the controls were not on such high doses such that TD symptoms are masked. Other significant problems realised were the difficulties in determining the exact duration on antipsychotic therapy, the participant's definitive HIV status and exposure histories to alcohol and smoking. For the later, it was felt that the patients were with-holding their true histories as they know that smoking and alcohol consumption are discouraged by medical professionals. This reduced the available predictor variables for analysis.

Due to time constraints, the AIMS test was administered once though it would have been desirable to administer it on at least two occasions to avoid bias. At the same time, this might have helped in avoiding the problem of attrition with subsequent tests. The study results are expected to be valid in black psychotic patients exposed to haloperidol and/or FD.

Evidence of interactive effects of *BDNF* and *DRD3* polymorphs;⁵⁰ *MnSOD Ala9Val* and *DRD3 Ser9Val* polymorphs amongst others prove that these effects may be important in TD aetiology.⁴⁶

Since *MnSOD Ala9Val* may be involved in the development of TD and other involuntary movements in black psychiatric patients exposed to antipsychotics,⁷⁹ it is a candidate genetic polymorphism to study with *CYP2D6*. A statistically powered pharmacogenetic case-control study on *CYP2D6*17* and *MnSOD Ala9Val* could be done to determine if an interaction of these genotypes has a relationship with tardive dyskinesia occurrence. *DPP6* is another candidate gene for such an association study. It has recently been correlated to TD occurrence in Japanese study and replication of this is needed in other races.⁸¹ As shown in the reviewed literature, *CYP2D6* is associated with TD occurrence in other races with different alleles involved.

The other issue that could be addressed in such an investigation is the use of multiplex arrays to increase the number of genes assayed and analysed. More SNPs can then be studied and correlations analysed. The other variable that can be added to the study is the determination of steady state typical antipsychotic levels. This will help relate the reduced drug clearance to TD occurrence if present. In addition to the genetic factors explored above, the role of HIV and cannabis can also be investigated. One study has shown a protective effect with cannabis in TD aetiology.⁸² This coupled with the fact that cannabis is being considered a therapeutic alternative in TD management warrants verification of the above mentioned protective effect.

Over and above, this is a useful pharmacovigilance tool to assess, understand and help prevent or manage the risk of TD in the antipsychotic exposed populations. If the pathophysiology of drug induced TD is elucidated and understood, necessary precautions can be instituted. These may include policies aimed at formulating antipsychotic prescription algorithms that take into account the patient's genotype thus, aiding in achieving a safe target dose. This is especially so given the advent of personalised medicine. Some regulatory bodies, such as the USA's FDA are encouraging inclusion of genetic information on package inserts. This will help in optimising drug therapy through identification of response and/or

adverse effects in patients.⁸⁰ This idea will also help in discovering further aids towards rational drug prescribing principles.

Conclusion

Based on the findings of the present study, there is no association between the major reduced function, *CYP2D6*17* genotype and tardive dyskinesia in black psychotic patients (Zimbabwean). Other factors such as exposure to chlorpromazine and age at drug initiation had a significant contributory effect in the development of tardive dyskinesia. Sex and duration of treatment had no role in the development of tardive dyskinesia.

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Appendix I: PATIENT DETAILS QUESTIONNAIRE

Participant name:

Hospital study entry number:

Hospital:

Hospital number:

Study reference number:

Case Control

1. Current age
2. Age at first psychiatric diagnosis
3. Sex
4. Current marital status
5. Diagnosis:

| | |
|----------------------------|-----------------------------|
| Schizophrenia | |
| HIV induced Psychosis | Temporal lobe epilepsy |
| Bipolar affective disorder | Substance induced psychosis |
| Psychotic disorder NOS | Other |
6. Duration of TD:

| | |
|----------------------|--|
| Insufficient details | |
|----------------------|--|
7. Exposure to: 1) Haloperidol

| | |
|--|--|
| 2) Fluphenazine Decanoate (FD) | |
| Others: CPZ Thioridazine TFPZ Benzhexol Atypical antipsychotic | |
8. Age at first antipsychotic treatment
9. Duration on typical antipsychotic

| | |
|---------------------------------------|--------------|
| Changed to atypical antipsychotic Y/N | |
| Periods off medication: YES or NO | if YES, why? |
10. Dose

| | |
|----------------|--|
| CPZ dose years | |
|----------------|--|
11. Current medications
12. Prior history of EPSEs: YES or NO
13. Smoking: YES or NO
14. Alcohol: YES or NO

| | |
|--------------------------|--|
| if yes, AUDIT score..... | |
|--------------------------|--|
15. AIMS score.....
16. Reported HIV status

Appendix II: AIMS (Abnormal Involuntary Movements) SCORE QUESTIONARRE

Clinician **Ref number** **Date** **Score**

Code: 0 = none; 1 = minimal; 2 = mild ; 3 = moderate; 4 = severe

| Movement rating: | | RATER | | RATER | | RATER | | RATER | |
|-------------------------|--|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | | DATE | DATE | DATE | DATE | DATE | DATE | DATE | DATE |
| Facial & oral movements | 1.Muscles of facial expression | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| | 2.Lips and perioral area | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| | 3.Jaw | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| | 4.Tongue | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| Extremity movements | 5.Upper limb(arms, wrists, hands, fingers) | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| | 6.Lower limb(legs, knees, ankles, toes) | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| Trunk movements | 7.Neck, shoulders & hips | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| Global movements | 8.Severity of abnormal mvts. | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| | 9.Incapacitation | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| | 10.Patient's awareness | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 | 0 1 2 3 4 |
| Dental status | 11.Current problems with teeth | YES | NO | YES | NO | YES | NO | YES | NO |
| | | YES | NO | YES | NO | YES | NO | YES | NO |
| | | YES | NO | YES | NO | YES | NO | YES | NO |
| | 12.Do mvts disapp. with sleep | YES | NO | YES | NO | NO | NO | YES | NO |

Appendix III: AUDIT SCORE TEST

Ref number..... Date

| Questions | Scoring system | | | | | Your score |
|--|----------------|-------------------|-----------------------------------|-----------------------|-----------------------------------|------------|
| | 0 | 1 | 2 | 3 | 4 | |
| How often do you have a drink containing alcohol? | Never | Monthly or less | 2 – 4 times per month | 2 – 3 times per month | 4+ times per month | |
| How many units of alcohol do you drink on a typical day when you are drinking? | 1 - 2 | 3 - 4 | 5 - 6 | 7 - 9 | 10+ | |
| How often have you had 6 or more units if female, or 8 or more if male, on a single occasion in the last year? | Never | Less than monthly | Monthly | Weekly | Daily or almost daily | |
| How often during the last year have you found that you were not able to stop drinking once you had started? | Never | Less than monthly | Monthly | Weekly | Daily or almost daily | |
| How often during the last year have you failed to do what was normally expected from you because of your drinking? | Never | Less than monthly | Monthly | Weekly | Daily or almost daily | |
| How often during the last year have you needed an alcoholic drink in the morning to get yourself going after a heavy drinking session? | Never | Less than monthly | Monthly | Weekly | Daily or almost daily | |
| How often during the last year have you had a feeling of guilt or remorse after drinking? | Never | Less than monthly | Monthly | Weekly | Daily or almost daily | |
| How often during the last year have you been unable to remember what happened the night before because you had been drinking? | Never | Less than monthly | Monthly | Weekly | Daily or almost daily | |
| Have you or somebody else been injured as a result of your drinking? | No | | Yes, but not during the last year | | Yes, but not during the last year | |
| Has a relative or friend, doctor or other health worker been concerned about your drinking or suggested that you cut down? | No | | Yes, but not during the last year | | Yes, but not during the last year | |

Scoring: 0 – 7 lower risk; 8 – 15 increasing risk;

16 – 19 higher risk; 20+ possible dependence

Overall Score

Appendix IV A: English consent form

SUBJECT INFORMED CONSENT

Ref no.

PROTOCOL TITLE: The relationship between CYP2D6 polymorphisms and Tardive Dyskinesia in black Zimbabwean psychotic patients on typical antipsychotics – a pilot study

PRINCIPAL INVESTIGATOR: Dr JT Masuka MBChB DMH (0772295092)

SUPERVISORS: Prof CFB Nhachi PhD

Dr WO Mangezi MBChB DMH MMed(Psychiatry)

PROJECT DESCRIPTION

You are kindly being asked to volunteer for a research study conducted by an MSc student in Clinical Pharmacology at the University of Zimbabwe. The study will help to find a way of avoiding some adverse effects of antipsychotic medication in susceptible patients. It will be carried out on adults attending Harare Psychiatric Unit and Parirenyatwa Annexe hospitals.

YOUR RIGHTS

Before you decide whether or not to volunteer for this study, you must understand its purpose, how it may help you, and what is expected of you. This process is called informed consent.

PURPOSE OF RESEARCH STUDY

The study is to determine the relationship between CYP2D6 genotypes (inherited traits) and the occurrence of the unexpected or dangerous reactions (adverse effect) Tardive Dyskinesia (abnormal body movements associated with antipsychotics). It also aims to relate the blood levels of haloperidol and/or fluphenazine decanoate (mental illness treatments) to these inherited traits in patients taking these medications.

PROCEDURES INVOLVED IN THE STUDY

You will be asked questions through questionnaires and then 2 X 5ml (2 X teaspoon equivalent volumes) samples of blood will be drawn for genotyping and serum drug level determination at AiBSt laboratories in Harare. Abnormal movements will be measured by one of the questionnaire tests – the AIMS test. No other tests will be done except for the tests agreed to above. The samples will be kept in the AiBSt Biobank for the duration of the study and disposed of within 30 days of completion of the study.

DISCOMFORTS AND RISKS

Apart from slight of discomfort giving blood, no other discomfort is anticipated. Going through the interview may take you about 40 minutes.

POTENTIAL BENEFITS

You may get no direct benefit from being in this study. You or others may benefit in the future from information about CYP2D6 genotypes and their effects on TD learned in this study. You also may get some personal satisfaction from being part of this research.

STUDY WITHDRAWAL

You may choose not to enter the study or withdraw from the study at any time without loss of benefits entitled to you.

CONFIDENTIALITY OF RECORDS

Every effort will be made to protect participant privacy and confidentiality to the extent possible. No information identifying you will be published without your permission.

PROBLEMS OR QUESTIONS

Please ask questions about this research or consent now. If you have any question in future please ask.

AUTHORIZATION

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I know being in this study is voluntary. I choose to be in this study. I know I can stop being in the study and I will not lose any benefits entitled to me. I will get a copy this consent form. (Please initial all the pages of the consent form).

Client Signature

Date

Client Name (printed)

Caregiver/Consultant's Name

Caregiver/Consultant's Signature

Date.....

Researcher Signature

Date.....

Witness Signature

Date.....

Initials_____

Appendix IV A: Shona consent form

GWARO RETENDERANO

Ref no.

PROTOCOL TITLE: The relationship between CYP2D6 polymorphisms and Tardive Dyskinesia in black Zimbabwean psychotic patients on typical antipsychotics – a pilot study

PRINCIPAL INVESTIGATOR: Dr JT Masuka MBChB DMH (0772295092)

SUPERVISORS: Prof CFB Nhachi PhD

Dr WO Mangezi MBChB DMH MMed(Psychiatry)

ZVINOENDERANA NECHIRONGWA

Munokumbirwawo kubatsira muchirongwa chirikuitwa nemudzidzi wepaUniversity yeZimbabwe ari kuita Masters yeClinical Pharmacology zvinoita kuti awane gwaro iri. Chirongwa ichi chichaitwa muvarwere vanorapwa muzvipatara zveHarare Psychiatric Unit neParirenyatwa Annexe.

KODZERO DZENYU

Musatimapa sarudzo kuti mopinda muchirongwa here kana kuti kwete, tinoda kukutsanangurirai chinangwa chechirongwa, kubatsirikana kwamungaita kubva pachiri, njodzi nezvino tarisirwa kubva kwamuri. Ukundiko kunonzi kupa mvumo manzwisisa chirongwa.

CHINANGWA CHECHIRONGWA

Donzo rechirongwa nderekuona kuti magenes anonzi CYP2D6 anoenderana sei nechitiko chinonzi Tardive Dyskinesia (chirwere chekufamba-famba kwemitezo yemuviri) uye neuwandu hwehaloperidol ne fluphenazine decanoate (mishonga inorapa zvirwere zvepfungwa) muvarwere varipa mishonga iyoyi.

ZVICHAITWA MUCHIRONGWA

Muchabvunzwa mibvunzo mushure mezvo mozotorwa netsono rinokwana ma5ml maviri (ropa rinokwana mateaspoon maviri) rekutarisa genotype uye uwandu hwemushonga muropa renyu mumaraboretori eAiBSt muHarare. Mafambiro emutezo wemuviri wenyu asina kutaranuka achaerwa nemibvunzo inonzi AIMS test. Hapana zvimwe zvakaita seHIV zvichavhenekwa kunze kwezvamabvumira muchirongwa chino. Ropa rinotorwa richachengetwa muraboretori eAiBSt panguva yedzidzo kusvika mazuva anokwana makumi matatu kubva panopera chirongwa ichi robva raraswa.

ZVIKANGAIDZO KANA NJODZI YAMUNGASANGANA NAYO

Hatitarisire kuti pangave nenjodzi yamungasangane nayo kunze kwekuswinyiwa zvishoma shoma pamunenge muchitorwa ropa.Kubvunzwa mibvunzo kunogona kukutorerai maminetsi anokwana kuita makumi maviri.

ZVAMUNGAWANA KUBVA MUCHIRONGWA

Hapana mibairo yamuchawana kupinda muchirongwa. Zvichabuda muchirongwa ichi zvinogona kuzokubatsirai imi nevamwe mune ramangwana paruzivo rweTardive Dyskinesia. Munokwanisa kunzwa kugutsikana mumoyo pakuva muongororo ino.

SARUDZO YOKUBUDA MUCHIRONGWA

Sarudzo ndeyenyu kuva muchirongwa chino kana kusapinda.Kana mapinda muchirongwa munokwanisa kubuda machiri cheronguva musingarasikirwe nezvamunofanira kuwana.

KUCHENGETEDZEKA KWEZVOSE ZVATAURWA NEMI

Zvose zvamuchataura muchirongwa nezvenhoroondo yenyu zvichachengetedzwa pakahwanda. Hakuna pachanyorwa nezvenhoroondo yenyu musina kukumbirwa mbvumo.

MIBVUNZO KANA ZVICHEMO

Sunungukai kubvunza mubvunzo pamusoro pechirongwa chino kana kubvuma kupinda muchirongwa. Mukaita mibvunzo maererano nechirongwa chino munokwanisa kubvunza makasunguka.

KUBVUMA KUPINDA MUCHIRONGWA

Ndaverenga kana kuverengerwa bepa rino rechirongwa chirikuitwa. Ndanzwisisa njodzi nezvandingawana muchirongwa chino. Ndanzwisisa kuti isarudzo yangu kupinda kana kusapinda muchirongwa. Ndasarudza kupinda muchirongwa. Ndanzwisisa kuti ndinogona kubuda muchirongwa chero nguva ndisingarasikirwe nezvandinofanira kuwana. Ndichapiwa bepa resarudzo rino. (Isayi mavara okutanga emazita enyu papeji yoga-yoga)

Runyoro rwenyu

Zuva ranhasi.....

Zitarenyu (rakanyorwa)

Zita remuchengeti/rachiremba mukuru

Runyoro rwemuchengeti/rwachirembe mukuru

Zuva ranhasi.....

Zita rearikuitisa chironywa

Zuva ranhasi.....

Runyoro rwemufakazi

Zuva ranhasi.....

initials_____