UNIVERSITY OF RWANDA

CORRELATION BETWEEN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY SEVERITY AND CYP3A4 VARIATIONS IN RWANDAN CANCER PATIENTS RECEIVING PACLITAXEL TREATMENT

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By

Jean Paul TUYISENGE

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Supervisor: Dr. Ismail Abiola ADEBAYO, PhD

Co-Supervisors: -Mr. Alexis RUGAMBA, PhD(c)

-Dr. Jean Paul NSHIZIRUNGU, PhD

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DECLARATION

I do hereby affirm that this project submitted in full fulfilment of the requirement of the Master's degree in Biotechnology, College of Sciences and Technology, is my original work. It has not been submitted for any academic award elsewhere. Furthermore, all referenced or cited information has been fully acknowledged in the list of references provided

Jean Paul TUYISENGE

DEDICATION

I dedicate this dissertation to the almighty God for being with me all over this journey of studying. My dedication to this work goes to our lovely parents, brothers, sisters, lecturers, professors and our friends who kept giving us all means of support that lead us to the accomplishment of this research project.

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Summary

Chemotherapy-induced peripheral neuropathy (CIPN) is a common and distressing complication experienced by cancer patients undergoing paclitaxel therapy. This study investigates the correlation between CIPN severity and genetic variations in the CYP3A4 enzyme among Rwandan cancer patients receiving paclitaxel-based chemotherapy. A cohort of 90 patients, predominantly female (96.5%) with breast cancer (88.37%), was enrolled at Butaro Cancer Center; 86 completed all clinical visits.

Patients were followed across four chemotherapy cycles, with neuropathic symptoms systematically assessed at each visit using standardized scoring tool, EORTC QLQ-CIPN20 scale tailored for CIPN severity. Blood samples were collected for genetic analysis, and CYP3A4 genotyping was conducted using Infinium Global screening Array, a robust and validated approach for single nucleotide polymorphism detection.

Genotyping revealed that 67.4% carried the wild-type allele (AA), 27.9% were heterozygous mutants (AT), and 4.7% were homozygous mutants (TT) of CYP3A4. Longitudinal assessment of neuropathic symptoms over four visits demonstrated a significant increase in CIPN severity, particularly after the second chemotherapy cycle. Linear mixed-effects modeling showed a statistically significant effect of CYP3A4 genotype on CIPN severity($p<0.001$), with homozygous mutants exhibiting markedly higher symptom scores compared to wild-type carriers ($p<0.001$). No significant difference was observed between heterozygous mutants and wild-type patients.

These findings highlight a potential genetic predisposition contributing to CIPN severity and underscore the importance of incorporating CYP3A4 genotyping in clinical oncology practice. Early and systematic neuropathy monitoring is recommended to optimize paclitaxel treatment and improve patient quality of life. Further studies in diverse African populations are warranted to validate these results.

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Acronyms

UR: University of Rwanda

CST: College of Science and Technology

CMHS: College of Medicine and Health Sciences

IARC: International Agency for Research on Cancer

CIPN: Chemotherapy Induced peripheral neuropathy

EORTC QLQ-CIPN20: European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Chemotherapy-Induced Peripheral Neuropathy 20-item scale

NCI-CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events

CYP3A4: Cytochrome P450 family 3 subfamily A member 4

CYP2C8: Cytochrome P450 Family 2 Subfamily C Member 8

ABCB1: ATP-binding cassette, subfamily B, member 1

IRB: Institutional Review Board

SPSS: Statistical Package for the Social Sciences.

GSA: Infinium Global Screening Array

Chapter 1. Introduction

This chapter covers the definitions of key terms, background, problem statement, hypothesis, objectives both general objective and specific objectives, and significance of the study.

1.1. Background

Cancer remains a major public health concern, impacting society and economies significantly(Bray et al., 2024). Global estimates in 2022, showed about 20 million cancer cases with 9.7 million deaths from the International Agency for Research on Cancer (IARC) and lung cancer accounting as the most frequently diagnosed cancer in 2022, followed by cancers of the female breast, colorectum, prostate and stomach respectively (Bray et al., 2024). These numbers highlight not only the growing incidence of cancer but also the heightened need for efficient prevention strategies, early detection, and treatment interventions. The progress in diagnostic technologies and therapeutic approaches has led to better cancer care outcomes by allowing earlier detection and providing a broader range of treatment options(Pulumati et al., 2023). Chemotherapeutic agents continue to be a mainstay of treatment for a wide variety of malignancies, despite the emergence of targeted therapies and immunotherapies. The increasing survival rate among cancer patients is evidence of progress in oncology; however, survivors often face chronic treatment-related complications (Mols et al., 2014). These complications can adversely affect their post-treatment quality of life, with chemotherapy-induced peripheral neuropathy (CIPN) being among the most commonly reported and debilitating side effects(Seretny et al., 2014). CIPN results from damage to the peripheral nervous system caused by neurotoxic chemotherapeutic agents. This condition is expressed by sensory symptoms like tingling, numbness, and a burning feeling, most commonly affecting the hands and feet. In severe cases, it can lead to motor dysfunction, gait instability, and even permanent

disability(Flatters et al., 2017). The incidence of CIPN varies considerably, reported to range between 12% and 96%, depending on the specific agent used, cumulative dose, treatment schedule, and individual patient characteristics(Park et al., 2013). Such high variability rates suggest an underlying interindividual susceptibility that has prompted research into genetic and molecular predictors.

Paclitaxel, A taxane-class chemotherapy drug, it is commonly employed in managing solid tumors such as breast, ovarian, and lung cancers. It works by attaching to the β-tubulin units of microtubules, stabilizing their structure, and consequently disrupting mitotic division in rapidly dividing cancer cells(Mukhtar et al., 2014)(Weaver, 2014a). However, this mechanism also affects non-cancerous cells, particularly neurons, leading to microtubule dysfunction and axonal degeneration in peripheral nerves(Chua et al., 2022). Studies have shown that up to 60–70% of patients treated with paclitaxel develop some form of neuropathy, with many experiencing persistent symptoms beyond the treatment period(Staff et al., 2020a).

The severity of CIPN is graded using standardized clinical tools such as the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE), which classifies neuropathy into five grades ranging from mild symptoms (grade 1) to life-threatening consequences (grade 5) (NCI, 2020) or European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Chemotherapy-Induced Peripheral Neuropathy 20-item scale (EORTC QLQ-CIPN20)(Rattanakrong et al., 2022). Despite the utility of such scales, they are often clinician-reported and may not capture the full spectrum of patient experiences, particularly subjective symptoms that affect quality of life and functional independence(Lee et al., 2024). The variability in susceptibility to paclitaxel-induced neuropathy has driven interest in pharmacogenomics the study of genetic determinants that influence drug response and toxicity(Johnson et al., 2023). A key area of focus in this regard is the cytochrome P450 (CYP450) family of enzymes, which

play a central role in the metabolism of various drugs. Among these, CYP3A4 is one of the most abundantly expressed isoforms in the liver and intestine, responsible for metabolizing more than half of all marketed drugs, including paclitaxel(Vander Schaaf et al., 2024).

Genetic polymorphisms in the CYP3A4 gene can alter enzyme activity, thereby affecting drug pharmacokinetics and clinical outcomes(Y. Zhang et al., 2024). Some variants result in reduced or null enzymatic function, leading to higher systemic exposure to paclitaxel and potentially greater neurotoxicity. Conversely, other variants may enhance metabolism, potentially reducing toxicity but also decreasing therapeutic efficacy(De Graan et al., 2013). Variants such as CYP3A4 1B, 22, and 1G have been studied in various populations, but findings remain inconclusive and often population-specific(D. Wang et al., 2011). Furthermore, CYP3A4 activity can be influenced by co-expressed genes, environmental factors (e.g., diet, smoking), and interactions with other medications, complicating the prediction of drug response. Though, identifying CYP3A4 polymorphisms associated with high-risk neuropathy profiles holds promise for personalizing chemotherapy regimens. Genotyping prior to therapy initiation could help oncologists tailor dosages or consider alternative regimens in patients predisposed to severe toxicity, ultimately enhancing safety and efficacy.

In low and middle-income countries (LMICs), including Rwanda, the burden of cancer is on the rise due to epidemiologic transitions, increased life expectancy, and improved diagnostic capacities(Stefan & Tang, 2023). However, access to molecular diagnostics, including pharmacogenetic testing, remains limited. There is a critical need for locally generated evidence to guide clinical decisions and policy formulation in oncology care(Stefan & Tang, 2023). Research focusing on the pharmacogenomic profiles of African populations is especially important given the high genetic diversity on the continent and the underrepresentation of African data in global genomic databases(Alimohamed et al., 2025).

In Rwanda, the implementation of chemotherapy regimens such as paclitaxel is increasingly common in tertiary hospitals and cancer treatment centers. However, the management of adverse drug reactions such as CIPN remains suboptimal, often limited to symptomatic treatments or dose reductions, which may compromise treatment outcomes. To date, no studies have worked systematically to evaluate the relationship between CYP3A4 polymorphisms and the severity of paclitaxel-induced neuropathy in Rwandan cancer patients. This represents a significant knowledge gap, particularly in the context of integrating precision medicine into the country's cancer control strategy.

This study, therefore, aims to assess the correlation between chemotherapy-induced peripheral neuropathy severity and CYP3A4 enzyme variations among Rwandan cancer patients receiving paclitaxel. The findings are expected to inform future clinical practice by identifying genetic markers associated with CIPN risk and contributing to the development of personalized cancer treatment protocols in Rwanda and similar resource-limited settings. Furthermore, this research could serve as a foundation for capacity-building in pharmacogenomics and contribute to broader efforts in equitable access to precision medicine across Sub-Saharan Africa

1.2. Problem statement

The area of cancer research and treatment is advancing, with the ongoing identification of specific biomarkers for different types of cancer(Passaro et al., 2024). Yet, despite the development of advancement in cancer diagnosis and early treatment, many cancer survivors express poor treatment outcomes due to the syndromes resulting from cancer treatment and may reduce quality of life (Eton et al., 2019; Lara-Morales et al., 2024). One

of the most troubling complications is chemotherapy-induced peripheral neuropathy (CIPN).

Peripheral neuropathy resulting from chemotherapy (CIPN) is a commonly and draining adverse effect in cancer patients, particularly those treated with paclitaxel, impacting up to 97% of patients with gynecological and urological cancers(Apellániz-Ruiz et al., 2015).While paclitaxel is the most commonly chemotherapeutic agent, but its use is frequently limited by the development of CIPN, which can lead to dose reductions, treatment suspensions, and a significant decline in patients' quality of life(McEvoy et al., 2023). The interindividual variability in CIPN severity suggests a potential genetic component, particularly involving genes that regulate drug metabolism(Zenatri et al., 2024). Cytochrome P450 3A4 (CYP3A4) is a major hepatic enzyme involved in the metabolism of numerous chemotherapeutic agents, including paclitaxel. While CYP2C8 is the principal enzyme responsible for paclitaxel metabolism, CYP3A4 plays a supportive yet substantial role(van Eijk et al., 2019). Genetic polymorphisms in CYP3A4 may alter enzyme activity, influencing drug clearance and leading to paclitaxel accumulation, thereby increasing the risk of neurotoxic side effects(De Graan et al., 2013).

Several studies have investigated the association between CYP3A4 polymorphisms and CIPN, with some identifying specific variants that correlate with an increased risk of neuropathy and treatment modifications(Diaz et al., 2018)(McEvoy et al., 2023). For instance, alleles associated with reduced or absent enzyme function have been linked to heightened paclitaxel toxicity(De Graan et al., 2013). However, findings across the literature remain inconclusive, with some studies reporting no significant association between CYP3A4 genotype and CIPN severity(Rodwin et al., 2022). These discrepancies underscore the need for further investigation, particularly within underrepresented populations such as those in sub-Saharan Africa.

In Rwanda, where the oncology services are expanding and access to chemotherapeutic agents is increasing, there remains a paucity of pharmacogenomic data regarding treatment outcomes. Exploring the relationship between CYP3A4 genetic variants and the severity of CIPN in Rwandan cancer patients has the potential to inform personalized medicine approaches. Such research could help identify individuals at elevated risk for severe neuropathy, enabling clinicians to implement early risk-mitigation strategies, improve treatment adherence, and ultimately enhance patient outcomes and quality of life.

1.3. Hypothesis

We hypothesize that specific genetic variants of CYP3A4 are associated with increased severity of CIPN in Rwandan patients undergoing paclitaxel chemotherapy.

1.4. Objectives

1.4.1. Main objective

To assess the correlation between the severity of CIPN and the genetic variation in cytochrome CYP3A4 in patients on paclitaxel chemotherapy

1.4.2. Specific objectives

- To assess the severity of chemotherapy induced peripheral neuropathy in patients undergoing chemotherapy using the National Cancer Institute's Common Terminology Criteria for Adverse Events European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Chemotherapy-Induced Peripheral Neuropathy 20-item scale (EORTC QLQ-CIPN20)
- To identify genetic variations in the CYP3A4 gene among the participants confirmed for ovarian, breast and cervical cancer taking paclitaxel chemotherapy
- To determine the correlation between CYP3A4 genetic variations and the severity of CIPN

1.5. Significance of the study

The study was aimed to identify specific CYP3A4 genetic variants associated with increased CIPN severity. Where the findings may provide insights into the mechanisms underlying CIPN development and help identify patients at higher risk. Data obtained could also be used to personalize chemotherapy treatment strategies and potentially reducing the incidence and severity of CIPN and improving patient outcomes.

Chapter 2. Literature review

2.1. Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a prevalent and dose-limiting side effect of cancer treatment, particularly in patients receiving paclitaxel(Staff et al., 2017). The incidence is estimated up to 60% of all cancer patients under chemotherapy(Klein & Lehmann, 2021). While in all gynecological and urological cancer patients, paclitaxel account 97% of neuropathy cases with severe cases of 33%, that significantly impairing quality of life and often necessitating dose reductions or treatment discontinuation(Klein &

Lehmann, 2021). Genetic variations in drug-metabolizing enzymes, such as CYP3A4, are hypothesized to influence paclitaxel metabolism and contribute to interindividual variability in CIPN severity(De Graan et al., 2013). This review synthesizes the current literature on the correlation between CYP3A4 genetic polymorphisms and CIPN severity in cancer patients undergoing paclitaxel chemotherapy.

CIPN Clinical manifestation, impact and pathophysiology

CIPN manifests as sensory disturbances, including numbness, tingling, and pain, primarily in the hands and feet. Motor and autonomic symptoms may also occur. These symptoms can persist long after chemotherapy completion, significantly impairing quality of life and daily functioning. CIPN often requires lowering the chemotherapy dose or stopping treatment altogether, which can negatively impact its overall effectiveness (Colvin, 2019). The pathogenesis of CIPN is multifactorial. Paclitaxel disrupts microtubule dynamics, essential for axonal transport, leading to neuronal damage. Additionally, mitochondrial dysfunction, oxidative stress, and inflammation contribute to neuronal injury. Recent studies have highlighted the role of ion channels and neuroinflammatory pathways in CIPN development (Kacem et al., 2024)

Risk factors for CIPN development

Risk factors for CIPN include cumulative dose, pre-existing neuropathies, diabetes, age, and genetic predispositions. Notably, genetic polymorphisms affecting drug metabolism and neuronal function have been implicated in CIPN susceptibility(Rodwin et al., 2022)

2.2. Paclitaxel, discovery and development

Paclitaxel is a famous chemotherapeutic agent, representing a significant advancement in cancer treatment(Sharifi-Rad et al., 2021). Initially isolated from the bark of the Pacific yew tree (Taxus brevifolia), and has evolved into an essential drug for treating a wide spectrum of cancers, including breast, ovarian, and non-small cell lung cancers(Ejeta et al., 2024).

The discovery and development journey of paclitaxel began in 1962 as part of a large-scale screening program initiated by the U.S. National Cancer Institute (NCI) in collaboration with the U.S. Department of Agriculture, aiming to identify novel plant-derived anticancer compounds(Wani & Horwitz, 2014), Where the NCI collected samples from over 30,000 plant species, one of which was Taxus brevifolia, a coniferous tree native to the Pacific Northwest of the United States. An extract from the bark of this tree demonstrated potent cytotoxic activity, eventually leading to the isolation of the active compound, paclitaxel (Gärditz & Czesnick, 2024). Despite its promising biological activity, challenges related to its low natural abundance and poor water solubility initially hindered development. These issues were later addressed by the development of a semisynthetic production method using Taxus baccata needles and by formulating the drug with the solubilizer Cremophor EL(S. Zhang et al., 2023)

Paclitaxel mode of action

Paclitaxel's mechanism of action differs from many other chemotherapeutic agents. It promotes the polymerization of tubulin into stable microtubules and inhibits their depolymerization, leading to the disruption of normal mitotic spindle function (Weaver, 2014b).This arrest of cell division in the G2/M phase induces apoptosis in rapidly dividing

cancer cells. Additionally, paclitaxel has been shown to exert antiangiogenic effects and modulate immune responses, contributing to its anticancer efficacy(Zhou et al., 2023)

Paclitaxel Clinical Applications, efficacy and limitations

Paclitaxel has demonstrated remarkable clinical efficacy against a broad range of malignancies. It was first approved by the U.S. Food and Drug Administration (FDA) in 1992 for the treatment of ovarian cancer, and its indications have since expanded to include breast cancer, Kaposi's sarcoma, and non- small cell lung cancer, among others(Sati et al., 2024) (Bernabeu et al., 2017). In breast cancer, paclitaxel is often used in adjuvant and neoadjuvant settings, improving survival rates in patients with early and advanced disease (Hirmas et al., 2024). It is also a standard component of combination regimens, such as with carboplatin in ovarian cancer and with cisplatin in lung cancer(Mansoor et al., 2023). Furthermore, the development of nanoparticle-based formulations like nab-paclitaxel, an albumin-bound paclitaxel has improved drug delivery and reduced toxicity, offering better therapeutic outcomes in several cancers (Cucinotto et al., 2013).

Despite its efficacy, paclitaxel is associated with limitations, particularly the development of resistance. Mechanisms of resistance include overexpression of drug efflux pumps such as P-glycoprotein (MDR1), alterations in tubulin isotypes, and defects in apoptotic pathways (Alalawy, 2024). Moreover, paclitaxel-induced peripheral neuropathy (PIPN) remains a major dose-limiting side effect, often resulting in treatment discontinuation(Staff et al., 2020b). Ongoing research is investigating genetic markers, such as polymorphisms in CYP2C8 and CYP3A4 enzymes, that influence paclitaxel metabolism and patient response, with the aim of developing personalized treatment strategies(Apellániz-Ruiz et al., 2015)(De Graan et al., 2013)

2.3. Role of CYP3A4 in metabolism of paclitaxel

CYP3A4 is an important enzyme in the cytochrome P450 family that helps break down and process nearly half of the medications commonly used in clinical practice through oxidative metabolism. It is mainly and most abundant cytochrome located in the liver and small intestine(Ince et al., 2013). For paclitaxel, a commonly used chemotherapeutic agent in the treatment of various solid tumors such as breast, ovarian, and non-small-cell lung cancers, its metabolism involves both CYP2C8 and CYP3A4 enzymes(Bidstrup et al., 2003). While Although paclitaxel is mainly broken down by CYP2C8 into 6α-hydroxypaclitaxel, CYP3A4 also plays a smaller role by producing minor metabolites such as 3'-p-hydroxypaclitaxel. These metabolites can undergo further biotransformation; specifically, CYP2C8 and CYP3A4 jointly contribute to the formation of 6α,3'-p-dihydroxypaclitaxel, another inactive derivative (Y. Wang et al., 2014). Even if CYP3A4 is not the primary enzyme involved in paclitaxel metabolism, its role gains clinical significance due to individual differences in enzyme expression and activity, which can affect the drug's absorption, effectiveness, and potential for toxicity(Y. Zhang et al., 2024).

2.4. Studies explored the relationship between CYP3A4 and chemotherapy-induced peripheral neuropathy

Apart from paclitaxel-induced peripheral neuropathy being dose dependent or being influenced by various clinical conditions that have been suggested as risk factors, genetic variations have been studied to be neuropathy development risk factor(Apellániz-Ruiz et al., 2015)(Baldwin et al., 2012). A study by whole-exome sequencing to reveal defective CYP3A4 variants predictive of paclitaxel dose limiting neuropathy in cancer patients study utilizing whole-exome sequencing was carried out by Apellániz-Ruiz M et al. in 2015 to identify defective CYP3A4 variants that may predict paclitaxel-induced dose-limiting neuropathy in cancer patients (Apellániz-Ruiz et al., 2015).It was the first to describe a genetic marker associated with paclitaxel treatment modifications caused by neuropathy CYP3A4 defective variants that may provide a basis for paclitaxel treatment individualization(Apellániz-Ruiz et al., 2015). The study showed that patients with certain

genetic changes were more likely to develop severe neuropathy. Specifically, those with loss of function changes had twice the risk, and those with missense changes had a slightly higher risk compared to those without these changes(de Jong et al., 2023). Concluding that, these genetic markers could serve as predictive indicators for paclitaxel treatment modifications due to neuropathy(Apellániz-Ruiz et al., 2015).

Another significant variant, CYP3A422, has been linked to decreased enzymatic activity and has been studied for its association with CIPN. Through study research by de Graan et al. demonstrated that female carriers of the CYP3A422 allele were at an increased risk for developing CIPN during paclitaxel therapy, although this association was not observed in male patients within their exploratory cohort(De Graan et al., 2013). Though, in a validation cohort, both male and female carriers exhibited an elevated risk, suggesting that this variant may be relevant across genders(Johnson et al., 2023).

Additionally, a study carried out between March 2011 and June 2015 among female patients with operable breast cancer receiving paclitaxel treatment investigated the association between taxane-induced neurotoxicity and single-nucleotide polymorphisms (SNPs) in several genes, including CYP3A4(Kus et al., 2016). By evaluating taxane-induced neurotoxicity during the treatment, referred to the National Cancer Institute Common Toxicity Criteria version 4.03 prior to each cycle and then they compared to the genotype results and severity of neuropathy. where the genotype CYP3A4 392 AA/AG had significantly higher risk for grade ≥2 neurotoxicity and was concluded to be taken as predictors of neurotoxicity while using taxane chemotherapy(Kus et al., 2016).

2.4. Genetic Predictors of Chemotherapy Toxicity

Pharmacogenetic studies have identified several genetic variants influencing chemotherapy-related adverse effects. CYP3A4 polymorphisms have been implicated in altered paclitaxel clearance, suggesting a potential link to CIPN risk (McEvoy et al., 2023). Genetic studies on chemotherapy toxicity have also revealed that polymorphisms in other enzymes, such as CYP2C8 and ABCB1, may interact with CYP3A4 to influence drug

metabolism and toxicity(*Pharmaceutics-16-01407-V2,* n.d.).However, the direct relationship between CYP3A4 genetic variants and neuropathy severity remains underexplored, highlighting the need for further investigation.

2.5. Molecular techniques towards SNPs detection

Among the techniques validated so that the variants CYP3A4 can be identified, DNA extraction by QIAamp DNA Mini Kit was employed and the genotyping using Global Screen Array.

Principle of DNA extraction using QIAamp Mini Kit

The QIAamp DNA Mini Kit uses silica-membrane technology to purify DNA from a variety of biological sample types. The core principle is that, under certain conditions, DNA binds selectively to the silica-gel membrane in the presence of chaotropic salts, while contaminants are washed away. Elution with a low-salt buffer or water releases highly pure DNA for next use in the tests like PCR, qPCR, genotyping(Qiaamp et al., 2007). QIAamp DNA Mini Kit (designed for rapid purification of an average of 6 µg of total DNA (e.g., genomic, viral, mitochondrial) from 200 µl of whole human blood and up to 50 µg of DNA from 200 µl of buffy coat, 5 x 106 lymphocytes, or cultured cells that have a normal set of chromosomes). The procedure suitable for use with whole blood treated with citrate, heparin, or EDTA(Qiaamp et al., 2007).

Materials and reagents

The manual extraction process utilizes a set of standardized reagents and materials provided within the kit, along with some additional laboratory supplies. The key reagents include Buffer AL, a lysis buffer containing chaotropic salts to disrupt cells and denature proteins; Buffer AW1 and Buffer AW2, which are wash buffers formulated to remove contaminants such as proteins, salts, and other cellular impurities; and Buffer AE, used as the elution buffer to release the purified DNA from the silica membrane of the spin column. Additionally, QIAGEN protease (or alternatively proteinase K) is used to digest proteins

during the lysis step. Absolute ethanol (96–100%) was required to facilitate DNA binding to the column membrane during the binding phase. The extraction procedure to be conducted uses QIAamp Mini spin columns and 2 ml collection tubes provided in the kit, which enable DNA purification through a silica membrane under centrifugation. Other essential laboratory materials include 1.5 ml sterile microcentrifuge tubes, pipettes with aerosol-resistant tips, vortex mixer, microcentrifuge capable of speeds up to 14,000 rpm, and a heating block or water bath set at 56 °C. All reagents ae used according to the manufacturer's instructions, and DNA is eluted in nuclease-free conditions to ensure purity and integrity for downstream applications(Qiaamp et al., 2007).

Principle for of the Infinium GSA technology genotyping technique

The core principle of the Infinium GSA technology relies on the use of silica bead-based microarrays, in which each bead is covalently attached to a specific oligonucleotide probe that targets a known genetic locus. Genomic DNA is first enzymatically fragmented to an optimal size range and then subjected to whole-genome amplification to increase DNA yield. The amplified DNA is enzymatically fragmented again, followed by precipitation and resuspension in a hybridization buffer. The DNA is then hybridized to the bead chip under precise conditions, allowing specific binding of target sequences to complementary probes immobilized on the array surface.

Following hybridization, the arrays undergo a single-base extension reaction. At each SNP locus, a single fluorescently labeled nucleotide complementary to the polymorphic base is incorporated. Each nucleotide is tagged with a distinct fluorescent dye, enabling discrimination between alleles. After washing and staining steps to remove unbound reagents and enhance signal clarity, the array is scanned using the Illumina iScan system. The iScan instrument detects and quantifies the fluorescence signal at each probe location, and the intensity data are processed using GenomeStudio or other genotyping software to call genotypes at each target site(Illumina, n.d.).

Materials and reagents

GSA genotyping workflow is performed using reagents and instrumentation supplied by Illumina, in accordance with the Infinium HD Assay Ultra protocol. Materials used include the Infinium GSA BeadChips, hybridization buffers, isothermal amplification reagents, fragmentation buffer, precipitation and resuspension solutions, staining reagents, and single-base extension (SBE) mix containing allele-specific labeled nucleotides. And instrumentation is composed of the Illumina iScan system for high-resolution array scanning, the Illumina Hybridization Oven for consistent hybridization conditions and also temperature-controlled incubators, microcentrifuges, vortex mixers, thermal cyclers, and magnetic stands.

Chapter 3. Methodology

3.1. Study design

A prospective cohort study was conducted, enrolling patients diagnosed with cancer and receiving paclitaxel-based chemotherapy, and participants were followed along 4 visits, assessing progress of chemotherapy induced peripheral neuropathy.

3.2. Study area

Study was conducted in BUTARO Hospital, a level two teaching hospital located in Northern Province, Burera District. Holding a national cancer center, a center of excellence that offers spectrum of diagnostic oncology and treatment services, including chemotherapy, surgery, pathology laboratory and palliative care.

3.3. Study population and sample size

From confirmed cancer patients under chemotherapy paclitaxel. Using Sample size estimation in Correlation Studies according to Hulley et al., 2013

A sample size formula for estimating the required number of subjects to detect a significant correlation (r) between two continuous variables which are the CIPN severity scores and CYP3A4 enzyme variants.

$$n = \left(\frac{Z_{\alpha/2} + Z_{\beta}}{r} \right)^2 + 3$$

Where:

n = Required sample size

$Z_{\alpha/2}$=Standard normal value for significance level or z-score (e.g., 1.96 for $\alpha = 0.05$, confidential interval 95%)

Z_{β}=Standard normal value for power (e.g., 0.84 for 80% power)

r= Expected correlation coefficient between two variables

For detecting a correlation of r=0.3(hypothetical for moderate correlation) between CIPN severity scores and CYP3A4 enzyme variants, with 95% confidence and 80% power, a sample size of 90 participants was required. So, the 90 participants were enrolled in the study with 86 of them participated completely to the study.

3.4. Sampling strategy

Purposive sampling method was employed in the study to recruit eligible cancer patients receiving paclitaxel chemotherapy at Butaro Cancer treatment center in Rwanda. As there was defined population who are cancer patients, taking paclitaxel chemotherapy and willing to participate in the study along 4 visits. Moreover, the limited number of participants was also factor.

3.5. Inclusion and exclusion criteria

Inclusion criteria: Patients diagnosed with cancer, starting paclitaxel chemotherapy treatment.

Exclusion criteria: Patients with pre-existing neuropathies prior to chemotherapy

3.6. Data collection

Participants accepted to be part of the study were explained the purpose of the study, the timeline and the conditions of the study and allowed to sign the consent form. **(Appendix1).** After signing the consent form, the participants were considered for the first visit for data collection and informed the next visits timetable.

3.6.1. Demographic and Clinical Data

Information on age, sex, cancer type, chemotherapy regimen, clinical symptoms' data related to CIPN were collected using questionnaire (**Appendix 2**).

CIPN severity assessment

CIPN was assessed at baseline and with each drug cycle visit. The EORTC QLQ-CIPN20 which is a standardized tool for identifying, describing and grading adverse events related to cancer, will be used to grade sensory neuropathy(Rattanakrong et al., 2022).

From developed standardized questionnaire, the questions placed in order to accurately assess CIPN caused by paclitaxel among breast, cervical and ovarian cancer patients: and then findings of the symptoms were scored as not at all **o** score, a little with **1** score, quit a bit **2** scores, very much with **3** scores. The symptoms assessed through series of 18 questions from 20 of the EORTC QLQ-CIPN20 scale(Yeo et al., 2019).

"Did you have tingling fingers or hands? Did you have tingling toes or feet? Did you have numbness in your fingers or hands? Did you have numbness in your toes or feet? Did you have shooting or burning pain in your fingers or hands? Did you have shooting or burning pain in your toes or feet? Did you have cramps in your hands? Did you have cramps in your feet? Did you have problems standing or walking because of difficulty feeling the ground under your feet? Did you have difficulty distinguishing between hot and cold water? Did you have a problem holding a pen, which made writing difficult? Did you have difficulty manipulating small objects with your fingers (for example, fastening small buttons)? Did you have difficulty opening a jar or bottle because of weakness in your

hands? Did you have difficulty walking because your feet dropped downwards? Did you have difficulty climbing stairs or getting up out of a chair because of weakness in your legs? Were you dizzy when standing up from a sitting or lying position? Did you have blurred vision? Did you have difficulty hearing?"(Rattanakrong et al., 2022)

And this CIPN severity assessment was done at the beginning of chemotherapy throughout 4 visits during chemotherapy uptake; the grades of severity ranged 18-30 as mild, 31-42 moderate and 43-54 as severe CIPN.

The records of 90 participants of the overall scores were recorded, 86 participants were able to undergo 4 visits, and genotyping of the 86 samples was carried out.

3.6.2. Sample collection

86 DNA samples were collected from participants after 4^{th} visit, whole blood collected in EDTA tubes and were subjected to DNA extraction and then genotyping.

3.6.3. DNA extraction

During DNA extraction, the QIAamp Mini Kit was used to manual extracting the DNA. Manually to have a good control of each step of DNA extraction and to maximize the yield. So, Qiagen Mini kit is validated for whole blood and was employed in the study.

Procedure followed for manual DNA extraction

In this study, DNA was extracted from blood samples, following the manufacturer's protocol. Briefly, 20µl of QIAGEN protease (or proteinase K) was first pipetted into the bottom of a 1.5 ml microcentrifuge tube. A 200 µl blood sample was then added to the tube. In cases where the sample volume was less than 200 µl, phosphate-buffered saline (PBS) was added to reach the required volume. Subsequently, 200 µl of Buffer AL was added, and the mixture was thoroughly vortexed for 15 seconds to ensure complete lysis. The tube was incubated at 56 °C for 10 minutes to facilitate cell lysis and the release of nucleic acids.

Following this, 200µl of absolute ethanol (96–100%) was added and mixed again by vortexing for 15 seconds.

The resulting lysate was transferred to a QIAamp Mini spin column placed in a 2 ml collection tube, and centrifuged at approximately 6,000 × g (8,000 rpm) for one minute. The waste debris(flow-through) was discarded, and the column was moved to a new collection tube. For the first washing step, 500 µl of Buffer AW1 was added to the column, followed by centrifugation at the same speed for one minute. After discarding the flow-through, a second wash was performed using 500 µl of Buffer AW2 and centrifugation at 20,000 × g (14,000 rpm) for three minutes to ensure the removal of residual contaminants and salts. To further dry the membrane, an optional additional spin at 14,000 rpm for one minute was performed.

At the end, the column was placed in a clean 1.5 ml microcentrifuge tube for DNA elution. Between 50 and 200 µl of Buffer AE or nuclease-free distilled water was applied directly to the membrane, followed by incubation at room temperature for one minute and centrifugation at 6,000 × g (8,000 rpm) for one minute to elute purified DNA. The extracted DNA was then stored at −20 °C until further analysis by genotyping had to be done.

3.6.4. Genotyping

We employed the Global screening Array (GSA); a high-throughput microarray based genotyping platform widely used for comprehensive genome-wide analysis of single nucleotide polymorphisms (SNPs), insertion-deletion polymorphisms, and copy number variants in human DNA. The array is built upon Illumina's Infinium assay chemistry and bead chip technology, offering robust and reproducible performance across diverse populations and genetic loci(Illumina, n.d.).

Following amplification, the DNA was enzymatically fragmented into uniform pieces of optimal size using a proprietary fragmentation reagent. The fragmented DNA was then

precipitated using alcohol-based precipitation, washed, and resuspended in a proprietary hybridization buffer. The prepared DNA was dispensed onto the Infinium GSA BeadChip and hybridized overnight at 48°C in the Illumina hybridization oven. During this process, the fragmented DNA anneals to its complementary oligonucleotide probes immobilized on the silica beads distributed across the array surface.

Post-hybridization, unbound and non-specifically bound DNA was removed through a series of stringent wash steps. The beadchip was then subjected to single-base extension (SBE), a process in which DNA polymerase incorporates one fluorescently labeled nucleotide at each hybridized locus. The incorporated nucleotide is specific to the base present at the SNP position, allowing allele discrimination. Each of the four nucleotides carries a distinct fluorescent dye, enabling detection of homozygous and heterozygous genotypes.

After the extension and staining reactions, the bead chips were scanned using the Illumina iScan system, which excites and detects the fluorescent signals emitted from the labeled nucleotides at each bead location. The iScan system generates high-resolution images of the beadchip, and the signal intensity data were converted into genotype calls using Illumina's GenomeStudio software. The software algorithm assesses signal intensity ratios to determine genotype classifications (AA, AT, or TT) at each of the genomic loci represented on the array. The resulting genotype data were subjected to further analysis for association with CIPN phenotype.

3.7. Reliability and Validity

The methods of data collection were valid and reliable for instance; EORTC QLQ-CIPN20 is the common method used in assessing the severity of CIPN through questions addressing the symptoms of the neuropathy, has been validated and reliable for several studies(Rattanakrong et al., 2022)(Yeo et al., 2019). And the GSA genotyping technology used is validated with consistent studies reporting 99.9% concordance between GSA genotypes and high confidence reference datasets for SNP detection(Russell et al., 2022).

3.8. Data analysis

Data were analyzed using IBM SPSS Statistics Version 25, where the analysis involved descriptive and inferential statistics, with the primary model of interest being the Linear Mixed Model (LMM) to report repeated measures of CIPN symptom severity over time on multiple visits to the same patients, while accounting for both fixed effects of genotype and time.

3.9. Limitations

The sample size was not fully achieved, some participants failed to complete 4 visits due to reachability barriers. Difficulty in completing the participants in the same period ranges also delayed data collection period.

3.10. Ethical consideration

Ethical approval was obtained from the Institutional Review Board (IRB). Informed consent was secured from all participants, ensuring confidentiality and voluntary participation.

Chapter 4. Results

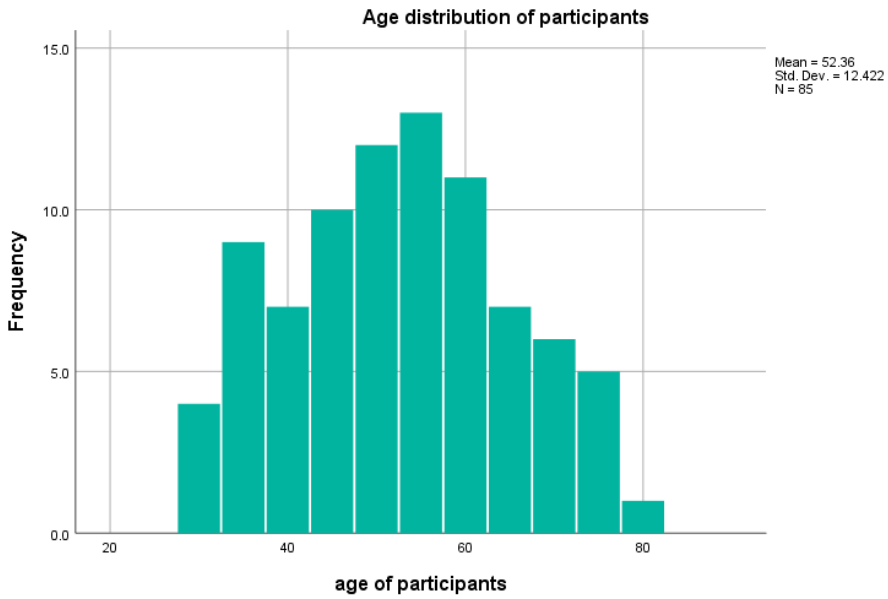
4.0. Introduction

To assess the correlation between chemotherapy induced peripheral neuropathy severity and the enzyme CYP3A4 variations in cancer patients taking paclitaxel in Rwanda; the total of the 90 participants were enrolled in the study at Butaro Cancer facility, but 86 of them completed the four (4) visits. The demography and cancer types were assessed, the data on CIPN severity were recorded through scores of the symptoms and genotype data recorded and correlation determined using linear mixed models in SPSS, the outputs presented in Tables, figure and graphs.

4.1. Participant Demographics and Baseline Characteristics

Age and sex distribution of participants

Among the participants 82/86 (96.5%) were female and the 3/86(3.5%), and the age ranged 30 to 81 years with mean of 52 ± 12 years.



Graph 1. *Representing the age distribution of the participants*

Table 1. Sex-Based Demographic Profile of Participants

Sex	Frequency	Percent	Valid Percent	Cumulative Percent
female	83	96.5	96.5	96.5
male	3	3.5	3.5	100.0
Total	86	100.0	100.0	

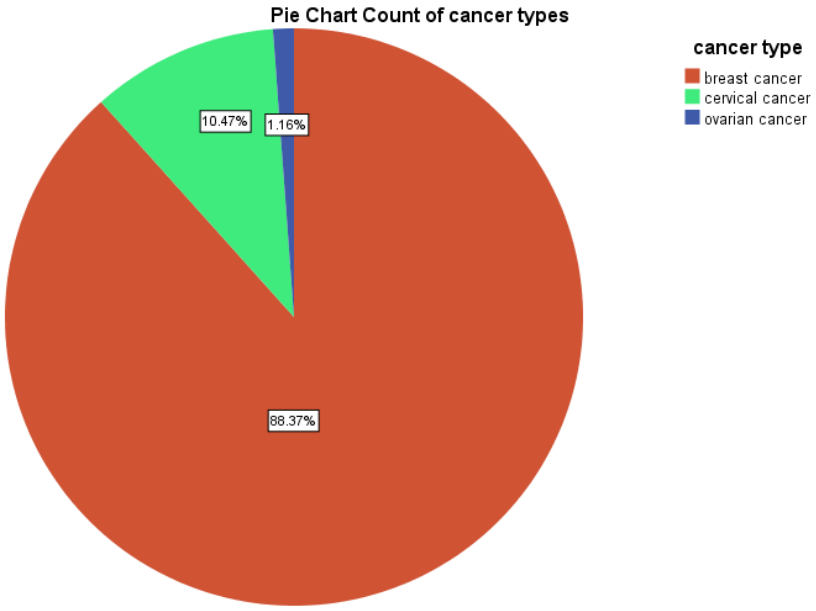


Fig.1: Cancer type distribution in participants

The pie chart showing the cancer type distribution of the participants in the study where breast cancer accounts the 88.37%, followed by cervical and ovarian cancer with 10.47% and 1.16% respectively.

Genotyping results of CYP3A4 variants and their association with CIPN

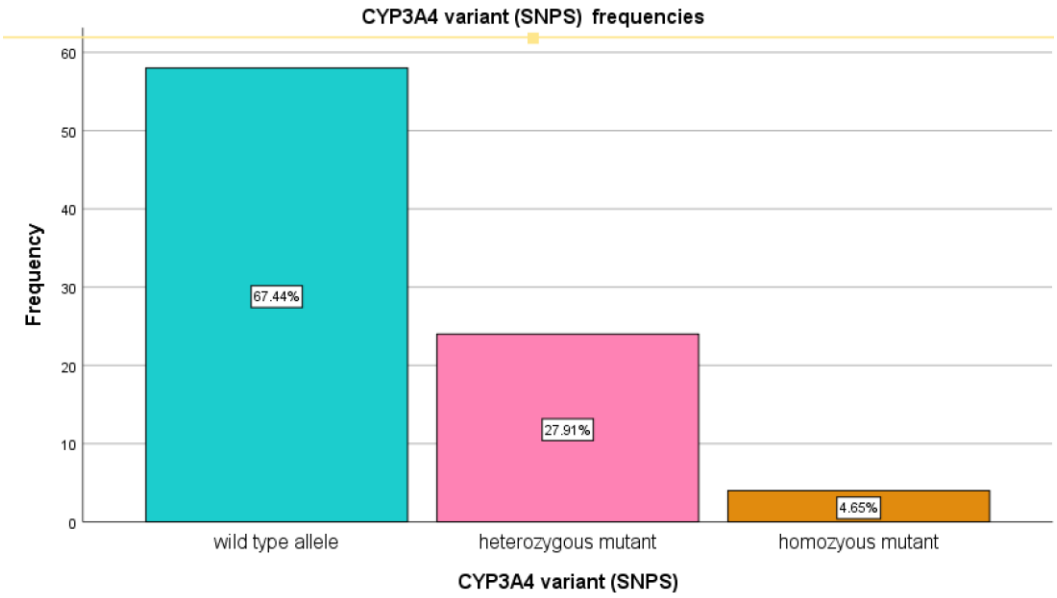


Fig.2: CYP3A4 Variant (SNPS) frequencies

*Wild type allele (**AA**) found to be 67.44%, heterozygous mutant (**AT**) 27.91% while homozygous mutant (**TT**) was 4.65% of the CYP3A4 enzyme*.

After genotyping, the wild-type allele (coded as AA) of the CYP3A4 enzyme was identified in 67.44% of the participants, followed by 27.91% of heterozygous mutants (AT), and 4.65% which were homozygous mutants (TT) for CYP3A4.

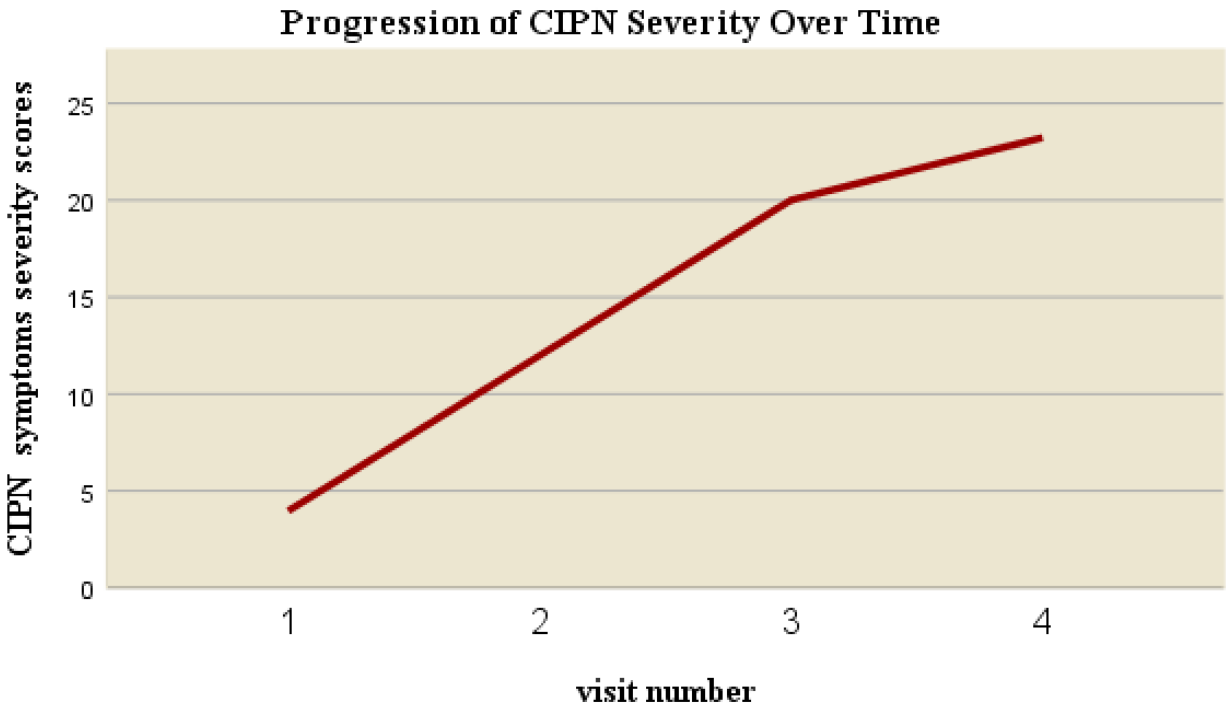


Fig.3: Trend of Chemotherapy-induced peripheral neuropathy symptoms

Illustrates the trend in chemotherapy-induced peripheral neuropathy (CIPN) symptoms severity scores among cancer patients receiving paclitaxel, recorded across four successive clinic visits. The y-axis represents the mean severity scores of CIPN symptoms, while the x-axis denotes the visit number, ranging from the first to the fourth visit.

As illustrated in the fig.3., there is a clear upward trend in CIPN severity over time. The mean symptom severity score was lowest during the first visit, indicating minimal neuropathic symptoms at the early stage of chemotherapy administration. A substantial increase is observed between the first and second visits, suggesting the early onset and progression of peripheral neuropathic effects. This rise continues through the third and fourth visits, although the rate of increase slightly tapers off after the third visit.

This trend aligns with the known dose-dependent neurotoxicity associated with paclitaxel, supporting the temporal relationship between continued drug exposure and worsening of peripheral neuropathy symptoms(Johnson et al., 2023). These findings underscore the

clinical relevance of monitoring CIPN longitudinally and further justify the investigation into potential genetic modulators such as CYP3A4 enzyme variations that might influence individual susceptibility to neuropathy during treatment.

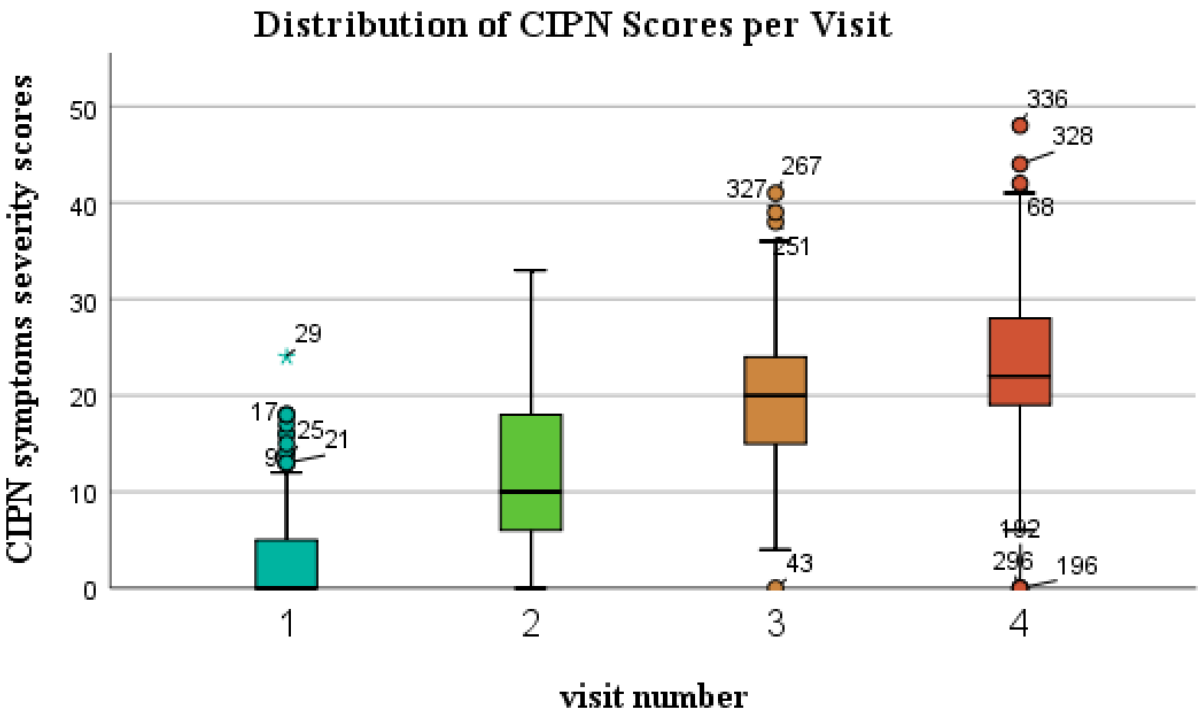


***Figure 4**: Distribution of CIPN Symptom Severity Scores Across Four Visits*

Figure4 presents a boxplot distribution of chemotherapy-induced peripheral neuropathy (CIPN) symptom severity scores recorded at four different clinical visits among cancer patients receiving paclitaxel. The y-axis represents the total CIPN severity scores derived from patient-reported symptoms, while the x-axis displays the sequential visit numbers.

The boxplots illustrate both the central tendency and variability of CIPN scores over time. A clear upward shift in the median scores is evident from Visit 1 through Visit 4, indicating a general increase in neuropathy severity with continued chemotherapy cycles. The interquartile ranges (IQRs) also expand progressively, reflecting growing heterogeneity in patient experiences of CIPN symptoms. At Visit 1, the scores are relatively low with a narrow IQR and minimal outliers. By Visit 2, both the median and spread of scores increase, showing a broader distribution of symptom severity. This trend becomes more pronounced in Visit 3 and Visit 4, where the boxplots demonstrate higher medians, wider IQRs, and the presence of numerous outliers. Suggesting that while the majority of patients experience worsening symptoms, a subset may develop particularly severe neuropathy. Remarkably, several extreme values (outliers) appear at Visits 3 and 4, emphasizing interindividual variability in CIPN progression, possibly influenced by genetic factors such as CYP3A4 enzyme variations. These findings further reinforce the importance of personalized monitoring and pharmacogenomic assessment in managing chemotherapy-induced side effects

Assessment of correlation of CIPN and CYP3A4 variations

A linear mixed-effects model was used to assess the longitudinal association between CIPN severity and CYP3A4 genotypes across four treatment visits. The model included fixed effects for visit number, genotype, and their interaction, with a random intercept for participant ID to account for repeated measures. An AR(1) covariance structure was applied to model within-subject correlations over time.

Type III Tests of Fixed Effects revealed that both "Genotype" (CYP3A4 variation) and "visit" have a statistically significant effect on CIPN scores per visit, as indicated by their p-values (Sig.)($p < 0.05$). The F-statistic for Genotype is 13.792, and for visit is 101.773(**Table2**). While the the estimated coefficients for each level of the fixed effects, Genotype," compared to the "Genotype=2" (wild type allele, which is set to zero as it's

redundant), "Genotype=0" (homozygous mutant) had a statistically significant positive effect on CIPN scores (Estimate = 12.139375, p<.001). "Genotype=1" (heterozygous mutant) did not show a statistically significant difference from the wild type (Estimate = -0.186, p = .889)(**table 3**)

Table 2. *Overall Significance of Fixed Effects (CYP3A4 Genotype and Visit) on CIPN Severity*

Parameters/Source	**Numerator df**	**Denominator df**	**F-factor**	**p-value**
Intercept	1	0.000	471.760	0.000
Genotype	2	112.830	**13.792**	0.000
Visit	3	232.562	101.773	0.000

***Table 3**. How CYP3A4 genotypes and visit time-interval associated with CIPN Severity*

Parameter	Estimate	Std. Error	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Intercept	-2.230921	1.132100	0.050	-4.460916	-0.000927
[Genotype=0]	**12.133284**	2.351123	**0.000**	7.475440	16.791128
[Genotype=1]	-0.186861	1.105808	0.866	-2.377547	2.003824
[Genotype=2]	0[b]	wild type	wild type		
Visit	6.554331	0.387904	**0.000**	5.791023	7.317640

Table3 Shows intercept at baseline level (not significant), homozygous mutants have **12.13** points higher CIPN severity than wild type, No significant difference from wild type for the heterozygous mutant genotype=1 from the reference wild type genotype=2. And for each increasing visit, CIPN increases by 6.55 points on average.

Results show that: Homozygous mutant patients experience significantly more severe CIPN symptoms than wild-type patients. Heterozygous genotype is not significantly different from wild type and CIPN severity worsens over time if you consider the positive visit coefficient.

Table4*.Estimation on CYP3A4 variants associated with the severity of CIPN.*

CYP3A4 variant (SNPS)	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
homozyous mutant	26.417	2.266	0.000	-239.733	292.568
heterozygous mutant	14.124	0.926	0.000	-5.724	33.971
wild type allele	14.278	0.601	0.000	9.363	19.193

This table 4 provides the estimated marginal means of CIPN scores for each CYP3A4 variant. The homozygous mutant has the highest mean CIPN score (26.417), followed by the wild type allele (14.278) and heterozygous mutant (14.124). this reveal CIPN severity differs across the genotype groups.

Chapter 5. Discussion

The study investigated the correlation between chemotherapy-induced peripheral neuropathy (CIPN) severity and variations in the CYP3A4 enzyme among cancer patients undergoing paclitaxel-based chemotherapy in Rwanda. The findings provided both clinical and pharmacogenomic insights into the progression of CIPN and highlight the potential genetic factors that may contribute to individual differences in susceptibility to this distressing side effect. In enrolled 90 cancer patients at Butaro Cancer facility, with 86 completing all planned clinical visits. The cohort was predominantly female (96.5%), with ages spanning 30–81 years (mean 52±12). Most participants had breast cancer (88.37%), while cervical (10.47%) and ovarian cancers (1.16%) were less common.

CIPN Progression and Symptom Burden Over Time

While looking the CIPN symptoms scores along the 4visits; there was significant increase in this study over the four treatment visits. Both the mean symptom scores and the variability in patients' experiences rose steadily with continued chemotherapy exposure. The boxplot trends revealed not only a shift toward higher symptom scores but also a widening spread of data over time, reflecting growing heterogeneity among patients. This progressive pattern aligns with earlier studies showing that CIPN develops in a dose-dependent manner and typically worsens with each additional cycle of taxane-based chemotherapy. For instance, Hershman et al. (2014)(Hershman et al., 2014) reported that the incidence and severity of peripheral neuropathy increase notably after three or more cycles of paclitaxel, leading in some cases to dose reduction or treatment discontinuation. Similarly, Čermák V. et al in 2020 emphasized that repeated exposure to microtubule-targeting agents like paclitaxel results in cumulative damage to peripheral nerves(Čermák et al., 2020). The current findings reinforce the importance of longitudinal monitoring of neuropathy symptoms throughout chemotherapy, particularly in settings where resource constraints may delay symptom recognition and management. They also highlight a need to better understand which patients are more vulnerable to severe or early-onset CIPN.

CYP3A4 genetic variants and their association with CIPN Severity

The analysis revealed that CIPN severity was not uniform across all patients, but was significantly influenced by their CYP3A4 genotype. Patients who were homozygous for the mutant allele (TT) experienced significantly higher CIPN symptom scores($p<0.001$) compared to those with the wild-type allele (AA), even after adjusting for repeated measures across visits. In contrast, heterozygous individuals (AT) showed no significant difference from the wild-type group, suggesting a recessive or threshold-based genetic effect. These results are biological reasonable, The CYP3A4 enzyme plays a central role in the hepatic metabolism of paclitaxel. Genetic polymorphisms that reduce CYP3A4 activity may impair paclitaxel clearance, leading to increased systemic exposure and, consequently, a higher risk of neurotoxicity(Klyushova et al., 2022). Previous pharmacogenomic studies, such as those by Hertz et al. (2013), have highlighted associations between paclitaxel clearance rates and CYP3A family variants, although the specific contribution of CYP3A4 polymorphisms remains an area of ongoing investigation(Hertz & McLeod, 2013).

Moreover, the work by Chan A et al. (2019) emphasized that interindividual variability in CIPN is often linked to genetic variants in drug-metabolizing enzymes and transporters, reinforcing the current study's focus on CYP3A4(Chan et al., 2019). The absence of a significant effect in heterozygotes may indicate that a single functional allele is sufficient to maintain adequate metabolic activity under typical dosing conditions, consistent with findings from pharmacokinetic modeling studies(De Graan et al., 2013)

The observed genotype-dependent differences in CIPN severity carry important clinical implications. In low-resource settings such as Rwanda, where genetic screening is not yet routine, patients at high genetic risk for drug-related toxicities may be particularly vulnerable to preventable complications. Introducing targeted pharmacogenetic screening, even in a pilot or research-based capacity, could enable early identification of high-risk patients and guide more personalized treatment plans

Chapter 6: Conclusions and recommendations

6.1. Conclusion

This study investigated the association between genetic polymorphisms of the CYP3A4 enzyme and the severity of chemotherapy-induced peripheral neuropathy (CIPN) in Rwandan cancer patients undergoing paclitaxel treatment. Utilizing a prospective cohort design, CYP3A4 variants were genotyped, and neuropathy severity was longitudinally assessed using the EORTC QLQ-CIPN20 scale over four chemotherapy visits. The findings demonstrated a statistically significant relationship between CYP3A4 genetic variations and CIPN severity. Specifically, patients carrying the homozygous mutant genotype (TT) indicated markedly higher neuropathic symptom scores compared to those with the wild type genotype (AA), indicating a probable recessive genetic effect. No significant difference in neuropathy severity was observed between heterozygous mutants (AT) and wild-type patients, suggesting that a single functional allele may be sufficient for normal paclitaxel metabolism. Moreover, neuropathy symptoms progressively worsened with repeated chemotherapy cycles, confirming the cumulative dose-dependent neurotoxic effect of paclitaxel.

This study supports the pivotal role of CYP3A4 as a key enzyme responsible for paclitaxel metabolism and highlights how genetic variations resulting in reduced enzymatic activity may increase systemic drug exposure and consequent neurotoxicity risk. Above all, this research is among the first to document such pharmacogenomic associations studies in Rwanda, highlighting a critical data gap for African populations to be represented in genomic databases.

Given the increasing cancer burden and expanded adoption of paclitaxel chemotherapy in Rwanda(Bray et al., 2024), these results have clinical and public health significance.

Understanding the impact of CYP3A4 polymorphisms on treatment toxicity can facilitate the advancement of precision oncology in the region, promoting safer and more effective chemotherapy management tailored to patients' genetic profiles. Moreover, the findings underscore the necessity of integrating pharmacogenomic insights into national cancer control strategies to improve patient quality of life and treatment adherence.

6.2. Recommendations

Referring to the findings the recommendation include; further research across diverse African populations and balancing gender, to clearly understand and document the contribution of CYP3A4 variants in development of CIPN. Also, healthcare providers should adopt systematic longitudinal neuropathy assessments throughout the chemotherapy course, with clinical attention after the second cycle when symptom escalation is most pronounced. Enhanced early detection facilitates timely symptom management, dose adjustments, and referrals to supportive care services aimed at preserving patient functional status and quality of life. Furthermore, to incorporate CYP3A4 genetic screening into oncology care services for patients scheduled to receive paclitaxel.

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Appendices

2. Raw data

		Partipants Demo,Visit symptoms scores and genotype data									
No	**PID**	**sex**	**age**	**cancer type**	**visit 1**	**visit 2**	**visit 3**	**visist4**	**Average-visits scores**	**Genotype**	
1	BU-040	female	58	breast cancer	4	13	14	19	12.5	1	**AA=1**
2	BU-041	female	58	breast cancer	11	33	15	19	19.5	1	**AT=2**
3	BU-042	female	73	breast cancer	16	24	20	19	19.8	2	**TT=0**
4	BU-043	female	52	breast cancer	12	33	16	25	21.5	1	
5	BU-045	male	70	breast cancer	16	21	28	35	25.0	2	
6	BU-046	female	54	breast cancer	13	4	21	27	16.3	1	
7	BU-047	female	58	breast cancer	13	28	9	18	17.0	1	
8	BU-049	female	49	ovarian cancer	24	16	31	35	26.5	2	
9	BU-052	female	55	breast cancer	14	22	31	36	25.8	1	
10	BU-054	female	57	breast cancer	18	2	14	22	14.0	2	
11	BU-055	female	55	breast cancer	17	7	0	21	11.3	2	
12	BU-057	female	68	breast cancer	14	10	25	35	21.0	2	
13	BU-058	female	58	breast cancer	0	8	24	41	18.3	2	
14	BU-059	female	64	breast cancer	8	12	20	26	16.5	1	
15	BU-060	female	48	breast cancer	2	27	20	27	19.0	2	
16	BU-062	female	49	breast cancer	10	8	24	-	14.0	2	
17	BU-063	female	42	breast cancer	3	31	36	42	28.0	0	
18	BU-066	female	51	breast cancer	15	4	11	25	13.8	1	
19	BU-067	female	37	breast cancer	10	19	14	12	13.8	2	
20	BU-068	female		breast cancer	13	17	25	33	22.0	2	

21	BU-071	female	54	breast cancer	18	18	36	18	22.5	2	
22	BU-072	female	55	breast cancer	18	15	24	35	23.0	2	
23	BU-073	female	63	breast cancer	12	15	28	36	22.8	2	
24	BU-074	female	69	breast cancer	0	8	23	21	13.0	2	
25	BU-075	female	40	breast cancer	0	0	7	6	3.3	1	
26	BU-076	female	50	breast cancer	2	24	13	22	15.3	2	
27	BU-077	female	45	breast cancer	12	24	21	-	19.0	2	
28	BU-078	male	74	breast cancer	0	2	5	26	8.3	2	
29	BU-079	male	74	breast cancer	10	12	22	8	13.0	1	
30	BU-080	female	39	breast cancer	0	12	14	21	11.8	2	
31	BU-081	female	33	breast cancer	2	10	32	20	16.0	1	
32	BU-082	female	38	breast cancer	0	3	20	29	13.0	1	
33	BU-083	female	52	breast cancer	3	20	21	33	19.3	2	
34	BU-084	female	81	breast cancer	3	3	25	13	11.0	2	
35	BU-085	female	58	cervical cancer	0	8	21	-	9.7	2	
36	BU-086	female	44	breast cancer	1	10	21	-	10.7	2	
37	BU-087	female	30	breast cancer	0	2	4	6	3.0	2	
38	BU-088	female	44	breast cancer	2	4	18	22	11.5	1	
39	BU-089	female	48	breast cancer	0	6	26	19	12.8	2	
40	BU-090	female	48	breast cancer	3	2	22	30	14.3	1	
41	BU-091	female	45	breast cancer	0	18	21	32	17.8	2	
42	BU-092	female	44	breast cancer	0	3	17	22	10.5	2	
43	BU-093	female	60	breast cancer	0	6	27	16	12.3	2	
44	BU-094	female	53	breast cancer	2	17	21	21	15.3	2	

45	BU-095	female	36	breast cancer	4	14	13	22	13.3	2	
46	BU-096	female	51	breast cancer	0	6	12	19	9.3	1	
47	BU-097	female	71	breast cancer	0	7	14	13	8.5	1	
48	BU-098	female	62	cervical cancer	0	15	20	0	8.8	2	
49	BU-099	female	62	cervical cancer	0	16	32	0	12.0	1	
50	BU-100	female	64	breast cancer	0	5	11	17	8.3	2	
51	BU-101	female	47	breast cancer	0	0	28	9	9.3	2	
52	BU-102	female	65	breast cancer	0	21	20	10	12.8	2	
53	BU-103	female	44	breast cancer	0	20	26	25	17.8	1	
54	BU-104	female	37	breast cancer	0	6	21	32	14.8	2	
55	BU-105	female	53	breast cancer	0	6	21	18	11.3	2	
56	BU-106	female	68	breast cancer	0	9	14	32	13.8	2	
57	BU-107	female	65	cervical cancer	5	9	9	22	11.3	2	
58	BU-108	female	53	breast cancer	2	10	20	22	13.5	2	
59	BU-109	female	34	breast cancer	0	5	21	21	11.8	1	
60	BU-110	female	48	breast cancer	0	7	18	23	12.0	2	
61	BU-111	female	59	breast cancer	0	14	18	24	14.0	2	
62	BU-112	female	62	cervical cancer	0	0	7	20	6.8	2	
63	BU-113	female	52	cervical cancer	0	21	38	23	20.5	2	
64	BU-114	female	55	breast cancer	0	10	20	23	13.3	2	
65	BU-115	female	38	breast cancer	0	9	17	19	11.3	2	
66	BU-116	female	35	breast cancer	0	4	25	14	10.8	2	
67	BU-117	female	46	breast cancer	2	28	41	41	28.0	0	
	BU-118	Female	36	breast cancer							

68	BU-121	female	54	breast cancer	0	0	24	37	15.3	2	
69	BU-123	female	57	cervical cancer	0	10	16	19	11.3	2	
70	BU-124	female	31	breast cancer	0	9	20	26	13.8	2	
71	BU-125	female	37	breast cancer	0	18	7	34	14.8	1	
72	BU-126	female	57	breast cancer	0	13	17	24	13.5	2	
73	BU-127	female	43	breast cancer	0	8	13	0	5.3	2	
74	BU-128	female	62	breast cancer	0	10	19	24	13.3	2	
75	BU-129	female	40	cervical cancer	0	7	13	22	10.5	2	
76	BU-130	female	72	breast cancer	0	18	16	27	15.3	1	
77	BU-131	female	74	breast cancer	4	13	20	19	14.0	1	
78	BU-132	female	74	breast cancer	1	5	24	28	14.5	2	
79	BU-133	female	63	breast cancer	0	19	21	21	15.3	2	
80	BU-134	female	65	breast cancer	0	6	16	19	10.3	2	
81	BU-136	female	32	breast cancer	2	27	39	44	28.0	0	
82	BU-137	female	45	breast cancer	0	4	24	25	13.3	1	
83	BU-138	female	30	breast cancer	0	15	30	48	23.3	0	
84	BU-139	female	42	cervical cancer	0	0	15	19	8.5	1	
85	BU-140	female	33	breast cancer	0	8	15	20	10.8	2	

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NATIONAL ETHICS COMMITTEE / COMITE NATIONAL D'ETHIQUE

E-mail: info@rnecrwanda.org
Web site: www.rnecrwanda.org

Ministry of Health
P.O. Box. 84
Kigali, Rwanda.

FWA Assurance No. 00001973
IRB 00001497 of IORG0001100

13 November 2024.

Principal Investigator: Sofia Birgersson (SB), PhD
Study Coordinator: Rugamba Alexis (AR), PhD student

ANNUAL RENEWAL APPROVAL NOTICE: NO.605/RNEC/2024.

Study Title: **"Impact of genetic variation in metabolic enzymes on paclitaxel treatment and clinical outcome in cancer patients in Rwanda"**

After review of the protocol and progress report during the RNEC meeting of 26th October 2024 where quorum was met the **requested annual renewal was approved.**

Please note that approval of the protocol and consent form both English and Kinyarwanda version is valid for **12 months**.

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrollment of participant
3. All consent forms signed by subjects should be retained on file. The RNEC may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the RNEC in a timely fashion and before expiry of this approval.
5. Failure to submit a continuing review application will result in termination of the study.
6. Notify the Rwanda National Ethics committee once the study is completed.

Sincerely,

Date of Approval: 26th October 2024

Expiration date: 25th October 2025

Dr. Vedaste NDAHINDWA
Chairperson, Rwanda National Research Ethics Committee.

C.C.
- Hon. Minister of Health.
- The Permanent Secretary, Ministry of Health

RWANDA NATIONAL ETHICS COMMITTEE R.N.E.C P.O BOX:84 KIGALI

The Rwanda National Research Ethics Committee, (RNEC), was established by Law N°. 015/2022 of 29/06/2022 Relating to Research on a Human Being in its Article 4.